EFFECT OF VARYING CONCENTRATIONS OF PARTIALLY DEHULLED AND EXTRUDED SUNFLOWER-MEAL ON GROWTH PERFORMANCE AND SENSORY ATTRIBUTES OF POST-JUVENILE ATLANTIC SALMON (*Salmo salar*)

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
(FOOD SCIENCE)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
February 2002

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ABSTRACT

Commercially available sunflower meal (SFM) was subjected to extrusion processing and then tail-end dehulling (using multiple vibrating screens) to nutritionally upgrade the protein content to 40.9% on a dry weight basis (db) and improve its digestible energy content. A basal and four test diets were formulated by replacing fishmeal protein in the dietary mix with increasing equal increments of nutritionally upgraded SFM (maximum dietary concentration 27%; db). The resulting five diets (~49% protein and ~18.5% lipid; db) were fed to fifteen groups of post-juvenile Atlantic salmon (n=50/group; 3 groups/diet) over an 84-day period. The performance of Atlantic salmon (initial mean weight ~ 116 g) was judged by the following parameters: weight gain, specific growth rate, feed intake, feed efficiency, protein efficiency ratio, percent protein deposited, gross energy utilization, and percent survival. Also, proximate analyses of both whole bodies and muscle samples (fillets) were undertaken on salmon from day 0 and 84. Atlantic salmon fed the basal diet (0% SFM) and the four test diets (contained SFM) did not differ significantly ($p > 0.05$) with respect to any of the preceding performance parameters.

To determine the effect of using SFM in the diet on the flesh quality attributes of Atlantic salmon, sensory evaluation (14 panelists) was conducted over 9 sessions (after 4 one-hour training sessions) to test salmon quality attributes of aroma, flavor, foreign flavor and texture, using a descriptive sensory ballot. Sensory panel results indicated that salmon – aroma, flavor and texture were similar ($p > 0.05$) across the five diets. However, fillets from salmon fed diets A (0% SFM) and E (27.1% SFM) and diets B (6.8% SFM) and E were significantly ($p < 0.05$) different, in perceptible foreign flavors, respectively. Salmon fillet color, quantified using Salmofan™ and Hunter Lab *L*, *a* and *b* scores, did not differ ($p > 0.05$) across the diets. In addition, Salmofan™ scores were positively correlated ($r = 0.86$) with Hunter (a) scores. Headspace gas chromatography (GC) of volatile compounds collected with solid phase micro-extraction fibers (SPME) was conducted using cooked salmon fillets. Results from GC were analyzed by the principal component similarity (PCS) technique and they further corroborated those obtained from foreign flavor analysis by a sensory panel.

In conclusion, the incorporation of ≤ 27.1% of SFM in the basal diet by partial replacement of premium quality fishmeal resulted in no adverse effects on growth of Atlantic salmon reared in seawater. But foreign flavors perceived by panelists were significant ($p < 0.05$) in the fillets from salmon fed diet E (27.1% SFM). Hence, the highest dietary SFM concentration used in the present study appeared to contribute towards some foreign flavors in the flesh, but further research is needed to confirm and possibly extend this finding if higher dietary concentrations of SFM are employed.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF APPENDICES

ACKNOWLEDGEMENTS

This dissertation is not the work of one person. Many people have contributed so generously and helped compile it and I wish to express my sincere gratitude to them all.

To Dr. B.J. Skura, Professor at Food Science, U.B.C. and my supervisor, my heartfelt gratitude for his invaluable advice and counsel. My many thanks for his timely feedback towards avoiding potential problems during the course of my work.

To Dr. D.A. Higgs, my co-supervisor and Professor at Department of Fisheries and Oceans Canada at West Vancouver Laboratory, my special thanks for providing me with the necessary research facilities. His wise counsel and positive enthusiasm along with loads and loads of encouragement has greatly helped bring this project to fruition. Thanks to him for having the patience of a saint while being confronted with a barrage of questions from my side.

To Mahmoud Rowshandeli, I am indebted for his day to day assistance, practical advice and his sustained commitment to guide me; which helped me immensely each step of the way.

To my friends and collegues at D.F.O., West Vancouver Laboratory – Nahid, Joanna, Chris, Nancy and Janice, my thanks for welcoming me into their circle and extending their cooperation, help and support. A very special thanks to Allison whose cheerful countenance and invaluable assistance helped maintain the momentum of my work.

To all my friends at Food Science, U.B.C. – Mareike, Parastoo, Judy, Ranjani, Hiro, Grace, just to name a few, my heartfelt thanks for their enthusiastic participation in my sensory panel and giving me unconditional support (especially during trying times) at every step of the way. Special thanks to Brenda, Sherman and Val for their quick and timely technical assistance whenever I got stuck and felt that the end of world was at hand. Thanks a lot! A very special thanks to Dr. S. Nakai and Sooyeun for helping me wholeheartedly with PCS and GC analysis.

My cordial thanks to Mr. Bakhshish Dosanjh and Mr. Jason Mann from E.W.O.S. Canada Ltd., Surrey for providing me with the opportunity to work on this fascinating project.

Navneet Gill

CHAPTER 1

GENERAL INTRODUCTION

On a global scale, farmed salmon is increasingly becoming an important source of high quality food for fish consumers. This holds especially true for aquaculture in BC because the seasonal availability of wild salmon is unable to keep pace with the rising demand for salmon and salmon related products.

Salmon require diets that are high in protein concentration since they depend on proteins to meet a portion of their daily energy requirements and they are unable to utilize high dietary levels of digestible carbohydrate. Until recently, fishmeal was used extensively as the main dietary source of protein in the feed for cultured salmon. But this resulted in a significant cost, as premium quality fishmeal is an expensive commodity. Hence, considerable resources have been expended to evaluate various alternative sources of animal and plant protein. In regard to the latter, oilseed protein sources are readily available as inexpensive meals which have been used to partially (meals) or completely (some concentrates) replace fishmeal in diets for farmed salmon.

Lately, the potential of using sunflower meal has been investigated. Sunflower meal (SFM) is an inexpensive protein and fiber-rich by-product from the sunflower oil processing industry. Despite the wide-scale availability of SFM, its high fiber content has discouraged its extensive use in monogastric diets. Therefore, SFM has to be processed further before it can be incorporated into the diets for carnivorous fish species. Tail- and front-end dehulling procedures partially decrease the high fiber content of SFM, and thus they potentially can increase the extent to which the protein source can be included in diets for carnivorous fish. In the present study, an attempt was made to nutritionally upgrade SFM before its incorporation into the diets for Atlantic salmon. Thus Atlantic salmon in seawater were fed diets containing graded concentrations of fiber-reduced and extruded SFM by replacement of premium quality fishmeal in the basal diet.

Thereafter, fish growth, feed intake and efficiency as well as protein and energy utilization, survival and body composition were evaluated in relation to diet treatment over an 84-day period. The fiber-reduced SFM was subjected to high temperature extrusion processing, before its dietary incorporation, to simulate the conditions that it

would be subjected to, if the diets were processed by extrusion processing (the diets in this study were steam pelleted). It was also of interest in this study to determine the effect of dietary SFM concentration on the quality attributes of Atlantic salmon fillets. Therefore, the present study had two major objectives:

The first objective was to determine the acceptable dietary concentration of pre-extruded and fiber-reduced SFM for farmed Atlantic salmon. Previously, no study had assessed the nutritive value of SFM for Atlantic salmon owing to the high fiber concentration in this oilseed-protein source. Hence, it was hoped that the implementation of a simple and economical tail-end dehulling procedure would adequately nutritionally upgrade SFM so that it could comprise a major source of dietary protein for Atlantic salmon with attendant reduction in diet cost. If this approach proved to be successful, as opposed to a procedure that would necessitate a radical change of machinery and/or processing operations in the sunflower crushing plant, then it would be easy to use the existing seed processing technology.

The second major objective was undertaken to determine whether a trained sensory panel could detect differences in the sensory attributes of the fillets obtained from Atlantic salmon fed the basal diet (fishmeal based, no SFM) or one of four test-diets (SFM ~ 7 to 27% of dry diet) in the feeding trial. Attributes like aroma, flavor, foreign flavor and texture were judged using descriptive sensory analysis. Independent measurements of color using instrumental and visual means before and after frozen storage of salmon fillets also helped to determine whether there were any possible changes in color of the fillets due to time-temperature effects during frozen storage.

In addition to the above, headspace gas chromatography (GC) was also conducted on the fillets from Atlantic salmon given the preceding dietary treatments to determine if there was any 'pattern similarity' of the chromatograms across the diets. In this regard, the data were processed using the PCS (principal component similarity) technique to evaluate the effectiveness of GC to distinguish between the

patterns of volatile compounds emanating from the fillets of Atlantic salmon previously fed different diets.

Collectively it was hoped that all of the information gathered from this study would lead to more cost effective salmon culture and to further studies aimed at assessing the potential for including sunflower protein products in the diets of Atlantic salmon.

CHAPTER 2

REVIEW OF LITERATURE

(2.1) FISH NUTRITION

2.1.1 Energy

All life processes in fish, from physical activity to metabolic reactions and growth, require energy (New, 1997; Tacon, 1997). The energy requirements of poikilotherms are in general about 10-30 times lower than those of mammals of equivalent weight (Brett and Groves, 1979). This is because salmonids have an efficient excretory system and do not expend energy in converting ammonia to another form like urea or uric acid before excretion. Also, salmonids do not need to maintain a constant internal body temperature. Hence, considerable energy is saved, simply by allowing the body temperature to equate with the environmental temperature.

Routine metabolism, or the energy expended by the fish during unrestricted spontaneous activity (Brett, 1995), is affected by water temperature and fish size. Water temperature is very important because, salmonids being poikilotherms, vary their metabolic rate in direct relationship with the prevailing temperature. By contrast, the metabolic rates of salmonids per unit weight decrease as the fish increase in size. In relation to the foregoing energy expenditures, it is important that formulated diets provide sufficient bioavailable or digestible energy (DE) to meet not only the needs of salmon for maintenance and voluntary activity, but also the requirement to achieve their maximum growth potential at different stages of their life history when they are held under different environmental conditions (Higgs *et al.*, 1995b). There is also a need to avoid a deficiency or excess of dietary non-protein energy (lipid and carbohydrate) or in other words to maintain an optimal balance between digestible protein and energy in the diet when the fish are fed to satiation.

In general, salmonids are more efficient in utilizing their feed than terrestrial animals. According to Asgard and Austreng (1995), salmonids retain 30% of the gross energy from their feed as compared to 16% for pigs and 12% for chickens. Salmonids also retain 30% or more of dietary protein whereas comparable values for chickens and pigs are

about 18% and 13%, respectively. In salmonids, energy is mainly derived from the metabolism of lipids and proteins whereas carbohydrates play a comparatively smaller role in this regard. Dietary energy first satisfies the routine maintenance of metabolic activities before being used for growth. A deficiency of dietary non-protein energy will decrease fish growth due to enhanced use of proteins (amino acids) for maintenance metabolism in preference to growth (Cho *et al.*, 1985).

An excess of dietary DE in relation to protein energy may depress growth if protein intake is sub-optimal for maximum growth. The DE content of formulated diets for juvenile and post-juvenile Atlantic salmon, immediately following transfer to seawater, and *Oncorhynchus* species should range from 15-19.6 MJ/kg diet (NRC, 1993; Higgs *et al.*, 1995b). Higher dietary levels of DE than 19.6 MJ/kg appear to be required by Atlantic salmon throughout most of their seawater residency period for maximum growth and feed efficiency. Hence, the energy requirements of salmonids change in relation to the life history stage of the fish and the prevailing environmental conditions.

2.1.2 Proteins

2.1.2.1 Protein requirements

Proteins provide amino acids, which are the building blocks used for tissue repair and synthesis of new tissue. Moreover, amino acids may be used as an energy source and for gluconeogenesis (carbohydrate production) when the dietary intake of carbohydrate is low. Proteins along with lipids are the main dietary energy sources for salmonids, because salmonids preferentially catabolize amino acids and fatty acids rather than glucose to meet their energy needs (Cho and Kaushik, 1985; Luquet and Watanabe, 1986). Since proteins are a major dietary energy source for salmonids, the dietary protein requirements are usually expressed relative to the dietary DE content rather than gross dietary energy content or on a relative or percentage of diet basis (McCallum, 1985).

Salmonid growth can be compromised by a variety of factors like inadequate dietary energy, excess ratio of dietary protein to energy or excessive lipid in the diet (Higgs *et al.*, 1995b). Thus for optimal growth and protein utilization in salmonids, the amount of digestible protein must be in balance with the amounts of other dietary energy-yielding nutrients i.e., lipids and carbohydrates as well as the overall DE content of the diet (Higgs *et al.*, 1995b). Atlantic salmon and *Oncorhynchus* species require an estimated 35-47% digestible protein (dry weight basis) in their diet for optimal growth (NRC, 1993; Storebakken and Austreng, 1987; Higgs *et al.*, 1995b) if the preceding conditions are met. In addition to life history stage, water salinity is known to influence the dietary protein requirement on a relative basis. Protein requirements when expressed as digestible protein (DP) in relation to DE are usually in the range of 21-26 g/MJ for all species of *Oncorhynchus*; this is similar to the range of DP over DE observed in the prey taxa for *Oncorhynchus* species (NRC, 1993; Higgs *et al.*, 1995b).

It is very important that the dietary protein be highly digestible and that it provide an optimal balance of amino acids (see 2.1.2.2 below) relative to the needs of salmon. This minimizes waste nitrogen excretion and the amount of expensive protein that is needed from imported premium quality fishmeal and other protein sources, of animal and plant origin, in the diet to meet the protein requirements of the salmon for growth. Protein efficiency ratio (PER; weight gain/protein intake) is often used in many studies to indicate the overall efficiency of the fish in utilizing dietary protein for growth. For rainbow trout ingesting commercial feeds, PER usually ranges from 1.2 to 2.2 (Pfeffer, 1982) and generally the range for salmon species extends from 2.4 to 2.6 (Anderson *et al.*, 1997).

2.1.2.2 Protein quality

Besides the amount of dietary protein, protein quality is also an important factor to consider for economical use of dietary protein. Protein quality refers to how well the available levels and proportions of amino acids from a protein source agree with species requirements. Fish require 10 amino acids, called essential or indispensable amino acids

(EAA), which cannot be synthesized (or synthesized in adequate quantities) to support their maximum rates of growth (Cowey, 1994). The EAA for fish include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Appendix A, Table 6). Tyrosine has a sparing effect on phenylalanine, while cystine in fish diets spares methionine (NRC, 1993). Excess methionine (Ingham and Arme, 1977) and a high ratio of leucine to isoleucine (Nose, 1979) depress fish growth.

If the essential amino acids are not in optimal levels or are not in balanced proportions with respect to each other, growth will be compromised even if the overall level of protein in the diet remains satisfactory. An optimal dietary balance between the essential and non-essential amino acids is also important to minimize the use of nitrogen from the essential amino acids to synthesize non-essential amino acids. Different studies (Holm and Walther, 1988; Wilson and Cowey, 1985; Arai, 1981 and Ogino, 1980) indicated that large amounts of free amino acids in the diets for salmonids make the feed inferior to a similar feed having a similar amino acid composition and content in the form of intact proteins. This is because free amino acids are absorbed faster than those obtained from the intact proteins during digestion and the different rates of amino acid uptake could result in an imbalance in the amino acid proportions at the sites of tissue protein synthesis. This, in turn, leads to reduced utilization of dietary protein for elaboration of new tissue (Murai *et al.*, 1987).

The amino acid requirements of fish have been determined frequently under conditions of sub-optimal fish growth (Cowey, 1988). Further, they are known to be influenced by fish size (age), sex, developmental stage, and the reproductive status of the fish. In fact, amino acid levels in the diets for salmonids should reflect the amino acid composition of the whole body in growing salmonids as observed by Wilson and Cowey (1985) in the case of Atlantic salmon and rainbow trout. Although the essential amino acid requirements have been stated in many studies for rainbow trout, chinook and coho salmon (Cowey, 1988), there is only limited information available on the amino acid requirements for Atlantic salmon reared in seawater. A recent study by Berge *et al.* (1998) reported that lysine should be 16-18 g/kg dry diet for growing salmon with an initial weight of ~ 400g.

2.1.3 Lipids

2.1.3.1 Role of different types of lipids

Lipids in the diets of salmonids spare the use of proteins for energy thus enhancing the use of protein for growth. In addition, they act as carriers for carotenoid pigments like astaxanthin and fat-soluble vitamins (vitamins A, E, D and K). In addition, lipids furnish essential fatty acids (EFA) that are required by fish for optimal growth, health, and reproduction. Salmonids obtain most of their dietary non-protein energy from the triacylglycerol (ester of 3 fatty acids and the alcohol - glycerol) fraction of lipids. Triacylglycerol stores in salmonids vary along the fish body and are also influenced by the species and size (age) of the fish as well as by the DE content of the feed consumed, the ratio of DP to DE, and the proportion of protein to lipid present in the diet. Other important factors include water salinity and temperature and a combination of the above-mentioned factors.

Diets for salmonids consist of a high amount of polyunsaturated fatty acids (PUFA), which are the major constituents in the marine oils (menhaden, anchovy, herring etc.) used in dietary formulations for farmed salmonids. PUFA consist of three major series, namely, n-9 (oleic series; parent acid oleic acid, 18:1(n-9)), n-3 (linolenic series; parent acid, linolenic acid, 18:3(n-3)) and n-6 (linoleic series; parent acid, linoleic acid, 18:2(n-6)). Highly unsaturated essential n-3 fatty acids (n-3 HUFAs) include EPA (eicosapentaenoic acid; 20:5 (n-3)) and DHA (docosahexaenoic acid, 22:6 (n-3)) and these must be supplied in the diet in sufficient amounts for optimal fish growth, health, cell and nerve structure and function, reproduction and survival, in addition to providing non-protein energy to the fish. They also act as precursors of eicosanoid compounds, which are essential for regulating many physiological processes in salmonids (March, 1992).

Plant oils like soybean oil, sunflower oil or canola oil are not rich in n-3 fatty acids and they do not contain n-3 HUFAs which is why marine oils must be included in the

diets for salmon (Higgs *et al.*, 1995b; Higgs and Dong, 2000). Genetically modifying the fatty acid composition in sunflower seeds has also given rise to sunflower seeds that have more than 40% linoleic acid content or seeds having less than 5% linoleic acid in the case of high oleic acid varieties (Higgs and Dong, 2000). But sunflower meal (produced after oil-extraction of sunflower seeds) has less than 1% lipid content. Hence, the fatty acid profile in sunflower meal does not significantly influence the lipid composition of diet formulations for cultured salmon.

Nevertheless, care must be taken to assess the sensory properties of raw/cooked fillets from salmon that are fed diets based on sunflower meal because of the possible adverse influence of lipid soluble components that are present in the residual oil of sunflower meal, especially on the odor and flavor of the muscle. Skonberg *et al.* (1993), for instance, reported that replacing herring oil with high oleic acid sunflower oil in diets for rainbow trout and coho salmon resulted in significant sensory differences. In this regard, the fillets from the fish fed the diets with sunflower oil had less fishy aroma than those from fish fed the diet with herring oil. Further, a study conducted by Waagbo *et al.* (1993) on Atlantic salmon that varied the dietary n-3 PUFA content by using different blends of soybean oil, capelin oil and sardine oil, found high rancid flavor, fattiness, juiciness, and taste intensity in the flesh from fish raised on diets with high n-3 PUFA content relative to those from fish fed diets with low n-3 PUFA content.

2.1.3.2 Fatty acid and lipid requirements

Salmonids are incapable of producing (n-6) or (n-3) fatty acids due to the absence of Δ 12 and Δ 15 desaturase enzymes that are required for the synthesis of linoleic acid and linolenic acid, the precursors of the n-6 and n-3 series of fatty acids, respectively (Henderson and Tocher, 1987). Salmonids are thus dependent on the dietary lipid sources to provide the correct levels and types of fatty acids from the preceding families to meet their respective essential fatty acid (EFA) needs. The body fatty acid composition of salmonids as well as that of their prey was used initially as a reference guide for designing the lipid

composition of their feed before there were precise determinations of their EFA requirements. But the exact amount of EFAs required by salmonids has been difficult to determine (Bezard *et al.*, 1994) because it depends on the quality of the lipid source, the ratio of n-3 to n-6 fatty acids in the feed, the stage of animal development and their metabolism.

Studies conducted on different salmonid species like rainbow trout (Castell *et al.*, 1972 a,b,c; Watanabe *et al.*, 1974 a,b), coho salmon (Yu and Sinnhuber, 1979) and chum salmon (Takeuchi et al., 1979) indicate that n-3 fatty acids are more important for normal growth whereas only small amounts of n-6 fatty acids are required for normal physiological functions in the different species. In general, the EFA needs of salmonids in freshwater are satisfied by 18:3(n-3) alone, a combination of 18:3(n-3) and 18:2(n-6) (chum salmon) or by n-3 HUFAs alone. The latter fatty acids may have higher essential fatty acid activity than 18:3(n-3), in the rainbow trout, or equivalent essential fatty acid activity in the case of coho salmon.

Present knowledge suggests that n-3 HUFAs should comprise 10% or more of the dietary lipid content to meet the EFA needs of salmonids in both fresh water and seawater (Higgs and Dong, 2000). Inclusion of at least 10% marine lipid from either marine protein sources (present in the form of residual lipid) and supplemental fish oil in formulated diets for salmonids should be enough to meet the EFA needs of salmonids (Pike *et al.*, 1990). Marine fish species by most estimates require between 1% and 4% (dry diet) of EPA and DHA as well as an optimal balance between levels of DHA and EPA in the diet for maximum growth and survival (Watanabe *et al.*, 1993; Sargent *et al.*, 1997).

At present, little is known specifically about the EFA requirements of Atlantic salmon. Ruyter *et al.* (2000) studied the effects of using either 18:2 (n-6), 18:3 (n-3) or an equal mixture of n-3 HUFAs on fry growth and survival. Their results indicated that n-3 (linolenic series) fatty acids should comprise at least 1% (dry weight) of the diet of Atlantic salmon fry. Moreover, a mixture of 20:5 (n-3) and 22:6 (n-3) in the diet enabled the

fry to grow faster than the fry fed a diet with only 18:3 (n-3). Further, dietary inclusion of 18:2 (n-6) did not have any significant effect on fry performance and the presence of n-3 fatty acids in the diet significantly reduced mortality.

Diets rich in n-6 fatty acids, but containing an adequate amount of n-3 fatty acids, have also been reported to induce severe cardio-myopathy in Atlantic salmon. This has been characterized by thinning of the ventricular muscle and necrosis. This situation has also led to increased levels of arachidonic acid (highly unsaturated member of the n-6 family) in the tissues at the expense of EPA (Bell *et al.*, 1991, 1993). In addition, sub-optimal levels and/or imbalanced ratios of EPA and DHA have been known to impair osmoregulatory function, pineal organ function and membrane function in Atlantic salmon (Bell *et al.*, 1996; Henderson *et al.*, 1996). Moreover, Thompson *et al.* (1996) showed that a low ratio of n-3 fatty acids to n-6 fatty acids in the diet for Atlantic salmon can result in the salmon being less resistant to infections from *Vibrio anguillarum* and *Aeromonas salmonicida*. Thus, the dietary ratio of n-3 to n-6 highly unsaturated fatty acids (EPA, DHA or arachidonic acid) seems important for proper immunological performance in Atlantic salmon and this is likely true in other salmonids as well.

Juvenile salmonids in freshwater generally need 15% or more lipid in their diet for optimum growth and feed efficiency, and Atlantic salmon above 200g appear to require 30-35% lipid in their diet for optimal performance (Helland *et al.*, 1997; Johnson and Hwan, 1991; Hillestad *et al.*, 1998). The majority of the dietary lipid has usually been provided by marine sources unless the cost of fish oil has been excessive owing to reduced global supply such as during El Niño events off coastal South America. During these times, less expensive alternative lipids of animal and plant origin have been used to some extent.

Due to the high content of PUFAs in diets for salmonids, caution must be taken to provide ample amounts of antioxidants in the diets to prevent lipid oxidation. Without adequate dietary levels of antioxidants, lipids can undergo oxidation through a chain reaction mechanism of free radical production, which ultimately can lead to rancidity,

as well as reduced bioavailability of some vitamins and amino acids (Castell, 1979). Natural antioxidants include vitamin E whereas other antioxidants that may be added to salmonid diets include ethoxyquin (santoquin), butylated hydroxytoluene and butylated hydroxyanisole.

2.1.4 Carbohydrates

2.1.4.1 Role and requirements

Carbohydrates, when broken down to glucose, serve as important fuel for the brain, nervous system, gonads, and erythrocytes. The absorption of glucose in salmonids also stimulates insulin production.

Diets for salmonids are rich in proteins and lipids whereas the concentration of digestible carbohydrate is usually less than 15%. This is because salmonids have limited capacity for metabolically utilizing digestible (bio-available) carbohydrate. This may be a consequence of low levels of digestible carbohydrate and concomitant high levels of protein and lipid in the prey of wild salmonids, especially if they enter the marine environment during part of their life history (Higgs *et al.,* 1995b). The glucose needs of salmonids are therefore met primarily through the process of gluconeogenesis. In fact, salmonids have a low glucose tolerance and a very limited capacity for insulin response after glucose administration. Hence a carbohydrate rich diet or high levels of digestible carbohydrate in the diet can lead to type 2 diabetes – non-insulin dependent diabetes mellitus (NIDDM). It is also known that besides breakdown of synchrony between insulin production and glucose absorption when dietary levels of digestible carbohydrate are high, salmonids have poor glucose phosphorylating ability, low glucose utilization rates and a small glucose distribution space relative to homeotherms (reviewed by Higgs *et al.,* 1995b). Thus, it is generally recommended that the digestible carbohydrate level in feed for salmonids should be $\leq$ 15% (Wilson, 1991; Satoh, 1991; Luquet, 1991).

Nevertheless, carbohydrates are still incorporated into fish feed because they act as a binder for other ingredients in the diet and additionally they stabilize the moisture in the feed during pelleting (Pike *et al.,* 1990). Also, from an economic perspective, it is desirable to include some carbohydrates in the diets for cultured salmon. Salmonids effectively absorb carbohydrates from pre-gelatinized starch, dextrin, and when they are in the form of monosaccharides. Cooked cereals (e.g., extruded wheat), and extruded oilseed meals (before and/or during diet pelleting) that have a large carbohydrate fraction, are used in the diets for cultured salmonids to spare the use of comparatively more expensive energy from lipid and protein to meet the energy needs of salmonids. Considerable starch gelatinization is achieved during extrusion and pelleting of diets. But to offset excess bioavailable levels of carbohydrate in the diet, Pike *et al.,* (1990) recommended that carbohydrate should not exceed 20% of the dry feed.

2.1.4.2 Fiber content

Plant protein sources (e.g., oilseed meals) also contribute crude fiber to the diet for cultured salmonids. Fiber content in fish feed is often measured using a 'crude fiber determination' method, which simply estimates the un-digestible carbohydrate in fish feed. There are two types of fiber present in plants – soluble fiber and insoluble fiber. Crude fiber measures the variable amounts of insoluble fiber like cellulose and lignin; but soluble fibers like hemicelluloses, hydrocolloids (gums, mucilages etc.) and pectins are not detected. Crude fiber in the diet is indigestible in salmonids and the presence of excess fiber in the diet may depress fish growth, feed digestibility and the bio-availabilities of some minerals and can reduce water quality (Hilton *et al.,* 1983; Higgs *et al.,* 1988). Hence, it has been recommended that crude fiber levels in diets for salmon and trout should not exceed 10% to avoid the preceding negative consequences.

Two other measures of the fiber content of animal diets include determination of acid detergent and neutral detergent fiber. Acid detergent fiber measures concentrations of lignin and cellulose, while neutral detergent fiber measures concentrations of lignin,

cellulose and hemi-cellulose. Neither of these latter methods, however, measures concentrations of pectin or hydrocolloids (Bennink, 1994). Unlike homeotherms, salmonids do not appear to have any need for indigestible fiber (e.g. hemicellulose, lignin) in the diet. Therefore, during diet formulation, every attempt is made to restrict this component of the diet to levels below that recommended above.

2.1.5 Vitamin and mineral requirements

Minerals and vitamins are required for the normal functioning of enzymes, hormones, osmoregulation and other bio-metabolic pathways operating within the fish to maintain good health. Fish can absorb minerals from their aquatic environment and additionally from their diets (NRC, 1993). Usually phosphates and sulfates are required in feed while others like calcium, magnesium, sodium, potassium, iron, zinc, copper and selenium are absorbed from the water (NRC, 1993). In a controlled environment, however, all the vitamin and mineral requirements should be included in the diet to offset any deficiency. NRC (1993) provides a list of adequate levels of minerals and vitamins for many species of fish. Since Atlantic salmon is not specifically mentioned in the NRC (1993) database, values for rainbow trout, chum salmon or Pacific salmon are generally used as a reference guide for salmonids.

2.1.6 Digestibility

The first task in evaluating the biological potential of including a feed ingredient in the diet of fish is to measure its digestibility. This commonly entails feeding groups of fish a reference diet as well as one or more test diets which contain a known percentage of the test ingredient (usually ~30%) followed by collection of the feces from fish fed the reference diet and the test diet(s). In the above procedure, an indigestible indicator such as chromic oxide is included in all diets at the same concentration (usually 0.5%) and then concentrations of this indicator compound in the diets and fecal samples are determined (e.g. according to Fenton and Fenton, 1979) together with the dietary nutrients of interest.

Subsequently, an equation (Cho *et al.,* 1982, 1985) is used to assess the digestibility of the nutrients and energy in the reference and test diet(s) and then through the use of another equation (Forster, 1999) the digestibility of the nutrients and energy in the test ingredient(s) can be determined.

Several procedures are used for collection of fish feces and none of these are without some error. Cho *et al.* (1975, 1982, 1985), for example, developed a settling column method for fecal collection which was subsequently modified successfully for use on salmon by Hajen *et al.* (1993 a, b). This procedure does not require killing the fish. Moreover, it minimizes fish stress, which can lead to negative nitrogen balance as well as the leaching of water-soluble nutrients. In general, the results obtained for protein and energy digestibility of ingredients based upon the preceding procedure have compared favorably with those obtained using feces collected by intestinal dissection, which perhaps gives the best estimates of digestibility, but necessitates killing the fish.

Once apparent digestibility coefficients (ADC) of the feed ingredients are known, these values can be used to formulate a balanced diet for the fish to support maximum growth. Subsequent evaluation of feed efficiency, weight gain, specific growth rate, protein efficiency ratio etc. can be calculated to measure fish performance supported by the dietary formulation (Cho *et al.,* 1982). Thus several ingredient combinations can be tested to find a desirable combination. The ADC value for protein in high quality fishmeal usually varies between 92 and 95.3% (Pike *et al.,* 1990). Most plant protein sources, except wheat gluten, corn gluten meal, oilseed protein concentrates and isolates, have lower ADC values for protein than those of premium quality fish meal in salmonids (Cho *et al.,* 1985; Anderson *et al.,* 1992; NRC, 1993; Hajen *et al.,* 1993b; Higgs *et al.,* 1996; Mwachireya *et al.,* 1999; Storebakken *et al.,* 2000). Similar findings have been obtained for the bioavailabilities of amino acids in protein sources for salmonids (Anderson *et al.,* 1992; Storebakken *et al.,* 2000).

2.1.7 Aquaculture

Salmon farming has grown to become one of the world's largest industries with more and more countries producing vast amounts of farmed salmon. The total world aquaculture production has jumped from 13 million tonnes in 1991 to 42.8 million tonnes in 1999. China is the leading country in this field with its contribution of over 67% of the total global aquaculture production, followed by India (6.7%) and Japan (3.1%) (FAO, 1999).

At the species level, finfish contributed about 50.2% of total aquaculture production in 1999, followed by mollusks (23.7%), aquatic plants (22.1%) and crustaceans (3.7%). Atlantic salmon production in 1999 was approximately 797,560 million tonnes (Figure 2.1), a 15.9% increase since 1998, and there has been an average increase of 27.2% per year in production since 1984 (FAS-USDA, 2001). Much of the massive increase in global salmon production has resulted from large-scale production increases by Norway and Chile (New, 1999).

