

THE INFLUENCE OF RATION LEVEL AND SWIMMING SPEED ON SENSORY  
ATTRIBUTES, GAS CHROMATOGRAPHIC PROPERTIES, INSTRON TEXTURE PROFILE  
ANALYSIS AND PH OF COOKED MUSCLE FROM FARMED CHINOOK SALMON  
(*Oncorhynchus tshawytscha*) CULTURED IN SEAWATER

by

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## Abstract

Representative samples of post-juvenile chinook salmon were obtained from the Department of Fisheries and Oceans Canada - West Vancouver Laboratory. The fish were part of a study directed to assessing the influence of two ration levels (75% and 100% of maximum ration) and three swimming speeds (0.5, 1.0, and 1.5 body lengths/second) on growth, body composition and thyroid function of chinook salmon in seawater. All analyses in the present study were conducted using cooked muscle. Sensory analysis, conducted in 19 sessions (10 days), was performed by 6 trained panellists. The treated and reference samples, composed of randomly mixed slices of muscle from farmed chinook salmon obtained at a local fishmonger, were graded for 28 sensory attributes; 9 aroma, 10 flavour, 8 texture as well as "overall acceptability". After completing preliminary analyses of the sensory data, data from panellist 6 and the first panel session were eliminated due to excessive inconsistencies in the results. ANOVA revealed that 8 attributes were significantly affected by ration level. After standardising the significantly affected attributes' data, using a z-transformation to remove the panellist effect, one aroma term was no longer significant. Principal Component (PC) Similarity graphs using the standardised data clearly illustrated the effect of ration level on these sensory attributes. The effect of using a replacement panellist for panellist 5 on two occasions became apparent from a PC 1 vs. PC 2 graph of that panellist's data. Purge and trap extracts were used for gas liquid chromatographic analysis of volatile compounds from cooked salmon. An ANOVA of consistently appearing peaks revealed that 27 of these were significantly affected by either SS or RL. Principal Component Similarity graphs of data from these peaks showed a clear separation on the basis of RL but not SS. The Instron Texture Profile Analysis statistics differed sharply from the other results since they indicated that SS and not RL significantly affected the texture of the cooked salmon. The pH values

for cooked fish were significantly affected by RL. The results of this study, with the exception of those from Instron TPA, agreed with those of Kiessling et al. (1994a,b) who generally found that RL and not SS significantly affected the growth and whole-body and muscle proximate composition of chinook salmon in seawater.

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## **1. Introduction**

Changes in fish that result from constant high levels of swimming have long been observed. Recently, several researchers working on this topic, talking informally, agreed that fish kept in flowing water look subtly healthier, for instance their skin appeared shinier, flesh seemed firmer, also their eyes seemed to be brighter (Love, 1988). This is not a recent revelation. In 1650, Venner observed this phenomenon and wrote "...of sea-fish the best swimmeth in a pure sea, and is tossed and hoist with the wind and surges; for by reason of continual agitation it becometh of purer and less slimey sic substances" (Love, 1988). Certainly, a clear-cut demonstration of a favourable effect of exercise (swimming speed) on one or more quality attributes of the flesh of farmed salmon would be of interest to the salmon farming industry. This is because the market values of farmed salmon can approach their cost of production and consequently any approach that raises market value or reduces production costs is of importance for economic viability of the industry.

This thesis examines the effect on the eating quality of the cooked muscle of cultured chinook salmon of rearing these fish with different combinations of swimming speed and ration levels. Sensory analysis was used to quantitate the changes in aroma, flavour and texture attributes. Various forms of instrumental analysis were employed to obtain objective measurements of the treatment effects on the fish. Textural changes were quantified using the Instron Texture Profile Analysis and pH. The treatment effect on the flavour volatiles was quantified by gas liquid chromatographic analysis of purge and trap extracts.

## **2. Literature Review**

### **2.1 Exercise Level**

#### **2.1.1 Types of exercise experiments**

Three types of exercise tests have been performed on fish. These include sprint, sustained swimming and training. Sprint swimming, otherwise known as "Burst" swimming tests, are conducted by forcing the fish to swim against very high water velocities for a short time; usually measured in seconds. Sustained swimming tests, which have been the subject of the greatest amount of research, involve forcing the fish to swim for several hours (Davison and Goldspink, 1978).

The fish obtained from Fisheries and Oceans Canada, West Vancouver laboratory for my study had undergone sustained exercise training. In experiments of this type, the fish first go through a period of training where they swim against increasing currents until reaching the desired water velocity. Then fish maintain this swimming velocity for the duration of the experiment. The experimenter notes any adaptive change(s) that occur (Davison and Goldspink, 1978).

Most of the research involving this type of exercise training in fish has involved salmonids i.e. salmon, trout and charr. There are several reasons for this. First salmonids are reared commercially, they are easy to obtain and generally respond well to captivity. Second, their behaviour is predictable unlike many other species (Davison, 1989). Third, salmonids respond readily to training under artificial conditions (Davison and Goldspink, 1977; Greer Walker and Emerson, 1978). Finally, since salmonids are a commercially important group of fish, research funding is more readily available than for other types of fish (Davison, 1989).

### **2.1.2 Effect of exercise training on fish**

Fish do not respond to exercise training in the same way as mammals. Changes in fish do occur as a result of training, but they are comparatively modest. Training has been shown to affect fish growth rates as well as their ability to swim (reviewed by Davison, 1989).

Unfortunately, as noted by both Davison (1989) and Broughton and Goldspink (1978), due to their limited number, comparisons between studies are difficult. There are several factors that contribute to this problem. Some of the difficulty is due to differences in the studies with regards to the species, size, sex and life history of the fish (Broughton and Goldspink, 1978). There is also a diverse array of apparatus that has been employed. Other areas of disparity include the dissimilar durations and intensities of exercise between studies and in many studies there have been differences in the types of tests that have been used to detect changes. As a result, there is a great deal of variability in the results, and conflicting data are often presented (Davison, 1989).

### **2.1.3 Effect of water current on fish behaviour**

In many previous studies (Davison and Goldspink, 1977; Davison and Goldspink, 1978; Greer Walker and Emerson, 1978; East and Magnan, 1987; Houlihan and Laurent, 1987; Christiansen et al., 1989) the control fish have been held in calm water in an attempt to reduce the amount of energy required for locomotion. This protocol, however, has led to behavioural problems, such as increased aggressive responses as manifested by biting and fin-nipping. Christiansen and Jobling (1990) found that fish without bite marks grew significantly better than those with evidence of bite marks. Elevated plasma cortisol levels which are known to suppress growth (Pickering, 1990) and feed efficiency (Vijayan and Leatherland 1989), have also been found. In contrast, salmonids reared in water with a



current, school, swim less randomly, and they also exhibit less aggressive behaviour (Christiansen et al. 1989; Christiansen and Jobling 1990) and have lower levels of plasma cortisol, adrenaline, and noradrenaline (Woodward and Smith, 1985). This leads to questions whether the improved growth, noted in the studies with the still water controls, was a result of the exercise per se or was due to the increased energy demands and stress that accompany aggression in the control fish (Kiessling et al., 1994b).

Davison and Goldspink (1977, 1978) conducted two experiments that address this issue. These researchers studied the effect of different levels of exercise training on the growth of brown trout, a member of the salmonid family, and goldfish, a fish normally found in still water. In both of these experiments the control fish were held in still water. The results of these two experiments were dramatically different.

In the trout experiment, fish at the lowest swimming speed grew much more rapidly than the control fish. Moreover, they had large stores of glycogen and lipids and other physiological changes. The fish at the medium swimming speed had decreased food utilisation due to increased energy demands. Many fish at the highest swimming speed were unable to survive.

By contrast, in the goldfish study the fish subjected to exercise often grew less than the controls and they also consumed substantially more food. Surprisingly, most of the goldfish survived at the highest swimming speed.

These preceding results led the researchers to speculate that once the fish acclimates to a higher exercise rate, elevated levels of anabolic hormones such as thyroxine are produced. They theorised that the goldfish were unable to acclimate to the flowing water which resulted in the fish having elevated levels of stress-related hormones such as adrenaline, noradrenaline, and cortisol.

However, they never considered the possibility that the trout control may have also been under stress. Hence, they drew some incorrect conclusions from their work.

#### **2.1.4 Fuel use for fish locomotion**

The fish's use of fuels for locomotion can be quite complicated; protein, lipids as well as glycogen may be used as sources of energy (reviewed by Davison, 1989).

In fish, red muscle has an aerobic form of metabolism and is used for normal locomotion while the white muscle is used for burst (sprint) swimming, or when the fish is involved in strenuous exercise.

White muscle uses glycolysis or the anaerobic degradation of glucose to yield lactic acid and energy. The overall rise in lactate is accompanied by a fall in glycogen concentration following exercise (reviewed by Broughton and Goldspink, 1978).

The findings of Johnston and Moon (1980a, b) suggest that training produces a shift towards fat utilisation rather than use of glucose as an energy source. Davison and Goldspink (1977) had similar findings. They observed that brown trout when exercised at a slower swimming speed (1.5 body lengths / second (bl/s)) had elevated levels of both glycogen and lipids. The trout at the intermediate speed (3 bl/s) still had elevated glycogen levels, but the lipid levels had fallen. This suggests that lipids were the major source of fuel for the fish at that swimming speed.

White and Li (1985) also found that lipids were the primary source of fuel during training. They found that chinook salmon at all speed by ration level combinations, except the slowest swimming speed by highest ration level, experienced a net decrease in body fat. The fish exercised at 2, 3 and 4 bl/s, at both the 2.5 and 6 % ration levels (% dry body weight/day), exhibited greater decreases in their percentages of fat than noted for the unfed fish (0% dry body weight/day). Alternatively,

Kiessling et al. (1994b) found that the fat contents in the fillet and whole bodies of chinook salmon were not significantly affected by changing the swimming speed over the range of 0.5-1.5 bl/s.

Several studies indicate that protein may also serve as a source of fuel for fish in training experiments. In the Davison and Goldspink (1977) study on trout, the protein content of the fish decreased as swimming speed increased. This suggested that protein might have been utilised as a fuel source. East and Magnan (1987) obtained similar findings with brook charr. In another study, White and Li (1985) observed that chinook salmon forced to swim for 10 days without food, had decreased protein levels while their lipid levels were unaffected.

## **2.1.5 Effects of exercise training on fish**

### **2.1.5.1 Increased stamina and maximum swimming speed**

Fish, like athletes, must be subjected to a period of conditioning before the full expression of their capacity for swimming is realised (Farlinger and Beamish, 1978). It has been shown that training generally increases stamina and aerobic capacity (Hochachka, 1961; Farlinger and Beamish, 1978; Broughton et al. 1980) as well as maximum swimming speed (Davison and Goldspink, 1977).

Hammond and Hickman (1966) showed that conditioned fish can not only tolerate higher levels of blood lactate, but they can also remove lactate from the blood more quickly than unconditioned fish. Both total accumulations of muscle lactate during exercise and its subsequent rate of removal during recovery were found to vary directly with the degree of physical conditioning.

Hochachka (1961) demonstrated that trained fish could acquire an oxygen debt that was three times higher than that of untrained fish before becoming fatigued. He postulated that the increased ability of trained fish to resist fatigue was due to increased buffering capacity. This hypotheses was

based on the observation that the trained fish had higher haemoglobin levels. With respect to this, haemoglobin has two functions, first as a carrier for oxygen, and second as a buffer. Hochachka theorised that in trout, the primary function of the haemoglobin may be to act as a buffer, and the respiratory function might be secondary in nature during resting metabolism. However, during more extreme conditions, e.g., higher exercise levels, the respiratory function may become more important. Under these conditions the increased oxygen carrying capacity would be invaluable. Love (1988) also postulated that other buffers may be present in larger quantities in trained fish. In addition, Love (1988) speculated that if buffers, such as anserine, were present in greater than normal quantities, there could also be an effect on the flavour of the fish.

#### **2.1.5.2 Hypertrophy of fish muscle fibre**

When a fish swims for long periods of time, there are often significant changes in the number and diameter of red and white muscle fibres, which together result in increased muscle size (reviewed by Davison, 1989). Unlike mammals where the fibre number in the body remains constant, the fibre number in many species of fish increases during their lifetime, paralleling the increases in body size (Weatherly and Gill, 1981, 1984). Davison and Goldspink (1977) noted that the initial size of the muscle fibre in the fish will determine whether the fibre splits or enlarges. For instance, when they exercised fish with small fibres, there was an increase in fibre size. Conversely, when fish with large muscle fibres were exercised, there were concomitant increases in muscle mass that were due to an increase in fibre number. Similarly, Patterson and Goldspink (1976), working with saithe, observed that the muscle fibres split longitudinally once they reached a diameter of 1.2 micrometers.

Greer Walker and Pull (1973) observed that the extent of fibre hypertrophy in coalfish varied with swimming speed. Also, they noted that different muscle types became active as the swimming speed was changed. In this regard, Johnston et al. (1977), working with carp, noted that red muscle fibres were used predominately at lower swimming speeds. However, as the speed was increased, the red fibres became progressively less important, whereas the pink, and then the white muscle fibres became increasingly more active. Collectively, the studies on fish suggest that white muscle is used primarily when the speed is near or above the threshold for sustained speed. At lower speeds both the red and white muscles are used (Greer Walker and Pull, 1973). Greer Walker and Emerson (1978), in an experiment involving rainbow trout found that oxidative metabolism occurred predominantly in the red muscle at swimming speeds up to 1.4 bl/s. Beyond this, the white fibres became increasingly active. In another experiment, Johnston and Moon (1980a) subjected brook trout to a water current of 1 bl/s and noted that electrical activity occurred only in the red muscle. At water speeds above 1.8 bl/s, however, electrical activity was exclusively observed in the white muscle.

Changes in muscle fibre number and hypertrophy provide good indications of the different muscle types that are active at a given swimming speed (Greer Walker and Pull, 1973). Generally, red muscle fibres hypertroph to a greater extent than white fibres at any given swimming speed (Greer Walker and Pull, 1973; Davison and Goldspink, 1977). Kiessling et al. (1994b) found that the red muscle of chinook salmon trained at 1.5 bl/s showed significant fibre hypertrophy (25%) in the rostral region of the fish. Also, they observed in another experiment that fish that swam at their critical swimming velocity every other day for 120 days had doubled the total red muscle area in their caudal region. Kiessling et al. (1994b) speculated that the dissimilarity in red muscle area between rostral and caudal regions was probably due to the differences in swimming patterns between the two experiments.

In situations where swimming speed is excessive, muscle fibre hypertrophy may only be evident to a small extent or not at all. For example Greer Walker and Emerson (1978) observed that red muscle fibre hypertrophy decreased when trout were forced to swim between 2 and 3 bl/s. They speculated that the reason for this response was due to the fibres contracting above their optimal frequency.

Other experiments have not shown any significant increase in muscle mass in relation to swimming speed. Davie et al. (1986), for example, did not find any alteration in the ratio of total muscle to total body weight of trout that had been forced to swim continuously at a low swimming speed for 200 days. They did, however, observe an increase in the proportion of red fibre muscle.

## **2.2 Physiological factors in fish affected by ration level**

### **2.2.1 The effect of ration level on fish size**

Level of dietary intake can have a profound effect on numerous aspects of fish physiology. Not unexpectedly, an increase or decrease in ration level can significantly affect fish size (weight and length). Kiessling et al. (1991a) found significant differences in the weights of 1 - 2 year old rainbow trout maintained on ration levels of 50, 75 and 100% (100% ration level defined as the ration level required for optimum growth). Numerous other studies on various fish species have also shown that growth is positively correlated with increased ration level until feed intake becomes excessive (Kiessling et al., 1989a; Storebakken et al., 1991; Li and Lovell 1992). Kiessling et al. (1994b) also found that chinook salmon weight increased significantly as ration level was increased from 75% to 100% of maximum.

### **2.2.2 The effect of ration level on the fat content of fish**

Fish maintained on higher ration levels often have increased body fat content. Kiessling et al. (1994b) found that the fat content of chinook salmon on maximum ration was significantly higher than noted for those given 75% of maximum ration. Storebakken et al. (1991) and Kiessling et al. (1991a) also showed a trend towards increased fat accretion in trout as ration level was increased. In contrast, Kiessling et al. (1989a) did not find any significant effect of fixed rations (25%, 50% and 100%) on total fat levels in either the white or the red muscle tissue of trout. They did find, however, that the fat content in the dorsal muscle fat depot rose in direct relation to ration level.

### **2.2.3 The effect of ration level on the protein content of fish**

Changes in ration level generally alter the absolute but not the relative (%) amounts of protein in fish (Kiessling et al. 1991b; Storebakken et al., 1991). For instance, Kiessling et al. (1991b), noted that the increase in protein content of rainbow trout epaxial muscle was independent of ration level once the fish had reached 50g. They also found that a profound drop in muscle protein content only took place in mature fish, and that this was only in response to decreased feed availability in combination with prolonged physical activity.

### **2.2.4 The effect of ration level on fish muscle fibre size**

Fibre size has been found to be highly correlated with fish size (Kiessling et al. 1989b; 1991a) which, in turn, is significantly affected by ration level. In the study performed by Kiessling et al.

(1989b), significant changes were seen in both white and red muscle fibre areas. The white muscle fibre area was positively correlated with slaughter weight. Kiessling et al. (1989b) concluded from this observation that the pattern of growth was based mainly on enlargement of single fibres rather than on increases in fibre number. There was also a shift toward dominance of fibres with larger areas as fish weight increased. They went on to speculate that fibres also grew in length as ration level was increased.

#### **2.2.5 The effect of ration level on the muscle glycogen content of fish**

Glycogen content appears to be affected by ration level, but this relationship is less clear. Kiessling et al. (1989b), for example found that the glycogen levels in both red and white muscle of rainbow trout increased as ration level was varied between 50 and 100%. In the same experiment, however, the glycogen content of the white muscle from trout on the 25% ration level was observed to be significantly higher than noted for trout on the 100% ration. In an experiment performed by Storebakken et al. (1991), blood glucose levels were found to increase between the 0 - 1% ration levels but then dropped at the 2 % ration level. Finally, the data of Kiessling's et al. (1991b) did not reveal any effect of ration level on the glycogen content of trout.

#### **2.2.6 The effect of ration level on fish growth hormone levels**

Growth hormone levels tend to decrease as ration levels increase. Storebakken et al. (1991) found a 3.5-4.0 fold reduction in the circulating concentrations of growth hormone in trout maintained on a ration of 2% of body weight relative to those deprived of feed. Elevations in growth hormone levels in fish maintained on reduced levels of dietary intake have been linked to depressed fat content.



Indeed, it is generally accepted that there is an inverse relationship between growth hormone level and fat deposition in fish (Storebakken et al. 1991).

### **2.3 The combined effect of ration level and swimming speed on fish**

Several studies have evaluated the combined effect of ration level and swimming speed on fish growth and other aspects of performance. One of the most recent of these was conducted by Kiessling et al. (1994b), and fish from this study were used for this thesis. White and Li (1985) and Leon (1986), also conducted similar projects on this theme using juvenile chinook salmon and brook trout, respectively.

White and Li (1985) found that nearly all of the variation associated with growth could be accounted for by differences in level of dietary intake. In addition to the energy required for standard metabolism, which is constant at all swimming speeds, the amount of energy required for activity (swimming) increased steadily as swimming speed was raised. As a result, the fish had to ingest more feed (energy) at the higher swimming speeds to maintain their body weight. Kiessling et al. (1994b) arrived at the same conclusion.

### **2.4 Sensory testing**

#### **2.4.1 Quantitative Descriptive Analysis (QDA)**

Traditionally, each food manufacturing company employed one or a group of experts to perform sensory analysis. These expert(s) judgements were relied upon in all facets of production, from the choice of ingredients, to the choice of which products were ready for release into the

marketplace. In recent years, our society has become increasingly multicultural. Consequently, the markets have become much more complex and competitive. The judgements of the expert are still useful, but there are limits to one person's ability (Stone et al. 1974).

Around 1949, the Arthur D. Little Co. proposed the Flavour Profile Method (FPM) as a means of dealing with the complex world of food flavours (Anon. 1963). In this method, a small group of judges evaluates the product together in a conference style meeting. Before testing, the judges undergo some descriptive training. To accomplish this, a broad selection of references is presented to the judges to prepare them for subsequent evaluation of the intensity of flavour and aroma attributes in one or more test samples. FPM allows, in many cases, the successful replacement of the individual expert by the expertise of the group (Stone et al., 1974).

Quantitative Descriptive Analysis (QDA), developed by Tragon Corp., has brought additional improvements to sensory evaluation (Stone et al., 1974). In QDA, trained individuals identify and quantify the sensory properties of a product or an ingredient (Stone et al., 1974). This method uses an interval scale with anchor points located one half inch from each end. The panellist places a vertical mark at the point that she/he feels best represents the magnitude of the intensity of the attribute (Stone et al., 1974). Use of QDA makes it possible to statistically analyse data. For example, a researcher can perform a one or two way analysis of variance to analyse individual and group performance. He/She could also use principal component analysis (PCA) to determine which are the primary sensory variables and then redundant terms could be identified and removed (Rutledge and Hudson, 1990; Stone et al., 1974).

#### **2.4.2 Unstructured line scale**

Using an interval scale can lead to problems due to the panellist's difficulty in attributing the same psychological width to the various intervals on the scale. This problem can be alleviated to a large extent by using unstructured graphic scales that consist of a 10 cm horizontal line anchored at the ends. The panellist response is indicated by a vertical mark on the line (Giovanni and Pangborn, 1983). Unfortunately, as Gacula (1987) has pointed out, panellists have a tendency to underestimate the score at the lower end and overestimate the score at the higher end of the unstructured scale.

#### **2.4.3 The difficulty in the analysis of sensory data**

There are many differences between sensory and instrumental analyses (Table 1) that can create problems in data analysis. Gacula (1987) found that sensory data were among the most difficult scientific data to statistically analyse and interpret since there are often untested assumptions about the data and the analysis procedure. Some of the difficulties are as follows. First, panellists tend to use the scale differently. The data are relative, easily skewed, and very difficult to replicate (Gacula, 1987). Third, panellists are prone to fatigue, time-order effects, and subject to drifts (Pangborn, 1987). Even when panellists have been screened and well trained, there is still the chance that a panellist by treatment interaction will stem from differences in motivation, sensitivity or psychophysical response behaviour (Lundahl and McDaniel, 1990).

#### **2.5 The measurement of food texture**

Szczesniak (1963) defined texture as "the sensory manifestation of the structure of the food and the manner in which this structure reacts to the applied forces and specific senses involved being

**Table 1** Comparative behaviour of instruments and human subjects (Pangborn, 1987)

Instrumental	Sensory
Separator	Integrator
Univariate	Multivariate
Absolute	Relative
Fast	Slow
Calibratable	Difficult to calibrate
Precise	Subject to drift
Doesn't Fatigue	Fatigues, Adapts
No time-order effects	Time-order effects
Equal-interval Units	Unequal-interval units
Expensive to purchase and maintain	Expensive to hire judges
Cannot measure hedonics	Biased by hedonics
Cannot mimic sensory	Artificial to mimic instrumental

vision, kinaesthetics and hearing." In 1990 she simplified this definition to "how the food feels in the mouth on manipulation and mastication, and how it handles during transport, preparation, and on the plate" (Szczesniak, 1990).

Texture for many years has been considered by some to be an overlooked food attribute. There are several reasons for this. First, there has been a lack of government funding for research into food texture. A second problem has been that off-texture is not a signal that a food is unsafe, unlike attributes such as smell, colour and taste. Finally, changes in texture are often more difficult to accomplish and often affect other quality parameters such as taste; these changes can not just be "added from a bottle" whereas those related to aroma and flavour can (Szczesniak, 1990).

Attempts have been made to measure texture quantitatively since the 1860's. According to Szczesniak (1990), the first texture measuring device was developed in Germany by Lipowitz in 1861 which was an instrument, designed to quantify the consistency of jelly. Since then, other instruments, designed to measure textural qualities of various types of food, have been developed and these have evolved into the instruments used today. Some examples of texture measuring devices currently in use include, the Instron Universal Testing Machine, the General Foods Texturometer, and the Brabender Farinograph.

According to Szczesniak (1963), textural measurements can be grouped into three types of characteristics: (1) mechanical, (2) geometrical and (3) other characteristics. Mechanical characteristics result from pressure exerted on the teeth, tongue and roof of the mouth during eating. These characteristics include the hardness, cohesiveness, viscosity, elasticity and adhesiveness, etc. of the food. Geometrical characteristics are related to the size, shape, and arrangement of the particles

within a food (Brandt et al., 1963). The last group is 'other' characteristics which includes mainly moisture and fat; qualities concerned with lubricating properties of the food product.

Brandt et al. (1963) developed the Texture Profile Method (TPA), patterning it after the flavour profile method developed by Cairncross and Sjostrom (1950). They used the standard rating scales developed by Szczesniak (1963), and systematically examined various textural attributes, breaking them down into initial (textural attributes perceived on the first bite), masticatory (perceived during chewing), and residual characteristics (changes that occur during mastication). In TPA, additional scales can be added to enable the judgement of moisture and fat content (Brandt et al. 1963).

TPA requires that the panellists be trained thoroughly with respect to the texture classification system and the use of standard evaluation procedures for assessment of the product. Panellists must also become reliable in recognising and identifying the degrees of each characteristic (Brandt et al., 1963).

### **2.5.1 Instron Universal Testing Machine**

The Instron is an instrument designed to study stress-strain properties of materials (Bourne, 1982). In addition to food, it can also be used to study texture in other materials such as fabric, metals, wood, rubber, plastics, etc. (Bourne, 1982). With an assortment of accessories available (Bourne, 1982), this machine can perform various types of tests such as penetration, shear, bending, compression, and extension (Segars and Kapsalis, 1987).

The Instron generates both force-time and force-distance curves, allowing work function to be calculated in pounds, kilogram, or Newton's (Bourne, 1978). The curve(s) can become the basis for calculating various mechanical properties of the material. These values may be used to correlate or predict sensory response to texture (Segars and Kapsalis, 1987).

### **2.5.2 Instrumental Texture Profile Analysis (TPA)**

TPA was a major breakthrough in the quest to produce a machine that could imitate mastication. The General Foods Texturometer attempts to imitate mastication by twice compressing a bite sized piece of food to 25% of its original height; mimicking a person taking two bites. From this, a force-time curve is produced which captures the entire force history of this simulated masticatory action.

Several textural parameters can be determined from the force-time curve. Bourne (1978) described seven parameters; five measured and two calculated. The measured parameters include fracturability, hardness, cohesiveness, adhesiveness, and springiness. The two calculated parameters are gumminess and chewiness (Bourne, 1978). Firmness may also be calculated from the curve by measuring the maximum slope on each compression cycle (Durance and Collins, 1991).

#### **2.5.2.1 TPA on cooked salmon**

It is difficult to obtain meaningful, reproducible instrumental texture measurements on cooked fish. Most of the devices that are commonly used in the rheological testing of foods, even those that are used for red meat, are generally unsuitable for fish. During eating, almost all of the energy required to prepare the fish for swallowing is used for mastication. As a result, instrumental testing of fish samples needs to measure resistance of the muscle fibres to mechanical disintegration (Dunajski, 1979).

Durance and Collins (1991), and Reid and Durance (1992) examined textural changes of canned late run salmon by using Bourne's (1978) TPA and an Instron Universal Testing Machine (Model 1122, Instron Corp. Canton MA). In their experiments, a modified syringe was used to form cylinders of flaked fish of uniform size. Using samples composed of thoroughly flaked fish, they managed to gain a greater degree of homogeneity between replicates. Borderias et al. (1983) tested

both minced fish and intact fillets using various Instron attachments and found a higher coefficient of variation for the fillets relative to the minced fish. They speculated that this occurred because when the force of compression was applied to the fish fillets, the myotome layers slid away from the force. Hence, it was more difficult to obtain reproducible results in separate determinations.