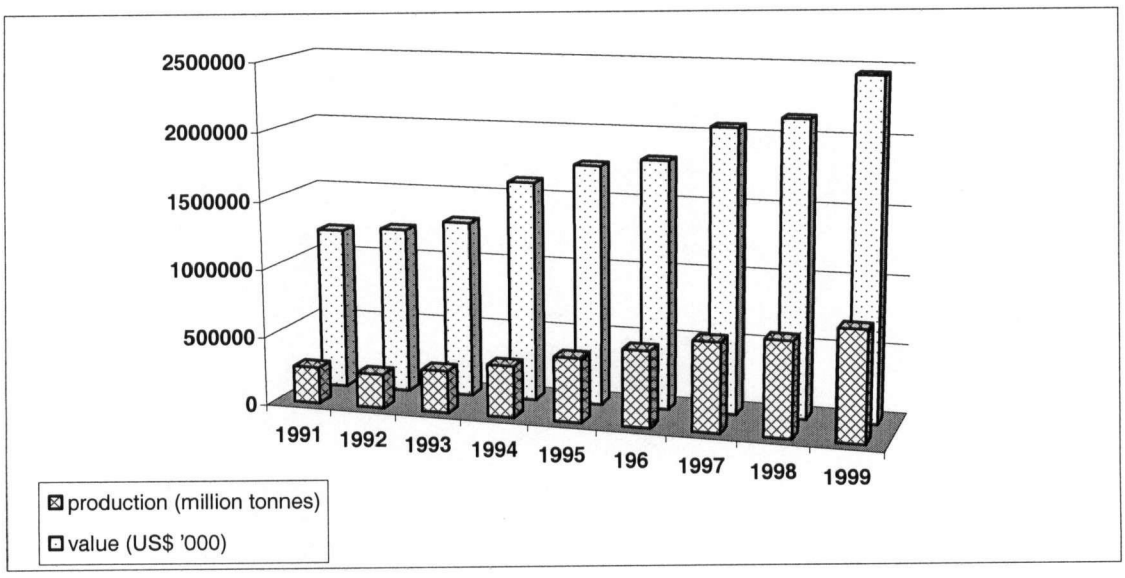

Figure 2.1: Worldwide aquaculture production of Atlantic salmon (*Salmo salar*) (FAO, 1999).

British Columbia (BC) has the distinction of being the fourth largest farmed salmon producer in the world (BCSFA, 2001). In B.C., farmed salmon production (Atlantic and Pacific spp.) increased from 6.6 thousand to 23.8 thousand tonnes from 1988-1995 (BCSFA, 2001). By 1996, farmed Atlantic salmon production in BC jumped to 17,050 tonnes from 11,300 tonnes in 1993 an increase of approximately 51% (BCSFA, 2001). As of the year 2000, there were 104 active salmon farms in BC operated by 11 companies.

Salmon production in B.C. for the year 2000 was approximately 43,440 tonnes (dressed weight), of which 82% was due to Atlantic salmon (35,680 tonnes) and 15% was from chinook salmon (6,370 tonnes; BCSFA, 2001). The salmon farming industry contributed 309 million dollars to the BC economy with a total export value of over 270 million dollars, making it BC's most significant agricultural export commodity. Over 85% of total BC farmed salmon is exported to the US and it is valued at $257 million, with the remaining 15% used within Canada (BCSFA, 2001).

BC contributes less than 5% of the world farmed salmon production. As the prices received by BC salmon farmers depend on world production from farmed and wild sources in relation to consumer demand, and presently production is excessive in relation to demand, the economic viability of salmon farming in BC is not very stable. Therefore, in order for BC salmon farmers to compete in the global market, production costs must be reduced. Since the dietary protein sources in feed for cultured salmon account for 50-66% of the total feed costs (Higgs, 1997), fish nutritionists are increasingly looking for ways to minimize this expenditure to enable salmon farming in BC to be more profitable.

2.1.8 Fish-feed for farmed salmon

Feed provided to farmed salmon is usually in the form of extruded pellets, which vary in size/dimensions depending on the size or life history stage of the fish being fed. Carbohydrates are added to feed formulations in the form of starch. The availability of

energy from starch depends largely on the type and dietary concentration of the starch used, and thermal processing conditions which influence the degree of starch gelatinization (Inaba *et al.*, 1963; Singh and Nose, 1967; Bergot and Breque, 1983). The protein component of farmed salmon diets usually consists mainly of fish meal (herring, anchovy, capelin, sardine, or menhaden meal; singly and in combination), which when included in the feed formulation along with other sources of protein of animal (poultry by-product meal) and plant origin (soybean meal or canola meal, corn gluten meal, wheat gluten meal, whole wheat) are sufficient to support maximum growth of salmonids.

Ideally, feed formulations contain enough energy from lipid and carbohydrate to permit the channeling of as much of the dietary protein into fish growth (protein synthesis) as possible. To decrease feed costs, it has been necessary to identify or develop alternative inexpensive protein sources to imported premium quality fish meal(s) for inclusion in formulated salmon diets. Oilseed protein products offer considerable potential in this regard.

2.1.8.1 Fishmeal

Approximately one-third of the annual world fish catch is used to produce fishmeal for animal feeding. The total fishmeal production for the year 1999/2000 was 6.26 million tonnes, an increase from the 1998/99 production of 5.80 million tonnes and from the 1998 production of 5.08 million tonnes (FAS-USDA, 2001; Figure 2.2). A preliminary production report by FAS-USDA (2001) quotes fishmeal production for 2001 as 5.93 million metric tones (Table II-I), a decrease from the previous year, and it further forecasts a decrease in production for the year 2001/2002 to about 5.82 million tonnes. Major producers of fishmeal are Peru (35%), Chile (11.8%), China (8.4%) and Japan (5.9%).

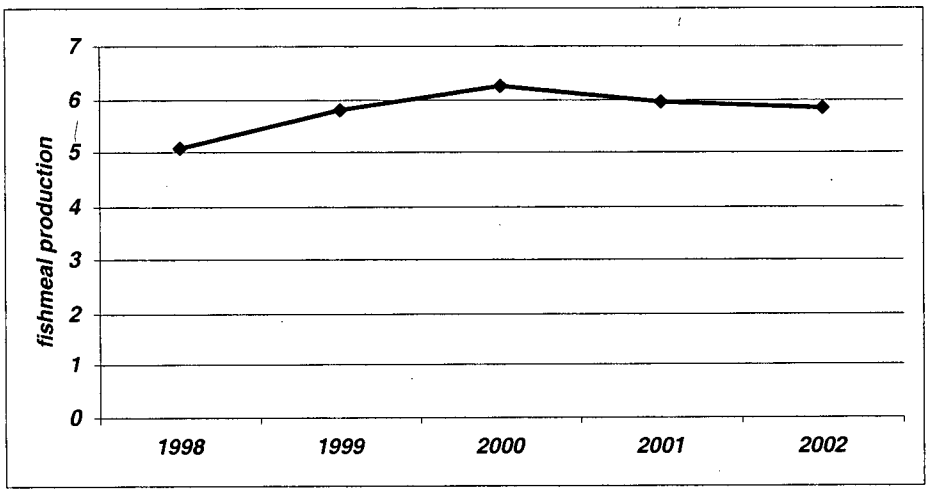

Figure 2.2: Temporal changes in global fishmeal production, expressed in million metric tonnes (FAS-USDA, 2001).

Future expansion of aquaculture will greatly tax global supplies of fishmeal and fish-oil, two key finite resources used as ingredients in fish feeds (Hardy, 1999); hence there is urgency to develop alternative sources of protein for use in fish feed. Indeed, the predicted rise in aquaculture production will require an additional 26 million tonnes of fish feed which, in turn, will necessitate an additional 7-9 million tonnes of protein (Hardy, 1999). Since salmonids require approximately 40% fishmeal in their diets and they represent the major sector of the aquaculture industry that consumes the greatest amount of fishmeal (70% consumption of total fishmeal produced), partial substitution of fishmeal in their diets with alternative proteins will have a major impact on the world trade of fishmeal (Hardy, 1999).

Fishmeal is the most expensive ingredient used in feed formulations for farmed salmonids, and the diets themselves account for 35-60% of the operating costs of salmon farming (Higgs *et al.*, 1995a; Higgs, 1997). The average cost of premium quality fishmeal (Hamburg cif: any origin, 64-65% protein) for the year 1999/2000 was US$407 per tonne, which rose to US $457 per tonne by June 2001 (FAS-USDA, 2001).

Table II-I: A preliminary report of the worldwide distribution of fishmeal production and consumption (in million tones) for 2000-01 (FAS-USDA, 2001).

Country	*Production*	*Consumption*
USA	**0.25**	**0.22**
EU	**0.50**	**1.05**
Denmark	0.32	0.15
France		0.13
Germany		0.08
Italy		0.08
Spain	0.05	0.15
UK	0.05	0.26
Iceland	**0.25**	**0.03**
Norway	**0.28**	**0.30**
Russia	**0.14**	**0.19**
Eastern Europe	**0.03**	**0.08**
Latin America	**2.89**	**0.67**
Chile	0.70	0.32
Peru	2.07	0.13
Middle East		**0.15**
Iran		0.11
South Africa	**0.08**	**0.10**
Asia	**1.33**	**3.02**
China	0.50	1.25
Japan	0.35	0.71
Taiwan	0.02	0.32
Thailand	0.38	0.47
New Zealand	**0.08**	
Other	**0.10**	**0.11**
Total	**5.93**	**5.92**

It should be mentioned that fishmeal is prepared from dried, ground tissues of whole marine fish (e.g., menhaden, anchovy or capelin). Also, fishmeals have good amino acid profiles and protein digestibility, but their quality depends highly on the processing methods (cooking and drying temperatures employed during meal manufacturing; variable quality and/or amounts of solubles added during press-cake stage) and the degree of freshness of the raw material used (Anderson, 1996; Higgs *et al.*, 1995a). McCallum and Higgs (1989) reported that high-temperature dried (more than 100°C) herring meal had dramatically reduced protein quality compared to low temperature-dried herring meal for chinook salmon. Thus a low-temperature dried (LT, preferably below 70°C) high quality fishmeal that contains an excellent amino acid profile is preferred (Pike et al., 1990). The degree of freshness of the raw material before processing can be quantified by measuring it's total volatile-nitrogen content which should be below 90 mg nitrogen per 100 g fish (Pike *et al.,* 1990). Thus, differences in – i) processing, ii) raw material composition and freshness, iii) drying temperatures used during meal production, iv) grinding and v) storage and transportation conditions have a dramatic impact on the quality and nutritive value of fishmeal for salmonids (Higgs *et al.*, 1995a).

Most fishmeal is highly palatable and digestible and contains high bioavailable levels of protein, lysine, sulfur amino acids, lipid (rich in n-3 HUFAs) and minerals. Thus it is generally used as a major protein source in starter diets when the needs of salmonids for high quality dietary protein are the highest. Although the protein and lipid concentrations in fishmeal may vary, respectively, from 55-72% and from 2-12%, NRC (1993) defines a good quality fishmeal as having a protein content of more than 68%, a lipid content of less than 10%, an ash content of less than 13%, with 200 ppm of supplemental antioxidant.

All of the preceding nutritional attributes of fishmeal make it a difficult dietary commodity to replace with an alternate protein source, especially of plant origin, without some decline in fish performance.

2.1.8.2 Formulation problem

Salmonids are carnivores and thus they require a high dietary concentration of protein. Also, as mentioned previously, they have a markedly reduced ability to metabolically utilize carbohydrates as an energy source and their dietary crude fiber intake should not exceed 10%. Moreover, common and unique deleterious components are present in oilseeds and some of these may adversely influence digestibility or mineral metabolism in fish. Even some carbohydrates such as oligo-saccharides that may be present in oilseed protein products can be detrimental to fish performance. Soybean meal, for example, is better utilized as a protein source by salmonids after alcohol extraction of oligosaccharides than without. Also, several key amino acids (especially lysine, methionine and tryptophan) may be deficient in oilseed protein products. All of the preceding factors must be taken into consideration when replacing fishmeal, in formulated diets for salmonids, by oilseed protein products. Also, some oilseed protein products such as commercially available sunflower meal are much lower in protein content than fishmeal. Hence, it is not even theoretically possible to completely replace fishmeal in diets for salmonids with this protein source unless it is nutritionally upgraded through processing to enhance the level of protein and concurrently reduce the levels of anti-nutritional factors.

2.1.9 Search for alternative sources of protein

Attempts have been made to develop alternative sources of protein and energy as dietary ingredients for farmed salmon (Asgard, 1988; Higgs *et al.*, 1988). Alternative sources that have been tested have included single cell protein (SCP), fish silage, and grain/oilseed protein products as the potential sources of protein in fish feed (Hardy, 1999). SCPs include unicellular algae, fungi, bacteria, cyanobacteria and yeast. SCP has an excellent amino acid profile. Further, SCP has been cultured on a large scale and has great potential for widespread application. But very limited work has been done on the use of SCP as a protein source in fish feed (Perera *et al.*, 1995; Rumsey *et al.*, 1991 on rainbow trout).

Some attention has been directed to the use of fish silage in diets for salmonids and other fish species. Espe *et al.* (1999) found that inclusion of 15% fish silage (termination of hydrolysis after 3-7 days), a hydrolysed fishery by-product from fish processing wastes, in diets for Atlantic salmon did not adversely affect fish performance. Lall (1991) also noted a slight improvement in fish weight gain, protein efficiency ratio (PER) and net protein utilization when fish silage was incorporated into salmonid diets. Further, El-Sayed (1999) observed that the inclusion of formic acid-preserved fish silage blended with fishmeal (1:1) in the diet of Nile tilapia resulted in similar growth of tilapia relative to that noted for fish fed a pure fishmeal-based diet. Many additional studies (Hossain *et al.*, 1992; Fagbenro and Jauncey, 1993, 1994) demonstrated that lactic acid fermented fish silage-based diets for Nile tilapia resulted in no loss of growth with very good apparent digestibilities for dry matter, protein and lipid. Consequently, this latter source of protein has excellent potential as an alternative protein source for tilapia.

Amongst the grain by-products that have been used to replace fishmeal in fish feed, gluten meals derived from corn and wheat are mostly used in feeds for salmon, trout and shrimp. Corn gluten (contains 60% protein which is 97% digestible in rainbow trout; Sugiura *et al.*, 1998) can replace 25-40% fishmeal in the diet of rainbow trout without any adverse effects on the growth performance of the fish (Morales *et al.*, 1994; Weede, 1997). But corn gluten reportedly imparts a yellow color to the flesh of rainbow trout when it is present in the diet at levels of more than 5%. In the case of salmon, however, the addition of astaxanthin to the diet can mask the yellow color. Hence, corn gluten can comprise as much as 22.5% of the diet (Skonberg et al, 1998). Corn gluten is also less expensive than fishmeal at US$ 94 per metric tonne as compared to US$ 457 per metric tonne for fishmeal (FAS-USDA, 2001).

Wheat gluten meal contains 70-80% protein, which is also highly digestible (more than 88%) in salmon (Storebakken *et al.*, 2000)). Wheat gluten meal can replace up to 25% fishmeal in the diet of rainbow trout without adversely affecting fish growth performance (Weede, 1997). Higher replacements levels of fishmeal with wheat gluten meal

(20-40%) supplemented with lysine were found to be acceptable by Hardy (1999), with respect to supporting trout growth performance equivalent to that observed for fish fed a fishmeal-based diet.

As a general rule, alternative protein sources are selected on the basis of the following criteria:

i) Quality and nutrient composition of protein source
ii) Processing methods employed in its production
iii) Nutrient and energy digestibilities of protein source for intended species
iv) Type and amounts of anti-nutritional factors present (ANF)
v) Nutritive value of protein source for intended fish species
vi) Cost and supply

Many plant protein sources may be limiting in their essential amino acid profile (especially in lysine, and methionine content) and in fatty acids like EPA and DHA (Naylor *et al.,* 2000). Therefore, in these circumstances, the diets containing these plant protein sources may not be cost effective. Feed ingredients of plant origin may also be lower in protein content and digestibility compared to those of animal origin (Naylor *et al.,* 2000) and the unsaturated oils present in the oilseeds can be susceptible to oxidation if they are not adequately protected through the use of antioxidants. The presence of high amounts of carbohydrates, fiber and ANFs present new nutritional problems. Although plant-based feed ingredients, except some specially processed oilseed protein concentrates, do not have similar nutritive value to premium quality fishmeal on an equivalent protein basis for salmonids, their use is on the rise.

2.1.10 Oilseeds

World production of 7 major oilseeds will increase to 68.9 million tonnes in 2001-02 (FAS-USDA, 2001; Table II-II). Sunflower production is expected to fall by 10% in

Canada and the European Union due to the relatively improved price offered for canola (rapeseed) and due to unfavorable weather conditions in India and Australia. But the decline in sunflower production was offset by a bumper crop of soybeans in 2000, due to a high soybean-marketing loan offered to farmers in the USA. Meanwhile, Brazil and Argentina had good weather, which accounted for their increased production of soybeans (Hardy, 1999). The consumption of oil meals and oilcakes in 2001(until June) exhibited slow growth but it is expected to rise as the economic conditions and weather improve in Asian countries. The USA uptake is relatively constant but the EU demand will increase due to a stricter ban being imposed on the use of meat and bone meal in livestock and because of Germany integrating bio-diesel in its fuel supply.

Soybean meal has been used extensively in feed for salmonds and other fish species (Li *et al.*, 2000). It has a good essential amino acid profile (except for lysine and the sulfur containing amino acids, methionine and cystine) and protein content. The presence of anti-nutritional factors (protease (trypsin) inhibitors, anti-vitamins, phyto-hemagglutinins, lectins, saponins etc.) which lower protein digestibility limit its use; but these can be inactivated during thermal processing (Tacon, 1993). Soy protein concentrates (70% protein) can replace 70%-100% of the fishmeal in diets for rainbow trout and salmon but their high price prevents their widespread use (Kaushik *et al.,* 1995; Hardy, 1999).

Canola seed has low protein content (21-23%) due to high levels of oil, crude fiber and phytic acid. Rapeseed/canola protein concentrates (~61% protein), however, have reduced concentrations of all ANFs except phytic acid (Higgs *et al.*, 1995b). Total removal of phytic acid from rapeseed/canola protein concentrates enables total replacement of premium quality fish meal in diets for rainbow trout provided that the diets containing this plant protein product are concurrently supplemented with a palatability enhancer and some minerals, e.g., phosphorus and zinc (Prendergast *et al.*, 1994).

Table II-II: A preliminary report of the worldwide production and consumption of major protein meals in million metric tonnes (FAS-USDA, 2001).

Oilseed	*Production*		*Consumption*	
	2000-01	*2001-02*	*2000-01*	*2001-02*
Soybean	115	121	115	121
Cottonseed	11.2	12.0	11.2	11.9
Rapeseed	21.3	21.1	21.3	21.1
Sunflower seed	**9.59**	**9.00**	**9.62**	**8.90**
Fish	5.93	5.82	5.92	5.70
Peanut	5.50	6.13	5.47	6.07
Copra	1.82	1.68	1.84	1.70
Palm kernel	3.65	3.76	3.50	3.69
Total	174	180	174	180

2.1.11 Sunflower

2.1.11.1 Conditions required for cultivation

Sunflower (*Helianthus annus* L.) is a composite (family *Compositae*) annual plant that is mainly grown in the warm temperate regions of the world. Sunflower grows well within the temperature range of 20-25 °C. Higher temperatures than 25 °C decrease the yield and oil content of the seeds. Although sunflower is drought resistant and adapts well to a wide variety of soils, the type of soil that is used for the production of wheat and maize is ideal for production of this oilseed.

Sunflower has one of the shortest growing seasons relative to other oilseeds with the early maturing varieties ready for harvesting in just 90-120 days after planting. By contrast, late maturing varieties require about 120-160 days before harvest.

2.1.11.2 Nutrient composition

Different grades of sunflower meal (SFM) are classified according to the oil content that is present within the seed cultivars before processing (i.e., low, medium, and high oil content). Seeds of high oil content cultivars are black with thin hulls, which adhere tightly to the kernels whereas seeds of low oil content cultivars have relatively thick hulls that are loosely attached to the kernel (Vaughan, 1970; Park *et al.,* 1997). The high oil content cultivars are used for extraction of sunflower oil while the comparatively more expensive low oil content cultivars are primarily used for human consumption.

The average fatty acid composition of the oil fraction in the seed is 40-75% linoleic acid and 15-25% oleic acid. Protein content is about 15-20%. The proportions of shell and kernel range from 25-30% and 70-75%, respectively depending on the cultivar. About 42.5% oil is obtained by pre-press and solvent extraction from seeds of normal moisture content (9%). Hulls contain about 74-90% highly indigestible carbohydrates with lipids, proteins and minerals comprising the rest. The indigestible carbohydrates consist of cellulose (29-32%), lignin (25-30%) and hemi-cellulose (pentosans, 25-31%). A schematic representation of processing sunflower seed is illustrated in Figure 2.3.

2.1.12 Sunflower meal (SFM)

The 'meal' is a byproduct of the oil-extraction process and consists of defatted matter and is rich in protein (30-40%) but low in lysine (less than 1%). Low temperature processing is often used to prevent denaturation of lysine and other amino acids.

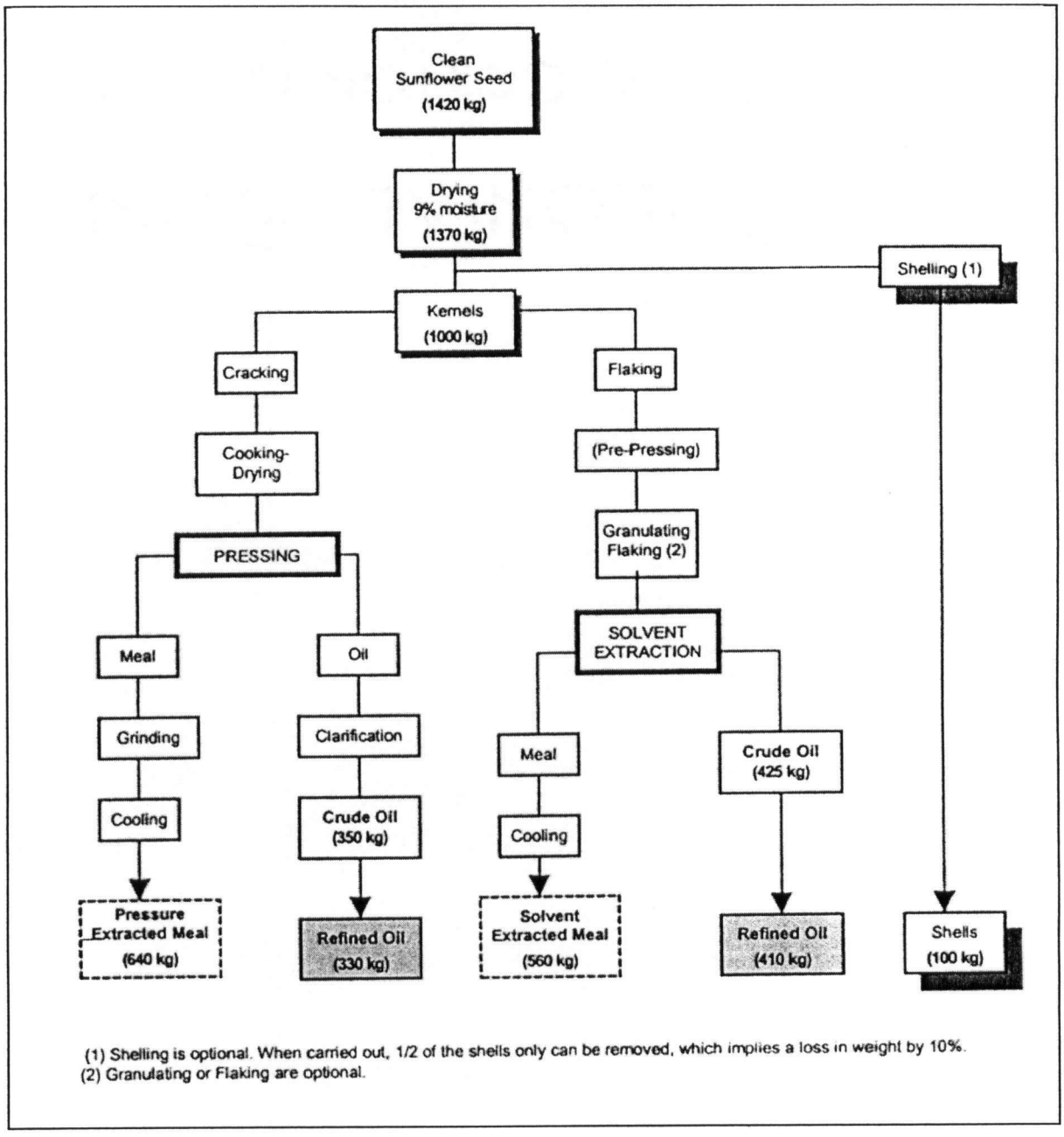

Figure 2.3: Schematic representation of the two main methods of processing sunflower seed (FAO/EBRD Agribusiness handbook, 1999).

A processing plant of 1000 tonnes/day capacity of sunflower for oil-extraction, costs approximately US$ 50 million. The high investment is because of a) the explosion proof electrical equipment that is required due to hexane fumes, b) the price of sunflower seed and c) the cost of fuel.

SFM from the solvent extraction process contains less than 1% residual oil, while the oil content is much higher after pressing. Whole SFM has only about 25% crude protein. SFM compares very well with other oilseed meals in all respects except for its high fiber and ash content, which bring down its bio-available energy content. The protein and fiber contents in SFM are inversely related. Dehulled SFM has a protein content in excess of 40% along with 13% or less of crude fiber. Defatted SFM is high in potassium, phosphorus, and magnesium with moderate amounts of calcium. It is also very low in iron, zinc, sodium, manganese and copper content (Nwokolo, 1996).

Partial dehulling produces SFM that has 30-35% protein and in excess of 20% crude fiber, which makes it unsuitable for use in monogastric animal feeds without further processing. Thus upgrading the protein content and reducing the fibre content of SFM is essential before SFM can be successfully incorporated into monogastric animal diets. At present, the high fiber content meals are used in ruminant diets to balance or supplement their protein content. The nutrient content in SFM varies with – i) the quality of the seed, ii) the oil-extraction method that is used and iii) the extent of dehulling of the seed prior to oil extraction.

World production of SFM is approximately 9.6 million tonnes per year (FAS-USDA, 2001) with Argentina and China being the main producers. The USA exported up to 11,046 tonnes of sunflower meal to Canada in the year 2000-01 (until June, 2001; Figure 2.4). In fact, sunflower meal is the fastest growing oilseed commodity in the US (FAS-USDA 2001, Figure 2.5) and worldwide. SFM price depends on its protein content. The Grain and Free Trade Association (GATFA) Trading Rule No 100 is most frequently applied in the world trade of SFM. It specifies a minimum protein- plus fat-content, with the specific protein/fat level at the time of sale.

The average sunflower seed prices in 1999-2000 were US$ 168 (US farm price, USDA) in the U.S.A and US$ 214 for the world (Rotterdam CIF, US/Canada Prior; oil world). In the year 2000/01 the average price of sunflower seed was US$154 for the U.S. and US$ 201 (until June, 2001) for the world. In the case of SFM, the prices in the year 1999-2000 were US$83 for the US (Minneapolis FOB, 32% protein; USDA) and US$102 for the world (Rotterdam CIF and oil world).

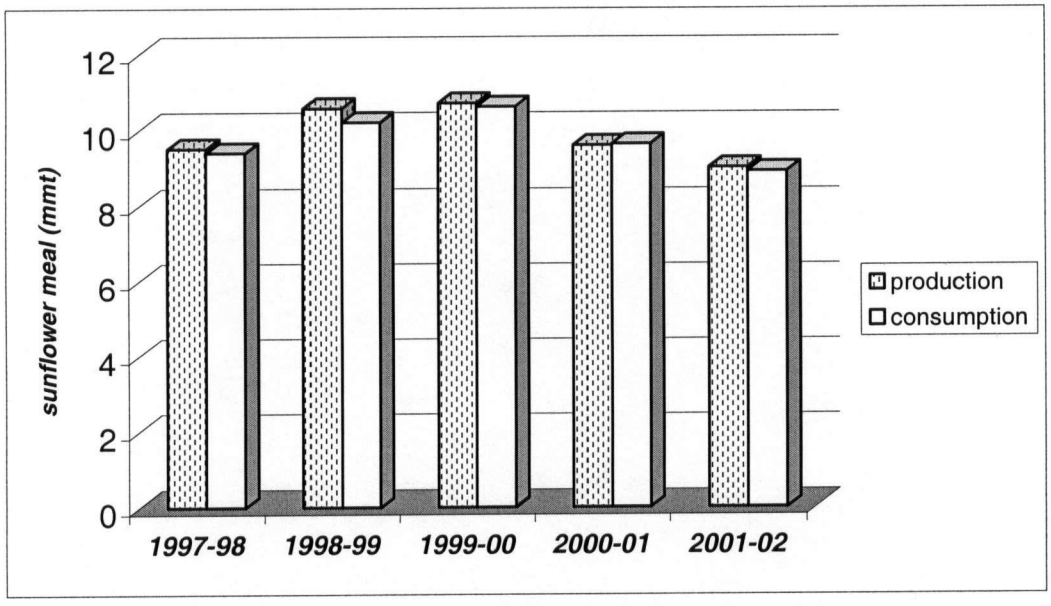

Figure 2.4: Worldwide production and consumption of sunflower meal in million metric tonnes (FAS-USDA, 2001).

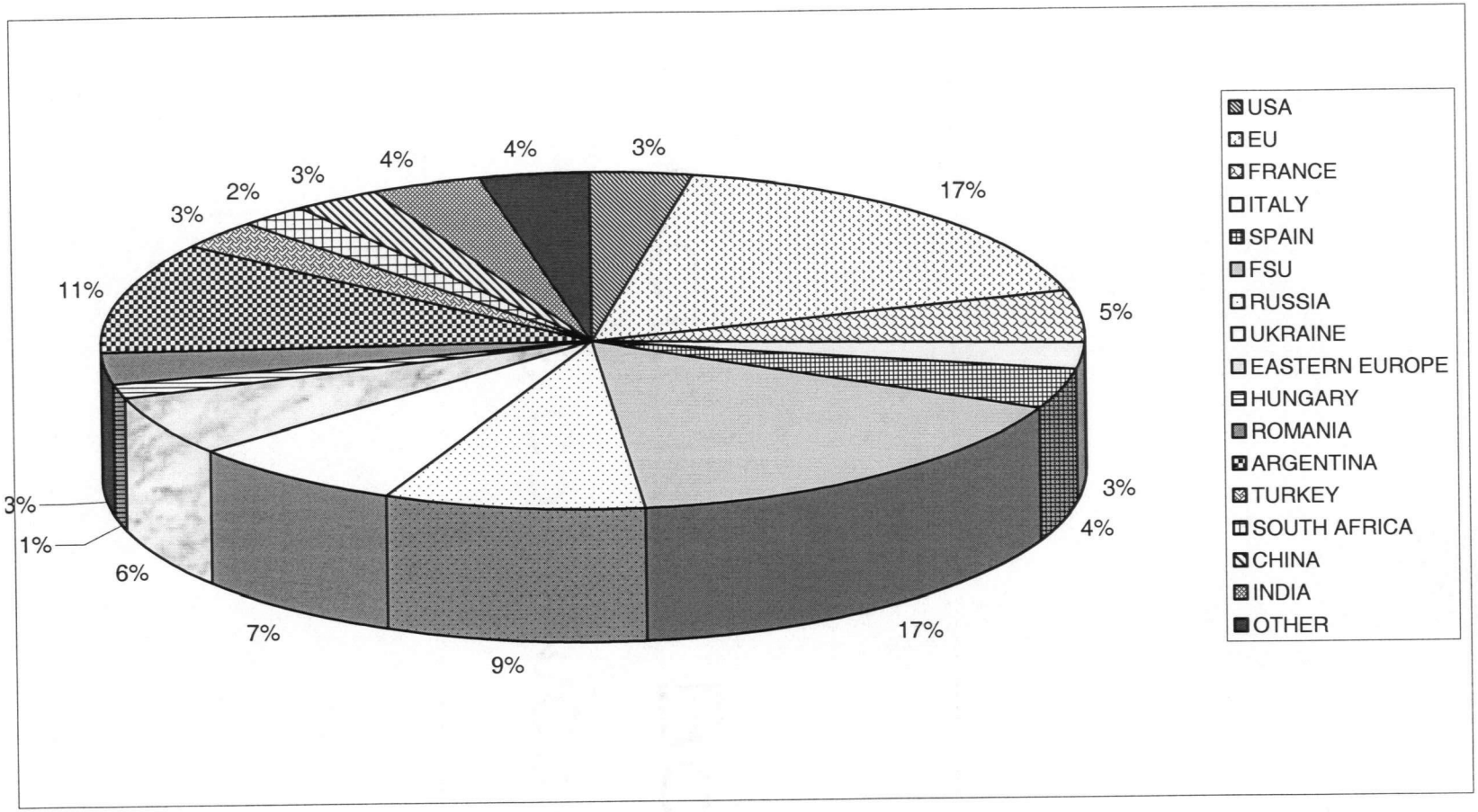

Figure 2.5: Preliminary breakdown of sunflower meal (SFM) production by different countries in million tonnes for 2000/01 (FAS-USDA, 2001).