## **2.6 The factors affecting the texture of cooked fish**

### **2.6.1 The effect of pH on the texture of cooked fish**

Love (1988) and Dunajski (1979) both stated that the pH of fish muscle is probably the most important factor affecting the rheological properties of a given muscle. Love (1988) postulated that muscle from exercised fish would have a lower post-mortem pH due to an elevated glycogen content. He went on to theorise this would lead to firmer muscle texture. Feinstein and Buck (1984) found a linear relationship between pH and the texture of flounder but only in the head section of the fish. They also looked for a relationship between pH and texture in cusk without success.

As with most animals, after the death of a fish, glycogen is degraded to lactic acid via the Embden-Meyerhof-Parnas glycolytic cycle. This causes the pH in the muscle to fall dramatically within the first several hours post-mortem. In most species of fish, the final pH is usually around 6.5 - 6.2, but is also can be as low as 5.4. As the pH approaches the isoelectric point of the myofibrillar proteins, there is a change in the ionisation of the polar groups of the protein molecules. Originally these were negatively charged but after death they become neutral. This causes a decrease in the repelling forces between proteins, resulting in a tightening in the protein structure. As the myofibrillar proteins become more concentrated, the muscle becomes increasingly tougher and drier. Dunajski (unpublished), for



example, noted that there was a 2.5 fold increase in the toughness of the fish as the pH changed from 6.7-5.7(Dunajski, 1979).

### **2.6.2 The effect of muscle fibre size on the texture of cooked fish**

Fibre size has also been found to affect the texture of fish muscle. Kanoh et al. (1988), in an experiment using yellowfin tuna, found that the fish had a firmer texture when the fibre diameter was less than that of ordinary muscle. Hatae et al. (1990), drew the same conclusion after examining the role of muscle fibre contribution to firmness in the cooked flesh of five species of fish. Dunajski (1979) reported an increase in the coarseness of the muscles when the diameter and length of fibres increased.

### **2.6.3 The effect of the level of connective tissue on the texture of cooked fish**

Unlike red meat, connective tissue in fish muscle is present in low quantities and hence does not play an important role in the texture of fish. Collagen is thermally denatured during cooking and as a result generally has very little influence on fish texture. The texture of muscle after cooking is more a consequence of the state of the myofibrillar proteins (Dunajski, 1979).

Hatae et al. (1990) did, however, report an effect of muscle collagen content on texture. In this regard, they observed that when cooked fish tissue was masticated the coagulated proteins tended to impede the sliding of the muscle fibres over each other. From this they concluded that, in fish with a lower muscle collagen content, the muscle fibres slide more easily over one another, resulting in a softer texture.

#### **2.6.4 The effect of the fat content on the texture of cooked fish**

Fat content can also affect the texture of fish samples. Samples of fish muscle with a higher fat content are often perceived as being more tender (Dunajski, 1979). Dunajski (1979) explained that, among other post-mortem changes in fish, the liquid neutral lipids are immobilised by the physical structure of the muscle. This tends to dilute the structural elements and decrease the overall mechanical strength of the fish meat. A higher fat content will also impart an oily mouthfeel (Szczesniak, 1963).

### **2.7 Gas Chromatographic flavour volatile analysis**

#### **2.7.1 Purge and trap analysis**

The principle behind purge and trap extraction is quite simple. First, the sample is placed in a sealed container that is flushed with an inert gas. This gas then passes through a trap containing a small amount of adsorbent, such as tenax, which retains the volatiles. Following the extraction, the trap is removed and adsorbed compounds are eluted with a small amount of solvent, and a sample of this is then analysed by gas liquid chromatography (GC) (Gilbert, 1990).

Heikes and Hopper (1986) outlined several advantages of this method. First, it is non labour-intensive and may be carried out unattended. Furthermore, it does not require highly specialised equipment (though now available); a suitable apparatus can be constructed quite simply with materials already available in most analytical laboratories. The extract is concentrated and relatively clean. The limits of detection that can be achieved are much lower relative to solvent extraction or static headspace analysis; both GC quantitation at low parts per billion and sub-parts per billion levels as well

as GC/MS confirmation are possible. Generally, this technique provides an inexpensive alternative to other methods (Olafsdottir et al., 1985).

There are conflicting opinions as to who originally developed purge and trap extraction. According to Gilbert (1990), this method was developed by Heikes in 1985 for the analysis of ethylene dibromide in grains. However, it is noteworthy that Josephson and co-workers published an experiment in 1983 that described the use of purge and trap extraction to identify aroma compounds from fresh white fish.

In recent years, this extraction method has been employed in several studies that have examined volatiles produced by several types of seafood. For example, Josephson et al. (1983, 1985, 1991) used purge and trap extraction to study the volatiles produced by fresh seafood such as fresh Whitefish, Great Lakes salmon and Atlantic and Pacific oysters. Shamaila et al. (1995) also used this technique to evaluate the volatiles in Pacific ocean perch (*Sebastes alutus*).

#### **2.7.1.1 The effect of fat content on purge and trap extractions**

Some studies have shown that the fat content of a food product undergoing purge and trap extraction affects the amount and types of compounds found, while others have not. Heikes (1985), in a study on the determination of ethylene dibromide (EDB) in table-ready foods, where various foods ranging from boiled cabbage to chocolate cake icing were spiked with EDB, did not find that fat content had an effect on the efficiency of the extraction. Persson and von Sydow (1973), on the other hand, in their study of the aroma of canned beef did find that fat content affected the amount and types of compounds found. It was also noted that when fat was added to some samples, some volatile compounds were more lipid soluble than others and consequently they were detected to a lesser

degree. Also, in those samples, some compounds such as straight chain aldehydes, furan and 2-methyl furan were detected in higher concentrations; possibly because fat is a precursor for those compounds.

#### **2.7.1.2 The choice of Tenax GC, a porous polymer, for use in purge and trap extraction**

Tenax GC (2,6-diphenyl-p-phenylene oxide polymer) is one of a group of porous polymers that are often used in research because of their ability to trap organic compounds. When the sample of gas is passed through the porous polymer, the organic compounds are retained and concentrated. Once these compounds are collected, they can either be thermally desorbed or eluted with a solvent (Butler and Burke, 1976; Olafsdottir et al. 1985).

Butler and Burke (1976), looking at the capacities and efficiencies of several porous polymers, concluded that no single porous polymer was universally suitable. One needs to examine the pros and cons of each and then choose the polymer that is most suitable for the application. Tenax GC has emerged as a widely used porous polymer for food, beverage, and environmental applications (Olafsdottir et al. 1985). It is particularly advantageous for samples consisting of only high boiling point components (Butler and Burke, 1976; Jennings and Fisoof 1977). This is due to this polymer's high temperature limit and relatively low retention volumes, which allow the trapped compounds to be desorbed more rapidly than from other adsorbents (Butler and Burke, 1976). In addition, it also has the advantage of having shorter recovery times (Jennings and Fisoof, 1977).

#### **2.7.1.3 The elution of adsorbed volatiles from porous polymers with ethyl ether**

Olafsdottir et al. (1985) examined the reproducibility and absolute recoveries of volatiles from Tenax GC desorbed with ethyl ether. They found that this method resulted in a variability in analysis of

less than 20% which is comparable to other adsorbents and solvents. Thus, this procedure was found to be suitable for many objectives and applications.

### **2.7.2 The relationship between Gas Chromatography (GC) data and quantity and intensity judgements from trained sensory panellists**

Persson et al. (1973 a, b) and von Sydow et al. (1970) were among the first researchers to present a clear-cut relationship between quality and intensity judgements from a trained panel and the GC/MS output for the product. Comparisons of these two sources of data are, however, not without their pitfalls. Van Gemert et al. (1987) found that the relationship between sensory analysis and chemical, physical and instrumental parameters was complex. The characteristic aroma of a food is often not the result of one compound alone, but rather, results from an interaction between several compounds. Consequently, an increase or decrease in only one odour compound might result in both increases and decreases in several of the sensory odour qualities (von Sydow et al., 1970).

There is a second difficulty in comparing these two types of data. Sometimes compounds that are highly correlated with flavour will not be flavour substances, although, more commonly they will be (Powers and Keith, 1968). At times this can occur if more than one compound co-elutes, or if the compounds responsible for the aroma are being adsorbed by an active site near the exit port and wash off when a major peak elutes (Williams and Tucknott, 1977).

## **2.8 Statistical analysis**

### **2.8.1 The box plot**

The box plot, first introduced in 1977, has proven to be an effective means of producing a visual summary of data. In this type of plot, there is a box that is divided with a horizontal line, and there are also two protruding "whiskers" that extend vertically from the top and bottom of the box. The box portion is comprised of the two middle quartiles of data, that is, the data that falls between the 25<sup>th</sup> and 75<sup>th</sup> percentiles. This interquartile range is computed as  $IQR = Q_{0.75} - Q_{0.25}$ , which serves to measure the amount of variation in the data. A horizontal line splits the box at the median (50<sup>th</sup> percentile). The lower whisker is defined as the smallest observation that is greater than or equal to the lower quartile minus 1.5 X IQR. Similarly, the upper "whisker" is defined as the largest observation that is less than or equal to the upper quartile plus 1.5 X IQR, which may be the upper extreme of the data. Any values that fall outside this range are considered to be outliers and are plotted as individual points (Ma, 1992).

### **2.8.2 Principal component similarity (PCS)**

PCS is a technique that was developed by combining principal component analysis (PCA), a data compression method which is based on identifying the most important directions of variability in a multivariate data space, with pattern similarity. In PCS, principal component (PC) scores are used for computing pattern similarity constants instead of using the original data directly (Vodovotz et al., 1993). Furtula et al. (1994b) considered PCS to be an extended version of PCA. PCS can utilise the information on variation of the PCA principal components for classification purposes (Vodovotz et al., 1993).

When Vodovotz et al. (1993) compared PCS with PCA and other types of multivariate analyses they found that they compared favourably. Also, they found that PCS had better resolution than PCA. PCS can also graphically illustrate a larger number of computed PC outputs than PCA (Furtula et al. 1994b). In PCA it is customary to use only 2 PC scores for a 2-dimensional (2-D) PC plot or three PC scores for a 3-dimensional (3-D) plot. However, portions of the original data that may have been important for classification can be ignored. This is particularly true in flavour analysis where it is not uncommon for seemingly minor compounds to play an important role in creating characteristic flavour notes. With PCS, results from numerous PC scores can be displayed graphically in a 2D figure, minimising this problem (Vodovotz et al., 1993).

PCS is most useful when the size of the data matrix is large (Furtula et al. 1994a). The advantages offered by PCS diminish as the number of PC scores for computation decreases (Furtula et al. 1994b).

### **3. Materials and Methods**

#### **3.1 Experimental conditions used in the rearing of salmon used in this study**

The fish for this research were obtained from the Department of Fisheries and Oceans, West Vancouver Laboratory, West Vancouver, B.C. A total of 660 salmon were used in the Fisheries and Oceans experiment. The all-female seawater-adapted hatchery-raised one year old Qualicum chinook salmon had been selected for uniform size and they originated from Sea Spring Salmon Farm Ltd. (Chemainus BC, Canada). Before commencement of the study, the fish were divided equally into 12 groups of 55 fish. Each group was placed into a separate outdoor fibreglass tank.

Each of the 4 m<sup>3</sup> circular tanks was fitted with a 1.5 m diameter inner fibreglass hoop to create a circular swimming channel that was 45 cm wide and 55 cm deep. One half of the tank was covered with plastic netting while the other half was covered with black nylon cloth. This allowed the fish to choose between dark and light areas. To create a current, the seawater was pumped into each tank through a vertically placed pipe that was equipped with three horizontally oriented pipes. All the tanks were designed with a flow through system and there was no recirculation of water (Kiessling, et al. 1994b).

A two-by-three factorial design with two ration levels (maximum ration = RL100, 75% of maximum ration = RL75) and three swimming speeds (SS), (0.5, 1.0, and 1.5 body lengths bl/s) was used (Table 2). Duplicate groups of fish were assigned to each of the six treatments.

The actual amounts of feed that the fish ingested varied between swimming speeds. As the SS level was increased, the fish on the RL100 protocol, which were fed to satiety, consumed more feed. At each SS, the RL75 fish were given 75% of the ration that was given to their RL100 counterparts (Kiessling, et al. 1994b).



**Table 2** Experimental design used by Kiessling et al (1994 a, b) to assess the influence of sustained exercise and two ration levels on growth of chinook salmon in seawater. A 2 X 3 factorial design was used with two ration levels and three swimming speeds and their treatment numbers have been used as identifiers in statistical analyses.

Treatment No.	Ration Level <sup>a</sup>	Swimming Speed (bl/s)
1	75	0.5
2	75	1.0
3	75	1.5
4	100	0.5
5	100	1.0
6	100	1.5

<sup>a</sup> Ration levels: RL100 (100% ration level) is a ration sufficient for satiation, RL75 is 75% of the RL100 at a given swimming speed

Careful records of daily feed waste were maintained in each case and these allowed accurate estimations of the actual rations consumed. All fish, irrespective of treatment, were fed 4 to 6 mm Biodry 2500 pellets (Bioproducts Inc., Warrenton, Oregon, USA). The mean levels (% of dry matter) of protein, lipid and ash in the Biodry pellets were 52.0, 20.2 and 12.6, respectively.

For the purposes of this thesis, five representative fish were removed from each tank at the end of the 212-day study. Subsequently, the fish were killed by a blow to the head. The salmon were then filleted, labelled, and vacuum packaged in mylar film. Thereafter, the packaged fillets were placed into a - 35°C freezer pending analysis months later. Fillets from the left side of the fish were used for sensory analysis, whereas those from the right side were reserved for instrumental analysis.

## **3.2 Sensory Analysis**

### **3.2.1 The selection of sensory panellists for QDA analysis**

Seven panellists, three men and four women, were recruited from the staff and students of the UBC Food Science Department. Although seven people were trained, only six people could be accommodated in any one sensory test due to the limited amount of sample. The extra trained person was available in the event that a regular panellist was unable to attend a session.

Interest in the experiment and the availability of personnel were the main criteria used for panel selection. It was also important that the selected panellists generally liked to eat salmon. The individual's experience on sensory panels was also considered to be an asset. It was fortunate that most of the panel members had some previous sensory panel experience.