2.1.13 Anti-nutritional factors (ANF) and sunflower meal

The presence of anti-nutritional factors (ANF) in fish feed is an important consideration because many feed ingredients are produced from grains, legumes and oilseeds. High temperature extrusion processing and solvent extraction usually help to destroy or remove most of the ANFs. Selective breeding can also help lower the ANF content of oilseed meals to acceptable levels in some cases (Dong *et al.,* 2000).

Unprocessed SFM contains high levels of chlorogenic acid (1.94 - 2.08 % of a total of 3-3.5% phenolic compounds), a tannin-like compound that inhibits the activity of digestive enzymes like trypsin, chymotrypsin, amylase and lipase (Cheeke and Shull, 1985). Methionine and choline need to be added to SFM in animal diets to counteract the effects of chlorogenic acid (Liener, 1980).

In a study by Delic *et al.* (1975), 2% inclusion of chlorogenic acid in the feed of mice resulted in decreased feed intake and reduced weight gain. Sunflower seeds contain arginase and trypsin inhibitors, which being heat labile, are inactivated during processing (Roy and Bhat, 1974). Chlorogenic acid is an ester of caffeic acid and quinic acid. Chlorogenic acid and caffeic acid are oxidized to ***o***-quinones by polyphenol oxidase in the plant. Once formed, these ortho-quinones react non-enzymatially to bind covalently with amino, thiol and methylene groups of amino acids and thereby these amino acids are rendered nutritionally non-available for digestion (Sosulski, 1979).

Thus it is important to include sufficient methionine and lysine in diets containing chlorogenic acid to offset the decreased bio-available levels in the feed. SFM is high in methionine but poor in lysine and threonine content. Thus concurrent supplementation of these two amino acids with SFM improves the amino acid balance of the feed.

2.1.14 Use of SFM in fish diets

There are no studies that have assessed the nutritive value of sunflower protein products, especially SFM, for Atlantic salmon. Other studies on this theme in salmonids have been conducted on rainbow trout. But even the results of those studies cannot be compared properly because of the different experimental methodologies that have been used between studies or because of the use of sunflower protein concentrates as opposed to SFM. Various studies also have not described the type of sunflower seed used to produce the test protein product(s) (e.g., high oil versus low-oil cultivars). Other differences in dietary factors such as the inclusion or absence of supplemental amino acids in the presence of SFM do not facilitate the comparison of the results among studies. Thus there is a definite need to conduct additional studies to assess the nutritive values of various sunflower protein products for different salmonid species.

Despite the aforementioned comments, several studies that are briefly outlined below suggest that sunflower protein products have good potential as dietary sources of protein in salmonids. Tacon *et al.* (1984), for instance, conducted a 150-day growth trial on rainbow trout in which 0%, 11% and 22% of solvent extracted soybean meal and wheat meal in the basal diet were replaced with solvent extracted SFM. Two additional diets that contained 36.5% SFM or 36.3% SFM + 2% L-methionine as well as 35% fishmeal but no soybean meal and wheat meal in each case were also evaluated. All diets contained 44-45% crude protein and 13-15% lipid. The results showed a progressive decrease in the apparent digestibility of protein and organic matter as the dietary level of SFM was increased. These trends were attributed to the high fiber content (24.7%) of the SFM that was used. Nevertheless, Tacon *et al.* (1984) did not observe any overall loss of fish growth performance and feed efficiency due to diet treatment. In addition, dietary methionine supplementation provided no additional benefits in fish performance. Thus, these researchers concluded that the major limiting factor for the use of SFM in feed for rainbow trout was its high fiber content.

In another study on rainbow trout, Scott *et al.* (1982) studied the effects of using partially dehulled SFM (35% crude protein, 17.5% crude fiber) to replace either 0 or 33% soybean meal in the diet. Each diet contained 30% fishmeal to maintain a dietary protein content of 38% in each, and the dietary levels of amino acids, vitamins and minerals either met or exceeded the respective requirements of these nutrients for rainbow trout. Fish performance (weight gain, feed efficiency, %mortality), at the end of the 3-month trial, indicated no effect of dietary substitution of SFM for soybean meal. Thus it was concluded that SFM could comprise 33% of the diet of rainbow trout by replacement of soybean meal without any adverse effects on trout performance. It is noteworthy, however, that there was no mention of the size of fish that were used in this study or of the culture environment (i.e., marine or freshwater).

Cardenete *et al.* (1991) evaluated SFM as a protein source in diets for rainbow trout by comparing the performance of fish fed a fishmeal-based diet to that of fish fed diets in which there was partial replacement of fishmeal with either soybean meal (32.6%) or SFM (42.6%) for a period of 45 days. All the diets were formulated to contain 40% protein and 11% lipid. No significant differences were observed due to diet treatment in terms of fish weight gain and specific growth rate. However, feed intake was significantly lower in fish fed both of the diets containing the oilseed protein sources relative to fish fed the fishmeal-based control diet. Protein and lipid digestibility coefficients for the SFM-based diet in trout were similar to those noted for the diet containing soybean meal but lower than those observed for the fishmeal-based diet. Probably this was the result of the higher fiber contents in the former two diets as well as the presence of other ANFs in the diet. Hence in that study, it was found that SFM could comprise over 40% of the diet of trout without significantly affecting fish growth. Cardenete *et al.* (1991), however, did not consider differences in amino levels between diets and there was no mention of fish size, culture environment (marine or freshwater), or of the source of SFM used.

In another study involving the assessment of sunflower meal, Sanz *et al.* (1994) compared the protein and energy utilization of rainbow trout over a 60-day period, when they were fed diets in which the fishmeal in the basal (control) diet was partially replaced by either either SFM or soybean meal. All of the diets in that study were formulated to contain 42% protein. The SFM based diet had higher protein and lipid digestibility for trout than the control diet. However, the ADC obtained for organic matter in the SFM-based diet was lower than that noted for the control diet due to the reduced digestibility of the carbohydrate fraction in the former diet. The study suggested that the lower digestibility of carbohydrates (% DE) in the SFM diet was offset by the higher digestibility of the protein and lipid in this diet. Hence, there was no effect on the overall growth performance of rainbow trout.

With respect to the assessment of the nutritive values of other sunflower protein products in trout, Stickney *et al.* (1996) compared the nutritive values of soybean, canola and sunflower protein concentrates in diets for rainbow trout over a 10-week period. In this regard, they formulated diets in which fishmeal was replaced partially by either 25, 50, 75 or 100% soybean protein concentrate; 25 or 50% canola protein concentrate; or 25, 50 or 75% sunflower protein concentrate. Control fish were fed a fishmeal-based diet without any inclusion of an oilseed protein concentrate. All the diets contained 40% crude protein and 13.8% lipid and they met the mineral, amino acid, and vitamin requirements of trout. Fish performance (growth rate, feed intake, % survival, ADC for protein) was measured during the study. The results indicated that 25 and 50% replacement of fishmeal with soybean protein concentrate or 25% replacement of fishmeal with sunflower protein concentrate did not significantly affect fish performance relative to control fish. Thus the study concluded that 25% of the fishmeal in the control diet could be successfully replaced with sunflower protein concentrate.

Comparatively few studies have assessed the potential for including sunflower protein products in the diets of warm water fish species. Sintayehu *et al.* (1996) demonstrated that SFM could replace 32% of the fishmeal protein without any

adverse affects on the growth performance of tilapia. Moreover, in an earlier study by Jackson *et al.* (1982) on tilapia, it was demonstrated that SFM could comprise 75% of the protein in the diet by replacement of fishmeal in the basal diet without any adverse effects on the growth performance or feed efficiency of the fish. More recently, Maina (2001) found that while low-fiber (less than 10%) containing SFM could replace 60% of the dietary protein without compromising the performance of tilapia, a high fiber (~24%) SFM could only contribute about 30% of the dietary protein in order to achieve growth performance comparable to the control.

(2.2) SENSORY PROPERTIES

Food quality is an arbitrary term generally signifying "fit-for-consumer consumption" (Sigurgisladottir *et al.*, 1997). The main advantage of fish farming over the wild fishery is the ability to control different quality attributes of fish to optimize their acceptance by the discerning consumer thus satisfying market preferences (Johnston *et al.*, 2000). The quality attributes of prime importance are – nutritional value, safety, flavor, color and the preservation and processing characteristics of the fillets (Haard, 1992). Alterations in the dietary compositions of feeds for farmed salmonids can influence the sensory attributes (texture, color, flavor, aroma etc.) of their flesh either positively or negatively, which, in turn, can affect the consumers' acceptance of the farmed product (Sigurgisladottir *et al.*, 1997).

Thus, the results from studies aimed at assessing the sensory attributes of fish may play a very important role with respect to the acceptance of a particular ingredient in the diet for farmed fish.

2.2.1 Sensory Panel

2.2.1.1 Facility

Sensory evaluations should be performed in a specially designed laboratory that has neutral colors on the walls and tables that have partitions. The latter minimizes visual contact and other communication between panel members (ISO 1985, 1994). In addition, samples should be served under red light to avoid any color bias.

2.2.1.2 Panel selection and training

Prospective panel members are recruited and following selection are trained. The panelists are first instructed in quantitative descriptive unstructured analysis and then they undergo a series of training sessions to familiarize them with the process. Through guided discussions general agreement is reached on the meaning of each of the terms used on the sensory ballot. This is done to minimize individual differences in perception (Poste *et al.,* 1991).

The training method described by Rutledge and Hudson (1990) provides useful guidelines in training panelists for sensory sessions. According to Rutledge and Hudson (1990), a round table format allows open and free discussions among the prospective panelists while working on developing a workable sensory 'ballot'. After marking the ballots, panelists usually discuss their scores until a consensus is reached on a particular attribute. This is the most important step because if everyone doesn't agree then the data from the panel will cease to have any meaning whatsoever. Training sessions also include optimization of the equipment like ovens, and cooking time for maximum control over variability. Practice sessions also help to teach all the panelists proper sensory assessing techniques like – sniffing the hot sample for aroma, clearing the palate in between samples, chewing the sample in order to evaluate flavor and texture, or abstaining from eating or drinking for at least an hour before the sensory session etc.

2.2.1.3 Descriptive sensory analysis

Quantitative descriptive tests are generally used as an analytical method to describe the texture and flavor profile of fishery products. (Brandt *et al.,* 1963; Iyer, 1972). Analytical descriptive procedures are concerned with different types and intensity of aroma and flavor factors and are often used in product development and quality assurance (Larmond, 1978). Descriptive analysis of fish can be reproducible and precise (Johnsen and Kelly, 1990) and can discriminate small flavor changes in the samples under investigation.

A continuous, unstructured scale is used for each of the attributes to be judged. It consists of a 15 cm long horizontal line that is anchored at either end with vertical bars that are labeled with a descriptive term. The bars are set 1.5 cm from each end. Judges record their evaluations with vertical lines drawn at points on the line to indicate their perception of the attribute. Structured scales, by contrast, are based on the assumption that equal distances between any two descriptors on the scale represent equal sensory intervals, which may or may not be the case. Also, panelists tend to avoid extreme ends of the scale and thus do not use the entire scale. Unstructured scales eliminate this problem of unequal intervals. They also avoid the use of the extreme ends of the scale. Open-end scales also allow the panelists freedom beyond the descriptive terms in order to gain more accuracy (Poste *et al.,* 1991). The raw scores are recorded as the distances between zero and the vertical line made by the panelist to indicate his/ her choice.

Composite samples of seafood are cooked and served to the panelists under red light to disguise color differences between samples. Unsalted crackers and distilled de-ionized water are used to rinse the mouth between samples. Usually, 3 replicates per sample are evaluated by the panelists. In order to avoid communication between panelists during the sessions, panelists are separately located in individual booths.

2.2.1.4 *Statistical analysis of results from the sensory panel*

Analysis of variance (ANOVA) is performed under the assumption that the scores are normally and independently distributed, treatment and environmental effects are additive and that the error variances are homogeneous. If the scores do not satisfy these assumptions then the deviation could result from taste fatigue (too many samples assessed at a given time) or taste adaption.

2.2.1.5 *Preparation of salmon samples*

The anterior and posterior portions of salmon fillets have different flavor intensities and different flavor profiles even amongst fish from the same tank (Sigurgisladottir *et al.*, 1997; Johnsen and Kelley, 1990). Since this could lead to a large standard deviation in the sensory evaluation scores, composite fish samples are often used, to reduce this effect. These composite samples are made by random combination of fish slices from different fillets and different regions of the fillets into one composite sample. This pooled sample technique ensures that replicates are true and are proper representatives of the sample population (Johnsen and Kelly, 1990). This helps reduce experimental variation due to differences between and within samples.

2.2.1.6 *Effect of using plant-based protein sources in fish diet on flavor*

Although the flesh quality of fish fed diets containing various plant protein sources has been studied, there is little information in this regard for Atlantic salmon, except for studies that have evaluated soybean protein products. In relation to the latter Bjerkeng *et al.* (1997) reported that replacement of 10% of the fishmeal in a high-energy diet (total dietary fat content was 32-39%) for Atlantic salmon with full fat soybean meal resulted in no significant changes ($p>0.05$) in flesh color (assessed instrumentally by CIE (1976) and visually with color cards), astaxanthin concentration, or sensory characteristics.

By contrast, total replacement of fishmeal with soybean protein products has been reported to change the sensory quality of the flesh of salmonids, especially flavor (Kaushik *et al.*, 1995). In this regard, the dietary inclusion of soy protein products may give rise to grassy, beany, bitter, or astringent off-flavors in the flesh (Morr and Ha, 1991). The 'green/grassy' odors could be due to the presence of C_6 -aldehydes and -alcohols probably produced by lipoxygenases (Morr and Ha, 1991; Hatanaka, 1993). These can, however, be removed along with the ANFs during processing (Morr and Ha, 1991; Nour *et* al., 1989; Rumsey *et* al., 1993, 1994). The presence of powerful flavor compounds in the plant protein and lipid sources that are used in diets for salmonids may cause off-flavors. Kaushik *et al.* (1995), for example, found that total replacement of fishmeal with soy protein concentrate caused rainbow trout fillets to taste more rancid. Replacing herring oil with sunflower oil gives a less 'fishy' aroma (Skonberg *et al.*, 1993) while replacement of herring oil with either menhaden oil or soybean oil in diets for Atlantic salmon resulted in no perceptible change in sensory quality of Atlantic salmon (Hardy *et al.*, 1987).

The quality of frozen fish during storage is affected by oxygen availability, presence of antioxidants, storage temperature and duration (Bjerkeng, 2000). In a study by Farmer *et al.* (2000), farmed Atlantic salmon exhibited no significant differences in terms of appearance, odor, flavor, texture, aftertaste, and overall acceptability after a storage period of up to 34 weeks at –25 °C before the commencement of sensory analysis.

In cases of inadequate storage temperature, di- or tri-methylamine are produced by endogenous enzymes, which cause the fillet to smell 'fishy' (Farmer *et al.*, 2000). But replacing dietary herring oil with sunflower oil enriched with oleic acid decreases fishy aroma because mono-unsaturated fatty acids are thought to decrease oxidative rancidity (Skonberg *et al.*, 1993). Laverty (1993) recommended bleeding the fish at sampling to avoid blood left behind which can give rise to a strong metallic taste and the bleeding of fish prior to storage helps decrease the likelihood of lipid oxidation in the stored salmon.

Refsgaard *et al.* (1998), in a study on the sensory and chemical changes in farmed Atlantic salmon during frozen storage, noted that metallic and bitter aftertastes increased during storage of individual fish fillets in aluminum foil and polythene bags at –10 and –20 °C for up to 34 weeks. The odors identified in stored salmon fillets were fishy, buttery, sweet, and green. The salmon color intensity decreased during the storage period but was found to be independent of the storage temperature.

2.2.2 Color

The conspicuous red/pink color of the flesh of salmon is due to the presence of carotenoid pigments (Shahidi *et al.*, 1998). Astaxanthin is the dominant carotenoid pigment (3-11 mg/kg; Skrede and Storebakken, 1986a) that is found in the flesh of wild salmon (Khare *et al.*, 1973; Schiedt *et al.*, 1985). Wild salmon absorb this carotenoid from their prey (crustaceans) because they cannot synthesize it themselves. The diets for farmed salmon are generally supplemented with synthetic astaxanthin and/or canthaxanthin with the concentration of carotenoid pigment(s) in their flesh at the time of marketing generally ranging from 4 –10 mg/kg (Torrissen *et al.*, 1989).

Since carotenoid pigments influence flesh color in salmonids and ultimately consumer acceptance (Johnston *et al.*, 2000; Skonberg *et al.*, 1998; Sigurgisladottir *et al.*, 1994; Gormley, 1992; Rounds *et al.*, 1992; Hatano *et al.*, 1987; Simpson *et al.*, 1981; Ostrander *et al.*, 1976), it is important to include optimal concentrations and forms of carotenoid pigments in diets for farmed salmon for cost effective pigmentation. Astaxanthin, has been reported by Storebakken *et al.* (1987) to be utilized more efficiently for flesh pigmentation than canthaxanthin or astaxanthin dipalmitate. Also, it should be mentioned that amongst all the salmonids, Atlantic salmon have the lowest efficiency in utilizing astaxanthin (March and MacMillan, 1996).

The most important commercial source of astaxanthin, Carophyll Pink, (Hoffmann La Roche, Basel, Switzerland), is a very costly feed ingredient and its dietary inclusion may account for more than 15-20% of the total feed cost (6-8% of the total production cost) of Atlantic salmon (Torrissen *et al.*, 1995). Moreover, usually less than 20% of the carotenoid pigment that is ingested is retained in the flesh of salmonids (Torrissen *et al.*, 1989; Storebakken and No, 1992).

When 5% of astaxanthin was provided in the diet of Atlantic salmon subsequent storage of the fillets at –25 °C for 5 months prior to color measurement resulted in no significant ($p > 0.05$) changes in color. Moreover, the correlation between the Natural Color Systems (NCS) and CIE (a*) scores was 0.78 (Skrede *et al.*, 1990).

Various packaging methods including air impermeable, oxygen-evacuated, and CO_2-enriched packages (vacuum bags), were used by Chen *et al.* (1984) for preservation of color in raw, sectioned fillets from rainbow trout during frozen (-20 °C) and refrigerated (1-2 °C) storage for 1, 6, 11 and 14 days. The flesh portion was used for sensory and chemical analyses. Highest pigment levels were seen in the air impermeable packed samples, whereas reduced pigment levels were observed in the vacuum-packed and CO_2-enriched samples (53% of the flesh pigment concentration was degraded) in 14 days at 1-2 °C. But no significant changes ($p > 0.05$) were reported between the packaging procedures when the fillets were stored for one month at –20°C.

No and Storebakken (1991) found that rainbow trout fillets at –20 to –80 °C had less than 5% loss of pigment over 6 months storage. Anderson and Steinsholt (1992) found that the redness of Atlantic salmon (gutted fish) stored at –35 °C was very stable. Sheehan *et al.* (1998) also noted that no significant changes in visual color scores or carotenoid pigment concentrations in Atlantic salmon (whole fish) occurred after 12 weeks of storage. The presence of antioxidants, like Vitamin E in the diet, also has been found to help and preserve the fillet color of Atlantic salmon during frozen storage (Bjerkeng *et al.*, 1999).

2.2.2.1 Sample location

Astaxanthin deposition in the flesh partially depends on the genetic origin of the salmonid and may vary with age, growth rate and state of sexual maturation (Torrissen and Naevdal, 1988; Torrissen *et al.*, 1984; Choubert *et al.*, 1997). Astaxanthin probably binds non-specifically to the hydrophobic sites on actomyosin (Hennmi and Hata, 1989). Nickell and Bromage (1998) suggested that distributional variations in both the number and size of muscle fibers could influence the number of binding sites for astaxanthin which in turn can result in varying visual color intensity in different parts of a fillet.

Astaxanthin concentration has been found to be 30-40% greater in the caudal part of the Atlantic salmon fillet than in the back and neck parts (Christiansen and Wallace, 1988; No and Storebakken, 1991). Refsgaard *et al.* (1998), however, reported that the astaxanthin concentration was 19% more in the caudal region of the fillet. In addition, McCallum *et al.* (1987) found an increase in carotenoid pigment concentration in the muscle towards the backbone of chinook salmon.

The lipid concentration in salmonid fillets decreases between the anterior and the caudal region and there also appears to be more concentration of lipid in the muscle along the lateral line (Hardy and King, 1989). A relatively high variation of fat content exists between fillets from individual fish within a population and it can range from 12-22% (Sigurgisladottir *et al.*, 1997). Lipid also has a diluting effect on color perception, since it tends to mask the pigmented muscle lying beneath the lipid (Christiansen *et al.*, 1995).

The sample location on the fillet (Figure 2.6) from which the different quality parameters are estimated, also varies from country to country. Hence, there is an urgent need to standardize the sampling techniques (Sigurgisladottir *et al.*, 1997). The Norwegian standard section, described by Sigurgisladottir *et al.* (1997) is thought to be the best available method because it varies proportionally with the size of the fish, *i.e.* the sample size gets bigger in larger fish.

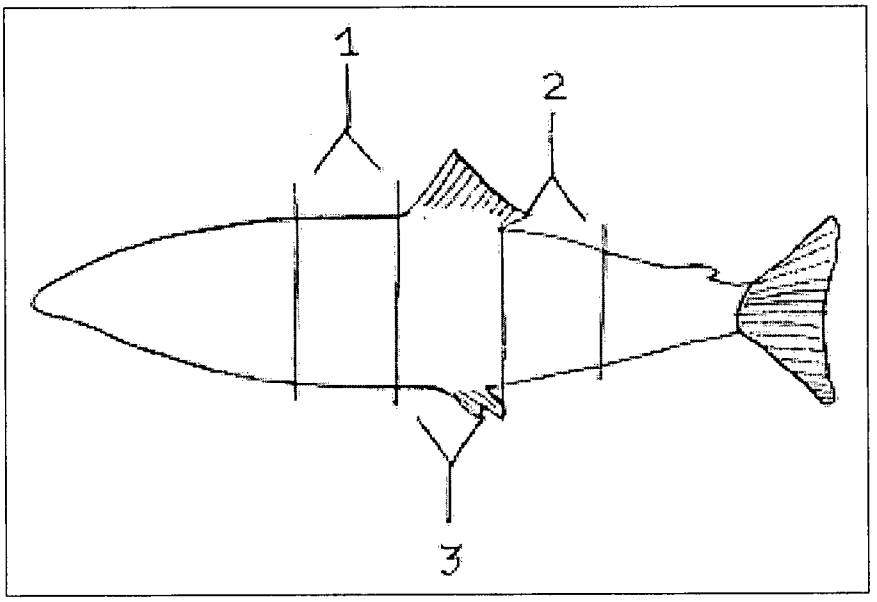

Figure 2.6: Different muscle sampling locations used in the salmon industry: 1) section normally used in France; 2) section recommended by Norway; 3) section commonly used in Europe (Sigurgisladottir *et al.*, 1997).

Recent studies reported an increase in the concentration and retention of astaxanthin in the flesh with increasing flesh lipid concentrations (Jensen *et al.*, 1998 in rainbow trout; Bjerkeng *et al.*, 1997 and Einen and Roem, 1997 in Atlantic salmon; Chan, 1999 in coho salmon), but not with increasing protein or carbohydrate concentrations in the fillets. Further, Bjerkeng *et al.* (1997) reported that fatter Atlantic salmon had more astaxanthin than leaner ones.

Bjerkeng *et al.* (1997) also reported that high dietary fat concentration helped facilitate astaxanthin absorption, leading to increased astaxanthin deposition, which

subsequently increased a* values in Atlantic salmon. Alternately, Sheehan *et al.* (1996) did not find any influence of dietary fat concentration on (a*) values for the flesh. The majority of studies, however, have found that increased dietary fat concentration enables salmon to deposit more astaxanthin in the muscle as shown by higher a* values (Bjeerkeng *et al.*, 1997; Tibaldi and Ballestrazzi, 1990; Torrissen *et al.*, 1990). Chan (1999) also reported a positive correlation ($r^2 = 0.68$) between fillet lipid content and astaxanthin concentration in post juvenile coho salmon reared in seawater. However, such positive correlations are not always found and additional studies are required to clarify the situation (Bjerkeng, 2000).

2.2.2.2 Visual methods

Visual assessments of color are crude estimates and are primarily meant to be used by fish farmers, fish-feed producers, processing plant operators and marketing organizations (Bjerkeng, 2000). The Salmofan™ color cards were developed by Skrede *et al.* (1990) for Hoffmann La Roche and they were based on flesh color investigations in Atlantic salmon. Generally, salmon with a flesh color score of 22-24 are considered to be less preferable for the consumer than those having a color score of 33-34 (Anderson, 2000).

2.2.2.3 Correlation between visual color assessment and astaxanthin concentration

In various studies on Atlantic salmon, the color card scores have correlated well, but nevertheless non-linearly, with the chemical determination of flesh astaxanthin concentrations. Christiansen *et al.* (1995) reported that $r^2 = 0.992$ between color card scores and astaxanthin concentration in the flesh of Atlantic salmon. There exists a positive relationship between Salmofan™ scores and muscle fiber density (Johnston *et al.*, 2000). But due to the lack of correlation between muscle fiber density and astaxanthin concentrations (Little *et al.*, 1979), carotenoid pigment concentration alone cannot be used to predict color by visual means.

Johnston *et al.* (2000) showed that Salmofan™ scores were significantly related to the muscle fiber density in the myotomal cross sectional area between dorsal and mid-belly regions of Atlantic salmon fillets. Muscle fiber density in the fresh Atlantic salmon fillet correlated significantly and positively with the Salmofan™ scores (Johnston *et al.*, 2000). Since color is a function of both light scattering and absorption by the muscle (Offer *et al.*, 1989) and muscle fiber density affects this, fish having high muscle fiber density could show a tendency for improved color as assessed visually for a given astaxanthin concentration.

2.2.2.4 Instrumental methods

As the visual means of quantifying color is subjective, there is an increasing dependence on instrumental techniques like CIE and Hunterlab systems for consistent and accurate quantification and recording of color (Hunter and Harold, 1987; Bjerkeng, 2000). But the color data provided is good only as far as the equipment's calibration and environment. The light source and background affect measurement of color. Therefore, it is necessary to standardize the light source and the surrounding environmental conditions to obtain reliable and reproducible results (Hunter and Christie, 1978; Skrede and Storebakken, 1986b; Skrede *et al.*, 1990).

2.2.2.4.1 CIE model

The color appearance of any object depends on three components viz, light source (illuminant), the object itself and human vision/photocell (Fairchild, 1998). In order to standardize the illuminant, the Commission Internationale de l'Eclairage (CIE), in 1931, established 'Standard Illuminants', which have spectral lines that are similar to that of natural light sources and are reproducible in the laboratory. They are called illuminants A, B and C where A is defined as light from an incandescent source, B represents direct sunlight and C the average sunlight from the total sky. In 1966 another series of illuminants 'D' was

adopted. D illuminants, besides completely and accurately describing daylight, also define a complete series of yellow and blue colored temperatures. D illuminants are identified from the first two digits of their color temperature. Thus D_{65} stands for a D illuminant at 6500 K temperature (Hunterlab, 1983).

Once the illuminant has been standardized, the interaction of its radiant light with the object is characterized in terms of radiometric quantities (reflection, absorption or transmission), which is measured spectrophotometrically or colorimetrically. According to this model, any color C can be described as:

$$C = R\,(r) + G\,(g) + B\,(b)$$

where the color C is matched by (r) units of Red, (g) units of Green and (b) units of Blue. Here, the red, blue and green spectral colors are defined using their precise wavelength in the spectrum (Chamberlin, 1951):

Red R = 0.7000 microns
Green G = 0.5461 microns
Blue B = 0.4358 microns

Thus the terms RGB indicate the amount of three primary colors required to match the color C of the given object and are referred to as 'tristimulus values'. The disadvantage of using the RGB tristimulus values for color matching to a specific object was that certain values had to be negative in order to match a particular color stimulus. Since negative light cannot be added in real situations, another set of tristimulus values were developed by CIE and called theoretical/mathematical tristimuli XYZ.

Using the XYZ model helped eliminate negative values in the color matching functions, thus making the color functions easier to deal with mathematically. The X and Z are chosen such that they produce no luminance response thereby leaving all of the luminance response to Y. This is a major improvement over the RGB model because the

luminance is not taken into account there. Thus the imaginary XYZ produce all positive color matching functions.

The X,Y,Z data are usually plotted as x, y and z coordinates where:

$$x = X / X+Y+Z$$

$$y = Y / X+Y+Z$$

$$z = Z / X+Y+Z$$

and where x + y + z = 1, so usually the spectrum is plotted two dimensionally on an *x,y* plot (Figure 2.7). Although XYZ tristimuli are mathematical values, they can be expressed in terms of RGB as:

A unit of red **R** = 0.490 units of **X** + 0.177 units of **Y**

A unit of green **G** = 0.310 units of **X** + 0.812 units of **Y** + 0.010 units of **Z**

A unit of blue **B** = 0.200 units of **X** + 0.011 units of **Y** + 0.990 units of **Z**

Thus the color of each wavelength in the spectrum can be expressed as a sum of different amounts of XYZ. The XYZ tristimulus values can combine in three dimensions to correspond to light–dark, red-green, and yellow-blue responses, and ultimately define color (Fairchild, 1998). The CIE (L*, a*, b*) tristimulus values are defined as:

$$\mathbf{L}^*_{(\text{lightness})} = 116\,(Y/Y_0)^{1/3} - 16$$

$$\mathbf{a}^*_{(\text{red to green})} = 500\,[\,(X/X_0)^{1/3} - (Y/Y_0)^{1/3}\,]$$

$$\mathbf{b}^*_{(\text{yellow to blue})} = 200\,[\,(Y/Y_0)^{1/3} - (Z/Z_0)^{1/3}\,]$$

where XYZ and $X_0Y_0Z_0$ are the tristimulus values for the sample and the white reference, respectively.

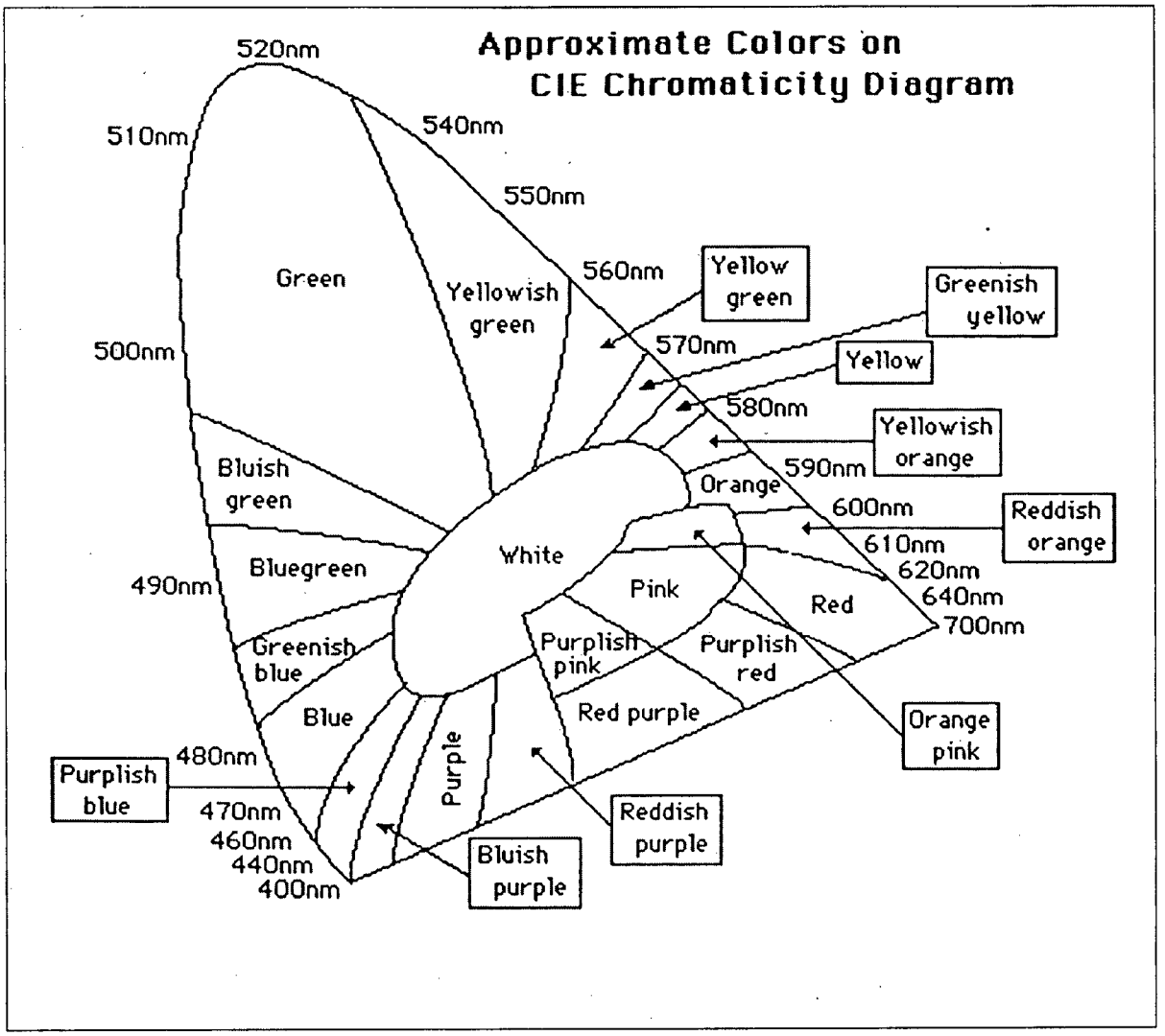

Figure 2.7: CIE chromaticity diagram. Approximate color regions are depicted and are rough categories rather than a precise color statement. The 'white' region represents the area where the three primary colors are present in equal amounts (www.hyperphysics.phy-astr.gsu.edu/hbase/vision/cie.htm).