### **3.2.2 Sensory panel training**

Panel training was carried out over a three week period, during which the panellists met five times. Training consisted of first gathering descriptive terms from the panellists in a round table format followed by eliminating the less distinguishable or redundant terms. That procedure produced 27 terms to describe the aroma, flavour, and texture, as well as the overall acceptability of the salmon. Agreement was then reached amongst the panellists regarding the scoring of those attributes.

Both wild and farmed Spring (chinook) salmon were used in the training sessions. These fish were purchased at a local fish market and they were of similar size to the experimental fish. The purchased fish were thought to be the best sources of the characteristic flavour, texture, and aroma extremes needed for training. The wild salmon were assumed to have been much more active and less well fed than their pen-reared counterparts.

The market fish were filleted. Subsequently, the fillets were vacuum packaged in a barrier film, and then frozen at -35 °C until the day before they were needed for analysis. At that time they were placed in a -4 °C freezer to partially thaw overnight. Samples of approximately the same size (approximately 1 cm by 3 cm) were wrapped in foil, and baked at 190 °C for approximately 15 minutes, or until they were cooked before being presented to the panellists.

### **3.2.3 The selection of sensory attribute terms**

The panellists were asked to list as many aroma, taste and texture notes as they could detect in the cooked salmon samples. When a panellist observed a flavour note, all the panellists would retaste the samples, looking for that sensory note. They then discussed their individual observations. This list

of attributes that was compiled was subsequently reviewed by the panel for the purpose of eliminating the terms that were either ambiguous, or redundant.

The terms were defined by the panellists to ensure that all the panellists were measuring the same sensation. Whenever it was possible, reference samples for the attributes were provided to aid in clarifying the terms for the panellists. For example, boiled milk, boiled potato, and seaweed samples were provided to the sensory panel as a reference for the corresponding aroma terms.

#### **3.2.4 Ballot familiarisation by sensory panellists**

Additional training sessions were necessary to allow the panellists to become familiar with using the ballot. After selection of the terms for the study, a sensory ballot was produced and copies were presented to the panellists. They were then given fish samples and asked to rate them using this ballot and then discuss their scores. This procedure enabled assessment of whether each panel member was using the same intensity scale in the prescribed manner.

One source of disagreement in the panellists responses occurred when one or more of the panellists did not have the same conceptualisation of a particular descriptive term. In this situation, every effort was made to clarify the term in question. This was sometimes accomplished by producing a reference, or by having the panellists discuss the sensation amongst themselves. If it became apparent that a term was ambiguous, or that there would never be any real agreement among panellists, the term was discarded.

### **3.2.5 The use of composite samples**

The training sessions also provided a forum for the panellists to express any ideas that they felt could improve the panel's performance. One suggestion was to construct composite samples from each fish, by combining slices from the anterior, middle and posterior sections of the fillet before cooking. During the course of the first few training sessions it had become apparent that the intensities and profiles of the sensory attributes changed substantially between the different portions of the fillet; the anterior portion being much more flavourful than the posterior portion. This finding is in agreement with Johnsen and Kelly (1990) who found that anterior and posterior portions of fish could have quite disparate flavour profiles.

### **3.2.6 Sensory panel set-up**

Steps were taken to eliminate as many sources of error as possible that may have influenced the panellists' perceptions. For instance the panellists were asked to refrain from various activities such as drinking coffee, wearing after-shave, or perfume (Rutledge and Hudson, 1990). Since the appearance of the fish was not being tested, the sensory testing was conducted under red lights to mask any variation in appearance and, thus reduce the risk of panellist bias. To avoid any carry over of flavours from one sample to another, the panellists were given distilled water and unsalted crackers to help cleanse their palate between samples. As much as possible, background sound was kept to a minimum to prevent this from disturbing the panellists.

### **3.2.7 Sensory panel session scheduling**

Sensory tests were performed on ten different days. All six treatments were represented on each of these days. Originally it was planned to have one session per day during which all the treatments and a reference would be rated. After the first session, however, it was found that having the task of assessing samples from six treatments and a reference for 28 attributes at one sitting resulted in some of the panellists becoming fatigued and making errors. Steps were then taken to adapt the procedure to reduce panellist error. In this regard, it was decided that three of the treatments would be selected at random for the morning session, and the remaining three were set aside for the afternoon. A reference was evaluated by the panellists at both the morning and afternoon sessions.

Sometimes, a panellist would be unable to attend one of the two sessions on a given day. When this situation arose, the panellist(s) would rate both sets of samples at the session they attended. To accomplish this the panellist was given a short break following the scheduled sensory panel session and then he/she was presented with the samples from the session that could not be attended.

All the sensory panels were carried out between June and August, 1992. Seven sensory panel days were carried out in a two and a half week period. This was followed by a three week break. After the break, the panellists participated in a training session to ensure consistency in judgements between the two periods. The three remaining sessions were then held over a one week period.

### **3.2.8 The preparation of samples for sensory panels**

On the day before a panel session was to take place, one fillet from each of the six treatments was selected at random and transferred along with packages of reference fish, from the -35°C freezer

to one set at  $-4^{\circ}\text{C}$ . This allowed the fillets to partially thaw overnight so that they could be sliced the next day on a meat slicer without becoming too mushy.

The fillets samples were removed from the  $-4^{\circ}\text{C}$  freezer just prior to being sliced using a Hobart meat slicer to a thickness of approximately 3 mm. During the slicing of each fillet, at least 6 slices were taken from each of the three sections referred to above i.e., anterior, middle and posterior. Slices from each section were then randomly distributed into six piles of slices on pieces of aluminium foil, and efforts were made to ensure that the six samples were as similar as possible. The six aluminium foil sheets were labelled previously with a three digit random code and samples from the same fillet had the same number inscribed, using a permanent ink felt marker. The dull side of the foil was always on the outside. Care was taken to pile the slices from the various sections so that the skin faced the same direction. The samples would, once cooked, have the appearance of a solid piece of fish.

Samples for both the morning and afternoon sessions were prepared in the morning before the first panel sitting. The afternoon samples were stored in a  $4^{\circ}\text{C}$  cooler prior to being cooked and presented to the panel.

The foil wrapped samples were placed on a foil pan and placed in a  $190^{\circ}\text{C}$  oven for 15-20 minutes. Then the cooked samples were served to the panellists as promptly as possible. Frequently, it was difficult to have all six panellists assembled when the samples were ready, even when they were notified just prior to the fish being served. When it was known that a panellist was going to be a few minutes late, the samples were left over the vent from the oven to keep them warm.

### **3.2.9 The reference samples used during sensory panels**

The reference consisted of a composite sample from the fillets of numerous farmed Spring (chinook) salmon that had been obtained commercially from a local seafood market. These fillets were sliced to uniform thickness using a meat slicer. After this, the slices were divided into four groups: the anterior, anterior and back midsections, and posterior of the fillet. The slices from the corresponding sections of all the fillet were pooled together and mixed thoroughly. Following this they were vacuum packaged with each bag containing at least 12 slices of fish. The packages were numbered either 1, 2, 3 or 4 depending on which section of fillets had been enclosed. All of these packages were placed in a -35°C freezer. One package from each section was removed the day before a sensory panel session and subsequently these were placed in a -4°C freezer over night.

On the morning of the sensory panel day, composite samples were prepared from the fish slices. Slices from each section were evenly distributed to twelve samples i.e., six reference samples for the morning and six for the afternoon session. The reference samples were wrapped in foil and labelled with an "R".

### **3.2.10 The sampling procedure employed by panellists**

The sensory tests were performed in the sensory panel room located in the UBC Food Science Building.

The panellists first rated the reference and then the treatment samples in random order for the aroma attributes. The reference sample, and then the treatment samples were judged on the remaining attributes. Testing all the samples first for aroma helped to insure that all the samples would be close



to the same temperature. This sequence was important because as the fish cools the amount of volatiles given off decreases.

The panellists were asked to take a fork full of fish, including portions from all the slices in the composite sample. This forkful of fish was then placed in the mouth and chewed. The panellists then evaluated the sample for the various taste and texture attributes. If necessary, the panellist could take a second or third forkful. The panellists then either expectorated or swallowed the fish. Distilled water was provided to the panellists to rinse their mouths, and unsalted crackers were provided to help cleanse the palate between samples.

#### **3.2.11 Generation of numerical scores from the sensory ballot judgements**

The ballot (Fig. 1) used by each of the panellists consisted of a 10 cm unstructured scale anchored with a term at both ends for each of the attributes being tested. The panellists were asked to indicate their score by placing a vertical line through the scale at the appropriate spot. A numerical score could then be generated by measuring with a metric ruler from the left side of the scale to the point where the panellist's line crossed the line.

### **3.3 Instrumental analysis of cooked salmon samples**

#### **3.3.1 Preparation of salmon samples for instrumental analysis**

Due to time and equipment constraints, it was only possible to perform instrument analysis on a maximum of two samples per day. Late in the afternoon on the day prior to analysis, the salmon fillets were placed in a freezer that was set at -4°C. This allowed them to thaw slightly overnight which

**Figure 1** Sensory ballot that was used to evaluate samples of cooked, farmed chinook salmon

Aroma Profiling Score Sheet

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Sample Number: \_\_\_\_\_

Instructions

1. PERFORM AROMA PROFILING ON ALL SAMPLES BEFORE MOVING ON TO FLAVOUR AND TEXTURE PROFILING
  2. Open the foil wrapper and smell contents, flake the fish if necessary to release more of the aroma
  3. Mark the horizontal lines with a vertical line to indicate the intensity of each of the odours listed.
  4. Record any meaningful observations either on the bottom or the back of the page.
4. DOUBLE CHECK TO MAKE SURE THAT THE SAMPLE NUMBER RECORDED ON THE SCORE SHEET IS CORRECT.

Seaweed	-----	none	very seaweed
Boiled milk	-----	none	strong
Boiled potato	-----	none	strong
Lemony	-----	none	very lemony
Sour	-----	none	very sour
Fishy	-----	none	very fishy
Chickeny	-----	none	very chickeny
Oily	-----	not oily	very oily
Fresh	-----	old	fresh

## Flavour Profiling Score Sheet

### Instructions

1. Taste the sample (note: texture analysis can be performed simultaneously)
2. Mark the horizontal lines with a vertical line to indicate the intensity of each of the flavour terms listed below.
3. Write any additional comments on the bottom or back of the page.

Flavour	-----	weak	intense
Fishy	-----	none	very fishy
Earthy	-----	none	very earthy
Papery	-----	none	very papery
Bitter	-----	not bitter	very bitter
Sour	-----	not sour	very sour
Lemony	-----	not lemony	very lemony
Salty	-----	not salty	very salty
Spicy	-----	not spicy	very spicy
Brothy	-----	not brothy	very brothy

## Texture Profiling Score Sheet

### Instructions

1. Chew sample.
2. Mark horizontal line with a vertical line to indicate the intensity of each of the texture terms listed.
3. Write any additional comments on the bottom or back of the page.
4. PLEASE RECHECK AND MAKE SURE SAMPLE NUMBER IS CORRECT.

Moistness	-----	dry	very moist
Powderiness	-----	not powdery	very powdery
Flakiness	-----	not flaky	very flaky
Firmness	-----	firm	soft
Chewiness	-----	not chewy	very chewy
Cohesiveness	-----	loose mass	compact mass
Adhesiveness	-----	not sticky	very sticky
Mushiness	-----	not mushy	very mushy
Overall Acceptance	-----	extreme dislike	extreme like

facilitated easier handling. On the day of the test, the fillets were skinned and chopped into 1 cm<sup>3</sup> cubes. To ensure that there would be sufficient sample for a minimum of three replicates of each treatment for GC, Instron, and pH analysis, all the fish cubes from the same treatment were pooled. These cubes were mixed thoroughly to ensure that the samples were uniform. To avoid having any refreezing of the samples, the instrumental analyses were performed on the same day that the fillets were removed from the freezer.

The fillets were divided by weight into the sample sizes that were required for the various experiments. These samples were wrapped in aluminium foil, dull side out, and stored in a 4 °C cooler until required. At that time the foil packages were placed in a preheated 190 °C oven and baked until the fish muscle was no longer translucent (15-20 minutes).

### **3.3.2 GC headspace analysis of cooked salmon samples**

GC Headspace analysis, after using a purge and trap extraction, was performed on the cooked samples. Due to limitations in the amount of fish available from each treatment, it was only possible to perform this analysis in triplicate.

A series of experiments that were designed to select the most appropriate conditions for this purge and trap extraction were performed before the test samples were analysed. The five variables examined were: sample size, extraction temperature, extraction time, nitrogen flow rate, and the amount of Tenax packed into the traps. To determine the right level, samples were extracted at various levels within a realistic range e.g. extraction times of 1, 2, 3, and 4 hours were used. The four variables not being examined in a particular experiment were kept at a moderate level (which, coincidentally, turned out to be the levels chosen for this study). Once the results from these

experiments were graphed, the best level was determined based on the level of volatiles extracted and the size of the increase in recovery between levels. For choice of temperature and the extraction time, the risk of sample loss due to moisture build-up in the Tenax GC also was taken into account. It was concluded that the best conditions for conducting this extraction were: a sample size of 200 g, an extraction temperature of 70 °C, an extraction time of 3 hours, a flow rate of 50 ml/min, and 120 mg Tenax GC. (conditions used are summarised in Table 3).

Three 200 g foil packages of the chopped fillets were prepared from each treatment and these were placed in a 190 °C oven until cooked (approximately 20 minutes). Once cooked, the fillets were gently flaked with a fork and then promptly deposited, along with any liquid that was released during cooking, into prewarmed 1 L extraction vessels (Wheaton, Millville, NJ).

The six extraction vessels were maintained at 70 °C with a waterbath (Haake FS). The water passed from the waterbath through plastic tubing into a copper pipe that connected all the extraction vessels in parallel. The water exited from the vessels in a similar fashion and was recirculated back to the waterbath. This was designed to ensure that all the vessels would be maintained at the same temperature.