2.2.2.4.2 Hunterlab model

Since CIE scales do not provide uniform intervals of perceived color in a three dimensional space (a large portion of the scale is dominated by green while reds are virtually condensed), scientists developed the so-called uniform color scales, i.e., a color space wherein equal distances approximately represent equal magnitude of color differences. These are called opponent colors (L, a, b type) scales based on opponent colors theory (you cannot see opponent colors together i.e., red and green or blue and yellow) given by Ewald Hering in 1930 and refined by G.E. Muller in 1930. The actual Hunterlab uniform color space was devised by Richard Hunter in 1942. Its rectangular coordinates are:

$$\mathbf{L}\ (\text{lightness}) = 100\ (Y/Y_0)^{1/2}$$

$$\mathbf{a}\ (\text{red to green}) = 175[\ X/X_0 - Y/Y_0\ ] * \left\{ \frac{0.0102\ X_0}{Y/Y_0} \right\}^{1/2}$$

$$\mathbf{b}\ (\text{yellow to blue}) = 70\ [\ Y/Y_0 - Z/Z_0\ ] * \left\{ \frac{0.00847\ Z_0}{Y/Y_0} \right\}^{1/2}$$

where XYZ and $X_0Y_0Z_0$ are the tristimulus values for the sample and the illuminant, respectively. Tristimulus values are the amounts of 3 matching lights required to match the shade being tested (Hunter and Harold, 1987).

Both spectrophotometers and tristimulus instruments (colorimeters) are used to measure color. While spectrophotometers generate wavelength by wavelength analyses of light reflecting or transmitting properties of objects, tristimulus instruments utilize filters and measure color in X,Y,Z or L,a,b values. Tristimulus colorimeters provide more precise and less expensive methods for routine inter-comparisons. In these systems (CIE and Hunterlab), color is described by the following parameters: L, a, b; where L = lightness or darkness, +a = redness, -a = greenness, +b = yellowness and –b = blueness of the sample.

2.2.2.5 *Correlation between the instrumental results and astaxanthin concentrations*

Schmidt and Idler (1958) found that Hunter (a) values were a good measure of visual red color of raw and processed sockeye and Atlantic salmon. Due to dissimilar nutritional status, stress, fish size, sexual maturation and environmental factors, flesh pigmentation in salmonids can vary between fish fed diets with the same astaxanthin concentration for a similar duration (Bjerkeng, 2000). Non-linear relationships have been reported between flesh astaxanthin concentration and various instrumental measurements of color in many studies on salmonids (Skrede and Storebakken, 1986b; Christian *et al.*, 1995; Choubert *et al.*, 1997; Nickell and Bromage, 1998).

2.2.2.6 *Correlation between the two instrumental techniques for quantifying color*

The different color systems (CIE and Hunterlab), that are used to quantify color in food products, all demonstrate that the value (a), the redness component, is proportional to the carotenoid pigment concentration (Schmidt & Idler, Hunter 'a' value, 1958; Saito, 1969; Choubert, 1982; Choubert *et al.*, 1997; Skrede and Storebakken, 1986b; Storebakken *et al.*, 1987; Hatlen *et al.*, 1998). Both systems, Minolta Chromameter (chromameter presents values L_{xy} where x and y are chromaticity coordinates of CIE x,y Chromaticity Diagram, and L is the luminance) and Hunterlab Labscan sphere spectrocolorimeter, have been found to be equally well suited for measuring salmon flesh color (Skrede and Storebakken, 1986 a,b). Baardseth *et al.* (1988) found a good relationship between color measurements obtained using CIE and Hunterlab for all the food commodities examined (pulses, cereals, dairy products etc.). Direct comparisons were difficult because separate regression equations had to be established for each food of interest.

2.2.2.7 *Correlation between the instrumental and visual techniques of quantifying color*

Christiansen *et al.*, (1995) demonstrated that although there was a linear correlation between the Roche Color card and CIE 1976 (a*) scores in Atlantic salmon, the color cards were not good predictors of astaxanthin content in the muscle of individual fillets because of high color variability across the length of the fish. In 1998, Storebakken *et al.* found that Salmofan™, Roche color card and CIE 1976 (a*) values were linearly correlated ($0.46 < r^2 < 0.51$) with the carotenoid pigment concentration in Atlantic salmon fillets. The carotenoid concentration in the fillets ranged from 3.3 – 4.3 mg/kg and this range corresponded to Salmofan™, Roche color card and CIE 1976 (a*) values of 25 – 26, 14 – 15 and 7.8 – 9.5, respectively.

When astaxanthin concentrations in fillets of Atlantic salmon exceed 6-8 mg/kg flesh, the eye becomes saturated leading to inaccurate perception of color hues. This leads to a plateau in color perception even with an increase in pigment concentration (Johnsen and Wathne, 1990; Bjerkeng, 2000). This inaccuracy mirrors the leveled off (a*) values at high astaxanthin concentrations (4-6 mg/kg) in the fillet (Bjerkeng, 2000). In this case, nonlinear relationships between CIE 1976 (L*,a*,b*) and carotenoid pigment concentration have been observed to give a higher correlation (Christiansen *et al.*, 1995; King, 1996, Bjerkeng *et al.*, 1997).

Many mathematical models have been used to describe the relationship between visual and instrumental assessment of color (Hatlen *et al.*, 1998). The models differ according to the experiment that they were based on. Little *et al.* (1979) found that multiple regression analyses which included L*, a* and b* values could be used to match instrumental to visual color evaluation. According to Little *et al.* (1979) carotenoid pigment concentration was not a good predictor of color by itself since the fat content affects the visual appearance of the samples having equal astaxanthin concentration. Schmidt and Cuthbert (1969) used a ratio of reflectances at different wavelengths (R_{650}/R_{510}) to evaluate color and then matched these with the color card scores of Pacific salmon. They further

developed an instrument for sorting salmon color along the production line based on this reflectance ratio (Francis and Clydesdale, 1975).

Since visual perception of salmon flesh color can vary at a given concentration of astaxanthin (Torrissen *et al.*, 1989; Christiansen *et al.*, 1995), common methods of evaluating color like the color card (Skrede *et al.*, 1990) and instrumental analysis (Skrede and Storebakken, 1986b; Christiansen *et al.*, 1995) should be applied together. Since both the CIE 1976 and Hunter Lab systems have limited abilities for accurate assessment of color when concentrations of carotenoids in the fillet exceed 4-6 mg/kg, those instruments should be restricted to the measurement of color in small fish or fish having less than 5 mg/kg of flesh astaxanthin (Einen and Skrede, 1998; Bjerkeng, 2000).

2.2.3 Texture

Firmness or texture is a very valued sensory attribute both for the food industry involved in the mechanical processing of fillets and for the consumer of fishery products (Dunajski, 1979; Haard, 1992). Fillet texture is influenced by the intrinsic muscle structure with its connective tissue matrix, and the various post-mortem changes associated with slaughter and storage (Fauconneau *et al.*, 1995, 1997). Although collagen fibers contribute towards the texture in raw fish they are relatively unimportant after cooking (Hatae *et al.*, 1986). The muscle fibers provide the major texture perception after cooking (Dunajski, 1979).

Juiciness is related to water holding capacity and lipid content of the fillet and seems to be influenced by the fatty acid composition of Atlantic salmon flesh (Waagbo *et al.*, 1993). Johansson and Kiessling (1991) demonstrated that starved rainbow trout were less juicy due to the reduced lipid content of the fillet. Lipid content and distribution can affect the perception of texture; lipids have a softening effect (Haard, 1992). Texture appears to be harder towards the tail side of the fillets (Sigurgisladottir *et al.*, 1994) and varies according to the size and distribution of myotomes (Dunajski, 1979).

2.2.4 Headspace gas chromatography (GC)

2.2.4.1 GC analysis

Volatile components from food often reflect its quality (Girard and Durance, 2000). Gas chromatography (GC) has been successfully used for a long time for the determination of volatiles in food. But the analysis cannot be conducted on the sample directly without some kind of sample preparation. Sample preparation usually includes homogenization of the sample to permit concentration and accessibility of the volatiles before analysis. Due to instrumental constraints only a small sample of volatiles can be injected. Therefore, usually volatiles present in the head space at a concentration of more than 10^{-7} g/L head space volume are detected using a FID (flame ionization detector). FID responds very well to the presence of organic compounds and so it is widely used as a detector for food volatiles (Reineccus, 1998).

Two types of volatile collection techniques are used – static and dynamic head space analysis. Static headspace sampling is generally used for rapid analysis of a major component; for example, measurement of hexanal as an indicator of oxidation (Seo and Joel, 1980). Dynamic headspace analysis involves concentration of the headspace volatiles, followed by trapping and de-sorption onto a GC column. The concentration of volatiles is usually accomplished by sample agitation and the addition of salt, which increases the headspace vapor pressure and thereby ensures maximum availability of the volatiles for adsorption onto the trap. Portable samplers act as adsorbent traps to trap water-free volatiles from the headspace and generally consist of synthetic porous polymers like tenax or SPME (solid phase micro extraction) fibers (Reineccus, 1998). An SPME unit consists of a fused silica fiber coated with stationary phases like – carbowax (CW), polyacrylate or polydimethylsiloxane (PDMS). These phases can be mixed with solid adsorbants like divinylbenzene (DVB) polymers or templated resins. The concentrated adsorbed volatiles are subsequently de-sorbed from the fiber directly inside a heated GC injection port.

2.2.4.2 *Fish volatiles*

Salmon in its raw, fresh state has a mild aroma arising from carbonyls and alcohols produced from the enzymatic degradation of fatty acids (Josephson *et* al., 1991). Cooked salmon whether pan-fried, baked or roasted, develops an odor typical of heat treatment (Girard and Durance, 2000). A typical salmon aroma is the sum total of the effects of sulfurs, aldehydes and ketones generated from the dehydrogenation of fatty acids, carotenoids and amino acids (Girard and Durance, 2000). Sensory changes during frozen storage are largely due to the formation of secondary oxidation products like aldehydes and ketones (Refsgaard *et al.*, 1998).

Refsgaard *et al.* (1998), in a study on the sensory and chemical changes in farmed Atlantic salmon during frozen storage, demonstrated that cooked salmon is characterized by a high intensity of earthy, sweet, sour, and fish oil notes. They also observed that metallic and bitter aftertastes increased during storage of individual fish fillets in aluminum foil and polythene bags at –10 and –20 °C for up to 34 weeks. The odorants identified in stored salmon fillets were fishy, buttery, sweet, and green. But the authors failed to mention the cooking method used for their study. That study also showed a correlation between metallic aftertaste in cooked samples and hexanal ($r = -0.304$) as a variable. Similarly, a bitter taste in cooked samples correlated ($r = 0.521$) with the ratio of 20:1 (n-9), a methyl ester of monoenoic fatty acid, measured before and after storage.

2.2.4.3 *Principal Component Similarity (PCS)*

Large amounts of data produced during food analysis makes it imperative to use multivariate analyses for efficient and meaningful data processing. Principal component analysis (PCA) is usually the multivariate technique of choice to condense the data to analyze the interrelationships between the many variables involved. PCA relies on the

premise that a few factors (unrelated to the original data-variable) can explain most of the variance observed in the data. These unrelated variables are called principal components (PC) and once the relevant PCs are chosen, only these are analyzed which simplifies the analytical procedure and leads to easier interpretation of the data. Another multivariate technique based on the PCA model is called principal component similarity (PCS) analysis. Both PCA and PCS are used when detailed information is not available and many variables are involved. Their strength lies in detecting outliers or anomalies and the ability to identify groups. PCS differs from PCA in a few ways that make it more suitable.

(1) PCS uses a linear regression of "deviations in PC scores" on "variance due to the PC scores" as the basis of classification (Vodovotz *et al.*, 1993) while in PCA, similarity coefficients are plotted directly from the PC scores. This linear regression is then presented as a PCS scattergram (a plot of "slope" vs. "coefficient of determination"). This results in PCS utilizing more than three PC scores for classification, which leads to a classification ability superior to that of PCA (Nakai *et al.,* 2000; Horimoto *et al.*, 1997).

(2) PCS can detect anomalies like outliers and also it can find the causes of grouping data, which can further be extended to the possible discovery of new groupings (Nakai *et al.*, 2002). This can be used for classifying consumers during market-research when no other information (such as age, sex, income, education) about the consumers are known. In this way, PCS can be used as a predictive model.

(3) PCS also has the advantage of changing/rotating the reference and the number of PC scores that are used. It is also possible to create multiple/different classifications because the difference in slopes is greater for fewer PC scores than in the case of inclusion of more PC scores. These multiple comparisons are useful to understand the complete picture and also when one particular scattergram is unable to provide meaningful results.

PCS demonstrates similarity between samples using the principal component (PC) scores instead of the original chromatographic data directly. The peak areas obtained from the GC chromatogram of the samples are usually standardized (relative to the internal standard used) and then subjected to the PCS program. A linear regression analysis of the deviations of PC scores of an unknown relative to those of the reference sample versus the variability accounted for by the PC scores is used to select the PC scores that have eigenvalues ≥ 1.0, since they contribute the most to the variation (Kaiser rule). Alternately, a graphical representation called 'scree-test' is also used to determine the number of PC scores to be included for the analysis. Here, eigenvalues are plotted against their respective PC scores and the cut-off point is determined where the slope levels off. Thus PCS can effectively reduce the number of original variables needed for analysis to just a few 'principal components', that are obtained after linear combinations, which can then be used to identify the points that contribute the most to the observed variation in the data.

GC followed by principal component similarity (PCS) analysis can also be employed as an analytical tool in quality assurance of food (Ogihara *et al.*, 2000). It has been used successfully as an initial step to crudely classify contaminating bacteria in beef (Ogihara *et al.*, 2000), as well as salmon and hamburgers (Nakai *et al.*, 1999) as a primary step before any other sophisticated identification assays have been employed. It has also been used successfully to classify mango samples based on the volatiles assessed by GC (Vodovotz *et al.*, 1993). PCS was demonstrated to be more advantageous than PCA for evaluating progressive changes during cheese aging (Furtula *et al.*, 1994a, b), detecting spoilage (Nakai *et al.*, 1999) and finding unexpected results (new type of beany flavor in soymilk, (Wang *et al.*, 1998).

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CHAPTER 3

EFFECT OF VARYING DIETARY CONCENTRATIONS OF PARTIALLY DEHULLED AND EXTRUDED SUNFLOWER-MEAL ON GROWTH PERFORMANCE OF POST-JUVENILE ATLANTIC SALMON (*Salmo salar*)

ABSTRACT

This study assessed the nutritive value of nutritionally upgraded (partially dehulled and extruded) sunflower meal (SFM) for Atlantic salmon (*Salmo salar)* in seawater. The proximate compositions of whole body and muscle along with energy utilization were also studied. Fifteen groups of 50 (initial mean weight ~ 116 g) Atlantic salmon were held in 4000 L outdoor fiberglass tanks supplied with running (25-40 L/min), filtered, oxygenated (D.O. 7.0 - 8.5 mg/L) and ambient temperature (11.0 - 12.1 ^{o}C) seawater (salinity, 29- 31 ppt). During the 84-day feeding trial, triplicate groups of salmon were fed one of five diets formulated to have equivalent digestible protein and energy content. LT Anchovy meal comprised 68.2 % of the basal diet. SFM progressively replaced in equal steps, up to 33.0% of the digestible protein provided by LT Anchovy meal in the basal diet, or it comprised up to 27.1% of the diet on a dry weight basis. All four test-diets that contained SFM supported similar salmon performance to the basal diet (contained no SFM) in terms of growth, feed intake, feed efficiency, protein and energy utilization and percent survival.

Hence, this study demonstrated that one third of the digestible protein provided by the premium quality fishmeal protein in the basal diet could be replaced by digestible protein from partially de-hulled, extruded SFM without any adverse effects on the growth rate (ranged from 1.39 to 1.45 % per day) and protein utilization of feed (protein efficiency ratio values ranged from 2.40 to 2.56) of Atlantic salmon, under the conditions used in this study. Also, the muscle and whole body proximate compositions of the salmon were unaltered by dietary treatment. An inverse relationship was observed between the digestibility of energy in the diets and the dietary SFM concentration. This trend was probably due to increasing proportions of indigestible carbohydrates originating from the nutritionally upgraded SFM, but it is noteworthy that none of the diets differed significantly in digestible energy content.

3.1 INTRODUCTION

It is a well known fact that fish and fish products are one of the major sources of nutrition for humans. As the world's population continues to increase, the global consumption of fish products has also grown mainly through the expansion of aquaculture to keep pace with the growing demand for aquatic protein. The latter has occurred because global fish capture from both marine and fresh water resources has reached a plateau (New, 1997) and it may indeed decline due to erratic global climatic changes and more frequent phenomena like *El Niño*. High quality fishmeal is widely known to be the best source of dietary protein for farm raised salmonids (Pike *et al.*, 1990), but it is often very expensive, especially during *El Niño* events.

In B.C., high quality fishmeals for manufacture of salmon diets mainly originate from South America. However the world wide supply of fishmeal is finite, and most likely the cost of fishmeal will increase in the future due to the increased demands that are being placed on this valuable commodity. This trend will reduce the profitability of salmon farming in B.C. since, typically, feed accounts for 35 – 60 % of the production costs. Moreover, the protein sources (mainly fishmeal) account for about half of the diet cost (Higgs, 1997). Therefore suitable alternative sources of protein, preferably from less costly nutritionally upgraded plant sources are needed for cost effective salmon culture.

There is some information on the nutritive values of plant protein sources in diets for Atlantic salmon in fresh and seawater. To date, most studies have assessed the nutritive values of soybean meal and soybean protein concentrate (Storebakken *et al.*, 1998; Stickney *et al.*, 1996; Rumsey *et al.*, 1993; Berge *et al.*, 1999). Other studies have been conducted mostly on rainbow trout fed diets containing different concentrations of oilseed meals like soybean meal, cottonseed meal, rapeseed meal and canola meal (Hardy and Sullivan, 1983; Tacon *et al.*, 1984; Viola *et al.*, 1983) or oilseed protein concentrates (Storebakken *et al.*, 1998; Stickney *et al.*, 1996). Wheat gluten meal (Sugiura *et al.*, 1998;

Thodesen and Storebakkan, 1998; Watanabe *et al.,* 1993) and corn gluten meal (Cho and Kaushik, 1990; Watanabe *et al.,* 1997).

Sunflower protein products have not been evaluated in the diets for Atlantic salmon, even though numerous studies have been conducted on rainbow trout (Tacon *et al.,* 1984; Martinez, 1986; Cardenete *et al.,* 1993; Sanz *et al.*, 1994; Stickney *et al.,* 1996). Collectively, these have shown that sunflower meal may comprise up to 41.8% of the dietary protein without compromising trout performance (*i.e.,* growth, feed efficiency and survival).

The carbohydrate fraction in most plant-protein sources is generally not very digestible. The varying bioavailability of carbohydrates in them depends on factors like the proportion of indigestible hulls in the meal, the degree of starch gelatinization or other factors. The often high concentrations of the indigestible fiber fraction in oilseed meals after oil extraction can be decreased by front-end dehulling of oilseeds (Jones, 1979) before further processing or tail-end dehulling of oilseed meals (McCurdy and March, 1992) and there is an attendant increase in the protein concentration. Solvent extraction (aqueous and/or alcohol) is one means that can be used to reduce the indigestible carbohydrate fraction that is present in the oilseed protein products before their use in any diet. Enhanced carbohydrate bioavailability can also be accomplished by high temperature extrusion processing of oilseed meals and this may also concurrently decrease levels of phytic acid and other deleterious components (Satoh *et al.*, 1998). These approaches have not been applied to nutritionally up-grade sunflower meal (SFM) for salmonids.

Consequently, the objective of this project was to assess the nutritive value of tail-end dehulled and extruded SFM for Atlantic salmon in sea water. Accordingly, the growth performance of Atlantic salmon fed diets, containing one of four concentrations of nutritionally upgraded SFM by progressive replacement of premium quality fishmeal in a basal diet on an equivalent digestible protein basis, was compared to that of salmon fed a fishmeal-based basal diet for 84 days. At the end of this growth trial, salmon were sampled for body and muscle compositional analyses. It was hoped that the results from this study

would lead to more cost effective formulation of diets for Atlantic salmon and to additional studies aimed at improving the nutritive value of sunflower protein products for salmon and other aquatic species.

3.2 *MATERIALS AND METHODS*

3.2.1 Experimental salmon

Post-juvenile Atlantic salmon were obtained in January 2000 from NorAm Hatchery near Campbell River on Vancouver Island, B.C. They were held in outdoor 4000 L fiberglass tanks that were supplied with filtered, oxygenated, ambient temperature salt water at the West Vancouver Laboratory until the digestibility and growth trials could be conducted.

3.2.2 Experimental Facility

The growth trial along with a digestibility (bio-availability) experiment on Atlantic salmon (*Salmo salar*), were conducted on the premises of The Department of Fisheries and Oceans, West Vancouver Laboratory. Subsequent biochemical analyses associated with the above-mentioned experiments were also completed at the same location.

3.2.3 Experimental protocol

A schematic representation of the experimental protocol is depicted in Figure 3.1.

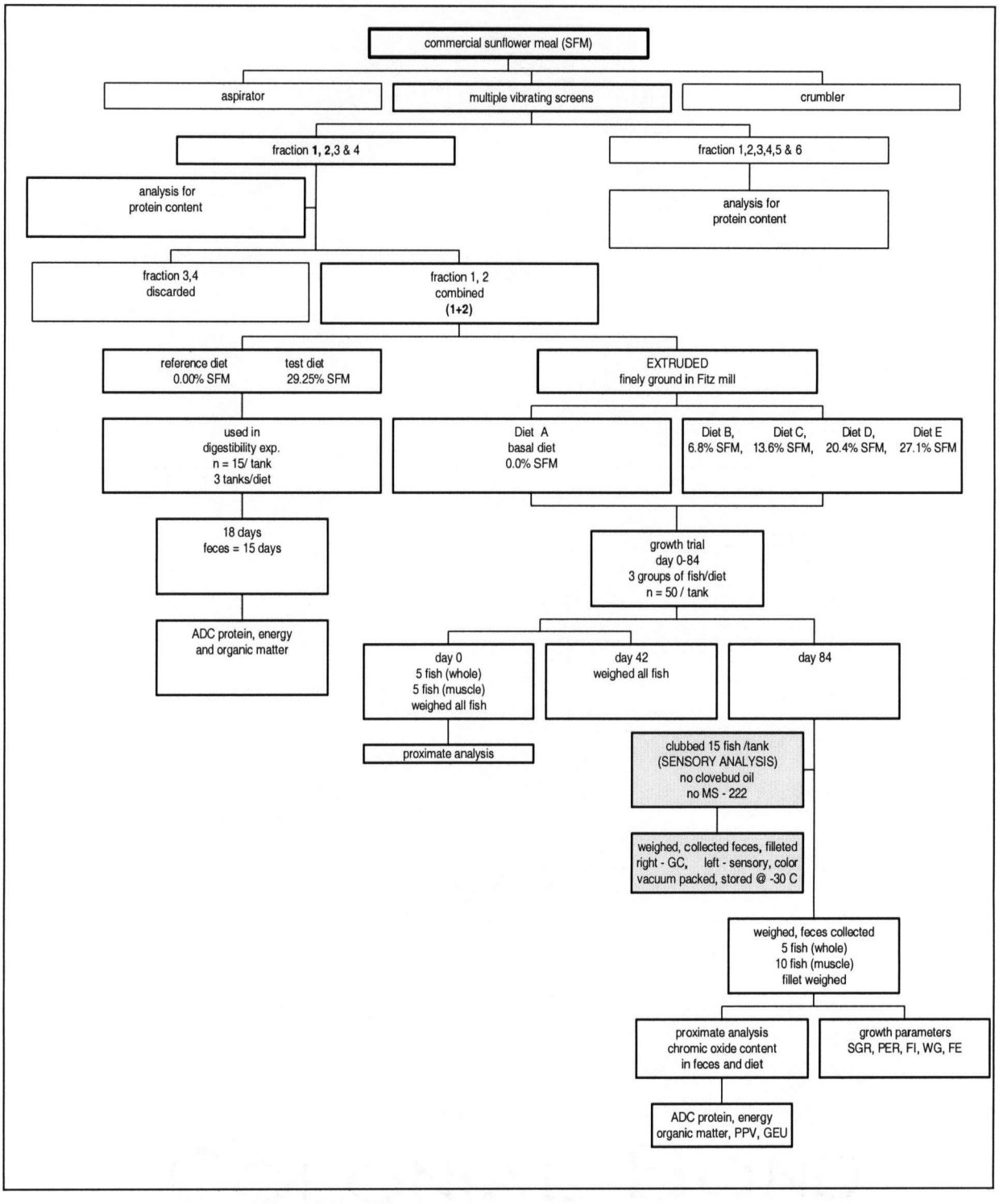

Figure 3.1: Schematic representation of the experimental protocol followed in this study. Shaded boxes represent the fillets used in sensory analysis (Chapter 4, Figure 4.1).

3.2.4 Preparation of partially de-hulled sunflower meal

A total of 500 kg of commercially prepared sunflower meal (SFM) was purchased from *Cargill* group of industries, U.S.A. This commercially available SFM (originated from non-genetically modified hybrid seeds that had high oil content i.e., ~40-45% before processing, with a high content of linoleic acid in the oil) contained a lot of hulls rendering it unsuitable for use in the diets for Atlantic salmon. Attempts were therefore made to improve its quality by partially de-hulling the meal using the procedures described below.

3.2.4.1 Crumbler

A laboratory scale crumbler (Manufactured by W.W. Grinder Corp., Wichita, Kansas) that was modified by motorizing both of the two corrugated rollers (one had a fixed speed of 300 rpm, while the other had any possible speed between 0 and 1200 rpm) was used in an attempt to separate the hulls from the meats through a 'shearing' action. The rotational speed of the variable speed roller was adjusted between 300 and 1016 rpm and several gap widths between the rollers were tested. However, all possible combinations of speeds and gap sizes between the rollers did not improve the de-hulling of SFM.

3.2.4.2 Aspirator

This device, operating as a cyclone-system, was specially constructed at the D.F.O. in an attempt to remove the hulls from the meats of SFM through air-classification. The latter procedure is based on the dissimilar densities between the hulls and the meats after the SFM has been ground to a common particle size. Poor success was achieved using this procedure and there was no significant separation of the hulls from the meat fraction.

3.2.4.3 Multiple vibrating screens

Manufactured by Kason®, the vibrating screen separator (Appendix A, Figure A.3) (Separator Engineering, Montreal, Canada) consisted of five mesh-screens - size

16M, 20M, 30M, 60M and 100 M. The screens were used to separate the commercial SFM into 6 fractions, as given in Table III-I. The protein concentration in each fraction was determined according to micro-kjeldahl method (Higgs *et al.,* 1979) and the results are provided in Table III-II. Since fractions 1, 2 and 3 had higher protein concentrations than the other fractions, another attempt was made to fractionate the commercial SFM by using 3 screens instead of 5. Thus 4 fractions were obtained by using the 20 M, 30 M and 40 M screens as shown in Table III-III. Fractions 1 and 2, which had the desired level of protein, were combined. This combination was found to be suitable for use in this project.

3.2.5 Fish - feed formulations

3.2.5.1 Preparation of reference and test diets for digestibility experiment

Of the six tanks allocated for the experiment, alternate tanks were chosen to represent the test and control groups. While the fish in the control group were fed a reference diet, the fish in the test group were fed the test diet, which was composed of 29.85% test ingredient (partially de-hulled SFM; combination fraction (1+2), refer to Table III-III) and 69.65% basal diet on an air-dry basis. Chromic oxide (0.5%) was added to the basal and the test diets as the indigestible marker. Both the reference and test diets were manufactured at the West Vancouver Laboratory. Table III-IV shows the ingredient and proximate compositions of the reference diet used in the digestibility experiment. Appendix A, Tables A.1 and A.2 provide the compositions of the vitamin and mineral supplements respectively, which were used in the preparation of the basal diet.

The moisture content of each diet mash was equalized to a level of 9% before cold pelleting. Both diets were cold pelleted using a 3.18 mm die and subsequently they were stored in a humidity controlled (Relative humidity 25%) feed room, in plastic air-tight containers at 14 - 15 ^{o}C until required.

TABLE III-I: Percent yield of different fractions of SFM obtained after multiple screening of commercial SFM (22.8 kg) using 5 screens.

Fraction #	*Screen #*	*Screen size (micron)*	*Weight (kg)*	*% Yield*
1.	100 M	140	0.460	2.37
2.	60 M	233	2.41	12.4
3.	30 M	516	2.42	12.4
4.	20 M	864	5.12	26.4
5.	16 M	1130	5.45	28.0
6.	Top Discard		3.58	18.4
			19.4	99.9 %

TABLE III-II: Percentages of ash, moisture and protein in each of the six fractions obtained after multiple screening of commercial SFM.

Fraction #	*% Ash*	*% Moisture*	*%Protein*	*Protein (db)**
1.	8.00	9.26	38.0	41.9
2.	6.88	12.0	37.6	42.8
3.	6.34	11.3	35.6	40.1
4.	5.40	11.1	28.4	31.9
5.	5.00	11.6	26.7	30.2
6.	6.02	12.1	30.5	34.7

*db= dry basis

TABLE III-III: Percent yield of different fractions of SFM obtained after multiple screening of commercial SFM (22.8 kg) using 3 screens. Percentages of ash, lipid, moisture and protein in the fraction used are also given.

Fraction #	*Screen #*	*Screen size (micron)*	*Weight (kg)*	*% Yield*
1.*	**40 M**	381	3.38	15.3
2.*	**30 M**	516	2.94	13.4
3.	20 M	864	7.56	34.3
4.	Top Discard		8.18	37.1
			22.1	100

Fraction #	*% Ash*	*% Lipid*	*% Moisture*	*%Protein*	*Protein(db)*
SFM					
(1+2)combined	7.35	2.63	7.58	37.8	40.9

* fractions 1 and 2 were combined and used in the diets for both digestibility and growth experiments

TABLE III-IV: Ingredient- and proximate- compositions of the reference diet used in the digestibility experiment conducted on Atlantic salmon (*Salmo salar*).

Ingredients	*(g/kg air dry basis)*
LT Anchovy meal	643.2
Blood flour (spray dried)	41.0
Wheat starch (pre-gelatinized)	80.9
Wheat starch (raw)	26.9
*Vitamin supplement	18.9
**Mineral supplement	18.9
LT Anchovy oil (stabilized,0.5g/kg santoquin)	122.4
Soybean lecithin	9.46
Choline chloride(60%)	4.73
Vitamin C (mono-phosphate, 42%)	3.38
Permapell	9.46
Finnstim™	14.2
DL-methionine	1.51
Chromic oxide	5.00
Proximate Composition	
Dry matter	879
Protein	453
Lipid	178
Ash	104

* refer Table A.1(Appendix A) ** refer Table A.2 (Appendix A)

- LT anchovy oil, Ewos Canada Ltd., Surrey, BC (from South America)
- Blood flour; spray-dried, American Protein Corporation, Manning, IA USA
- Pregelatinized wheat starch and raw wheat starch, Archers Daniels Midland, Montreal, Canada
- Soy lecitihin, Central Soy Company Inc., Fort Wayne, Indianna, USA
- Choline chloride, Van Waters and Rogers Ltd., Abbotsford, BC
- DL- methionine , Sigma Chemical Co., Missisuaga ON
- Ascorbic acid monophosphate (42%), BASF Canada Inc., Abbotsford, BC
- Permapell (lignin sulphonate binder) - Georgia Pacific, Bellingham, WA, USA
- Finnstim (diet palatability enhancer; > 90% betaine as well as free amino acids especially those with branched chains), Cultor Ltd., Finnsugar Bioproducts, Helsinki, Finalnd
- Chromic oxide, BDH Laboratory Supplies, Poole, England

3.2.5.2 *Preparation of diets for the growth trial*

The partially de-hulled SFM ($SFM_{(1+2)}$; refer to Table III-III) was sent to the Abernathy Technology Center, Longview, WA for extrusion. The conditions under which the SFM was extruded are provided in Appendix A, Table A.7. Thereafter, the extruded SFM was ground to a flour-like consistency in a Fitz mill (Homoloid® Machine model JT, code J16, The Fitzpatrick Company, Elmhurst, IL, USA).