The internal standard used in this experiment was tetradecane (purity: 99+ %, Aldrich) that was dissolved in diethyl ether (spectranalyzed grade, Fisher Scientific)(1:10 v/v). After removing the tenax trap assembly, 20 microlitres of this standard was injected into the extraction vessel through the open side arm. The trap assembly was promptly replaced and the extraction vessel was closed tightly. The vessels were left to equilibrate for 30 minutes. After this time had expired, the volatile compounds that were produced by the cooked fillet were purged from the flask with prepurified N<sub>2</sub> gas (UHP grade, Linde Union Carbide) into the tenax GC trap.

**Table 3**      Conditions used in the extraction of cooked salmon using a purge and trap procedure

sample size	200 g
no. of replicates	3
extraction vessel temperature	70 °C
equilibrium time	30 min.
extraction time	3 hours
N <sub>2</sub> gas flow rate	50 ml/min.
adsorbent used	Tenax GC
amount of adsorbent	120 mg
internal standard	tetradecane
solvent used to elute traps	diethyl ether

Approximately 120 mg of Tenax GC (60/80 mesh, Alltech), a porous polymer, was packed into a glass tube (11.5 cm, 6 mm O.D., 4 mm I.D.), that was held in place between two plugs of glass wool. The Tenax GC had been conditioned prior to the extractions to remove contaminants. This involved holding the Tenax GC at 200°C with N<sub>2</sub> flowing through the tube at 30 ml/min for a minimum of 4 hours (Jennings and Filsoof, 1977). The narrow end of the trap was wrapped several times with teflon tape before attaching it to the extraction vessel to help ensure an air-tight fit.

After the 3 hour extraction was completed, the Tenax traps were removed and eluted with 2 ml diethyl ether (spectranalyzed grade, Fisher Scientific). The extracts were stored in 3.7 ml glass vials (screw top lid with septa) (Supelco), which were placed in a 4 °C cooler until required for GC analysis. At that time, the extract was concentrated by evaporating the ether using a gentle stream of nitrogen, until approximately 100-200 microlitres remained. One microlitre of this extract was then injected into a Varian 3700 gas chromatograph (Varian and Associates, Inc. Palo Alto, CA) set up and operated according to the specifications given in Table 4.

Relative amounts of each compound were then determined by taking a ratio of each peak area to that of the internal standard. These data were then subjected to an ANOVA (Systat 5.01, Systat Inc.), to determine if there were any treatment effects. Only peaks that consistently appeared in all of the chromatograms were examined.

### **3.3.3 Instron TPA analysis of cooked salmon samples**

Bourne's (1978) TPA method, based on the compression of the sample with the Instron Universal Testing Machine (Model 1122, Instron Corp., Canton, MA), was used to achieve an objective quantitative measurement of the cooked salmon texture. Cylinders of flaked salmon were



**Table 4** GC conditions used in the analysis of purge and trap extracts from cooked chinook salmon samples

GC	Varian 3700
Integrator	3390A Hewlett-Packard
Detector	Flame Ionisation
Split injection	100:1
Column	capillary
	SPB-1
	nonpolar
Column manufacturer	Supelco Inc.
Internal diameter	25 mm
Film thickness	0.25 micro meters
Column length	30 m
Initial temperature	50 °C
Time initial temperature held	5 min.
Rate of heating	5 °C/min.
Final temperature	220 °C
Injector port temperature	250 °C
Detector temperature	250 °C
He (UHP grade) flow rate	30 ml/min
Air (Zero Gas) flow rate	300 ml/min
H <sub>2</sub> (UHP grade) flow rate	30 ml/min
Volume of sample injected	1 µl

formed using a 60 ml syringe (2.6 cm internal diameter) with the end cut off at the zero line. Ten grams of the cooked, deboned, flaked fish were poured into the top of the syringe and then gently compressed with a flat bottomed plunger to form a cylinder 2 cm high. These fish samples were compressed twice with the Instron, between two parallel plates (approximately 14.8 cm diameter), to a height of 0.5 cm; 25% of their original height. This testing procedure creates a texture profile curve with two peaks from which numerous textural parameters may be measured. These include hardness, firmness, cohesiveness, chewiness and gumminess. Test conditions (Table 5) were selected after preliminary trials. The Instron was interfaced with a personal computer, using JCL6000 software, the force/deformation curves at a rate of 2 times per second were recorded. Prior to analysis, the Instron was calibrated by measuring the difference in load weight output with no weight and with a known weight. Quadruplet samples of each treatment were used in this portion of the experiment.

#### **3.3.4 pH measurement of cooked salmon samples**

The pH of the salmon was measured using the procedure outlined by Feinstein and Buck (1984). Samples were prepared by adding 3 g of fish muscle to 30 ml of deionized, distilled water and homogenised for approximately 10 seconds using a Kinematica GmbH homogenizer (speed setting 6).

pH was then measured using a Corning pH meter 220 (standardised to pH 7). These analyses were performed in quadruple.

**Table 5**      Conditions used for Instron measurements of minced cooked chinook salmon samples

Analysis	TPA
No. of replicates	4
Load cell	100 lb.
Crosshead speed	100 mm/min
No. of cycles of crosshead	2
Sampling rate	2 data points/sec.
Temperature	20°C (approximately)

### **3.4 Data Analysis**

#### **3.4.1 Analysis of sensory data**

##### **3.4.1.1 Exploratory analysis**

Prior to performing complex statistical analyses of the sensory data, some basic statistics were calculated. For example, for each panellist, the number of observations, the averages and standard deviations were tallied for the six treatments for each attribute. In addition, the data were also represented graphically in a series of boxplots. Boxplots were also constructed for the reference in the same manner.

Through this exploratory data analysis, portions of the data were found to be unacceptable due to an excessive number of inconsistencies. These included the first replicate (first sensory panel session) as well as all the data from panellist 6. These unreliable portions of the sensory data were subsequently removed prior to further analysis, leaving 5 panellists and 9 taste panel days (replicates).

##### **3.4.1.2 Principal component analysis (PCA)**

PCA was performed on the pooled data for the sensory aroma attributes, as well as the pooled flavour and texture data using Systat software. For each set of PCA data, a series of graphs of PC1 vs. PC2 were produced. Separate graphs were also produced by labelling data points with the treatment number, the panel day number, and the panellist number.

#### **3.4.1.3 ANOVA**

Several sets of ANOVAs, were performed on the sensory data. These included a two factor ANOVA on reference data that examined the effect of panellists, the day effect, and the interaction of these two factors. For the treated samples, all the sensory attributes were subjected to a three factor ANOVA, in which the main effects of ration level (RL), swimming speed (SS), and panellists (PAN) were assessed.

#### **3.4.1.4 Z-transformation of significant sensory attribute scores**

When ANOVAs were performed on the raw sensory data (treated samples), the panellist effect was consistently found to be highly significant. Additionally, for several of the attributes where a significant treatment effect had been uncovered, the panellist X treatment interactions were also significant. To remove the variation in the sensory data due to the panellist to panellist variation, a z-transformation was performed on all the sensory attributes that had been significantly affected by either RL or SS.

With respect to this, z-scores for a given sensory attribute were calculated in several steps. First, the data were sorted, so that the responses of each panellist could be identified. Following this, the averages and standard deviations of the responses of the individual judges were calculated from the raw data. The z-score for each response was then calculated by first subtracting the panellists average score from each response that she/he gave for that attribute, and then dividing the product by her/his standard deviation (Reid and Durance, 1992).

Subsequently, the transformed data were examined using ANOVA. Since the panellist to panellist variation had been eliminated, only a two way ANOVA examining the effects of the RL and SS was necessary.

### **3.4.2 Calculation of Instron TPA parameters**

#### **3.4.2.1 Calibration of results**

Prior to using the Instron, the instrument was calibrated daily by using a known weight. This was accomplished by taking measurements with the empty load cell for thirty seconds to establish a baseline, followed by placing a 1 kg weight on the inverted load cell for 30 seconds. This procedure was repeated three times in succession. The calibration factor was then calculated by taking an average of the scores recorded when the 1 kg weight was applied, and then subtracting the baseline score.

The test sample's data were calibrated by first subtracting the baseline value from the data and then dividing it by the calibration factor. These measurements were then converted into Newtons. This was accomplished by multiplying the scores by  $9.8 \text{ m/s}^2$ . The data were then used to measure or calculate Instron TPA measurements as outlined in Table 6.

#### **3.4.3 Calculation of TPA "Firmness"**

Firmness, the maximum slope of the compression cycle, was determined by measuring the maximum slope of the force curve. The slope was determined by calculating the distance the curve had risen on the y-axis divided by the distance it had covered on the x-axis. The slope was calculated for every 1 second interval on the curve.

#### **3.4.4 Calculation of peak area**

In order to measure cohesiveness and gumminess, it was necessary to first calculate the area under the curve. To accomplish this, the area of the "bites" was measured between the start of the curve and the peak force (the highest measured force). To calculate the area under the curve, the distance travelled on the chart for each reading was first determined. This was accomplished by first converting the chart speed to mm/s and then dividing it by the sampling rate, giving the distance travelled on the chart during each measurement. The area was then determined by the sum of this value multiplied by each point on the curve.

#### **3.4.5 ANOVA of Instron TPA and pH data**

A two way ANOVA, looking at the effect of swimming speed and ration level on various pH and TPA measurements was performed. When a significant result was found in swimming speed, having more than two levels, a Tukey test was also conducted to determine which levels were responsible for the significant differences. The data were analysed with the aid of Systat statistical software.

#### **3.4.6 Principal Component Similarity (PCS) analysis of Sensory, and GC headspace volatile data**

PCA, using Systat software, was performed separately on the sensory and GC headspace volatile data. The sensory data included only those sensory attributes that had been significantly affected by either RL or SS. These results had subsequently undergone a z-transformation to eliminate

**Table 6** Calculation of Instron TPA parameters

Texture parameters	Definition	Reference
Hardness 1	Peak force during the first compression cycle	(Bourne, 1978)
Hardness 2	Peak force during the second compression cycle	(Bourne,1978)
Firmness 1	Maximum slope of the first compression cycle	(Durance and Collins, 1991)
Firmness 2	Maximum slope of the second compression cycle	(Durance and Collins, 1991)
Cohesiveness	Ratio of positive force area during the second compression cycle to that of the first ( $A_2/A_1$ )	(Bourne, 1978)
Gumminess	Product of hardness X cohesiveness	(Bourne, 1978)



the panellist effect. By averaging the responses of all the judges for each replicate, a 5 fold reduction in the size of the data set was achieved, leaving 9 replicates for each treatment combination.

For the analysis of the GC data, only those peaks that consistently appeared and were significantly affected by either SS or RL were used. This resulted in 27 out of a possible 71 peaks being included in this analysis.

PCA was performed on these two sets of data, and this resulted in a print out for each, and the scores were saved. Starting with the first PC, the percent of total variance explained by the PC were added together until more than 90 % of the variance was accounted for; these are the PC that were used in the PCS. The scores from these PCs were copied into a data file to be imported into the PCS program. PCS produces the slope and coefficient of determination for each case, which were subsequently saved in a data file. After importing this file into a computer spreadsheet, the slope was graphed against the coefficient of determination.

## **4. Results and Discussion**

### **4.1 Sensory analysis of cooked salmon samples**

#### **4.1.1 Sensory panel reference samples**

##### **4.1.1.1 Purpose of reference sample**

At both the morning and afternoon sensory panel sessions, a reference sample was presented to the panellists along with the test samples. The reference samples were composite samples of small, randomly selected slices of fish from several 0.9-1.5 kg farmed chinook salmon. Using this method, a large number of fairly uniform samples was produced. Since an individual fillet from each treatment was used along with a reference sample for each of the nine panel days, it was essential to include reference samples. Without a reference it would have been difficult to distinguish from the test samples whether a statistical difference in the day was due to a true difference between the sessions or simply stemmed from fish to fish variation. Consistency between reference samples was also important as panellists have a tendency to grade the test samples relative to the reference (Giovanni and Pangborn, 1983). A summary of the sensory reference data is found in Table 7.

The reference was also used to help the panellists calibrate their responses. These reference samples were prepared for the panellists on several panel training sessions. At these training sessions, the panellists were able to interact with each other, discussing how and why they would give these samples a particular score, eventually reaching a consensus on the appropriate grade. During the sensory panels, these reference samples were rated prior to any of the treated samples. This allowed the panellists to calibrate their responses, between each other, as well as from session to session (Johnsen and Kelly, 1990). However, despite the use of the reference samples and training, there

**Table 7** Range, mean, and standard deviation (St. Dev.) of sensory attributes of the cooked, farmed chinook salmon reference samples (5 panellists, 9 panel days)

	Attribute	Range	Mean & St. Dev
Aroma			
	Seaweedy	0.7-7.0	3.75 ± 1.49
	Boiled milk	0-4.0	1.29 ± 0.89
	Boiled potato	0-5.8	2.51 ± 1.44
	Lemony	0-5.8	1.62 ± 1.25
	Sour	0-4.8	1.06 ± 0.93
	Fishy	0.1-8.9	3.59 ± 1.77
	Chickeny	0-7.0	1.76 ± 1.78
	Oily	0-5.2	1.28 ± 0.96
	Fresh	1.7-9.6	5.72 ± 1.44
Flavour			
	Flavour	1.0-9.1	5.43 ± 2.14
	Fishy	0.2-8.8	3.49 ± 2.06
	Earthy	0-5.5	1.30 ± 1.33
	Papery	0-7.4	2.01 ± 1.48
	Bitter	0-6.7	1.22 ± 1.16
	Sour	0-4.4	1.06 ± 0.84
	Lemony	0-7.7	1.65 ± 1.54
	Salty	0-4.1	1.53 ± 1.06
	Spicy	0-7.2	2.13 ± 1.54
	Brothy	0-7.7	2.15 ± 2.17
Texture			
	Moistness	0.5-9.0	4.89 ± 1.93
	Powderiness	0-8.9	2.55 ± 1.93
	Flakiness	0.1-8.3	3.55 ± 2.25
	Firmness	0.6-8.6	5.30 ± 1.71
	Chewiness	0.1-8.5	4.13 ± 2.18
	Cohesiveness	0.4-7.7	4.17 ± 1.83
	Adhesiveness	0-8.0	2.91 ± 1.85
	Mushiness	0-7.3	1.94 ± 1.58
	Overall	2.1-8.7	5.58 ± 1.37

would often be a wide range of responses received from the panellists for a given sample. The panellists, each with their own personal style, were consistent from session to session.