Four diets varying in their concentration of partially de-hulled and extruded SFM were prepared by modifying the basal (control) diet (Table III-V) such that **diet A** *basal diet* had 0% SFM, whereas test diets **B, C, D** and **E** had 6.78%, 13.6%, 20.4% and 27.1% SFM on a dry weight basis by replacement of 8.25%, 16.5%, 24.75% and 33% of the digestible protein provided by fishmeal in the basal diet on an equivalent protein basis, respectively. Care was taken to ensure that all diets had adequate levels of sulfur amino-acids, lysine, vitamins (Appendix A, Table A.3) and minerals (Appendix A, Table A.4) as the concentration of SFM was progressively increased in the diets. Also 55 ppm of the pigment, astaxanthin (Carophyll®, Hoffman-La Roche, Basel, Switzerland) was added to each diet to enhance flesh color. In addition, each of the five diets contained 0.5% chromic-oxide as an indigestible marker to help determine the dietary levels of digestible protein and energy.

All of the five dry diets were steam pelleted (Laboratory pellet mill #315003 manufactured by California Pellet Mill Co, San Francisco, CA, Crawfordsville, IN) with a 4.76 mm die and each was formulated to contain 20% digestible lipid and 44 % digestible protein.

All the diets were analyzed for amino-acid composition (AAA Laboratory, Mercer Island, WA) and crude fiber content (POS Pilot Plant Corp., Saskatoon, SK) and the results of these analyses have been provided in Appendix A (Tables A.5 and A.6; Table A.8). Dietary concentrations of chlorogenic acid (Appendix A, Table A.8) were also determined using a capillary electrophoresis method (POS Pilot Plant Corp., Saskatoon, SK).

TABLE III-V: Ingredient compositions of the basal and four test diets used in the growth trial conducted on Atlantic salmon (*Salmo salar*). All diets were formulated to contain 442g digestible protein and 19.2 MJ digestible energy/kg on a dry weight basis.

Ingredients	*g/kg dry weight basis*				
	Diet A (0.0%SFM)	*Diet B (6.78%SFM)*	*Diet C (13.6%SFM)*	*Diet D (20.4%SFM)*	*Diet E (27.1%SFM)*
Austral fish meal	450.3	413.1	376.0	338.8	301.7
Sunflower meal$^{\Phi}$	**0.00**	**67.8**	**135.7**	**203.5**	**271.4**
Blood flour (spray-dried)	50.0	50.0	50.0	50.0	50.0
Squid meal	70.0	70.0	70.0	70.0	70.0
Wheat gluten meal	80.0	80.0	80.0	80.0	80.0
Pre-gelatinized wheat starch	122	89.8	77.3	39.8	2.32
Raw wheat starch	20. 0	20.0	0.00	0.00	0.00
* Vitamin Supplement	20.0	20.0	20.0	20.0	20.0
** Mineral Supplement	20.0	20.0	20.0	20.0	20.0
Anchovy oil (stabilized)	121.8	123.5	125.3	132.0	138.8
Soybean lecithin	10.0	10.0	10.0	10.0	10.0
Choline Chloride (60%)	5.00	5.00	5.00	5.00	5.00
Vitamin C $_{\text{mono-phosphate (42\%)}}$	1.00	1.00	1.00	1.00	1.00
Carophyll pink (2%)	2.75	2.75	2.75	2.75	2.75
Permapell	10.0	10.0	10.0	10.0	10.0
Finnstim™	10.0	10.0	10.0	10.0	10.0
DL-methionine	1.82	1.88	1.94	2.00	2.09
Chromic Oxide	5.00	5.00	5.00	5.00	5.00
Santoquin	0.022	0.029	0.036	0.043	0.050

* refer: Table A.3 (Appendix A) ** refer: Table A.4 (Appendix A)

- Austral fish meal and LT anchovy oil (from South America), EWOS Canada Ltd., Surrey, BC
- Blood flour (spray-dried) and squid meal (APC 2300), American Protein Corporation, Ames, Iowa, USA
- Wheat gluten meal, Archer Daniels Midland/ Ogilvie Mills Ltd., Montreal, Canada
- Pregelatinized wheat starch and raw wheat starch, Archer Daniels Midland, Montreal, Canada
- Soy lecitihin, Central Soy Company Inc., Fort Wayne, Indianna, USA
- Choline chloride, Van Waters and Rogers Ltd., Abbotsford, BC
- Ascorbic acid monophosphate (42%), BASF Canada Inc., Abbotsford, BC
- Carophyll pink (2% free synthetic astaxanthin), Hoffman La-Roche, Basel, Switzerland
- Permapell (lignin sulphonate binder) - Georgia Pacific, Bellingham, WA, USA
- Finnstim (diet palatability enhancer; > 90% betaine as well as free amino acids especially those with branched chains), Cultor Ltd., Finnsugar Bioproducts, Helsinki, Finland
- DL- methionine, Sigma Chemical Co., Mississauga ON
- Chromic oxide, BDH Laboratory Supplies, Poole, England
- Santoquin, BC Packers Ltd., Steveston, BC

3.2.6 Experimental procedure

Prior to the digestibility experiment, all groups of salmon were acclimated to the experimental conditions for 10 days. During this period, the salmon were fed a diet of almost identical composition to the reference diet without the addition of chromic oxide. Thereafter, they were switched to the reference and test diet for the duration of the experiment (18 days).

3.2.6.1 Digestibility experiment

The digestibility experiment was conducted on 90 Atlantic salmon (70-145 g) that were distributed randomly into 6 tanks such that each tank held 15 fish. These specially designed (Hajen *et al.*, 1993a) indoor tanks have a capacity of 150 L (Appendix A, Figure A.4) and they are each equipped with a "Guelph" fecal collection column. The design of the digestibility tank ensured that the feces were swiftly carried into the settling column to minimize any leaching of soluble nutrients from the feces. Each tank was supplied with running (8 L/min), filtered, oxygenated (D.O. between 7.9 and 8.1 mg/L) and ambient temperature (10.7 to 11.1 $^{\circ}$ C) seawater from Burrard Inlet (salinity between 24.6 and 30.5 ppt). Additional aeration in each tank was provided by the use of a diffuser stone. Water salinity and dissolved oxygen concentrations were measured daily throughout the experiment.

A natural photoperiod was provided by a series of fluorescent lights (Vitalite, Durotest 40W) controlled by a photocell. The fish were acclimated to the experimental conditions for 10 days prior to the commencement of the experiment. During this time they were fed a highly digestible basal diet (formulated and prepared at the West Vancouver Laboratory) that was based mainly on premium quality fishmeal and anchovy oil.

Triplicate groups of salmon were assigned to each diet and each was fed by hand three times daily until satiation. The weight of feed that was dispensed to each group of salmon was recorded daily. The tanks were cleaned every afternoon an hour after the last

feeding to remove any uneaten feed and feces. On day-4 of the experiment, the feces from each tank were collected from the individual 'Guelph' columns attached to each tank before the first daily feeding. Care was taken to minimize fecal pellet disintegration. This procedure was repeated every morning for the remainder of the experiment (15 days) as described by Hajen *et al.* (1993b).

Daily records of fish mortality and environmental factors like dissolved oxygen levels, salinity and water temperature were maintained. The feces were freeze-dried as described by Hajen *et al.* (1993a,b) and subsequently they were stored at -20 °C pending analyses.

3.2.6.2 Growth trial

The growth trial was conducted on 750 vaccinated (against vibriosis and furunculosis) Atlantic salmon that had initial weights of 111.5 to 120.5 g. These fish were randomly and equally distributed into fifteen 4000L circular outdoor fiberglass tanks (Appendix A, Figure A.5) that were each supplied with running (25-40 L/min), filtered, oxygenated (D.O between 7.0 and 8.5 mg/L) and ambient temperature (11.0 to 12.1 °C) sea water (salinity between 29 and 31 ppt). Supplemental aeration was provided with an air-diffuser stone in each tank. The salmon were acclimated to the experimental conditions for 15 days prior to the first sampling, which marked the commencement of the experiment.

The experiment was conducted as a randomized complete block design as illustrated in Figure A.2 of appendix A. Each dietary treatment was assigned randomly to each tank within each block (n=3). The salmon were acclimated to the tanks and experimental conditions for a period of 15 days before being weighed (g ± SD) and injected intra-peritoneally with 'Oxyvet 100 LP' (Syndel Laboratory Ltd., Vancouver, BC) on day 0 of the experiment. A dosage of 20 µg/g body weight of Oxyvet was given to minimize the likelihood of bacterial pathogen outbreak.

The outdoor tanks were drained of water to a height of 30 cm and then clovebud oil (Hill Tech. Canada Inc., ON) diluted with 90% ethanol (1:9) was added to the water to provide a concentration of 40 ppm. Clovebud oil has a sedative effect on the fish (Keene *et al.*, 1998). Thereafter the fish were netted and carried to the wet-lab in aerated 250 L buckets filled with seawater. Salmon were anesthetized with 60 ppm of MS-222 (tricaine methane sulfonate; Syndel Laboratory Ltd., Vancouver, B.C.) in a container of aerated seawater, according to Kiessling *et al.* (1995). Once the salmon were anesthetized, they were swiftly weighed and then held in a recovery bucket that contained 250 L of aerated seawater. After all the salmon in a tank were weighed and held in the recovery buckets, they were transferred back to their respective tank before starting the sampling of another tank. Records of the number of deaths on a daily basis and the weights of salmon were maintained.

All salmon were fed their prescribed diets twice a day, beginning on day-1, until satiation over the 84-day experiment, except on days 0, 42 and 84 when the salmon were individually weighed following the aforementioned dual anesthetic treatment (clovebud oil and MS-222). On the days prior to sampling, the fish were fed only once, in the morning. Feed intake and water quality parameters (Appendix A, Figure A.1) – water temperature, salinity and dissolved oxygen content were recorded daily. Each of the tanks was cleaned by siphoning every afternoon, an hour after the last feeding. The uneaten pellets from each tank were removed and the mass (number of uneaten pellets multiplied by the mean pellet weight) was deducted from the daily feed dispensed (g) in order to obtain an accurate estimate of the actual daily ration consumed. On days 42 and 84, the tanks were pressure-cleaned to get rid of algae prior to the salmon being returned to the tanks after sampling

On day-0, a total of 10 fish common to all groups were sampled. Five of these fish were used for individual determinations of whole body proximate composition whereas the other 5 were filleted with the right and left fillets of each blended to prepare 5 composite samples for determination of muscle proximate composition.

On day-84, 15 additional fish were anesthetized as described above and weighed. Subsequently, they were intestinally dissected to obtain fecal samples and then 5 of these were sampled for individual determinations of whole body proximate composition and the remaining 10 fish were filleted for subsequent muscle proximate analyses. The fish fillets were pooled to give 5 composite samples, each comprised of 2 fillets each.

On day 84, 15 fish from each tank were randomly collected without anesthesia, for sensory analyses and fillet color determinations. The fish were killed with a sharp blow on the head before being weighed and dissected for collection of feces (Hajen *et al.,* 1993b). They were then filleted with the right fillet being rinsed in tap water to remove blood. Thereafter, each fillet was weighed and vacuum packed in oxygen impermeable bags (Food Pak Systems Ltd., Vancouver, B.C.; oxygen transmission rate, 2.3 cc/m^2.24hrs.atm; water transmission rate, 7.8 g/m^2.24hrs.atm) before being stored on ice. Each left fillet was rinsed with tap water to remove blood, weighed and used to visually determine color by comparing its color against Salmofan™ color cards before being vacuum packaged and stored on ice. The visual assessment of color was conducted using a color box that was painted white on all sides and equipped with special fluorescent tubes (Lithonia Lighting Canada, model-P220120LPF). All knives and cutting boards were rinsed with water before being sanitized with 200 ppm of sodium hypochlorite solution after filleting each salmon.

After fish sampling, all the vacuum packaged samples were stored at –20 °C. The feces from each replicate in a diet were pooled and freeze-dried.

3.2.7 Chemical Analysis

3.2.7.1 Digestibility experiment

The freeze-dried fecal samples were analyzed for concentrations of chromic oxide, protein and organic matter, while the SFM, basal diet and test diets were subjected to

proximate analysis to determine protein, ash, moisture and organic matter content as described by Hajen *et al.* (1993a,b).

In this regard, protein content was assessed by the Kjeldahl method using an autoanalyzer (Technicon Autoanalyzer II, Technicon Industrial Systems, Tarrytown, NY). The % nitrogen levels obtained from the autoanalyzer readings were converted to % protein content in each sample by using 6.25 as the conversion factor. Crude lipid content was determined using a modified method of Bligh and Dyer (1959) and Folch *et al.* (1957). In this regard, 4 g of blended sample was washed with 10 ml of chloroform (Anachemia, >99.8%) and 20 ml methanol (Anachemia, >99.8%). Then the mixture was homogenized (Sorvall Omni-mixer, Ivan Sorvall Inc., Norwalk, US) at ~ 10,000 rpm for 120 seconds followed by addition of 10 ml of chloroform to create a biphasic solution. The mixture was homogenized for 30 seconds and after addition of 8 ml of distilled water was homogenized for another 30 seconds. Thereafter, the mixture was filtered using Whatman No. 1 filter paper under vacuum and the filtrate was allowed to remain undisturbed in a 50 ml glass graduated cylinder to facilitate the separation into two phases. The volume of the lower phase (consisting of chloroform) was recorded and the upper layer (consisting of methanol) was suctioned off. Five milliliters of chloroform were pipetted onto a pre-weighed, pre-heated aluminum weigh boat which was then heated in a fume hood to allow evaporation of chloroform leaving a thin lipid coating from the sample in the weigh boat. The boat was then transferred to a drying oven (Isotemp Oven, Fisher Scientific, Pittsburgh, PA) at 100 $^{\circ}$C for an hour to remove any residual chloroform. After cooling in a dessicator, the weight gained by the boat was recorded to calculate the lipid content in 5 ml of sample.

To determine the moisture and ash content in the samples, homogenized fish and diet samples were weighed in pre-weighed, labeled crucibles and dried in an oven (Isotemp Oven, Fisher Scientific, Pittsburgh, PA) at 100 $^{\circ}$C for 16 hours. Thereafter, the air-dried samples were further ignited in a muffle furnace (Isotemp Muffle Furnace, Fisher Scientific, Pittsburgh, PA) at 600 $^{\circ}$C for 2 hours and the final weights of the crucibles were recorded to determine the ash content of the samples. The difference in weight before and

after air-drying was used to determine the moisture content in the samples. Chromic oxide in feces and diets was determined spectrophotometrically (Fenton and Fenton, 1979).

3.2.7.2 Growth trial

Pooled fecal samples from salmon fed each of the five diets were freeze-dried prior to analysis. Fecal samples as well as the five diets and the selected frozen whole fish and left fillets were analyzed for proximate composition according to the methods described by Higgs *et al.* (1979). In addition, fecal samples and diets were also analyzed for chromic oxide content as described by Fenton and Fenton (1979). Energy contents of the diets and fecal samples were measured using an adiabatic bomb calorimeter (IKA Calorimeter System C5001 duo control, Germany).

Crude protein content in the preceding samples was determined by multiplying percent nitrogen by 6.25. Carbohydrate or nitrogen-free extract (NFE) concentrations were calculated by difference, as follows:

$$NFE_{(db,\ for\ diets)} = 100 - (\%ash + \%crude\ fiber + \%\ protein + \%\ lipid)$$

$$NFE_{(wb,\ for\ whole\ body/muscle)} = 100 - (\%ash + \%moisture + \%\ protein + \%\ lipid)$$

3.2.8 Calculations

3.2.8.1 Digestibility experiment

Apparent digestibility coefficients (ADC) for crude protein in each diet were calculated according to the equation used by Cho *et al.* (1982).

$$ADC = \left[1 - \left\{ \frac{\%\ fecal\ protein}{\%\ dietary\ protein} \times \frac{\%\ dietary\ chromic\ oxide}{\%\ fecal\ chromic\ oxide} \right\}\right] \times 100$$

The apparent digestibility coefficient (ADC) for protein in the test ingredient (SFM) was calculated using the equation of Forster (1999).

$$\mathbf{ADC}_{protein} \text{ in } \mathbf{SFM} = [\{(a+b) * ADC_{protein} \text{ test diet}\} - \{(a) * ADC_{protein} \text{ ref. diet}\}]b^{-1}$$

(a) = protein contribution of reference diet to protein content of test-diet

(b) = protein contribution of test ingredient to protein content of test diet

(a+b) = level of protein (%) in test diet

3.2.8.2 Growth Trial

Apparent digestibility coefficients (ADC) for crude protein, organic matter and energy for each diet was calculated according to the equation used by Cho *et al.* (1982).

$$ADC = \left[1 - \left\{\frac{\%\text{ fecal nutrient}}{\%\text{ dietary nutrient}} \times \frac{\%\text{ dietary chromic oxide}}{\%\text{ fecal chromic oxide}}\right\}\right] \times 100$$

The growth performance of Atlantic salmon was measured by using the following indices.

- Weight Gain (WG) = final weight (g) - initial weight (g)
- Specific Growth Rate (SGR)=[{ln final weight – ln initial weight}/ # of days] * 100
- Feed Efficiency (FE) = wet weight gain (g)/ dry feed intake (g)
- Protein Efficiency Ratio (PER) = weight gain (g)/ protein intake (g)
- Dry Feed Intake (FI, g/fish) = Feed consumed (g, dry basis)/ # of fish
- Percent Protein Deposited (PPD) = [protein gain (g)/ protein intake (g)] * 100

- Gross Energy Utilization (GEU) = [energy gain (g)/ energy intake (g)] * 100
- % survival (%S) = [# of fish alive on last day/ original # of fish] * 100

The digestible protein and energy content of the diets were calculated as described below

- Digestible Protein (DP) = [% dietary protein (db) * ADC]
- Digestible Energy (DE) = [gross energy (MJ/kg dry weight) * ADC]

3.2.9 Statistical Analysis

All performance data like weight gain, specific growth rate, feed efficiency, total feed intake and protein efficiency ratio were subjected to a randomized block analysis of variance, ANOVA using the general linear model of SYSTAT (version 5).

Parameters expressed as percentages e.g. percent protein deposited, gross energy utilization, percent survival, apparent digestibility coefficients for protein, energy, and organic matter as well as whole body and muscle proximate constituents were first subjected to arcsine square root transformation as recommended by Snedecor and Cochran (1989) before ANOVA. This was done to achieve homogeneity of variance. The effects of block and diet in the ANOVA were considered to be significant when $p < 0.05$.

Transformed value (%) = arcsin (square root (value in % /100)) * (180 /3.142)

3.3 *RESULTS & DISCUSSION*

3.3.1 Digestibility Experiment

The percentages of selected proximate constituents in the reference and test diets along with SFM are provided in Table III-VI. The apparent digestibility coefficients for crude protein and energy in the preceding diets and test ingredient (SFM) are given in Table III-VII. SFM was estimated to have 87.9% digestible protein. This value enabled progressive replacement of the digestible protein provided by anchovy meal in the basal diet by an equivalent amount of digestible protein from SFM in the test diets used for the growth trial.

Mortality ranged from 4 % for fish on the reference diet to 10 % for those fed the test diets. Some loss of scales and development of pop-eye during the experiment was observed. Contributory factors were likely stress associated with sampling and an instance of accidental water drainage from a tank.

The digestibility experiment was only conducted to obtain an estimate of the apparent digestibility of protein in SFM. This was a preliminary step towards incorporation of SFM into the dietary formulations used for the subsequent growth trial on Atlantic salmon. Results from Table III-VII reveal that the ADC for protein in the SFM compared well with those obtained for other oilseed protein sources e.g., soybean meal when Atlantic salmon were used as the test species (Scott *et al.*, 1982).

TABLE III-VI: Levels of proximate constituents on a dry weight basis in the reference and test diet during the digestibility experiment conducted on Atlantic salmon (n=45/diet group) reared in seawater over an 18-day period.

Diets	*% Ash*	*% Dry Matter*	*% Lipids*	*% Protein*	*Gross energy(MJ/kg)*	*%Cr_2O_3*
Reference (0.0%SFM)	11.8	87.9	20.2	51.6	20.2	0.56
Test (29.85% SFM)	10.8	87.5	15.0	47.9	19.5	0.57

TABLE III-VII: Apparent digestibility coefficients for protein and energy in the reference and test diet using nutritionally upgraded SFM. The digestibility experiment was conducted on Atlantic salmon (n=45/diet group) reared in seawater over an 18-day period.

Diets	*ADC_{pr}*	*ADC_{energy}*	*DP*	*DE*	*DP/ DE*
Reference (0.0%SFM)	91.2	88.7	47.0	17.9	26.3
Test (29.85% SFM)	90.3	83.4	43.3	16.3	26.6

ADC = [1 - {(% fecal nutrient / % dietary nutrient) *(% dietary chromic oxide / % fecal chromic oxide) }] * 100

Digestible Protein (DP) = [% dietary protein (db) * ADC_{pr}]

Digestible Energy (DE) = [gross energy (MJ/kg dry weight) * ADC_{energy}]

ADC for protein in $SFM_{(1+2)}$ was calculated to be 87.9 % using Forster's (1999) equation.

3.3.2 Growth Trial

In nutritive value assessments, it is important to ensure that the basal diet supports excellent growth, feed efficiency and health of the animal species. Thus care was taken in this study to ensure that the salmon were fed a basal diet that was composed of premium quality ingredients that provided the required available levels and balance of nutrients and energy for their growth (NRC, 1993).

Likewise, attempts were made to ensure that the test diets contained adequate levels of all nutrients and energy for salmon growth through alterations of the supplemental amounts of lipid, vitamins, minerals and essential amino acids, especially sulfur amino-acids (Barnes and Kwong, 1965; Richardson, 1980-81). This is important when assessing the nutritive value of oilseed protein products relative to that of fishmeal. For instance, Spinelli *et al.* (1979) found that the mineral status of rainbow trout, fed soy-based diets, was affected. This was attributed to anti-nutritional factors associated with the fiber fraction of soybean meal. Lastly, the addition of 1.0% Finnstim to each of the diets was done in an attempt to maintain high diet palatability regardless of the level of dietary SFM.

In relation to the above, it was noted that the gross energy contents (MJ/kg) of the five diets used in this study were almost identical (ranged from 20.9 - 21.1 MJ/kg of dry feed; Table III-VIII). However the digestible energy (DE, Table III-IX) contents of the diets decreased as the dietary level of SFM was raised progressively, and ranged from 16.8 - 15.5 MJ/kg for diets A and E respectively. This trend did not affect the growth of the salmon because of the increased feed intakes (not significant) of the salmon fed all of the diets containing SFM (Table III-X). Both the crude protein (CP) and digestible protein (DP, Table III-IX) concentrations in the diets showed little change as progressively more SFM was substituted for anchovy meal in the basal diet. The CP ranged from 48-50.4% of dry matter while the DP concentration decreased (not significant, $p \geq 0.05$) from 43.1 to 41.1%. Except for Diet E, DP values for the diets were inversely related to the dietary concentration of SFM. Thus, the ratios of DP to DE (DP/DE) were similar in diets A to D (~25.5%) whereas in diet E, there was an increased ratio of DP to DE (27.9; Table III-IX).

TABLE III-VIII: Concentrations of proximate constituents, gross energy content (MJ/kg) and chromic oxide on a dry weight basis in the basal and four test diets used during the growth trial conducted on Atlantic salmon over an 84-day period (n=2/diet). %NFE was calculated by difference.

Diets	*% Ash*	*% Dry Matter*	*% Lipid*	*% Protein*	*%Crude fiber*	*%NFE*	*Gross energy(MJ/kg)*	*%Cr_2O_3*
Diet A (0.0%SFM)	9.25	90.8	18.2	49.3	0.45	22.8	21.0	0.52
Diet B (6.8 % SFM)	9.21	90.8	19.0	48.6	1.31	21.8	20.9	0.54
Diet C (13.6 % SFM)	8.87	91.1	18.8	48.7	2.16	21.5	20.9	0.53
Diet D (20.4 % SFM)	8.98	91.0	18.7	48.0	3.01	21.3	21.1	0.54
Diet E (27.1 % SFM)	9.27	90.7	18.8	50.4	3.65	17.9	21.0	0.55

NFE (db) = 100 – (%ash + %crude fibre + % protein + % lipid)

TABLE III-IX: Apparent digestibility coefficients for protein (pr), organic matter (org) and energy (energy), and concentrations of digestible protein (DP), digestible energy (DE) and the ratios of DP to DE in the basal and four test diets used during the 84-day growth trial conducted on Atlantic salmon.

	%					
Diets	*ADCpr*	*ADCorg*	*ADCenergy*	*DP*	*DE(MJ/kg)*	*DP / DE*
Diet A (0.0%SFM)	87.3	73.0	80.2	43.0	16.8	25.6
Diet B (6.8% SFM)	86.6	73.0	78.7	42.1	16.4	25.6
Diet C (13.6% SFM)	85.9	72.3	78.5	41.8	16.4	25.5
Diet D (20.4 % SFM)	85.7	70.3	76.7	41.1	16.2	25.4
Diet E (27.1 % SFM)	85.4	66.4	73.6	43.1	15.5	27.9

- ADC = [1- {(% fecal nutrient / % dietary nutrient) *(% dietary chromic oxide / % fecal chromic oxide)}] * 100
- Digestible Protein (DP) = [% dietary protein (db) * ADC]
- Digestible Energy (DE) = [gross energy (MJ/kg dry weight) * ADC]

TABLE III-X: Mean values for initial weight (IW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed efficiency (FE), protein efficiency ratio (PER), percent protein deposition (PPD), gross energy utilization (GEU) and percent survival (%S) measured during the 84-day growth trial conducted on Atlantic salmon (n=150/diet).

Diets	*IW* (g)	*WG* (g)	*SGR* (%/day)	*FI* (g/fish)	*FE* (g/g)	*PER* (g/g)	*PPD* (%)	*GEU* (%)	*S* (%)
Diet A (0.0%SFM)	113	250	1.39	206	1.21	2.44	46.0	47.9	99.3
Diet B (6.8% SFM)	117	270	1.42	215	1.26	2.56	47.5	47.1	96.0
Diet C (13.6% SFM)	117	277	1.45	230	1.20	2.44	46.7	46.5	98.7
Diet D (20.4 % SFM)	115	265	1.42	222	1.19	2.44	46.3	49.4	98.0
Diet E (27.1 % SFM)	118	268	1.41	220	1.22	2.40	45.8	49.4	99.3
Pooled SD	3.06	16.4	0.045	0.210	0.045	0.095	1.80	1.91	0.619
*P**	0.376	0.424	0.685	0.459	0.529	0.370	0.896	0.808	0.075

*all results were n.s for diet ($p > 0.05$) and block ($p>0.05$)

§ Arcsine transformed percentages for SGR, PER, %S, PPD and GEU were analyzed by randomized block ANOVA.

- Weight Gain (WG) = final weight (g) - initial weight (g)
- Specific Growth Rate (SGR) = [{ln final weight – ln initial weight}/ # of days] * 100
- Feed Efficiency (FE) = wet weight gain (g) /dry feed intake (g)
- Protein Efficiency Ratio (PER) = weight gain (g)/ protein intake (g)
- Dry Feed Intake (FI, dry basis) = Feed consumed (g, dry basis) / # of fish
- Percent Protein Deposition (PPD) = [protein gain (g)/ protein intake (g)] * 100
- Gross Energy Utilization (GEU) = [energy gain (g)/ energy intake (g)] * 100
- % survival = [# of fish alive on last day/ original # of fish] * 100

TABLE III-XI: Whole body proximate compositions (w/b) and energy contents (MJ/kg) of Atlantic salmon fed the basal and experimental diets during the 84-day growth trial. Measurements represent average values for each diet (n=30/diet).

Diets	*% Ash*	*% Moisture*	*% Lipid*	*% Protein*	*%NFE*	*Energy (MJ/kg)*
Day 0	2.36	75.5	5.85	14.4	1.89	4.58
Day 84						
Diet A (0.0%SFM)	2.81	71.5	8.54	17.3	0.00	7.51
Diet B (6.8% SFM)	3.53	71.3	7.99	17.0	0.18	7.26
Diet C (13.6% SFM)	3.29	71.4	8.24	17.7	0.00	7.47
Diet D (20.4 % SFM)	2.82	70.6	9.00	17.3	0.28	7.74
Diet E (27.1 % SFM)	3.25	70.9	8.95	17.3	0.00	7.68
Pooled SD	0.495	0.355	0.580	0.493	1.72	4.33
*P**	0.071	0.328	0.229	0.761	0.311	0.314

*all results were n.s for diet ($p > 0.05$) and block ($p>0.05$)

§ Arcsine transformed percentages for ash, moisture lipid, protein and NFE were analyzed by randomized block ANOVA.

NFE (wb) = 100 – (%ash + %moisture + % protein + % lipid)

TABLE III-XII: Muscle proximate compositions (w/b) and energy contents (MJ/kg) of Atlantic salmon fed the basal and experimental diets during the 84-day growth trial. Measurements represent average values for each diet (n=30/diet).

Diets	*% Ash*	*% Moisture*	*% Lipid*	*% Protein*	*%NFE*	*Energy (MJ/kg)*
Day 0	1.55	78.4	1.74	18.0	0.31	4.46
Day 84						
Diet A (0.0%SFM)	2.35	74.1	4.11	19.4	0.04	6.29
Diet B (6.8% SFM)	2.65	73.8	4.32	19.5	0.00	6.35
Diet C (13.6% SFM)	2.72	73.8	4.44	19.7	0.00	6.42
Diet D (20.4 % SFM)	2.44	73.6	4.79	19.1	0.07	6.45
Diet E (27.1 % SFM)	2.61	73.6	4.49	19.9	0.00	6.52
Pooled SD	0.379	0.290	0.463	0.466	1.19	2.84
*P**	0.281	0.596	0.264	0.696	0.264	0.614

*all results were n.s.for diet ($p > 0.05$) and block ($p>0.05$)

§ Arcsine transformed percentages for ash, moisture, lipid, protein and NFE were analyzed by randomized block NOVA.

NFE (wb) = 100 – (%ash + %moisture + % protein + % lipid)

The lower ADC values for protein, organic matter and energy in the test diets, as compared to those obtained for the basal diet may have occurred because oilseed meals like SFM generally contain crude fiber (Liener, 1980) and other indigestible carbohydrates, whereas fishmeal does not. Indeed, the dietary concentrations of crude fiber were inversely related to the ADC values obtained for protein, organic matter and energy in this study. The ADC for protein (85.4%, Table III-IX) in Diet E (27.1%SFM) was in the range observed by others where sunflower protein products were included in the diets for rainbow trout (Stickney *et al.*, 1996; Cardenete *et al.*, 1993; Tacon *et al.*, 1984). According to Rolls *et al.* (1978) fiber-rich diets appear to generally cause an increase in the intestinal micro-flora. This can probably explain the lower ADC values observed for protein in the test diets fed to Atlantic salmon.

The primary aim of this study was to determine whether growth, feed efficiency, protein utilization and survival of Atlantic salmon would be adversely affected when nutritionally upgraded SFM was incorporated into their diets up to 27.1% (db) by replacement of premium quality fishmeal.

At the beginning of the experiment, the initial mean weights of the groups were not statistically different (Table III-X). It is noteworthy that dietary treatment did not significantly ($p > 0.05$) influence weight gain, specific growth rate, feed intake, protein and energy utilization and percent survival of the salmon during the 84-day study even though up to one-third of the digestible protein provided by the fishmeal in the basal diet was replaced by digestible protein from SFM (Table III-X).

The inclusion of up to 27% SFM in the diet (db) also did not result in any obvious signs of pathology and the health of the salmon appeared excellent as judged by the high percent survival ($\geq 96\%$) of all groups in this study. The results on Atlantic salmon are consistent with the acceptable dietary concentrations of SFM found for rainbow trout (Tacon *et al.*, 1984; Martinez, 1986; Cardenete *et al.*, 1993; Sanz *et al.*, 1994). In this regard, no adverse effects have been observed on rainbow trout growth when partially de-hulled SFM in dietary concentrations of 33% (Scott *et al.*, 1982), 36.5% (Tacon *et al.*, 1984), 41.8%

(Sanz *et al.*, 1994) and 40% (Cardenete *et al.*, 1993) have been used. The absence of any toxic or deleterious effects of SFM in the salmon in this study also supports the findings of Scott *et al.* (1982), Tacon *et al.* (1984) and Sanz *et al.* (1994) who did not find any growth depressing or toxic substances in studies conducted on rainbow trout.