#### **4.1.1.2 Reference sample observations**

Significance for the day X panellist term interaction that appears in Table 8 is likely largely due to some of the unavoidable differences between panel sessions. It was not always possible to have the panellists start the panel at the same time. From time to time, a panellist was unavoidably detained and came to the session late. Occasionally a panellist was absent for one of the two sessions on a panel day. The panellist took a short break following the scheduled session he/she attended and then rated the samples from the missed session. The resulting differences in temperature and taste acuity may partially be responsible for the day X panellist interaction (Meilgaard et al., 1991; Larmond, 1977). It was also noted that on occasion a panellist did not comply with the request to abstain from eating lunch or consuming coffee prior to the panel session and this may have resulted in confusion or carry-over sensations with the samples (Rutledge and Hudson, 1990).

#### **4.1.2 Treated samples**

##### **4.1.2.1 Exploratory analysis**

The data set for this portion of the research was extremely large and required some exploratory data analysis. For each individual panellist, boxplots of each attribute were constructed (examples in appendix A). Tables giving each panellist's average, standard deviation, range and number of observations were also prepared (data not shown).

#### **4.1.2.1.1 Boxplots**

It proved very difficult to ascertain from the boxplots whether the treatments were significantly different from each other. Even in attributes where a significant difference between treatments existed, the wide variation in panellist rating styles masked evidence of the treatment differences.

These boxplots, however, did clearly show that despite training there was a large amount of judge to judge variation. The judges differed greatly in their style of rating of the attributes, varying widely in the range of values that they used (data not shown). They, however, appeared quite consistent with their individual styles of rating the attributes, using the same range and psychological distances between grading levels i.e. what one panellist would grade as a 0.3 cm, a second might score as 1.5 cm. Fortunately, according to Stone et al. (1974), it is not of critical importance that the individual panellists used different segment of the scale, as long as their individual performances were constant.

#### **4.1.2.1.2 Deletion of unacceptable data**

Upon examination of these results, it was decided that some of the data collected would not be used in any further analysis. All the data collected on the first day, as well as the contribution of panellist 6, were removed.

The data set collected on the first day was eliminated because it contained numerous panellist errors, mostly related to panellist fatigue. In the first panel session, it was wrongly assumed that all the panellists would be capable of rating each of the 7 samples (6 treatments and one reference) for the 28 sensory attributes without becoming fatigued. In subsequent sessions, the panel days were divided into morning and afternoon sessions. Three randomly chosen treatments and a reference were presented to

**Table 8** ANOVA results of judge and panel day effect on reference samples for 28 sensory attributes of cultured chinook salmon (5 panellists, 9 panel days)

Sensory attributes		F ratio			Mean square error
		Day	Panellist	Day X Pan	
Aroma					
	Seaweedy	1.748	30.992***	1.006	0.848
	Boiled milk	1.196	18.450***	3.748***	0.244
	Boiled potato	0.482	18.504***	1.454	1.069
	Lemony	0.682	27.416***	0.941	0.668
	Sour	0.261	3.494*	1.383	0.698
	Fishy	3.048**	5.303**	1.330	2.164
	Chickeny	1.740	38.885***	1.325	1.149
	Oily	2.576*	11.447***	1.233	0.486
	Fresh	2.109	18.584***	1.233	0.486
Flavour					
	Flavour	0.597	35.396***	0.762	1.762
	Fishy	0.501	19.857***	1.474	2.315
	Earthy	2.536*	47.925***	2.859**	0.418
	Papery	1.321	5.834**	1.233	1.449
	Bitter	0.978	5.266**	1.337	1.099
	Sour	1.145	0.681	0.972	0.704
	Lemony	1.209	12.622***	0.982	1.617
	Salty	1.805	42.830***	3.383***	0.308
	Spicy	0.853	20.321***	1.005	1.092
	Brothy	1.364	129.229***	0.931	0.715
Texture					
	Moistness	0.904	16.656***	0.579	2.361
	Powderiness	1.091	2.301	1.775*	2.469
	Flakiness	0.713	39.043***	1.100	1.817
	Firmness	2.331*	8.238***	1.206	1.842
	Chewiness	2.500*	67.577***	1.683*	1.232
	Cohesiveness	1.593	13.919***	0.884	2.039
	Adhesiveness	1.589	28.128***	1.776*	1.232
	Mushiness	1.857	3.148*	1.554	1.376
	Overall	1.567	24.223***	1.503	0.728

\* p<.05, \*\* p<.01, \*\*\* p<.001

the panellists in the morning with the remaining three treatments and a second reference offered at an afternoon session.

A large number of missing data points, as well as excessive variation in replicates (data not shown), made it necessary to omit the contribution of panellist 6 from the data set. The data from panellist 6 were actually a combination of data contributed by three people. Each of these three people had a personal style of rating the samples that varied greatly from one other. Since consistency and accuracy are so very important for panellists in this type of sensory analysis (Stone et al. 1974), it was decided not to include the data from panellist 6. Upon elimination of this data set a second set of summary statistics was calculated (Tables 9 - 15).

#### **4.1.2.2 The use of replacement panellists**

As this panel took place over a period of several weeks during the summer, it proved an impossible task to find six willing panellists who were able to commit to being present throughout the duration of the experiment. It was decided that back-up panellists, who had also completed the training sessions, would substitute for absent panellists. Fortunately, back-up panellists were only necessary for panellists 5 and 6.

Evidence of the substitution of a back-up panellist for panellist 5 on two panel days became very apparent during data analysis. In Figures 2-4 a set of data points, one from each treatment, was separated from the main cluster. This set was evident in the aroma and pooled texture variables (Fig. 2 and 4); no evidence of this set was readily apparent in the corresponding flavour graph (Fig. 3).

These irregularities in the data were further examined by producing a second set of these figures where the treatment number was replaced with the panel day number (Fig. 5-7). From these

**Table 9** Range, mean, and standard deviation (St. Dev.) of cooked, cultured chinook salmon sensory attributes (all treatments combined; 5 panellists, 9 panel days)

	Attribute	Range	Mean & St. Dev.
Aroma	Seaweedy	0-9.9	4.05 ± 2.17
	Boiled milk	0-6.1	1.26 ± 1.00
	Boiled potato	0-6.7	2.20 ± 1.47
	Lemony	0-6.3	1.44 ± 1.21
	Sour	0-6.3	1.37 ± 1.23
	Fishy	0-8.9	3.58 ± 1.92
	Chickeny	0-7.6	1.51 ± 1.47
	Oily	0-5.6	1.36 ± 1.03
	Fresh	0.6-9.6	5.26 ± 1.71
Flavour	Flavour	0.3-9.4	5.63 ± 2.07
	Fishy	0.1-8.8	3.42 ± 2.20
	Earthy	0-7.5	1.23 ± 1.26
	Papery	0-8.6	1.99 ± 1.64
	Bitter	0-7.1	1.32 ± 1.25
	Sour	0-7.1	1.27 ± 1.11
	Lemony	0-7.7	1.30 ± 1.67
	Salty	0-7.0	1.82 ± 1.28
	Spicy	0-8.8	2.35 ± 1.72
	Brothy	0-8.4	2.31 ± 2.13
Texture	Moistness	0.5-9.2	5.34 ± 1.92
	Powderiness	0-8.9	2.45 ± 1.97
	Flakiness	0-8.4	3.14 ± 2.04
	Firmness	0.5-8.8	4.63 ± 1.89
	Chewiness	0-9.0	4.13 ± 2.15
	Cohesiveness	0.2-9.0	4.14 ± 1.84
	Adhesiveness	0-8.3	2.76 ± 1.88
	Mushiness	0-8.6	2.43 ± 2.04
	Overall	1.9-8.9	5.48 ± 1.44



**Table 10** Mean sensory scores and standard deviation of cultured chinook salmon aroma attributes for each ration level X swimming speed treatment ( 5 panellists; 9 panel days)

Attribute	Ration			Level		
	75%			100%		
	Swimming			Speed (bl/s)		
	0.5	1.0	1.5	0.5	1.0	1.5
Seaweed	3.95 ±2.22	4.17 ±2.23	3.50 ±2.07	4.38 ±2.23	4.34 ± 2.46	4.52 ±2.63
Boiled milk	1.37 ±1.19	1.31 ±1.05	1.23 ±1.14	1.17 ±0.88	1.28 ±1.05	1.16 ±0.80
Boiled potato	2.28 ±1.56	2.19 ±1.61	2.36 ±1.50	2.06 ±1.50	1.84 ±1.26	1.89 ±1.29
Lemony	1.44 ±1.17	1.51 ± 1.33	1.26 ±1.14	1.34 ±1.07	1.56 ±1.33	1.17 ±1.00
Sour	1.56 ±1.29	1.36 ±1.23	1.06 ± 0.93	1.56 ±1.44	1.76 ±1.05	1.52 ±1.23
Fishy	3.44 ±1.99	1.46 ±1.71	3.46 ± 1.74	3.27 ±1.85	3.63 ± 2.20	4.09 ±2.18
Chickeny	1.49 ±1.28	1.30 ±1.12	1.75 ± 1.75	1.28 ±1.15	1.45 ±1.34	1.34 ±1.30
Oily	1.29 ±1.02	1.37 ± 0.97	1.37 ± 0.94	1.27 ± 0.99	1.51 ±1.29	1.51 ±1.04
Fresh	5.23 ±1.67	5.15 ±1.62	5.26 ± 1.30	5.24 ±1.98	5.00 ±1.98	4.78 ±1.88

**Table 11** Range of sensory scores of cultured chinook salmon aroma attributes for each ration level X swimming speed treatment (5 panellists; 9 panel days)

Attribute	Ration			Level		
	75%			100%		
	Swimming			Speed (bl/s)		
	0.5	1.0	1.5	0.5	1.0	1.5
Seaweedy	0.7-9.3	0.2-9.6	0.2-9.7	0.7-9.2	0.2-9.9	0-9.8
Boiled milk	0-5.8	0-4.6	0-6.1	0-4.3	0-3.8	0-3.4
Boiled potato	0-5.7	0-6.0	0.1-6.7	0-6.0	0-5.3	0-5.3
Lemony	0-6.3	0-6.3	0-6.1	0-4.9	0-5.6	0-4.1
Sour	0-5.3	0-5.7	0-4.0	0.1-6.3	0-5.6	0-6.3
Fishy	0-8.1	0.1-7.3	0.04-6.6	0.2-8.0	0.2-7.9	0.1-8.6
Chickeny	0-5.9	0-4.6	0-7.6	0-5.7	0-7.4	0-6.7
Oily	0-3.5	0-5.4	0-4.1	0-4.5	0-5.6	0-4.6
Fresh	1.9-8.6	1.5-8.9	2.3-9.6	1.2-9.1	1.2-9.5	0.6-8.9

**Table 12** Mean sensory scores and standard deviation of cultured chinook salmon flavour attributes for each ration level X swimming speed treatment (5 panellists; 9 panel days)

Attribute	Ration			Level		
	75%			100%		
	Swimming			Speed (bl/s)		
	0.5	1.0	1.5	0.5	1.0	1.5
Flavour	5.95 ±1.84	5.86 ±2.13	5.69 ±1.89	5.31 ±2.07	5.69 ±1.89	5.60 ±2.07
Fishy	3.41 ±2.08	3.31 ±2.25	3.33 ±2.40	3.49 ±2.15	3.33 ±2.40	3.50 ±2.26
Earthy	1.09 ±1.02	1.36 ±1.31	1.34 ±1.42	1.13 ±1.03	1.34 ±1.42	1.09 ±1.21
Papery	1.92 ±1.73	2.02 ±1.42	2.06 ±1.76	2.04 ±1.64	2.06 ±1.76	1.92 ±1.70
Bitter	1.46 ±1.39	1.52 ±0.94	1.52 ±1.19	1.13 ±1.16	1.52 ±1.19	1.49 ±1.66
Sour	1.47 ±1.54	1.16 ±1.00	1.25 ±0.96	1.25 ±1.00	1.25 ±0.96	1.46 ±1.22
Lemony	1.28 ±1.05	1.19 ±0.95	1.22 ±0.97	1.15 ±1.06	1.19 ±1.02	1.06 ±0.92
Salty	2.03 ±1.35	2.01 ±1.34	1.85 ±1.32	1.79 ±1.39	1.87 ±1.28	1.91 ±1.30
Spicy	2.48 ±1.72	2.38 ±1.69	2.64 ±1.68	2.42 ±1.88	2.22 ±1.81	2.40 ±1.78
Brothy	2.51 ±2.05	2.56 ±2.12	2.48 ±2.28	2.07 ±2.00	2.27 ±1.99	2.28 ±2.06

**Table 13** Range of sensory scores of cultured chinook salmon flavour attributes for each ration level X swimming speed treatment (5 panellists; 9 panel days)