Despite the progressive decrease in ADC values for protein and energy when the dietary level of SFM was increased, it is important to emphasize that generally the ratio of DP to DE in the diets for Atlantic salmon was similar (25.4 to 27.9). Since the salmon were fed to satiation, this permitted similar intakes of DP and DE, leading to similar feed efficiency and growth rates among all the groups of salmon in this study. Hence, all experimental diets in this study supported excellent weight gains of salmon over the 84-day period (> 3 times the initial body weight). Also, it should be mentioned that there was no effect of dietary treatment on whole body or muscle proximate compositions and gross energy content (Tables III-XI, III-XII). These findings further support the premise that all groups ingested similar amounts of DP and DE. This is because whole body proximate and muscle compositions in salmonids are known to be influenced by the proportions of available dietary energy originating from protein and lipid (Higgs *et al.*, 1995).

3.4 CONCLUSION

In conclusion, the present study demonstrated that digestible protein from partially de-hulled and extruded SFM could replace up to 33% of the digestible protein provided by fishmeal in a highly nutritional basal diet for Atlantic salmon in seawater, without affecting their performance, health and body composition adversely. Thus under the conditions of this study, it was found that nutritionally upgraded SFM could successfully comprise up to 27% of the dietary dry matter of post-juvenile Atlantic salmon. These results do not, however, permit the establishment of the maximum acceptable dietary concentration

of SFM in Atlantic salmon reared in seawater and this should be the subject of further research. Presumably, the acceptable dietary concentrations of SFM will be influenced by the concentrations and types of indigestible carbohydrates that are present which, in turn, will be affected by the processing methods that are used to produce the meal(s) under evaluation. The inclusion of inexpensive SFM in diets for Atlantic salmon will reduce salmon production costs. Additional savings may be realized by developing cost effective strategies for enhancing the nutritive value of sunflower meal even further from what was accomplished in this study. Strategies to achieve this are outlined in the section of this thesis entitled "Recommendations".

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APPENDIX A

TABLE A.1: Supplemental levels of vitamins included in the reference and test diets used in the digestibility experiment conducted on Atlantic salmon (*Salmo salar*).

Ingredients	*amount per kg dry diet*
D- Calcium Pantothenate	168.4 mg
Pyridoxine HCl	49.3 mg
Riboflavin	60.0 mg
Folic Acid	15.0 mg
Thiamine Mononitrate	56.0 mg
Biotin	1.50 mg
Vitamin B_{12}	0.09 mg
Menadione	18.0 mg
Vitamin E(DL α- tocopherol acetate)	300 IU
Vitamin D_3	2400 IU
Vitamin A(acetate)	5000 IU
Inositol	400 mg
Niacin	300 mg
BHT[ϕ]	22.0mg

[ϕ] BHT (antioxidant)
Aldrich Chemical Company Inc.,
Milwaukee, Wis., USA

- All vitamins
Van Waters and Rogers Ltd.,
Abbotsford, BC

TABLE A.2: Supplemental levels of different minerals in the reference and test diets used in digestibility experiments conducted on Atlantic salmon (*Salmo salar*).

Ingredients	*mg / kg dry diet (db)*
Mg as $MgSO_4.7H_2O$	400
Cu as $CuSO_4.5H_2O$	6.62
Fe as$FeSO_4.7H_2O$	100
Zn as $ZnSO_4.7H_2O$	90.0
Mn as $MnSO_4.H_2O$	75.0
Na as NaCl	1500
K as K_2SO_4	750
K as K_2CO_3	750
I as KIO_3	5.00
I as KI	5.00
F as NaF	5.00
Co as $CoCl_2.6H_2O$	3.00
Se as Na_2SeO_3	0.200

- All minerals
  Anachemia Science,
  Richmond, BC

TABLE A.3: Supplemental levels of vitamins included in the basal and four test diets used in growth trials conducted on Atlantic salmon (*Salmo salar*).

Ingredients	*amounts (per kg dry diet)*
D- Calcium Pantothenate	168 mg
Pyridoxine HCl	49.3 mg
Riboflavin	60.0 mg
Folic Acid	15.0 mg
Thiamine Mononitrate	56.0 mg
Biotin	1.50mg
Vitamin B_{12}	0.09mg
Vitamin K(menadione)	18.0 mg
Vitamin E	300 IU
Vitamin D_3	2400 IU
Vitamin A	5000 IU
Inositol	400 mg
Niacin	300 mg
BHT	22.0mg

- BHT (antioxidant)
  Aldrich Chemical Company Inc.,
  Milwaukee, Wis., USA

- All vitamins
  Van Waters and Rogers Ltd.,
  Abbotsford, BC

TABLE A.4: Supplemental levels of minerals included in the basal and four test diets used in growth trial conducted on Atlantic salmon (*Salmo salar*).

Minerals	*(mg / kg dry weight basis)*				
	Diet A	*Diet B*	*Diet C*	*Diet D*	*Diet E*
Na as $NaH_2PO_4.H_2O$	-	524	1048	1571	2096
Na as NaCl	1500	976	452	-	-
P as $NaH_2PO_4.H_2O$	-	706	1412	2118	2824
Mg as $MgSO_4.7H_2O$	400	400	400	400	400
Cu as $CuSO_4.5H_2O$	7	3	3	3	3
Fe as $FeSO_4.7H_2O$	100	100	100	100	100
Zn as $ZnSO_4.7H_2O$	119	104	88	73	58
Mn as $MnSO_4.H_2O$	75	67	59	51	43
K as K_2SO_4	1036	724	412	198	-
K as K_2CO_3	1036	724	412	-	-
I as KIO_3	5	5	5	5	5
I as KI	5	5	5	5	5
F as NaF	5	5	5	5	5
Co as $CoCl_2.6H_2O$	3	3	3	3	3
Se as Na_2SeO_3	0.2	0.2	0.2	0.2	0.2

- All minerals
  Anachemia Science,
  Richmond, BC

TABLE A.5: Amino acid compositions of the basal and four test diets used in the 84-day growth trial conducted on Atlantic salmon (*Salmo salar*).

	% (w / w; dry weight basis)				
Amino-acid	*Diet A*	*Diet B*	*Diet C*	*Diet D*	*Diet E*
Ala	2.91	2.90	2.77	2.81	2.83
Arg	2.82	2.91	2.92	3.04	3.18
Asp	4.28	4.34	4.23	4.35	4.87
Cys/2	0.53	0.58	0.58	0.61	0.63
Glu	8.03	8.40	8.53	8.70	8.98
Gly	2.64	2.61	2.58	2.63	2.69
His	1.49	1.48	1.42	1.46	1.51
Ile	1.94	1.96	1.90	1.94	1.93
Leu	3.94	4.04	3.90	3.98	4.17
Lys	2.59	2.56	2.39	2.38	2.40
Met	1.37	1.27	1.31	1.35	1.24
Phe	2.24	2.29	2.25	2.31	2.45
Pro	2.85	2.87	2.89	2.82	2.95
Ser	2.26	2.30	2.29	2.35	2.40
Thr	2.08	2.10	2.04	2.09	2.15
Trp	0.24	0.20	0.19	0.22	*
Tyr	1.61	1.61	1.56	1.56	1.62
Val	2.65	2.73	2.63	2.69	2.88

* repeat analysis was inconclusive because of a huge variation in the content of tryptophan. Another analysis is in progress.

TABLE A.6: Essential amino acid (EAA) compositions of the basal and four test diets used in the 84-day growth trial conducted on Atlantic salmon (*Salmo salar*) when compared to the amino acid requirements for chinook salmon (NRC, 1993).

	% of diet protein					
Essential Amino-acids	***NRC*†**	*Diet A*	*Diet B*	*Diet C*	*Diet D*	*Diet E*
Arg	**6.0**	5.7	6.0	6.0	5.9	6.3
His	**1.8**	3.0	3.0	2.9	3.0	3.0
Ile	**2.2**	3.9	4.0	3.9	4.0	3.8
Leu	**3.9**	8.0	8.3	8.0	8.3	8.3
Lys	**5.0**	5.3	5.3	4.9	5.0	4.8
Met+Cys	**4.0**	3.9	3.8	3.9	4.1	3.7
Phe+Try	**5.1**	7.8	7.4	7.8	8.1	8.1
Thr	**2.2**	4.2	4.3	4.2	4.4	4.3
Trp	**0.5**	0.5	0.4	0.4	0.5	*
Val	**3.2**	5.4	5.6	5.4	5.6	5.7

† *(40% protein in diet)*

* repeat analysis was inconclusive because of a huge variation in the content of tryptophan. Another analysis is in progress.

TABLE A.7: Extrusion conditions for sunflower meal that was used in the experimental diets for the 84-day growth trial conducted on Atlantic salmon.

	Time interval (min) at which measurements were taken						
	00	*04*	*14*	*24*	*34*	*44*	*59*	*74*
Feeder (rpm) start	18	18/19	17/18	17	17/18	17/18	17/18
Cylinder (rpm)	398	398	398	398	398	398	398
Water cylinder (kg/hr)	21.4	22.6	22.8	22.7	22.6	22.6	22.8
Steam cylinder (kg/hr)	8.8	8.9	8.9	8.9	8.9	9.0	8.6
Water extruder (kg/hr)	5.7	5.3	5.4	5.5	6.0	6.1	5.8
Steam extruder	-	-	-	-	-	-	-
Extruder (rpm)	581	577	573	577	577	578	576
Extruder (% load)	37	32	23	27	27	25	22
Cylinder temperature (°C)							
Head 1	43	57	64	66	67	68	76
Head 2	24	38	48	55	63	70	73
Head 3	24	36	49	126	140	142	144
Head 4	34	41	55	142	147	148	149
Head 5	59	56	91	135	138	139	139
Head 6	55	59	65	76	84	89	92
Head 7	44	62	68	77	85	90	89
Knife (rpm)	895	796	837	834	834	834	961

Fan on dryer (rpm) 780

Dryer temperature 82.2-87.2 ^{0}C

Temperature of feed coming out of the extruder was around 80 ^{0}C

TABLE A.8: Crude fiber and chlorogenic acid contents in the basal and four test diets used in the 84-day growth trial conducted on Atlantic salmon (*Salmo salar*).

	% diet (dry weight basis)	
	Crude Fiber	*Chlorogenic Acid*
Diet A	0.451	0.00
Diet B	1.31	0.033
Diet C	2.16	0.166
Diet D	3.01	0.216
Diet E	3.65	0.323

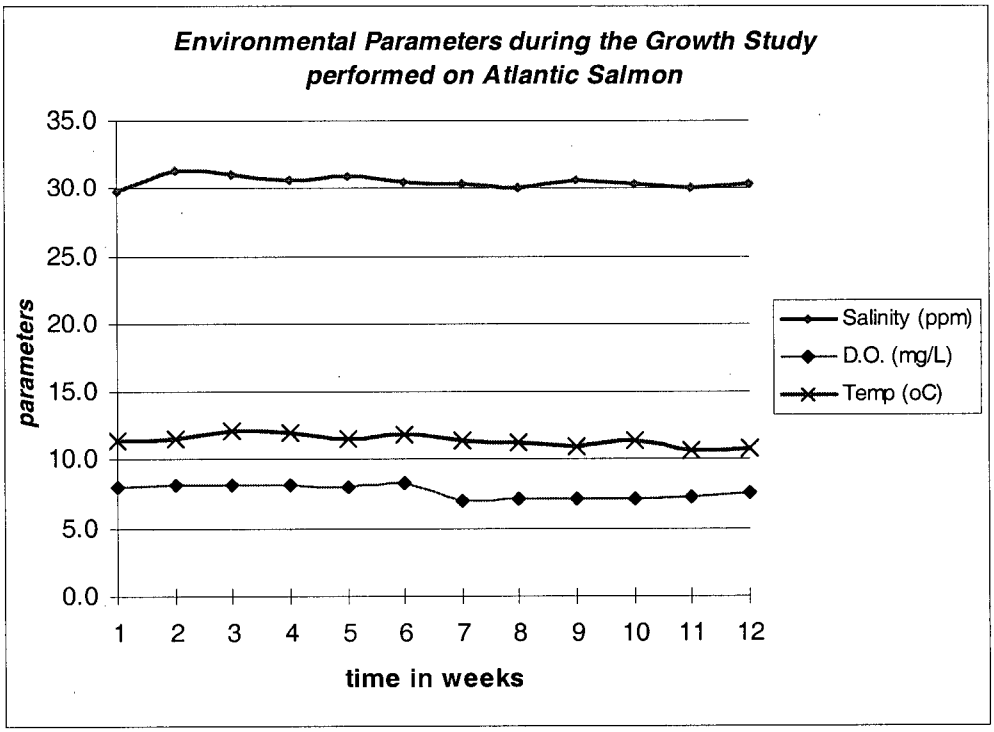


Figure A.1: Environmental conditions during the 84-day growth trial.

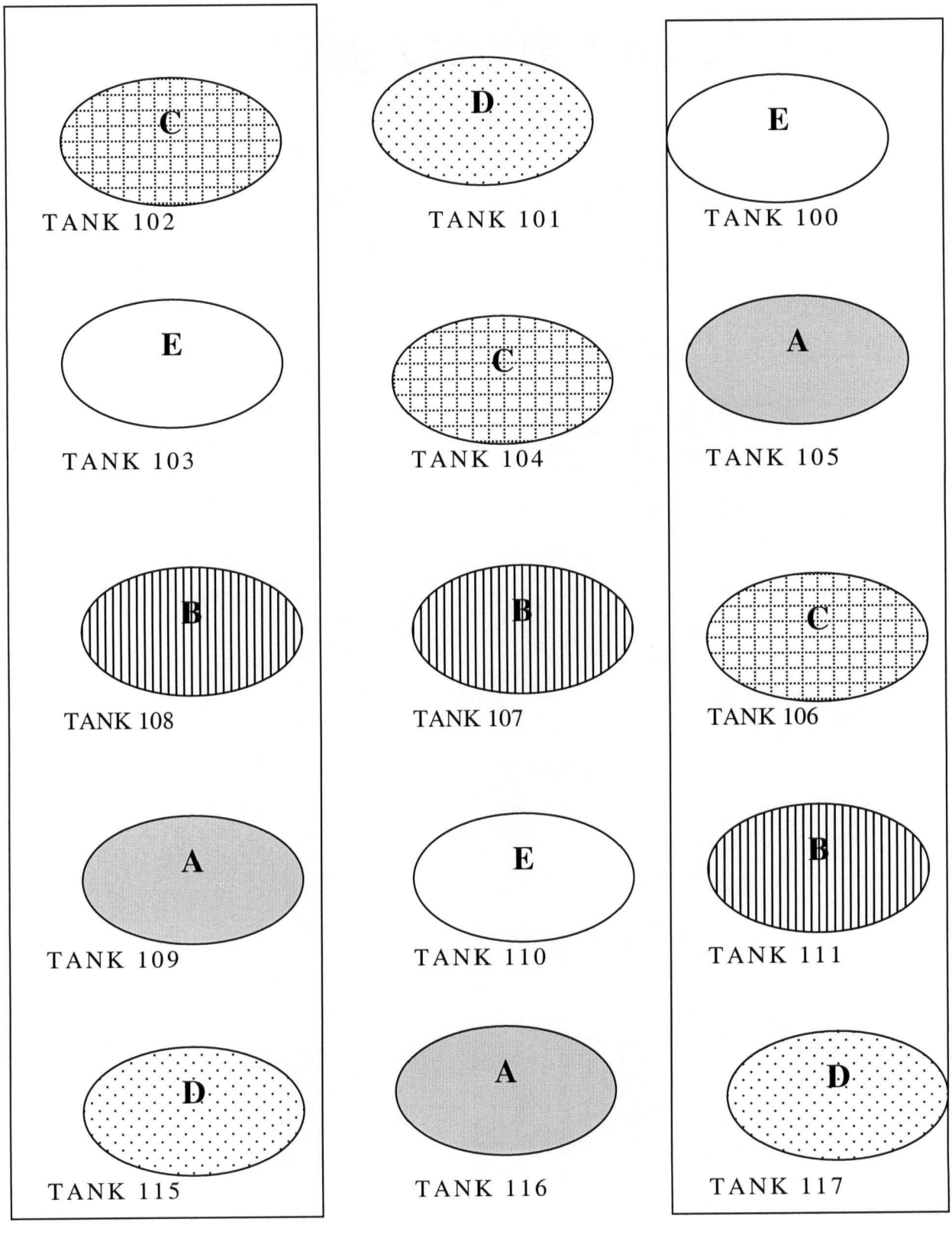


Figure A.2: Schematic illustration of the tank layout used during the 84-day growth trial conducted on Atlantic salmon.

Figure A.3: Multiple vibrating screens used to nutritionally upgrade the commercial SFM before being incorporated into diets used for the 84-day growth trial on Atlantic salmon.

Figure A.4: Specially designed digestibility tanks used for the digestibility experiment conducted on Atlantic salmon prior to the growth trial.

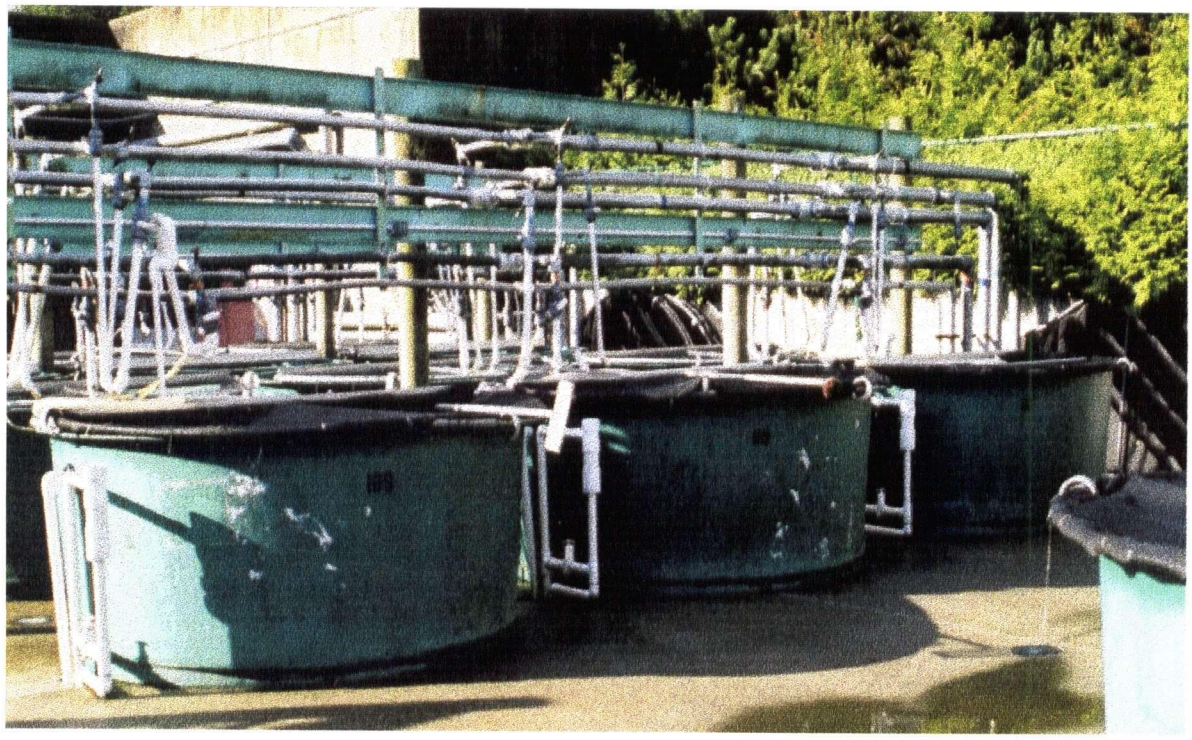


Figure A.5: Fiberglass 4000 L capacity outdoor tanks used for the 84-day growth trial conducted on Atlantic salmon.

CHAPTER 4

EFFECT OF VARYING DIETARY CONCENTRATIONS OF PARTIALLY DEHULLED AND EXTRUDED SUNFLOWER-MEAL ON SENSORY ATTRIBUTES OF POST-JUVENILE ATLANTIC SALMON (*Salmo salar*)

ABSTRACT

This study assessed the effect of nutritionally upgraded (partially dehulled and extruded) sunflower meal (SFM) on various flesh quality attributes of Atlantic salmon (*Salmo salar)* reared in seawater. During the 84-day feeding trial[§], triplicate groups of 50 (initial mean weight ~ 116 g) Atlantic salmon were fed five diets formulated to have equivalent digestible energy and protein concentration. LT Anchovy meal comprised 68.2 % of the protein in the basal diet. To formulate four test diets, SFM progressively replaced up to 33.0% of the digestible protein furnished by LT Anchovy meal in the basal diet or SFM comprised up to 27.1% of the diet on a dry weight basis. On day 84, 15 fish from each tank were killed with a blow to the head and were filleted. Each fillet was vacuum packed and stored at –30°C for sensory analyses at a later date. Color was measured using Salmofan™ and the Hunterlab system. A high correlation coefficient (r^2 = 0.747) was found between Hunter (a) values for redness and corresponding scores from Salmofan™ indicating that Salmofan™ values can be used as a good estimate for color measurements of Atlantic salmon.

A sensory panel consisting of 14 judges evaluated aroma, flavor, foreign flavor and texture of the salmon samples provided. Headspace gas chromatography, followed by the principal component similarity (PCS) technique, was conducted to analyze the headspace 'volatile patterns' in the flesh of the salmon fed the different diets. This study demonstrated that SFM could comprise up to 27.1% of the dietary dry matter by replacement of premium quality fishmeal without any adverse effects ($p \geq 0.05$) on aroma, texture and flavor of Atlantic salmon flesh. However, some noticeable foreign flavors ($p < 0.05$) were noted in the flesh of salmon fed the highest percentage of SFM (27.1% SFM). These results mirrored with those that were obtained by GC-PCS.

§ refer : Chapter 3

4.1 *INTRODUCTION*

Consumer acceptance of salmon depends on attributes like flavor, color, texture, freshness, and general appearance. Salmonid fillet color is generally regarded as the most important attribute towards determining a quality product (Sigurgisladottir *et al.*, 1997). Consumers perceive redder salmon as being fresher, better flavored and of a high quality. Redder salmon fillets also appear to command a higher price (Ostrander *et al.*, 1976; Hatano *et al.*, 1987; Gormley, 1992; Rounds *et al.*, 1992; Skonberg *et al.*, 1998) than less pigmented fillets. The fact that color plays such a decisive role towards consumer acceptance is why the farming industry pays such close attention to the astaxanthin levels in feeds given to farmed salmon.

For a crude albeit practical, quick, and correct 'everyday' assessment of salmon color in an industrial setting, the Roche Salmofan™ (Hoffmann-La Roche, Basel, Switzerland), an internationally recognized method for color measurement, is used all over the world. The Salmofan™ has a scale from 20-34 and it replaced the Roche Color Cards, which have a scale from 11-18. Color measurement cabinets that have controlled fluorescent lighting conditions and white background, are used to ensure consistency when Salmofan™ is used for color assessment.

For an objective measurement of color, the Hunterlab (L,a,b) system or CIE (1976) (L*,a*,b*) system can be used. Both systems make use of tristimulus values, to define color in a three dimensional space where- 'a' represents red/green, 'b' represents yellow/blue and 'L' represents lightness. Additional values such as H (hue) representing intensity of color and C (chroma) representing degree of saturation of a color are also used. Thus these five values in a three dimensional array of points are used to define all possible colors (Hunter and Harold, 1987).

Researchers using different color systems to objectively quantify color all agree that the value (a) or the redness component is directly related to the carotenoid pigment concentration in salmon fillets (Schmidt & Idler 1958, Hunter (a) value; Saito 1969, Choubert 1982, Skrede and Storebakken 1986a, Storebakken *et al.* 1987, Hatlen *et al.* 1998, CIE 1976 (a*) value). Both systems, Minolta Chromameter and Hunterlab Labscan sphere spectrocolorimeter, were found by Skrede and Storebakken (1986a) and Storebakken *et al.* (1987) to be equally well suited for measuring salmon flesh color.

Redness significantly contributes to the overall acceptance of cooked salmon (Sylvia *et al.*, 1995). The value (a) is often used as an indicator of the astaxanthin content in salmon flesh as it generally exhibits the best correlation with increasing carotenoid pigment levels (Hatlen *et al.*, 1998; Bjerkeng, 2000). This was demonstrated in the case of Atlantic salmon, by Wathne *et al.* (1998). In a study by Storebakken *et al.* (1998) on color measurements in Atlantic salmon, results obtained by the Roche Color card, CIE 1976 redness (a*) and Salmonfan™ scores were linearly correlated ($0.46 < r^2 < 0.51$) to the astaxanthin concentration in the fillets. At the same time nonlinear relationships between L*, a*, b* values and carotenoid pigment concentrations have been observed to give a higher correlation in cases when the salmon flesh carotenoid pigment concentration was very high (4-6 mg/kg) (Christiansen *et al.,* 1995; King, 1996; Bjerkeng *et al.*, 1997).

Since visual color perception can vary from person to person, this often results in different color descriptions at a given concentration of astaxanthin (Torrissen, 1989; Christiansen *et al.*, 1995) and therefore, common methods of evaluating color, like the color card (Skrede *et al.*, 1990) and instrumental analysis (Skrede and Storebakken 1986b; Christiansen *et al.*, 1995), should be applied together.

Several papers have addressed the impact of frozen storage on color of Atlantic salmon. According to Anderson and Steinsholt (1992) the redness of salmon stored at –35 °C was found to be higher than those stored at –13 °C prior to sensory evaluation. Sheehan *et al.* (1998) found that there were insignificant changes (loss of astaxanthin) in the color of salmon fed diets containing astaxanthin even after 12 weeks of frozen storage (-20

°C). In a study by Farmer *et al.* (2000) farmed Atlantic salmon exhibited no significant differences in terms of appearance, odor, flavor, texture, aftertaste, and overall acceptability when stored at -25 °C for up to 34 weeks (fish steaks in laminated vacuum pouches) before the commencement of sensory analysis.

Quantitative descriptive tests in sensory panel analyses are generally used as an analytical tool to evaluate the texture and flavor profiles in fishery products (Brandt *et al.*, 1963; Iyer, 1972). According to Rutledge and Hudson (1990) a round table format allows open and free discussions among the prospective panelists while they develop a workable sensory 'ballot'. After marking the ballots, panelists usually discuss their scores until a consensus is reached for a particular attribute. This is the most important step because if everyone doesn't agree then the data from the panel will cease to have any meaning whatsoever. Training sessions also include optimization of the equipment like ovens, and cooking time for maximum control over variability. Practice sessions also help in teaching all the panelists proper tasting techniques like sniffing the hot sample for aroma, clearing the palate in between samples or abstaining from eating or drinking for at least an hour before the sensory session etc. Analytical descriptive procedures are concerned with different types and intensities of aroma, and flavor factors are often used in product development and quality assurance. Descriptive analysis of fish, which can be reproducible and precise (Johnsen and Kelly, 1990), can discriminate small flavor changes in samples.

Gas chromatography (GC) has been successfully used in the past decade for the determination of volatile components in food. Dynamic headspace analysis involves concentration of the headspace volatiles, followed by their trapping and de-sorption onto a GC column. A typical salmon aroma is the sum total of effects of sulfur compounds, aldehydes and ketones generated from the dehydrogenation of fatty acids, carotenoids and amino acids (Girard and Durance, 2000). Sensory changes during frozen storage are largely due to the formation of secondary oxidation products such as aldehydes and ketones (Refsgaard *et al.*, 1998). GC followed by principal component similarity (PCS) analysis may be used as an analytical tool in quality assurance of food (Ogihara *et al;* 2000). PCS can effectively reduce the number of original variables needed for analysis to just a few

'principal components', obtained after linear combinations, which can explain maximum variability observed in the data.

The present study was aimed at assessing possible sensory changes in Atlantic salmon that had been reared in sea water and fed diets containing different concentrations of tail-end dehulled and extruded sunflower meal (SFM) by partial replacement of premium quality fishmeal in the basal diet. Sensory attributes like color, flavor, texture, and aroma of fillets from Atlantic salmon previously fed diets containing one of four concentrations of nutritionally upgraded SFM by progressive replacement of premium quality fishmeal on an equivalent digestible protein basis, were compared to those of fillets from salmon fed a fishmeal- based basal diet for 84 days (refer: chapter 3).

Salmon flesh color was measured visually with Salmofan™ and instrumentally with the Hunterlab or Labscan system. An attempt was also made to test any possible changes in fillet color during frozen storage using Salmofan™. A sensory panel, comprised of 14 judges, was selected to assess four sensory attributes– salmon aroma, salmon flavor, off flavor and salmon texture, using a continuous descriptive method (Poste *et al.*, 1991). Gas chromatography (after solid-phase micro-extraction of headspace volatiles) was conducted to assess the possible effects of diet treatment on the flavor volatile patterns from the cooked salmon.

4.2 MATERIALS AND METHODS

4.2.1 Experimental protocol

A schematic representation of the experimental protocol is depicted in Figure 4.1.

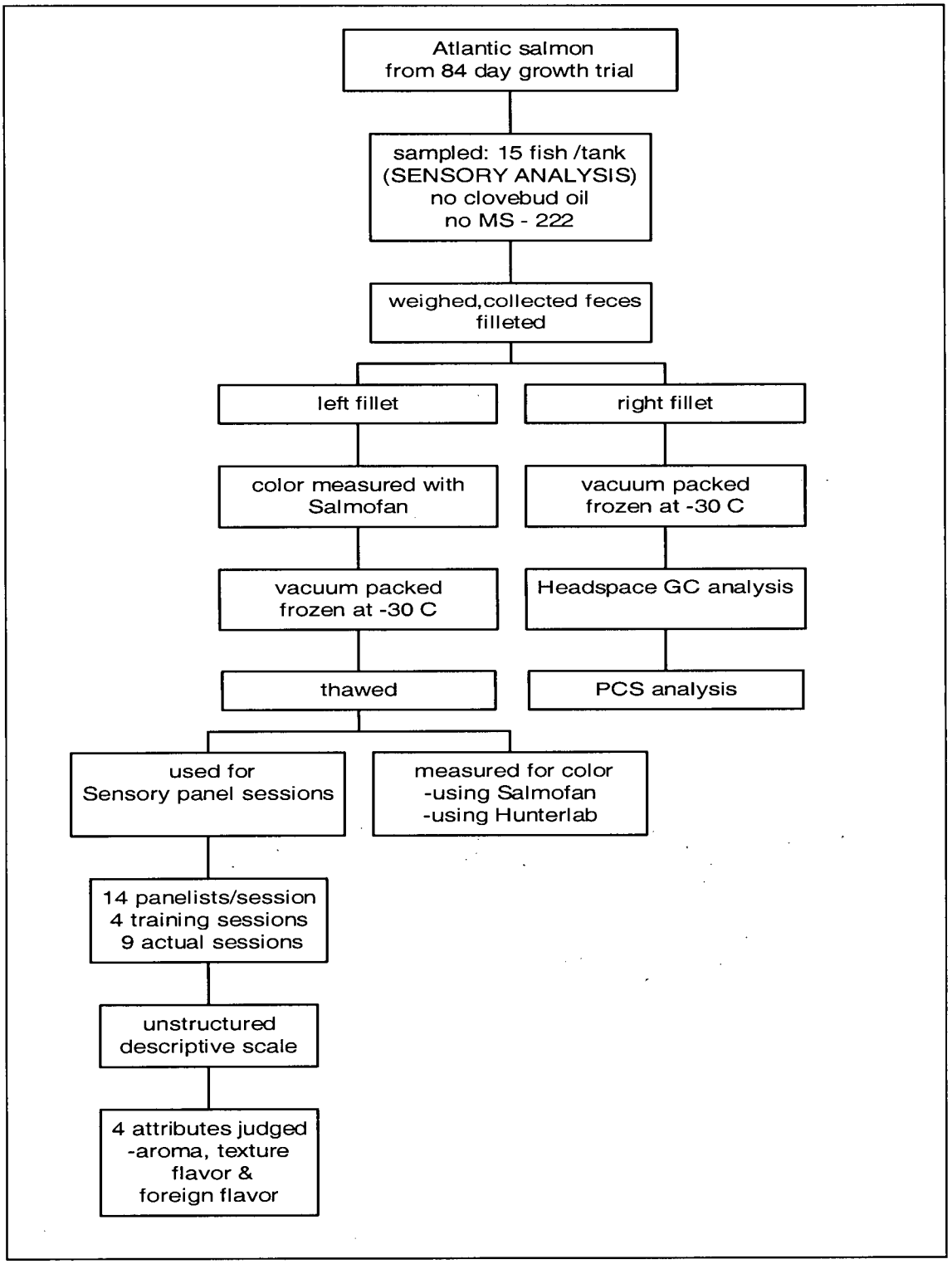


Figure 4.1: Schematic representation of the experimental protocol followed in this study.

4.2.2 Experimental salmon

Post-juvenile Atlantic salmon from the 84-day growth trial conducted at the West Vancouver Laboratory (described in chapter 3) were subsequently used for the sensory analyses described in this study.

On day 84, 15 fish from each tank were randomly collected with a net without anesthesia (MS222 or clovebud oil) for sensory analysis and killed by a blow on the head before being weighed and dissected (Higgs *et al.*, 1979) for collection of feces. Salmon were filleted, and the skin was removed before each fillet was washed with water to remove as much blood as possible. The cutting boards and knives used were sanitized with 200 ppm sodium hypochlorite between each fish.

The right fillets were rinsed with tap water to remove blood and then weighed before being vacuum packed in low oxygen-transmission bags (Food Pak Systems Ltd., Vancouver, B.C.; oxygen transmission rate, 2.3 cc/m^2.24hrs.atm at 25°C; water transmission rate, 7.8g/m^2.24hrs.atm at 25°C) and stored at –30 °C. They were later used for analyzing headspace volatiles using gas chromatography. The left fillets were rinsed with tap water to remove blood. Thereafter, they were weighed and used to visually determine color by reference to the Salmofan™ (refer: 2.5.1) before being vacuum packed in oxygen impermeable bags and then stored at –30 °C.

The same left fillets were later used for assessing color with Salmonfan™ and also for the sensory analyses after a period of approximately 6 months. Color measurements using Salmofan™ were conducted by the same operator on each occasion. This was done to determine if the vacuum packed fillets had any loss of color after 6 months of storage at –30 °C and if so, whether the color loss could be related to dietary treatment.

4.2.3 Sensory Analysis

4.2.3.1 Experimental Facility

All the sensory analyses were conducted in the Food Science building at The University of British Columbia, Vancouver, B.C., Canada. The sensory study was conducted on the fillets from Atlantic salmon that were randomly chosen for this study at the time of final sampling from the 84-day growth trial conducted at the Department of Fisheries and Oceans Canada, West Vancouver Laboratory (chapter 3). A specially designed sensory panel room, as described by Larmond (1978) and Poste *et al.* (1991) was used for the sensory sessions. This room had partitions between the subjects to minimize visual contact and other communication, neutral colors on the walls and tables and red lights to mask any color differences between the samples (ISO 1985, 1994). Also the room was air-conditioned.

4.2.3.2 Panel selection and training

The sensory panel, which was comprised of 14 judges (6 males and 8 females), was recruited from the Food Science program, at the University of British Columbia. The members were trained during 4 one hour long training sessions conducted one week prior to the commencement of the sensory trial. The training sessions were held on alternate days to avoid sensory fatigue amongst the panelists.

The training sessions were held according to the round table format as described by Rutledge and Hudson (1990) to encourage maximum participation from the panelists. Panelists were instructed in quantitative descriptive unstructured analysis. Through guided discussions agreement was reached on the meaning of each of the terms used on the sensory ballot by employing reference standards like cod, sockeye, Atlantic salmon (cooked at 190 ^{o}C for 4 minutes) and overcooked Atlantic salmon (cooked at 190 ^{o}C for 6 minutes) to indicate the extremes for every parameter. Panelists were encouraged to discuss their interpretation of the different terms to be used on the descriptive sensory ballot in order to familiarize them with the judging of the attributes. With the input gained from

these training sessions the actual descriptive unstructured sensory ballot was then modified to suit the study.

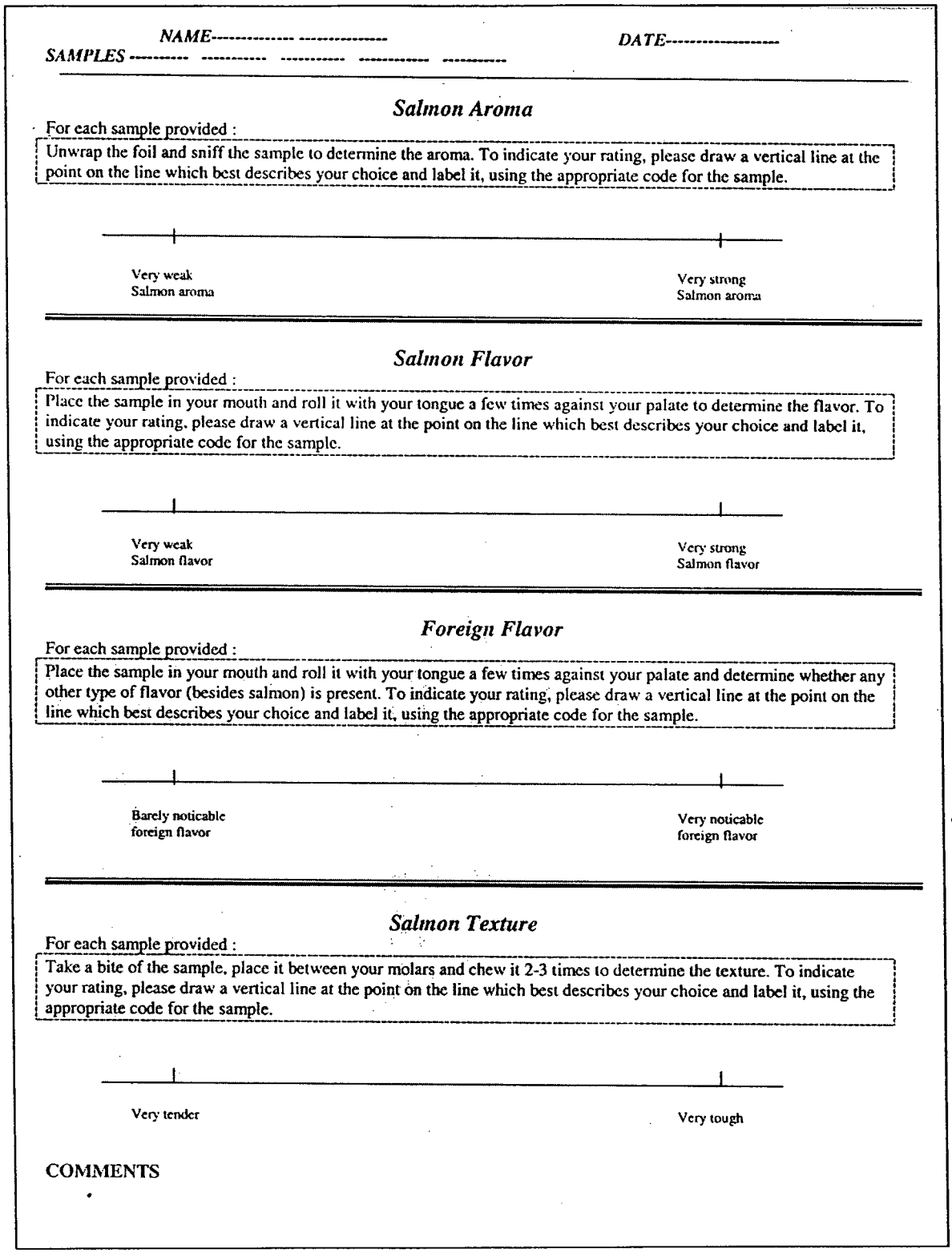
4.2.3.3 The Sensory Ballot

A descriptive sensory ballot (Figure 4.2), having a continuous, open-ended unstructured scale was used for each of the attributes. It consisted of a 15 cm horizontal line anchored at either end with points set 1.5 cm from each end and labeled with a descriptive term. The sensory ballot was based on an 'unstructured, continuous and open-ended' descriptive test (Poste *et al.*, 1991).

The panelists were asked to compare the five given samples for four attributes- aroma, flavor, foreign flavor, and texture and thereafter rate them using a continuous unstructured and open-end scale. Panelists recorded their evaluations by drawing vertical bars on the horizontal line. Each vertical bar was then labeled with the appropriate three-digit code to indicate the panelists' choice in each of the four attributes asked of them. Panelists were provided with instructions on the sensory ballot itself. Raw scores were recorded as the distances between zero and the vertical line made by the panelist to indicate his/ her choice.

4.2.3.4 Sensory Sessions

A total of nine sensory sessions were held on alternate days of the week over a three-week period. This included replicates per diet (3 composite samples/ tank with 3 tanks/ diet, for a total of n=9 for each panelist/diet group). Each panelist was provided with five, three-digit randomly coded, cooked salmon samples at the same time under red-light to disguise any color differences between samples. Bottled, non-carbonated mineral water and unsalted soda crackers were provided so that the panelists could cleanse their palate between samples.


NAME---------- ----------
DATE----------
SAMPLES ---------- ---------- ---------- ---------- ----------

Salmon Aroma

For each sample provided :

Unwrap the foil and sniff the sample to determine the aroma. To indicate your rating, please draw a vertical line at the point on the line which best describes your choice and label it, using the appropriate code for the sample.

Very weak Salmon aroma

Very strong Salmon aroma

Salmon Flavor

For each sample provided :

Place the sample in your mouth and roll it with your tongue a few times against your palate to determine the flavor. To indicate your rating, please draw a vertical line at the point on the line which best describes your choice and label it, using the appropriate code for the sample.

Very weak Salmon flavor

Very strong Salmon flavor

Foreign Flavor

For each sample provided :

Place the sample in your mouth and roll it with your tongue a few times against your palate and determine whether any other type of flavor (besides salmon) is present. To indicate your rating, please draw a vertical line at the point on the line which best describes your choice and label it, using the appropriate code for the sample.

Barely noticable foreign flavor

Very noticable foreign flavor

Salmon Texture

For each sample provided :

Take a bite of the sample, place it between your molars and chew it 2-3 times to determine the texture. To indicate your rating, please draw a vertical line at the point on the line which best describes your choice and label it, using the appropriate code for the sample.

Very tender

Very tough

COMMENTS

Figure 4.2: Sensory ballot used during the sensory trial in this study. The ballot has been scaled down in this illustration.

Panelists were also instructed not to eat or drink up to 30 minutes prior to the commencement of the sensory sessions, in order to minimize the possible introduction of any new bias. Panelists sat in individual booths to minimize communication amongst themselves. Panelists were also encouraged to refrain from wearing perfume/cologne on the days the training sessions and sensory sessions were conducted.

4.2.3.5 Preparation of cooked salmon fillet

The anterior and posterior portions of the fish, from the same tank, have different intensities of flavors and different flavor profiles (Johnsen and Kelley, 1990; Sigurgisladottir *et al.*, 1997). Since this could lead to a large standard deviation in the sensory evaluation scores, composite fish samples were used in order to reduce this effect. These composite samples were made by random combination of fish slices from different fillets and different regions into one composite sample. This pooled sample technique ensures that replicates are true representatives of the sample population (Johnsen and Kelly, 1990) in order to reduce experimental variation due to differences between and within samples.

Randomly chosen and individually wrapped right fillets of salmon from each of the dietary treatments were removed from frozen storage at –30 °C and thawed by placing them in an incubator (Forma Scientific refrigerated incubator, Mallinckrodt Inc., Marietta, OH, USA) at -10 °C overnight for easier handling on the next day. The partially thawed samples were then sliced to approximately 5 mm width using a meat slicer (Hobart Model 410 Slicer, Don Mills, ON).

Slices from 4 fish from the same tank were each separated into 3 sections – anterior, middle and posterior. To maintain homogeneity, equal numbers of randomly chosen slices from each of the 3 sections were combined to form a composite sample. These composite salmon samples from each dietary treatment were wrapped in aluminum foil, coded with 3 digit random numbers, and refrigerated at 4 °C until needed.

Samples were removed from the refrigerator 30 minutes prior to the scheduled sensory panel sitting. Upon the arrival of each panelist, samples were cooked for 4 minutes in a pre-heated (190 °C) oven (Kenmore deluxe cooking range) as was done by Shamaila *et al.* (1995). Foil-wrapped samples from all dietary treatments were presented to each panelist immediately after cooking.

4.2.4 Color measurements in raw fillets

4.2.4.1 Visual measurement

Salmon fillets were visually assessed for color using the Roche Salmofan™ (Hoffmann-La Roche, Basel, Switzerland) under standardized conditions. The latter involved the use of a color cabinet, painted white, that had a cool fluorescent discharge light (Ra > 90, temperature 6500K; Lithonia Lighting Canada, ON). Salmofan™ readings were taken by the same individual both at the time of final sampling (day 84 of the growth trial; refer: chapter 3) and at the time of sensory analyses (Table B.1, Appendix B) to obtain an estimate of possible loss of color during frozen storage. Dual lights were used in the color cabinet to eliminate any shadowing effect. Fillet color was measured at a position adjacent to the dorsal fin and level with the lateral line.

4.2.4.2 Instrumental measurement

The Hunterlab system was used to quantify the effect of diet treatment on the degree of flesh pigmentation in the fillets of Atlantic salmon (Smith *et al.*, 1992; Shamaila *et al.*, 1995, Christiansen *et al.*, 1995). In this regard, color was quantified using the Hunterlab Color Difference Reflectance Spectrophotometer (Hunterlab Labscan, Hunter Associates Laboratory Inc., Reston, VA) with a 1.27 cm aperture and a standard illuminant D_{65} to simulate daylight at a correlated color temperature ~ 6500 °K. The inner side of each fillet

was placed face down on the optically clear petri-dish (Fisher™ 100x15mm standard sterile disposable plastic petri-dishes; Fisher Scientific Co., Canada) and positioned over the viewing area so that the reading was taken from the area adjacent to the dorsal fin and above the lateral line. Each fillet was then rotated through 90° towards the right, to obtain four readings and the resultant average constituted the final value. The instrument was calibrated to zero by using a black tile and then standardized using a white tile. The final values for Hunter *L, a* and *b* scores (Tables B.2, B.3 and B.3; appendix B) were recorded for each fillet.

4.2.5 Headspace gas chromatography

Each salmon sample (a composite of 3 sections in a fish) weighing 5g was homogenized (Ultra Turrex T25BS1, IKA Labortechnik, IKA Works Inc., Willmington, NC) in 20 mL of distilled de-ionised water in a 40ml clear vial screw top, fitted with PTFE/Silicone septa (Supelco, Supelco Park, Bellefonte, PA) and cooked (the bottle was covered with foil for this step instead of having a plastic screw cap) at 190 °C for 4 min, as described by Shamaila *et al.* (1995). A 0.1% (prepared in absolute alcohol) solution of caproic acid ethyl ester (grade I, Sigma Chemical Co, St Louis, MO, USA) was used as an internal standard (2 μL) in order to standardize peak areas. The headspace volatiles from the cooked salmon were then adsorbed/trapped for 30 min at 90 °C (hot water bath, constant stirring) onto 75 μm CARBOXEN/PDMS (Carboxen/polydimethyl siloxane), partially cross-linked solid phase micro-extraction fibers (SPME fibers, Sigma-Aldrich Canada, Oakville, ON). Thereafter the manufacturer's directions (Supelco, Canada) were followed for volatile collection and release onto the column in the gas chromatograph.

Gas chromatography (dynamic head space analysis) was conducted according to the procedures of Ogihara *et al.* (2000) and Nakai *et al.* (1999). A model GC-9A (Shimadzu, Columbia, MD) with a medium polarity DB-624 column (30m length, 0.53 mm internal diameter, 3 μm stationary phase thickness; J&W Scientific, Rancho Cordova, CA) and a flame ionization detector (FID) was used. Thermal de-sorption of volatiles from the

SPME fiber took place in the injection port at 220 °C in a split-less mode by holding for 5 minutes. The flow rates for compressed air (UHP grade, Praxair Products Ltd., ON, Canada), helium (as the carrier gas; UHP grade, Praxair Products Ltd., ON, Canada) and hydrogen (UHP grade, Praxair Products Ltd., ON, Canada) were set at 500 mL/min, 50 mL/min and 50 mL/min, respectively. The column temperature started at 40 °C for 5 min and subsequently, this was raised by 3 °C/min to 200 °C and further raised to 220 °C at the rate of 10 °C/min followed by holding at 220 °C for a period of 20 min.

The GC operating conditions were worked out with test samples prior to running the salmon fillets collected from the growth trials. The GC patterns were monitored using a Chromatopac, C-R3A (Shimadzu, Tokyo). Data processing was conducted using principal component similarity (PCS) technique, as described by Vodovotz *et al.* (1993) (Appendix B, Table B.5).

The peak areas obtained from the GC chromatogram of the samples were standardized and then subjected to the PCS program (in this study: written in Visual Basic 4 for Windows 95). PC scores having eigenvalues ≥ 1.0, were selected for analysis, since they contribute the most to the variation.

These PC scores were then used for the linear regression analysis. Slopes versus coefficients of determination (r^2) are plotted as a PCS scattergram, which was then used to interpret the results. A brief summary of the PCS computation steps is provided in Table B.5 (Appendix B). Thus PCS can effectively reduce the number of original variables needed for analysis to just a few 'principal components', that are obtained after linear combinations, which can then be used to identify the points that contribute the most to the observed variation in the data.

4.2.6 Statistical Analysis

All calculations were subjected to a randomized block analysis of variance, ANOVA using the general linear model of SYSTAT (version 5). For sensory trial results, the effects of two factors namely, diet and block, were evaluated using a three-way ANOVA with panelists as the third factor. For each of the sensory attributes tested, both raw and transformed scores were examined. Analysis of variance (ANOVA) of raw sensory scores of all the four attributes indicated a significant ($p<0.05$) difference between the judges. Results were therefore subjected to z-transformation, a standardization technique recommended by Reid and Durance (1992) before ANOVA. Thus z transformed scores helped reduce the error arising from the innate tendency of each judge to preferentially use a portion of the continuous scale. Out of 14 panelists, one was excluded (prior to z-transformation) because of faulty marking on the sensory ballot provided. The effects of block, diet, panelist and the interaction between panelist and diet in the ANOVA were considered to be significant when $p < 0.05$.

Hunter (a) and Salmofan™ scores were also analyzed using ANOVA. In addition to this, the relationship between Salmofan™ and Hunterlab 'a' scores was investigated using a linear regression analysis.

The peak areas obtained from the GC chromatogram of the cooked salmon samples (3 samples/diet) were standardized and then they were subjected to the PCS program written in Visual Basic 4 for Windows 95. A linear regression analysis of the deviations of PC scores of the unknown relative to those obtained for the reference sample versus the variability accounted for by the PC scores showed that 3 PC scores had eigenvalues ≥ 1.0. These 3 PC scores were then used for the linear regression analysis. Slope versus coefficient of determination (r^2) was then plotted as a PCS scattergram (Vodovotz *et al.*, 1993).

4.3 *RESULTS & DISCUSSION*

4.3.1 Sensory assessment by a trained panel

Astaxanthin is not distributed evenly in the fish fillet. Christiansen and Wallace (1988) and No and Storebakken (1991) reported that the concentration of astaxanthin in the caudal part was 30-40% more than that in the back and neck parts while Refsgaard *et al.* (1998) reported an astaxanthin concentration that was only 19% more in the caudal region. Also, the anterior and posterior portions of fish sampled from the same tank are known to have different intensities of flavors and different flavor profiles (Johnsen and Kelly, 1990). Since all this variation could lead to a large standard deviation in the sensory evaluation scores, composite fish samples were used to reduce this effect. This pooled sample technique ensured that replicates were true, and it also helped to reduce the experimental variation due to differences between and within samples.

Evaluation of the raw scores for all the four parameters (Table IV-I) revealed that the scores of the panelists were significantly ($p < 0.05$) different. To eliminate the effect of panelists in assessing the different diets, z-transformed scores were analyzed by ANOVA and the results are provided in Table IV-II. Composite flesh samples from fish fed the basal diet (diet A) and each of the 4 test diets were not significantly different ($p > 0.05$) in terms of salmon aroma, texture, and flavor. However, significant differences ($p < 0.05$) were found between samples from salmon fed diet A (basal diet) and those from salmon fed diet E and also between samples from diet B salmon and E salmon for foreign flavor.

While the addition of partially dehulled and extruded sunflower meal in the diets up to 20.4 % of dry matter resulted in imperceptible differences in the scores for salmon aroma, texture, and flavor (Table IV- II), distinct foreign flavors (mainly bitterness) were noted primarily in the flesh of salmon fed diet E which had 27.1 % SFM by replacement of fishmeal. This finding is similar to the study by Kaushik *et al.* (1995) in

which total replacement of fishmeal with soybean products reportedly caused changes in the sensory quality of rainbow trout especially flavor (Kaushik *et al.*, 1995). In this regard, grassy, bitter or astringent off flavors were detected (Morr and Ha, 1991).

The use of plant protein and lipid sources in diets for salmonids can result in the presence of foreign flavors, due to the presence of flavor compounds that are most likely phenolic compounds. However, these off-flavors can be avoided by the removal of these anti-nutritional factors (ANF) during processing (Morr and Ha, 1991; Nour *et* al., 1989; Rumsey *et al.*, 1993, 1994). Since, in the present study, the sunflower meal was not treated for the removal of anti-nutritional factors, those factors may have contributed towards the foreign flavors noted in the salmon fillets. Since Diet E had the maximum concentration of SFM (27.1%), the off-flavors were most noticeable ($p < 0.05$) in the salmon samples obtained from this dietary treatment.

The foreign flavors reported by the panelists included bitterness, metallic aftertaste, cereal-like flavor, and fishiness (Figure 4.3). Laverty (1993) recommended bleeding the fish in running water at the final sampling to avoid any residual blood in the fillets, which could cause a metallic aftertaste. Fishiness meanwhile could possibly have been due to the anchovy oil that was used as the source of supplemental lipid during preparation of the diets used in this study. The percentage of the oil increased from diet A to E and this trend can be related to the increased incidence of fishy flavor observed by the panelists in samples obtained from fish fed diet E compared to those from diet A.

The grassy or cereal–like flavor may have resulted from the ANF in the SFM because the SFM used in this study was not processed for the removal of ANF. Since diet E had the maximum inclusion of SFM in this study, panelists could distinctly perceive foreign flavor in the salmon samples fed this diet. Bitterness was noted to be a random variable across all diet treatments and possibly this resulted from the accidental nicking of the gall bladder when the fillets and viscera were removed from the salmon.

TABLE IV-I: Means of raw scores for flesh aroma, flavor, foreign flavor, and texture. Sensory trials conducted on composite samples from Atlantic salmon that had been fed the basal diet and four test diets (n=117).

Diets	*aroma*	*flavor*	*foreign flavor*	*texture*
Diet A (0.0%SFM)	8.26	8.29	1.97	7.54
Diet B (6.8% SFM)	8.65	8.36	2.28	7.71
Diet C (13.6% SFM)	8.85	8.28	2.69	8.05
Diet D (20.4 % SFM)	8.68	8.06	2.70	7.89
Diet E (27.1 % SFM)	8.82	8.09	3.38	7.76

TABLE IV-II: Means of standardized scores (z-transformed values) for flesh aroma, flavor, foreign flavor, and texture. (Refer to Table IV-I for additional information)

Diets	*aroma*	*flavor*	*foreign flavor*$^{\Phi}$	*texture*
Diet A (0.0%SFM)	- 0.191	0.030	- 0.280^{b}	- 0.132
Diet B (6.8% SFM)	- 0.014	0.044	- 0.121^{b}	- 0.042
Diet C (13.6% SFM)	0.093	0.000	0.075 ^{a,b}	0.128
Diet D (20.4 % SFM)	0.015	- 0.054	0.033 ^{a,b}	0.064
Diet E (27.1 % SFM)	0.100	- 0.026	0.304^{a}	- 0.018
Pooled SD	0.696	0.663	0.647	0.598
P *	0.356	0.971	**0.002**	0.365

Aroma, flavor & texture were not significantly affected by diet ($p > 0.05$), block ($p>0.05$) and the interaction between diet and panelist (diet panelist; $p> 0.05$)

Φ foreign flavor was significant ($p < 0.05$) between diets A and E and also between diets B and E.

§ z-transformed scores for – aroma, flavor, foreign flavor and texture were analyzed by ANOVA.

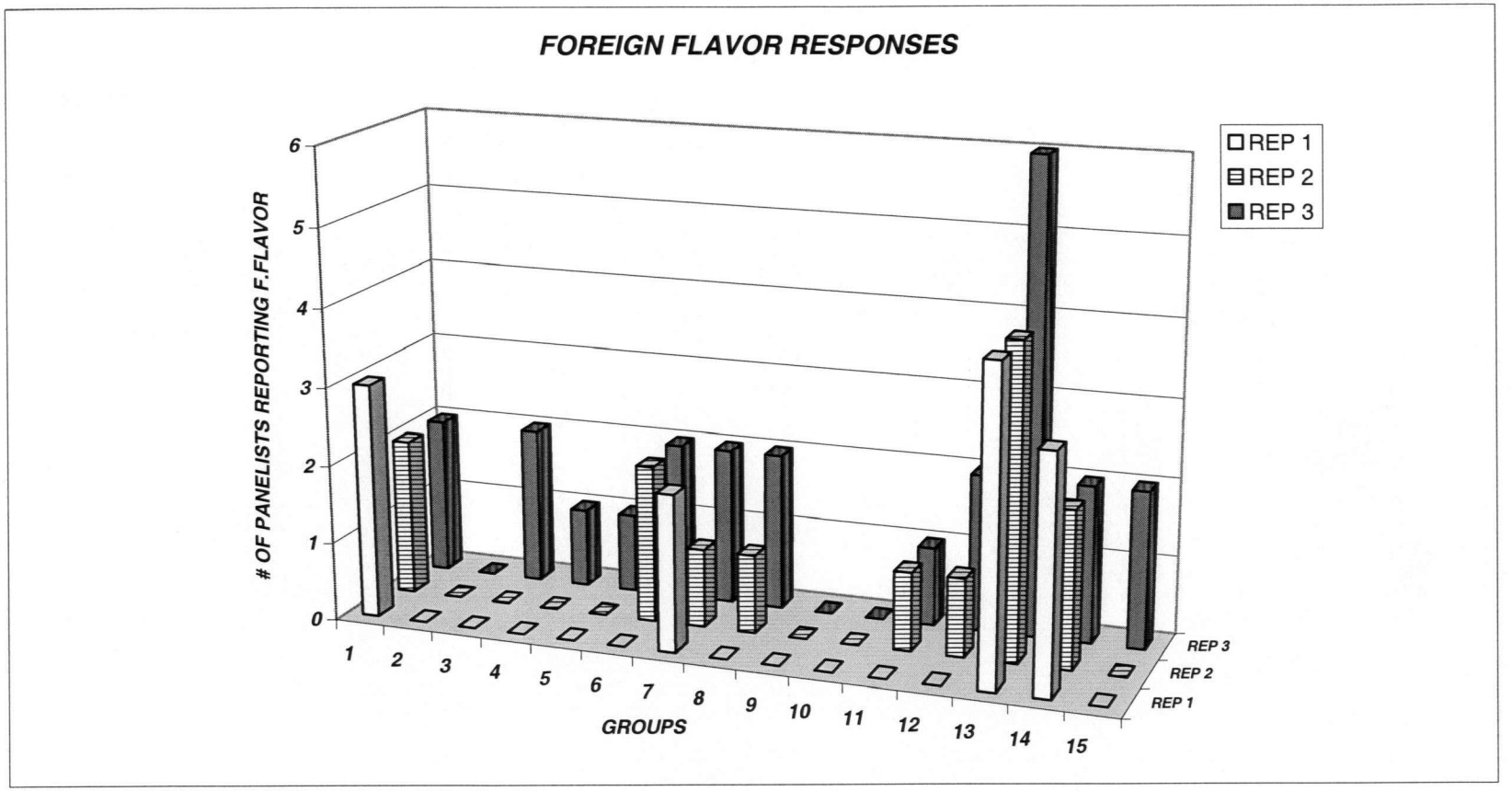


Figure 4.3: Number of panelists giving positive responses for 'foreign flavor' in the Atlantic salmon samples provided during the sensory trials. Triplicate samples from each tank were provided. Each dietary treatment consisted of triplicate groups: Diet A (0.0%SFM; groups 1-3), Diet B (6.8%SFM; groups 4-6), Diet C (13.6%SFM; groups 7-9), Diet D (20.4%SFM; groups 10-12) and Diet E (27.1%SFM; groups 13-15). ANOVA results revealed significant differences ($p < 0.05$) between diets A & E and between B & E for foreign flavor.

Alternately, bitterness may have stemmed from some component present in the SFM. No off-flavors were noted at the 10% level of substitution of soybean meal by Bjerkeng *et al.* (1997). This agrees with the present results, since no significant ($p > 0.05$) foreign flavors were detected by the panelists (Table IV-III) with respect to samples from fish fed diet A (0.0%SFM), B (6.8%SFM), C (13.6%SFM) and D (20.4% SFM). Refsgaard *et al.* (1998), in a study on the sensory and chemical changes in farmed Atlantic salmon during frozen storage, demonstrated that cooked salmon was characterized by a high intensity of earthy, sweet, sour, and fish oil flavors. They also noted that metallic and bitter aftertastes increased during storage of the individual fish fillets in aluminum foil and polythene bags at –10 and –20 °C for up to 34 weeks. The odorants identified in stored salmon fillets were –fishy, buttery, sweet, and green. But the authors failed to mention the cooking method used in their study. That study also found a negative correlation between the metallic aftertaste in cooked samples and hexanal (-0.304) as a variable. Similarly, a bitter taste in cooked samples was found to correlate (0.521) with the ratios of the fatty acid 20:1 (n~9) measured before and after storage.

3.2 Color Measurements

Salmofan™ scores for fillet color in Atlantic salmon (Table II-III) ranged from 22.6 (diet C) to 24.0 (diet E). Except for a slight dip in the color scores for fillets from salmon fed Diet C, there was an increasing trend in scores from diet A to diet E. A similar increasing trend in Hunter (a) values (Table IV-III) was observed in going from Diet A (7.74) to E (10.3) with a slight decrease in scores for fillets of salmon fed diet C (7.87). Although scores increased with the level of SFM in the diet, the dietary treatments did not differ significantly ($p > 0.05$) with respect to color scores, both using Salmofan™ and Hunter lab values for redness (a), when compared statistically using a two-way ANOVA. A study by Storebakken *et al.* (1998) on Atlantic salmon showed that Salmofan™ scores of 25-26 corresponded to CIE 1976 (a*) values of 7.8–9.5 which compared well with the results obtained in this study *i.e.* Salmofan™ scores of 22-24 corresponded with Hunter (a) scores of 7.7-10.3 (Table IV-III).

TABLE IV-III: Mean values of Salmofan™ scores (n=36) and Hunter values (n=36) for redness (a) for Atlantic salmon. Measurements represent the average of triplicate values for each diet. (Refer to Table IV-I for additional information).

Diets	*Hunter (a) values*	*Salmofan™ scores*
Diet A (0.0%SFM)	7.74	22.8
Diet B (6.8% SFM)	8.37	22.9
Diet C (13.6% SFM)	7.87	22.6
Diet D (20.4 % SFM)	9.25	22.9
Diet E (27.1 % SFM)	10.3	24.0
Pooled SD	0.942	0.772
P *	0.127	0.258

* all results were n.s with diet ($p > 0.05$) and block ($p>0.05$)

In a study by Bjerkeng *et al.* (1997), in which full fat soybean meal was substituted for fishmeal (total dietary fat was 32-39%) in a diet for Atlantic salmon at the 10% level, no significant changes for color (instrumentally using CIE 1976 (a*) values, visually with color cards), sensory characteristics or astaxanthin concentration were observed in muscle between the fish samples.

Different color systems that are used to quantify flesh color all show that the value (a) is directly related to the carotenoid pigment concentration (Schmidt & Idler, Hunter **a** value, 1958; Saito, 1969; Choubert, 1982; Skrede and Storebakken, 1986a; Storebakken *et al.*, 1987; Hatlen *et al.*, 1998). Both, reflectance spectro-photometric systems (*viz.* Minolta Chromameter and Hunterlab Labscan sphere spectro-colorimeter) were found to be equally well suited for measuring color in salmon flesh (Skrede and Storebakken, 1986a and 1986b). Baardseth *et al.* (1988) found a good relationship between color measurements obtained using CIE and Hunterlab for all the food commodities examined, but direct comparison was difficult and separate regression equations had to be established for each food of interest.

The degree of correlation between Hunter (a) values and the Salmofan™ color card scores was determined. A high degree of linear correlation ($r = 0.864$) between the two systems of color measurements (Figure 4.4) was observed. Christiansen *et al.* (1995) also observed a positive linear correlation between Roche Color card scores and CIE 1976 (a*) values. The correlation in the present study was found to be higher than that found in the study by Storebakken *et al.* (1987) on color measurements with Atlantic salmon. In this regard, the results obtained by Roche Color card, CIE 1976 redness (a*) and Salmonfan™ scores were linearly correlated ($0.46 < r^2 < 0.51$) to the astaxanthin concentrations in the fillets (Storebakken *et al.*, 1987).

In a study by Johnsen and Wathne (1990) a correlation coefficient of $r = 0.732$ was observed between Salmofan™ scores and astaxanthin content in Atlantic salmon fillets and $r = 0.706$ between the Hunter (a*) values and the astaxanthin contents in Atlantic salmon fillets. A significant but nonlinear relationship has been suggested between

astaxanthin concentrations and Roche color card scores (Christiansen *et al.*, 1995) as well as with instrumental color measurements (Skrede and Storebakken, 1986b; Christiansen *et al.* 1995; Choubert *et al.*, 1997; Nickell and Bromage, 1988, 1998). But this was found to hold true in cases when fillets were obtained from salmon that had been fed diets with very high levels of astaxanthin and the fillet astaxanthin concentrations varied between 6 and 8 mg/kg.