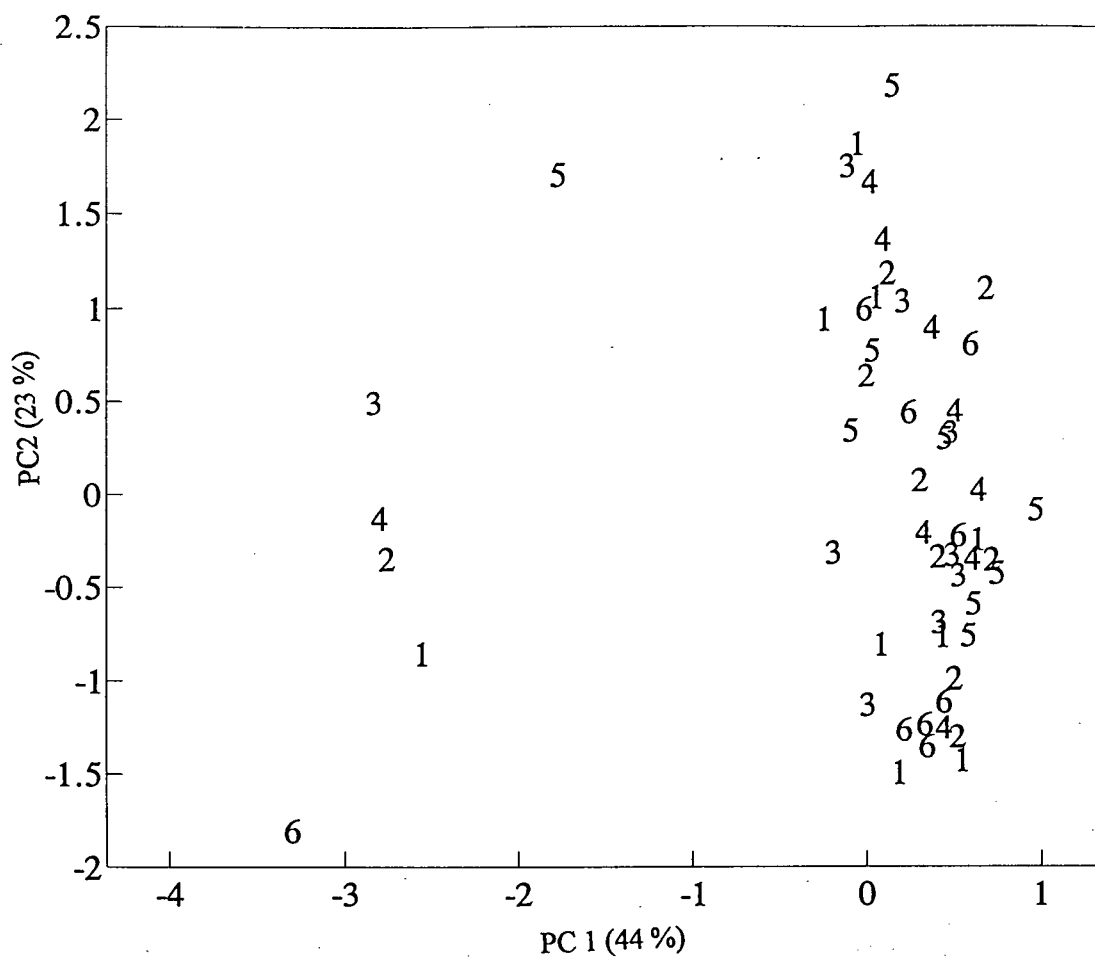
Attribute	Ration Level					
	75%			100%		
	Swimming			Speed (bl/s)		
	0.5	1.0	1.5	0.5	1.0	1.5
Flavour	0.6-9.4	1.2-9.1	0.4-8.8	0.9-9.0	0.4-8.8	0.4-9.1
Fishy	0.1-8.0	0.1-8.0	0.1-8.2	0.2-8.2	0.1-8.2	0.1-8.1
Earthy	0-4.3	0-5.1	0-7.3	0-4.7	0-7.3	0-4.6
Papery	0-7.5	0-6.2	0-7.0	0-6.7	0-7.0	0.2-8.5
Bitter	0-5.3	0-4.4	0-6.3	0-6.2	0-6.3	0-7.1
Sour	0-7.1	0-4.2	0-4.0	0-3.7	0-4.0	0-5.0
Lemony	0-3.5	0-4.0	0-3.9	0-5.1	0-4.0	0-3.3
Salty	0-5.6	0-5.8	0.1-5.2	0-7.0	0-4.6	0.1-5.4
Spicy	0.1-5.9	0-7.8	0.1-8.0	0-7.8	0-6.9	0.1-8.8
Brothy	0.2-7.4	0-7.8	0.2-7.3	0-7.1	0.1-8.4	0.1-7.7

**Table 14** Mean sensory scores and standard deviations of cultured chinook salmon texture attributes for each ration level X swimming speed treatment (5 panellists; 9 panel days)

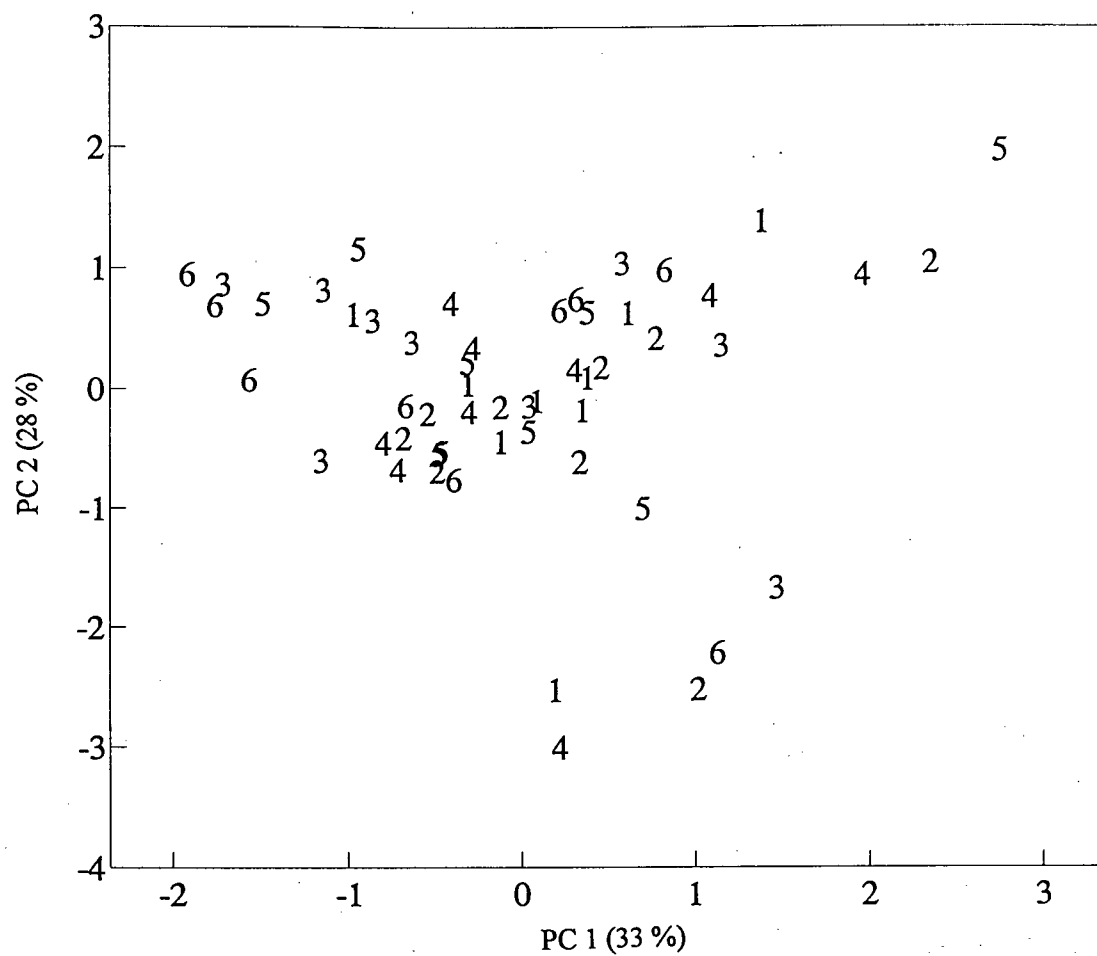
Attribute	Ration Level					
	75%			100%		
	Swimming			Speed (bl/s)		
	0.5	1.0	1.5	0.5	1.0	1.5
Moistness	5.27 ±1.90	5.52 ±1.76	5.04 ±1.93	5.48 ±1.67	5.98 ±2.02	5.66 ±1.94
Powderiness	2.50 ±1.93	2.33 ±1.88	2.45 ±1.94	2.42 ±2.02	2.26 ±1.99	2.49 ±2.11
Flakiness	2.92 ±2.12	3.21 ±2.05	3.04 ±1.79	3.07 ±1.77	2.95 ±2.08	2.88 ±1.83
Firmness	4.78 ±1.68	4.49 ±1.98	5.02 ±1.30	4.21 ±1.97	3.80 ±2.03	4.22 ±2.07
Chewiness	4.27 ±2.26	4.03 ±2.12	4.33 ±1.92	3.83 ±2.18	3.99 ±2.05	4.30 ±2.23
Cohesiveness	4.34 ±1.58	4.17 ±1.75	4.62 ±1.72	3.74 ±1.78	4.03 ±2.07	3.91 ±2.02
Adhesiveness	2.83 ±1.89	2.77 ±1.89	2.67 ±1.85	5.58 ±1.64	2.94 ±1.98	5.52 ±2.05
Mushiness	2.35 ±1.70	2.17 ±1.83	1.95 ±1.69	2.82 ±2.34	3.19 ±2.50	3.02 ±2.30
Overall	5.65 ±1.34	5.70 ±1.32	5.49 ±1.43	5.23 ±1.32	5.20 ±1.62	5.39 ±1.60

**Table 15** Range of sensory scores of cultured chinook salmon texture attributes for each ration level X swimming speed treatment (5 panellists; 9 panel days)

Attribute	Ration			Level		
	75%			100%		
	Swimming			Speed (bl/s)		
	0.5	1.0	1.5	0.5	1.0	1.5
Moistness	1.1-9.1	1.5-9.1	0.8-8.8	1.9-9.1	0.6-9.2	0.7-9.0
Powderiness	0-6.8	0-7.6	0-8.5	0-8.7	0-8.4	0-7.9
Flakiness	0.2-8.3	0-7.2	0-7.9	0.1-8.2	0.1-8.4	0-7.2
Firmness	1.5-8.0	0.6-8.8	2.2-7.5	0.6-7.7	0.5-7.9	0.6-8.4
Chewiness	0.1-8.3	0-9.0	0.7-8.5	0.3-8.0	0.2-8.8	0.1-8.3
Cohesiveness	0.8-8.5	0.4-7.6	0.5-7.7	0.2-7.4	0.4-9.0	0.3-8.2
Adhesiveness	0-7.4	0.1-7.7	0.1-7.0	0.1-7.8	0-8.2	0.1-8.3
Mushiness	0.2-7.4	0-8.6	0.1-6.8	0-7.9	0-8.1	0.2-8.0
Overall	3.4-8.7	2.7-8.9	2.0-8.5	1.9-8.6	2.2-8.5	2.2-8.5

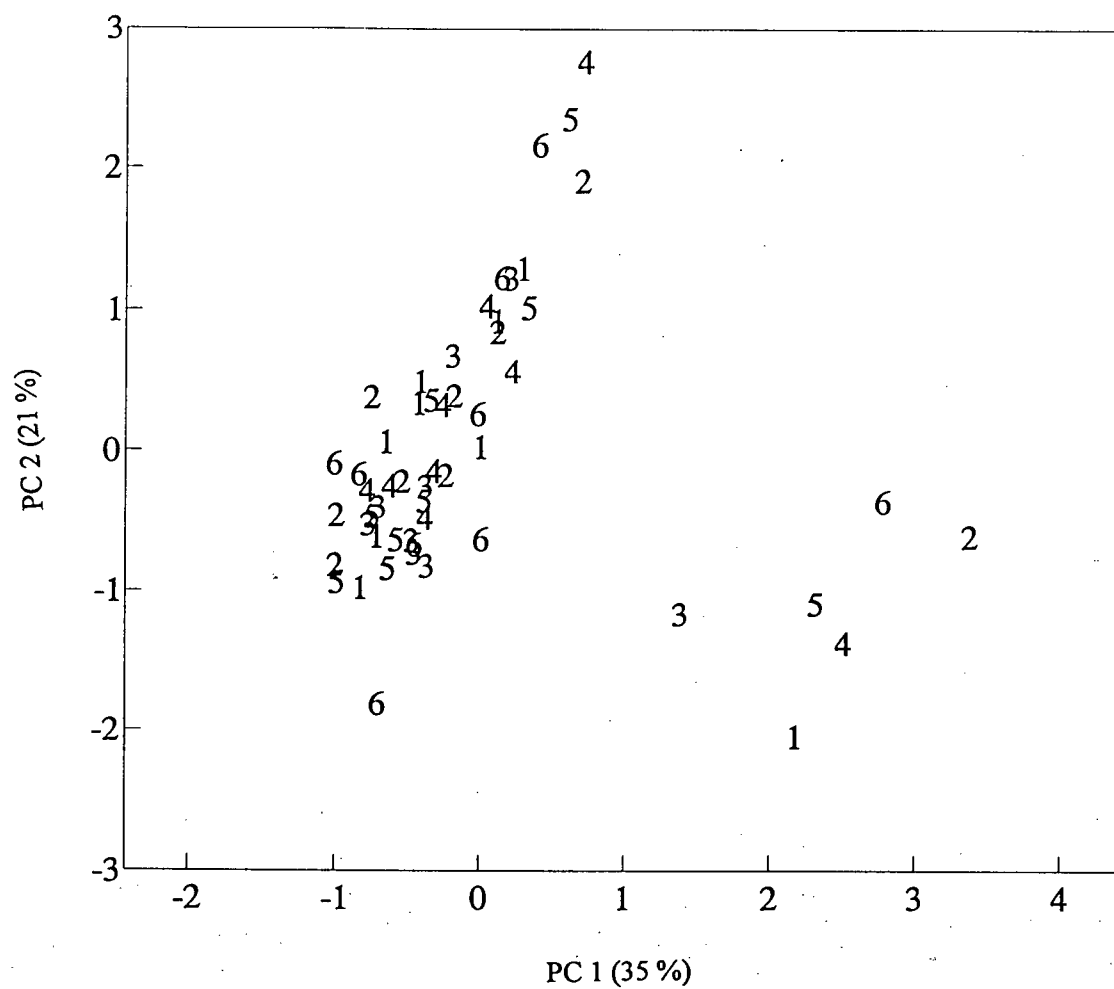


**Figure 2** PC 1 versus PC 2 using sensory aroma attribute scores from panellist 5, data points labelled with treatment numbers