Since Salmofan™ has been found to be less accurate when high levels of astaxanthin are present in the salmon fillet, simply expanding the color scale at the higher score levels would not help to solve this problem and improve the accuracy of the results. This is because the human eye is unable to distinguish between the color differences in this range of pigment concentration (Johnsen and Wathne, 1990). Also, as the visual color perception can vary at a given concentration of astaxanthin (Torrissen *et al.*, 1990; Christiansen *et al.*, 1995), common methods of evaluating color - like the color card (Skrede *et al.*, 1990) and instrumental analysis (Storebakken *et al.*, 1987; Christiansen *et al.*, 1995) should be applied together.

Higher dietary fat content results in more absorption of astaxanthin in salmon flesh, leading to higher a* values being observed in different studies (Bjerkeng *et al.*, 1997; Tibaldi and Ballestrazzi 1990; Torrissen *et al.*, 1990). The fillet lipid content can affect the perception of color and texture since increased lipid deposition has a diluting effect on flesh color (Christiansen *et al.*, 1995). In the present study, the lipid concentrations in the fillets between the groups did not affect the Hunter (a) scores because the lipid contents in the fillets were not significantly ($p > 0.05$) affected by the dietary treatments (refer: chapter 3).

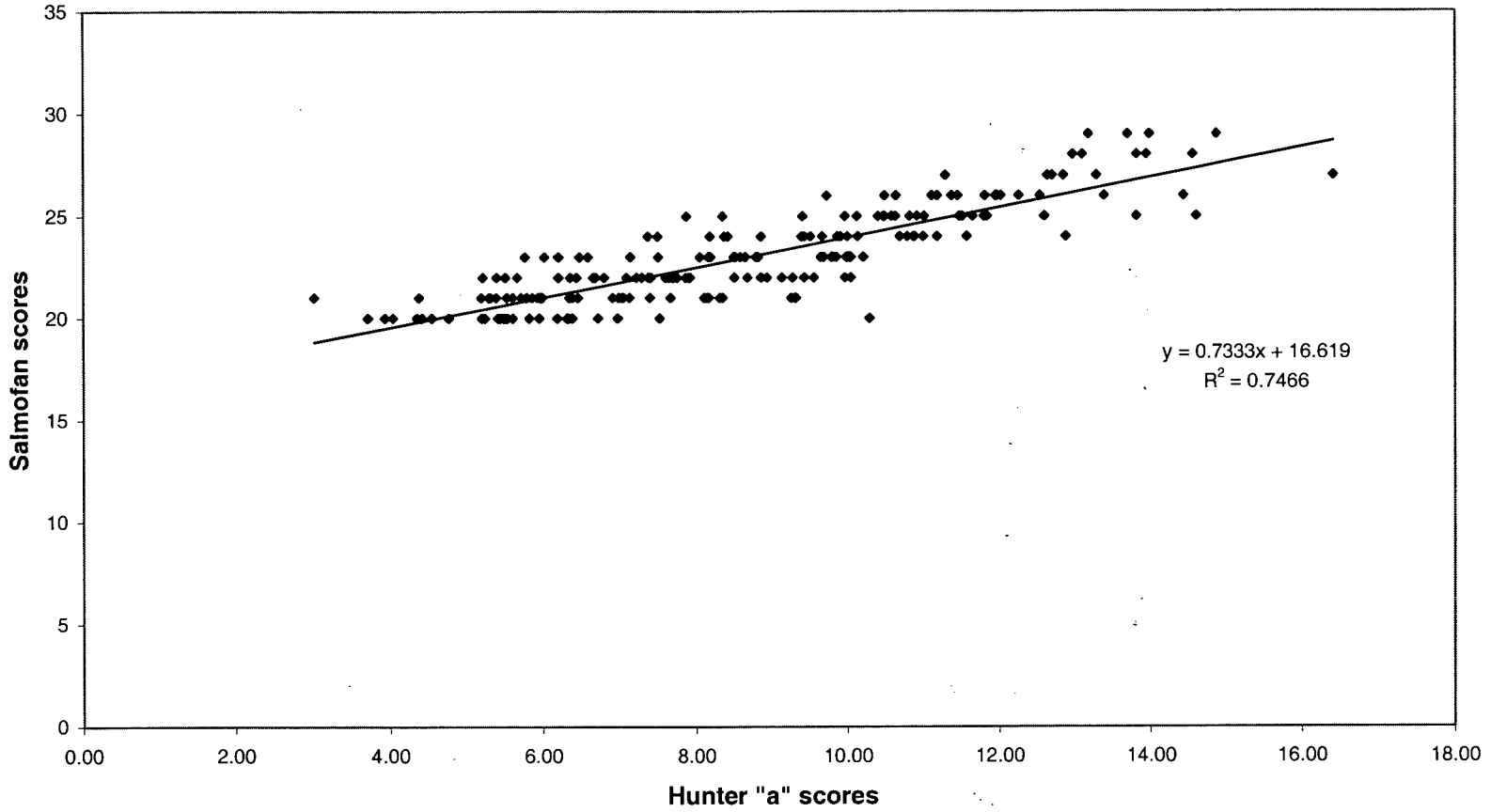


***Figure 4.4*:** Correlation between Salmofan™ and Hunter (a), redness scores obtained for Atlantic salmon samples that were assessed for color during the sensory trial (r = 0.864).

4.3.3 Effect of fillet storage conditions on color

In the present study, Salmofan™ readings were recorded for each fillet at the time of packaging the fillets (refer; chapter 3) and during the sensory trial with the scores related to before and after fillet storage, respectively. The readings were taken independently, by the same judge, at both measurement times. Although the color scores were not found to be significantly ($p > 0.05$) affected by different diet treatments at each time, a general decrease in the fillet color was observed between the measurement times, regardless of the dietary treatments.

This apparent decrease in color between the two measurement times could have resulted from several possible factors such as the oxygen transmission properties of the vacuum packaging material that was used for fillet storage, the presence of some oxidative changes in the fillet due to enzymatic activity and physical changes in the fillet micro-structure that affected the visual perception of color. Since no astaxanthin measurements were conducted we cannot pinpoint with certainty the cause of the temporal decrease in color.

Skrede *et al.* (1990) stored 90 fillets of Atlantic salmon that had been fed a diet containing 5% astaxanthin at –25 °C and measured the color within 5 months. They found an insignificant ($p > 0.05$) loss in the pigment levels. Farmer *et al.* (2000) studied the effect of storage of Atlantic salmon fillets at –25 °C for up to 34 weeks before commencement of sensory analysis. The only flavor altered by freezing was 'oily flavor', which was found to be less intense in frozen fish. But frozen storage did not alter aftertaste, texture, flavor, and odor. Hence, no perceptible sensory changes were found in Atlantic salmon during frozen storage. In addition, Storebakken and No (1992) found that rainbow trout fillets stored at –20 °C to –80 °C suffered less than 5% pigment loss during 6 months storage. Anderson and Steinsholt (1992) found that the degree of redness of Atlantic salmon fillets stored at –35 °C was very stable. Sheehan *et al.* (1998) also noted no significant changes in visual color scores or carotenoid pigment concentrations in Atlantic salmon fillets after 12 weeks of storage at -25 °C.

4.3.4 Volatiles as assessed using Gas Chromatography

The chromatograms of headspace volatiles obtained for fillets from the salmon fed diets A and E following GC analysis are shown in Figures B.1 and B.2 (Appendix B), respectively. Twenty-two peaks, that had areas constituting greater or equal to 1% peak area of the internal standard were used and they were consistently evident in the samples from fish fed all five diets. Accordingly, these peaks were chosen to carry out the principal component similarity analysis (PCS). The original data from the chromatogram were converted into PC scores (Appendix B, table B.6), which were further expressed as percentages that each PC score contributed towards the total variation (Appendix B, Table B.7). The results clearly indicated that the samples from fish fed diets E and A differed noticeably in their profiles as compared to profiles collected from samples from fish fed diets B, C and D, and these are shown in a scattergram (Figure 4.5 with diet A as reference; Figure 4.6 with diet E as reference). In addition, the plot of PC1 versus PC2 scores analyzed by PCA produced a scatter-plot showing similarities with that of PCS scattergram when diet E was used as the reference.

Since mass spectrometry was not conducted following GC, the individual volatile components could not be identified. But the GC chromatograms gave enough information to demonstrate the degree of similarity between salmon fed the 5 diets tested. In fact, a comparison of the PC scattergram (Figure 4.6) and the results obtained from the sensory panel for foreign flavors demonstrate a marked similarity. Both indicated that fillets from salmon fed diet E were perceptibly different from fillets from salmon fed diet A (reference/control diet). Salmon fed diet E were thus found to be noticeably different from salmon fed diet A, both by the sensory panelists and following the GC/PCS analysis. There is virtually no literature on the GC/PCS profiles for salmon fed diets containing proteins from a plant source. Thus it was not possible to compare the results of the present study with those from previous studies. The present findings suggest that additional studies should be conducted along these lines.

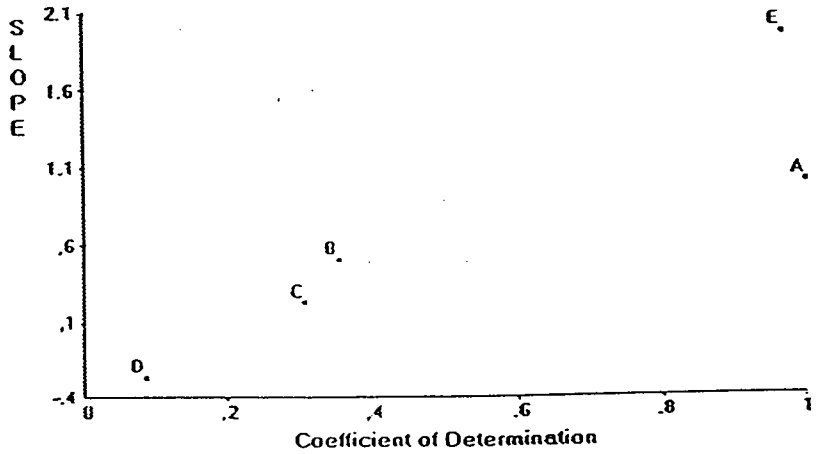


Figure 4.5: Scattergram showing the principal component similarity plot of samples from fish fed the five diets (n=3) obtained after GC/PCS analysis when diet A was used as a reference.

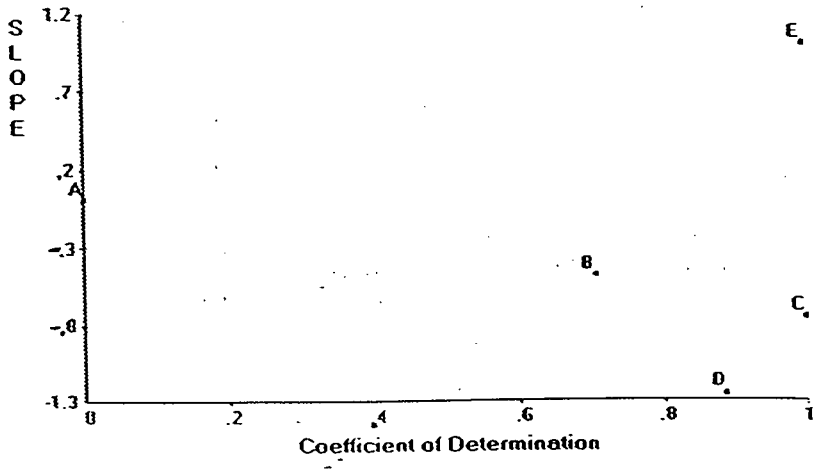


Figure 4.6: Scattergram showing the principal component similarity plot of samples from the fish fed five diets (n=3) obtained after GC/PCS analysis when diet E was used as a reference.

4.4 CONCLUSION

The present study demonstrated that digestible protein from nutritionally upgraded SFM (refer: chapter 3) could replace up to 33% of the digestible protein provided by fishmeal in a highly nutritious grower diet for post-juvenile Atlantic salmon in seawater without adversely affecting the aroma, flavor, and texture of fillets. However, it was noteworthy that in the flesh of the salmon which were fed the highest dietary level of SFM in this study (27.1% of dry diet) foreign flavors were clearly perceptible to the sensory panelists and also distinguishable by headspace GC. Therefore it is conceivable that higher levels of SFM in the diet of Atlantic salmon under the conditions of this study may have contributed to even more foreign flavors in the flesh of salmon. Further investigations should be conducted to identify the factor(s) in the nutritionally upgraded SFM that were responsible for foreign flavor development in Atlantic salmon fed diets containing elevated levels of SFM. Possibly, this adverse effect can be avoided using sunflower meal that has been processed further to remove the anti-nutritional factors that were responsible. In this regard, additional studies are needed to determine the best strategies for optimizing the cost effectiveness of dietary SFM.

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APPENDIX B

TABLE B.1: Salmofan™ scores for color measurements taken in Atlantic salmon (*Salmo salar*) fillets at the time of the sensory trials. The scores are presented for the fish sampled from each tank on day 84.

tank 100	tank 101	tank 102	tank 103	tank 104	tank 105	tank 106	tank 107	tank 108	tank 109	tank 110	tank 111	tank 115	tank 116	tank 117
24	23	20	27	20	24	28	25	23	24	28	25	26	20	24
22	25	25	25	20	26	29	20	24	21	24	23	23	21	22
26	20	26	24	23	23	23	23	21	25	26	21	25	27	25
24	23	21	25	26	23	25	22	22	20	25	22	22	29	24
29	22	22	22	20	25	23	25	23	27	22	22	24	21	23
24	25	21	23	22	28	20	23	24	20	21	21	22	23	22
26	21	24	22	26	21	21	22	24	22	26	22	26	25	24
20	20	21	26	22	24	21	21	26	21	23	27	24	20	21
26	22	21	28	23	21	21	21	22	22	23	21	25	21	21
23	24	21	22	23	24	23	26	28	24	21	20	25	22	21
24	22	21	22	22	27	23	25	21	20	23	20	22	24	20
22	26	25	25	21	20	20	27	20	23	20	21	20	22	20
					21				21			22	22	
					29				22			25	20	
					23				21			23	21	
									20				20	

TABLE B.2: Hunter 'L' values for Atlantic salmon (*Salmo salar*) fillets used in the sensory trials for color measurements. The scores are presented for the fish sampled from each tank on day 84.

tank 100	tank 101	tank 102	tank 103	tank 104	tank 105	tank 106	tank 107	tank 108	tank 109	tank 110	tank 111	tank 115	tank 116	tank 117
46.82	53.07	53.69	43.60	53.47	48.42	44.10	50.48	40.51	42.78	42.36	42.30	43.66	36.27	39.86
49.61	52.56	47.38	45.23	49.48	46.55	48.98	42.97	43.81	45.99	49.76	50.60	44.86	35.25	46.44
39.62	54.35	43.47	46.36	50.94	50.12	47.49	48.69	45.62	44.81	46.20	42.24	43.47	31.85	50.78
43.76	51.69	48.34	48.86	46.75	45.76	42.86	45.19	52.75	48.96	44.51	47.19	43.60	42.02	43.21
42.68	52.73	52.10	49.97	47.99	43.31	42.83	46.58	39.04	47.33	36.80	47.99	45.72	52.25	34.22
41.76	51.92	50.67	48.94	41.79	53.01	41.07	42.37	34.31	44.53	45.95	41.60	40.04	40.21	36.19
44.27	50.93	46.59	43.57	39.13	44.26	45.68	49.54	38.32	39.43	41.67	39.93	49.52	42.42	36.22
50.27	49.95	43.25	52.46	42.01	50.95	42.18	56.11	40.35	45.31	39.37	37.31	37.21	38.00	45.27
46.29	50.22	48.35	43.38	40.43	45.77	43.32	38.89	40.53	35.82	46.58	40.26	43.70	36.03	36.22
49.44	43.56	39.61	46.93	42.78	37.22	38.79	42.55	39.37	41.88	43.02	33.72	49.51	34.14	34.90
48.34	55.89	44.01	40.28	43.34	44.68	39.59	42.12	43.42	38.67	44.78	39.28	36.74	41.93	38.57
46.95	41.95	56.64	41.33	39.92	47.32	43.44	36.39	39.20	35.04	38.90	38.88	42.84	41.22	41.69
					39.68				35.69			43.25	44.37	
					44.46				49.44			46.64	52.12	
					44.82				48.44			42.32	54.11	
					43.85				52.14				46.56	

TABLE B.3: Hunter 'a' values for Atlantic salmon (*Salmo salar*) fillets used in the sensory trials for color measurements. The scores are presented for the fish sampled from each tank on day 84.

tank 100	tank 101	tank 102	tank 103	tank 104	tank 105	tank 106	tank 107	tank 108	tank 109	tank 110	tank 111	tank 115	tank 116	tank 117
7.49	8.16	3.93	16.41	4.77	9.51	13.10	11.65	9.69	10.87	13.82	12.60	13.39	5.41	11.57
7.28	8.35	9.97	10.82	5.51	11.45	14.87	10.29	10.14	5.97	10.69	8.82	9.82	5.38	9.14
11.97	5.53	10.64	12.88	8.05	8.65	8.80	6.20	6.34	11.84	11.95	5.31	11.80	12.85	14.60
9.40	10.00	9.26	11.01	10.49	6.47	10.47	5.21	7.60	6.71	13.82	6.43	8.94	13.70	11.00
13.18	9.43	5.50	6.65	6.38	9.41	7.50	10.63	8.19	12.64	10.05	6.68	10.89	7.04	9.99
8.37	11.47	5.86	8.50	7.08	14.55	5.95	6.02	9.67	5.49	9.33	5.52	7.69	9.79	9.97
11.11	6.90	8.42	6.79	9.73	5.29	7.12	8.51	9.91	8.86	12.54	7.40	11.18	10.49	9.43
3.71	4.42	7.02	11.37	5.66	7.36	8.35	3.01	12.26	5.99	9.86	13.29	10.00	4.55	8.11
12.02	7.90	5.93	13.95	7.13	5.71	6.45	5.19	6.35	7.75	10.21	7.40	10.13	6.38	6.98
8.59	10.79	5.96	9.28	5.76	8.18	9.65	11.81	12.97	8.86	9.31	5.60	10.92	7.37	8.15
11.80	9.56	5.60	9.97	7.92	11.29	10.03	10.40	8.17	5.82	10.05	5.44	7.21	9.87	6.19
7.64	14.43	11.51	10.58	7.66	4.35	5.20	12.70	5.48	8.59	7.52	8.31	6.97	8.68	6.33
					7.02				5.78			7.70	7.86	
					13.99				6.20			7.87	6.31	
					6.58				4.38			8.52	5.79	
					5.39				4.04				5.24	

TABLE B.4: Hunter 'b' values for Atlantic salmon (*Salmo salar*) fillets used in the sensory trials for color measurements. The scores are presented for the fish sampled from each tank on day 84.

tank 100	tank 101	tank 102	tank 103	tank 104	tank 105	tank 106	tank 107	tank 108	tank 109	tank 110	tank 111	tank 115	tank 116	tank 117
11.08	10.46	9.42	10.34	6.84	9.51	11.22	11.32	6.86	8.45	8.47	8.84	10.12	6.98	8.85
10.10	10.14	10.60	9.72	7.25	10.30	9.33	10.07	8.20	9.33	8.31	8.07	8.95	9.50	10.69
11.60	10.39	9.09	8.96	8.84	10.98	9.44	9.60	8.97	6.36	7.11	8.36	10.40	9.10	12.42
10.86	9.57	8.35	9.45	9.33	9.36	10.35	7.93	8.61	8.23	7.58	10.33	7.82	8.93	8.08
10.66	8.51	10.54	9.92	8.53	9.41	8.89	10.19	9.58	9.05	8.78	8.82	6.90	9.58	7.41
11.16	10.08	9.56	11.52	8.32	11.12	7.95	9.95	7.92	9.12	10.17	8.67	8.13	8.81	9.73
10.77	11.78	11.47	9.58	8.60	10.97	8.60	12.12	8.97	9.69	10.51	7.41	13.06	8.21	9.11
9.72	9.46	6.96	8.30	8.95	13.59	8.28	8.90	10.23	6.72	6.85	8.57	7.81	6.15	9.08
9.40	7.68	8.66	10.54	9.53	10.90	11.71	8.16	9.43	8.57	8.85	9.25	12.10	8.39	9.76
10.10	7.60	8.08	9.17	8.73	9.01	10.90	10.10	11.36	10.07	8.81	8.13	9.94	7.64	7.86
9.28	9.24	7.94	10.29	9.85	10.80	8.70	9.80	8.63	7.13	10.05	8.06	8.06	9.03	9.52
9.28	9.59	7.90	8.60	9.04	8.20	8.10	10.56	7.23	8.99	6.74	9.59	7.98	8.60	8.51
					10.90				6.30			8.78	12.94	
					11.43				6.56			10.81	12.39	
					9.31				12.52			11.37	10.10	
					5.69				12.26				10.99	

TABLE B.5: A brief outline of the computation steps followed during the 'principal component similarity' analysis.

- Apply PCA (based on correlation matrix) to the data for ***k*** variables and ***n*** samples to get ***k*** PC scores and ***k*** eigenvalues ***E.***

The PCS computer program calculates the following steps (1-2) for analysis of each sample:

1. $\mathbf{Y_i = V_i + [PC_i - PC_q]}$

   Where,

   Y = dependent variable

   V_i = independent variable = $100\ (1 - \sum_{(I-1)} P_i)$

   $P_i = E_i / \sum_{(I-k)} E_i$

   i = PC number

   q = reference

   A general rule of thumb is to discard E_i lower than 1.0 while selecting number of PC scores.

2. Perform linear regression of ***Y*** on ***V*** and compute correlation coefficient ***r*** and slope ***S*** for each sample.

- Plot ***S*** vs. r^2 for ***n*** samples to prepare a PCS scattergram .

(Vodovotz *et* al., 1993)

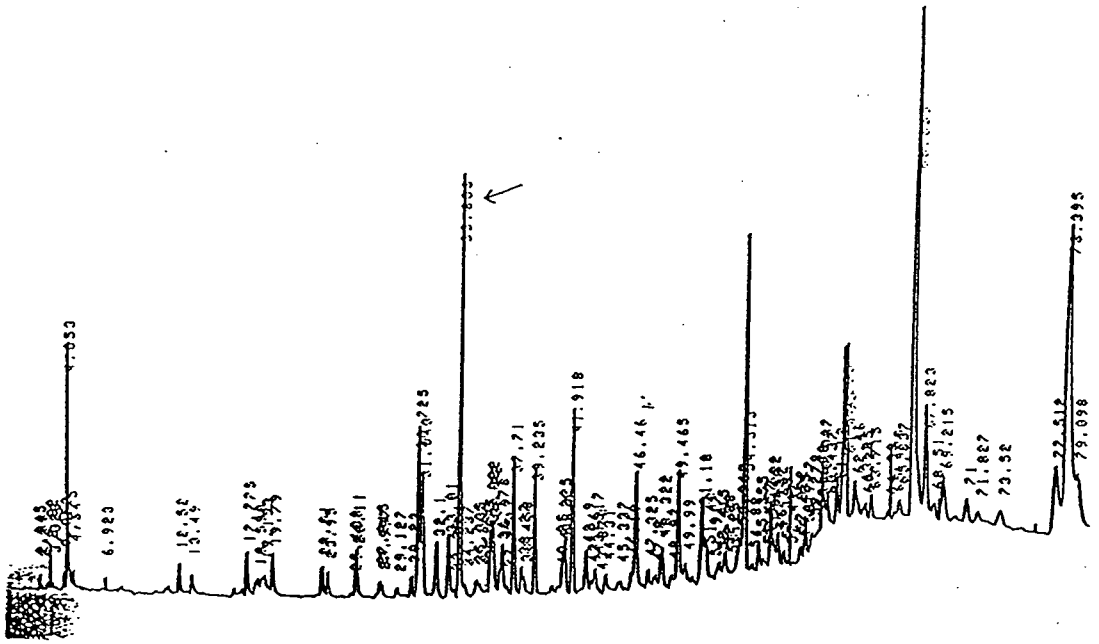

Figure B.1: A typical GC-chromatogram from the fillets of Atlantic salmon (*Salmo salar*) fed diet A. The internal standard peak is indicated with an arrow. Peaks beyond 60 min retention time were not included to minimize instrumental bias.

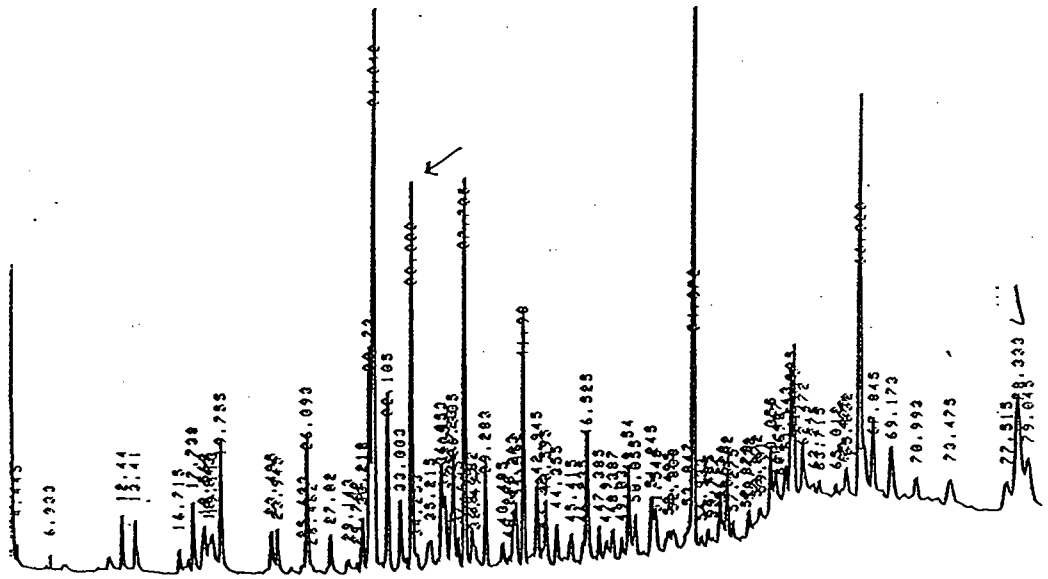

Figure B.2: A typical GC-chromatogram from the fillets of Atlantic salmon (*Salmo salar*) fed diet E. The internal standard peak is indicated with an arrow. Peaks beyond 60 min retention time were not included to minimize instrumental bias.

TABLE B.6: Principal components calculated for the GC analysis of the fillet samples of Atlantic salmon fed five diets for 84 days.

Principal component	*diet A*	*diet B*	*diet C*	*diet D*	*diet E*
PC 1	0.22	-0.26	-0.24	-1.23	1.52
PC 2	-1.77	0.50	0.48	0.21	0.59
PC 3	0.06	-0.55	1.70	-0.77	-0.45
PC 4	-0.11	-1.61	0.10	1.03	0.58
PC 5	0.00	0.00	0.00	0.00	0.00

TABLE B.7: Principal components with their respective eigen values, variance and % accumulation of the Atlantic salmon samples used for GC-PCS.

principal component	*eigen value*	*variance (%)*	*accumulation (%)*
PC 1	12.9813	61.8	61.8
PC 2	3.5971	17.1	78.9
PC 3	2.9001	13.8	92.8
PC 4	1.5216	7.24	100
PC 5	0.00	0.00	100

CHAPTER 5

CONCLUSION

&

RECOMMENDATIONS

CONCLUSION

The protein in diets containing up to 27.1% partially dehulled and extruded sunflower meal (SFM) was found to be well digested by Atlantic salmon, reared in seawater. The apparent digestibility coefficients for protein (feces collected by intestinal dissection) in the four test diets that contained from 6.8-27.1% SFM ranged from 85.4 to 86.6% and these values were not significantly different from that of the basal diet that contained no SFM (87.3 %). The apparent digestibility of energy in the test diet containing the highest amount of SFM by partial replacement of fishmeal in the basal diet was lower than that noted for the basal diet (73.6 versus 80.2%), but the difference was not significant. An 84-day growth trial, that was conducted to assess the performance (growth, feed intake and efficiency, and survival) of Atlantic salmon fed diets containing the aforementioned range in concentrations of SFM, demonstrated that SFM could comprise up to 27.1% of the dry diet or 23% of the dietary protein without compromising fish performance.

A sensory trial conducted to test the effect of diet treatment on four flesh sensory attributes viz., aroma, flavor, foreign flavor, and texture revealed that the salmon flesh samples taken at the end of the 84-day feeding trial were not significantly ($p \geq 0.05$) affected by diet treatment with respect to their aroma, flavor, and texture. Foreign flavors, however, were significantly ($p < 0.05$) pronounced in fillets from salmon fed the diet with 27.1% SFM relative to those from fish previously fed the diets without SFM or 6.8% SFM. Flesh color was also not significantly affected by the varying levels of SFM in the diet as assessed through visual and instrumental methods. Thus the highest dietary level of SFM used in the present study produced discernible foreign flavors in the fish.

Principal component similarity (PCS) analysis of the data (profile patterns) obtained from headspace gas chromatography (GC) of the volatile compounds that emanated from the cooked fillets from each diet treatment revealed that the GC/PCS procedure was able to detect differences amongst the headspace profiles due to diet

treatment. In this regard, the GC/PCS findings were similar to those found for the foreign flavor results that were obtained from the sensory panel trials.

RECOMMENDATIONS

The global aquaculture production of aquatic species is increasing progressively in an attempt to keep pace with the per capita needs of humans for aquatic protein due to world population growth. Consequently, there are increasing demands being placed upon the finite global supplies of fishmeal and oil. At some point in the near future, the current supply of fishmeal and fish oil will be inadequate to meet the demands for aquafeeds and other requirements (FAO, 2001). Increased demands for fishmeal in relation to supply are predicted to increase the cost of fishmeal and fish oil unless suitable alternative sources of fishmeal and oil are identified and/or developed. The use of nutritionally upgraded oilseed protein and lipid products offer considerable potential in this regard although there is still a need for a considerable amount of research in this area.

In this study, emphasis was placed on nutritionally upgrading the nutritive value of inexpensive commercially-produced sunflower meal (SFM) for salmon by reducing its concentration of fibre and concurrently raising its protein content through tail-end dehulling and through pre-extruding the meal to increase its digestible energy content. It was subsequently demonstrated that protein from this source of SFM could successfully comprise almost a quarter of the protein in diets for Atlantic salmon in seawater. Additional areas of study that likely will increase the acceptable dietary level of sunflower protein for salmon include the following:

- Determine the highest acceptable dietary concentration of partially dehulled and extruded SFM for Atlantic salmon since this was not determined in the present study. This long-term research could focus on the effect that SFM has on salmon physiology especially with respect to how salmon successfully adapt to a high fiber diet and how the final product (salmon fillet) quality is affected.

- Determine the bioavailable levels of amino acids in partially dehulled and extruded SFM (and in other sunflower protein products) as well as the amino acid requirements of Atlantic salmon to determine the most effective complementary protein sources and/or supplemental amino acids for use in diets based on sunflower protein products for salmon.

- Determine whether the removal of water soluble and/or ethanol soluble anti-nutritional factors that are present in partially dehulled and extruded SFM will further increase its digestibility and nutritive value for salmon and which approaches are most cost effective. The effect of the removal of ANF on salmon flesh quality should also be determined.

- Determine the biological and economical potential for using sunflower protein concentrates instead of meals in diets for Atlantic salmon. This work should consider different sources of non-genetically modified and genetically modified (GMO) sunflower seeds that have common as well as unique nutritional attributes e.g., seeds with reduced levels of phytic acid or enhanced concentrations of oleic acid and attendant low levels of linoleic acid in the oil. The use of GMO seeds should be explored albeit keeping in mind all possible risks and consequences to the salmon consuming diets containing protein and lipid products from GMO sunflower seeds and also to humans consuming the salmon that were previously ingesting these products in their diet.

In the aforementioned studies, it is recommended that PCS analyses of the profile patterns resulting from headspace GC of the volatile compounds that emanate from the cooked fillets of salmon given each of the diet treatments be performed. Thereafter, the extent to which these findings correlate to sensory measurements (i.e., aroma, flavor etc.) of the fillets shortly after the terminal time of fish sampling and then after several incremental periods of frozen fillet storage at different temperatures should be ascertained.

These data together with measurements of flesh fatty acid compositions and of compounds like hexanal, which cause bitterness and aftertaste, will establish whether the inclusion of any of the sunflower protein products in diets for Atlantic salmon at different concentrations influence the flesh quality and acceptability of the fillets for the consumer.