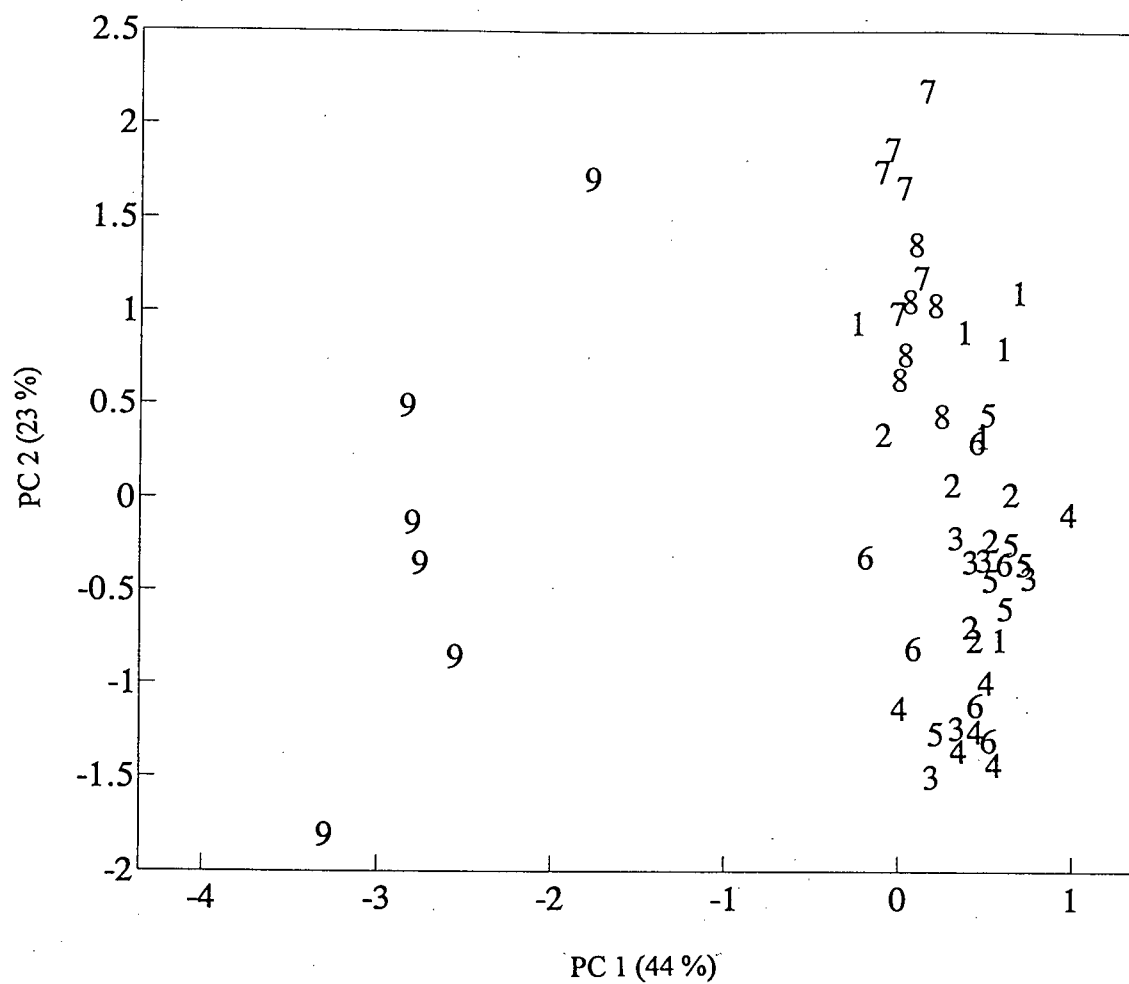


**Figure 3** PC 1 versus PC 2 of panellist 5 sensory flavour attribute scores, data points labelled with treatment numbers

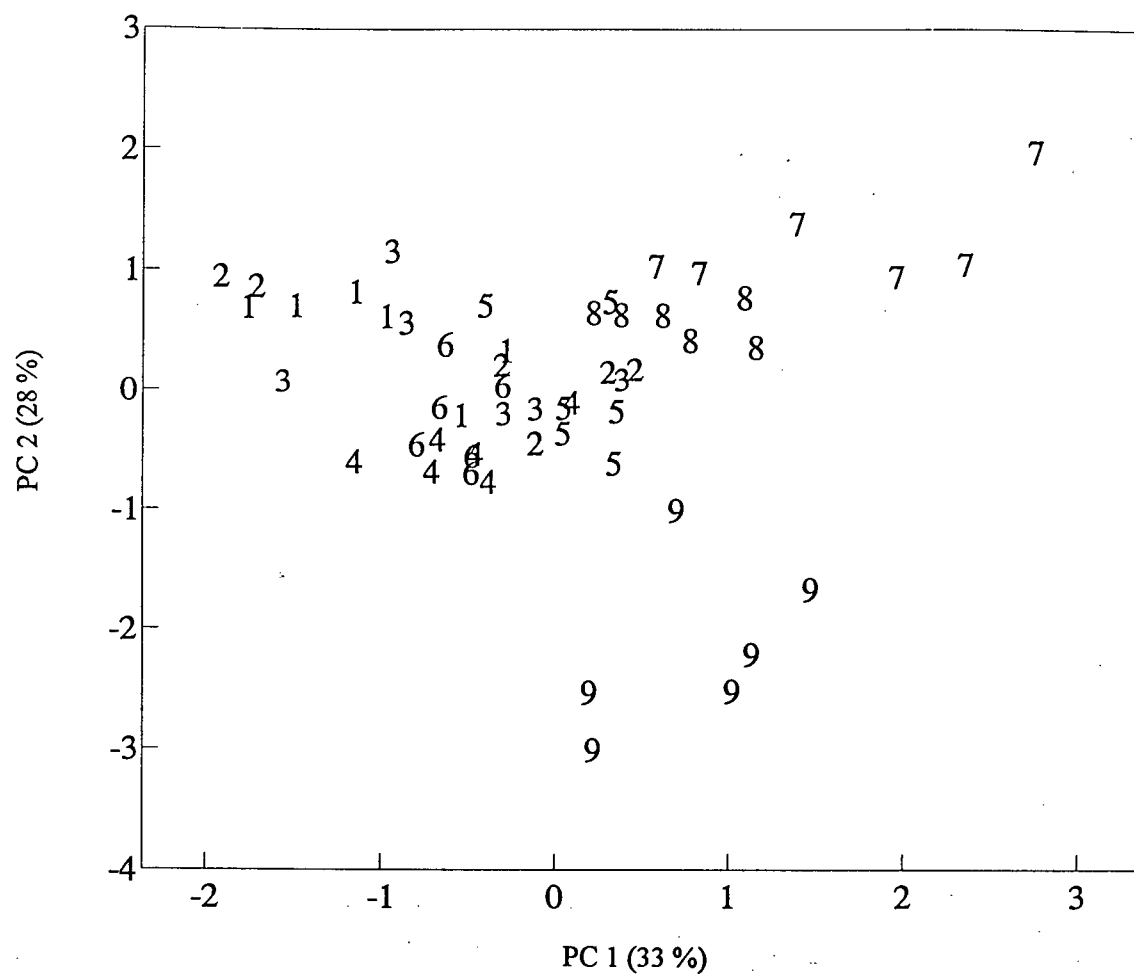


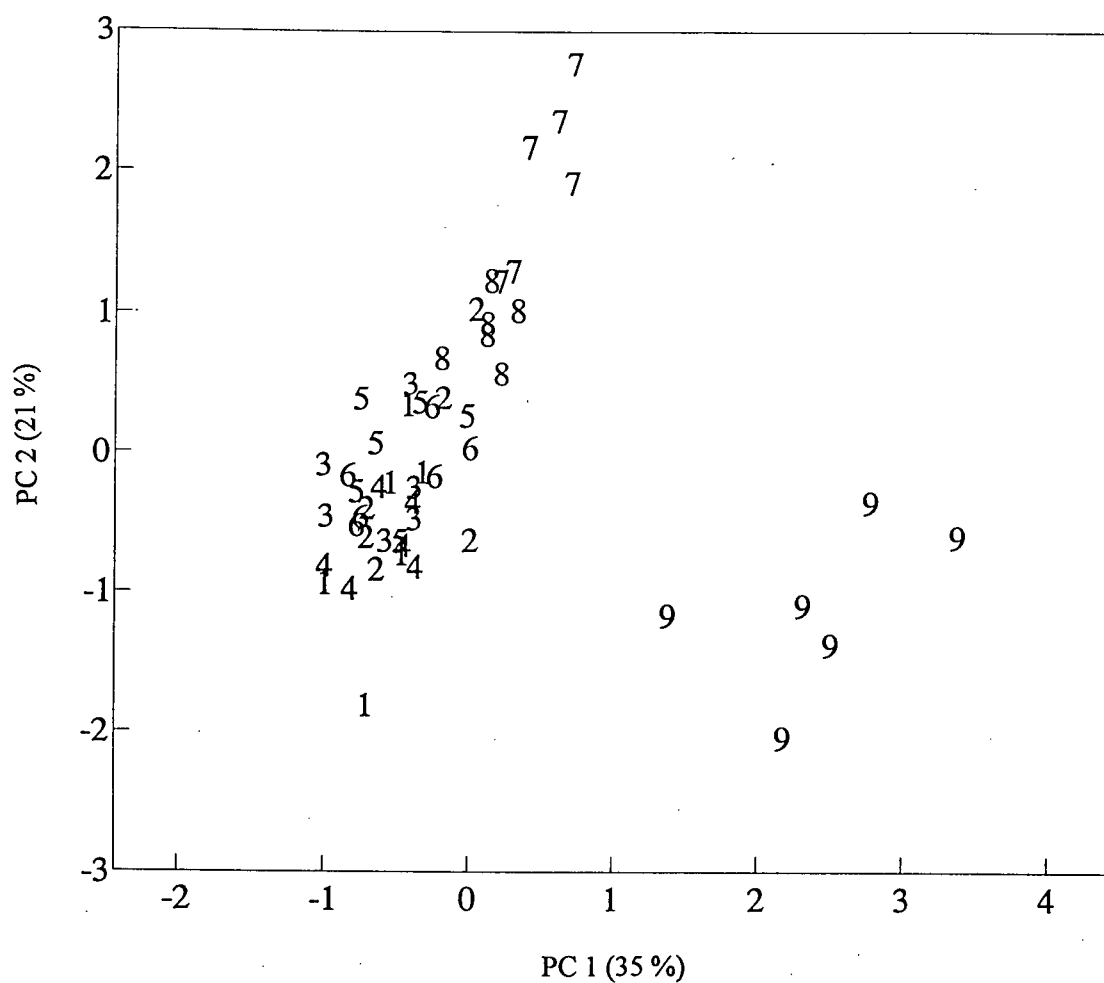


**Figure 4** PC 1 versus PC 2 of panellist 5 sensory texture attribute scores, data points labelled with treatment numbers



**Figure 5** PC 1 versus PC 2 of panellist 5 sensory aroma attribute scores, data points labelled with panel day number





**Figure 7** PC 1 versus PC 2 of panellist 5 sensory texture attribute scores, data points labelled with panel day number

figures it is apparent that the anomalies in the data are due to events on panel day 9. With the panel day hi-lighted a second cluster, this time in day 7, was also identifiable. Unlike the first set of graphs (Fig. 2-4), these irregularities were also evident in the flavour graphs. When notes recorded during the course of the sensory panel sessions were reviewed, it became apparent that in these two sessions a substitute panellist was used in place of panellist 5 (the same person on both occasions).

This irregularity in the judging attributed to one panellist, although unlikely to have a large effect on statistics such as the mean, standard deviation and range of responses, can lead to problems when testing for significant difference in ANOVA. For example, it could lead to panellist X day interactions. When attempting to standardize the data to remove panellist effect, the skewed average and standard deviation for that panellist will, in turn, skew the results.

#### **4.1.2.3 Summary statistics of treated samples**

The sensory data scores, generally, were quite low. Upon examination of the means (Tables 10, 12 and 14), it was apparent that most were at the lower end of the 10 cm scale; only four had averages over 5 cm, while 11 out of the 28 attributes had an average under 2 cm. In Tables 9, 11 and 13, the lower end of the range was often zero. On one or more occasions, this attribute was too faint to be discernible by at least one of the panellists.

All the salmon samples had a very delicate flavour. In informal discussions held with the panellists, they would often comment that the flavour notes were quite faint and difficult to quantify. In addition, they commented that they could not discern much of a difference among the six treatments. Many of the sensory attributes proved impossible for individual panellists to even detect in some samples, resulting in a score of zero.

#### 4.1.2.4 Three factor ANOVA of sensory attribute data

A three factor ANOVA was performed on all the attributes individually, to evaluate the contribution of SS, RL, and panellists as well as all the interactions (Tables 16-18). The panellist effect was highly significant ( $p < 0.001$ , or  $p < 0.01$ ) for all attributes. Even after training, it is quite common for the panellist effect to account for a large portion of the variation in the data. This variation stems from the subjective nature of this type of sensory evaluation and the individual differences between panellists (Stone et al. 1974).

There were a few attributes where the RL X PAN interaction was significant. In overall acceptability, there was also a significant SS X PAN interaction ( $p < 0.05$ ). Even in cases where panellists are screened and well trained, there is always a possibility that panellist by treatment interaction will occur due to differences in motivation, sensitivity or psychophysical response behaviour. This is especially true when the panel size is small (Lundahl and McDaniel, 1990) as it was in this experiment. Some confusion in scoring is acceptable, particularly in cases such as this where there is not a large degree of difference between samples (Stone et al. 1974).

Out of the 28 sensory attributes tested, no attributes were significantly affected by SS but eight were significantly affected by varying the RL. Of the aroma attributes tested, "Boiled potato," and "Sour" were significant at the  $p < 0.05$  level, and "Seaweed" at  $p < 0.01$ . Only one taste term, "Brothy" was found to be significant ( $p < 0.01$ ). Four texture attributes were significantly influenced: "Moistness" ( $p < 0.05$ ), "Firmness" ( $p < 0.01$ ), "Cohesiveness" ( $p < 0.05$ ) and "Mushiness" ( $p < 0.001$ ).

**Table 16** Summarised ANOVA results of ration level, swimming speed and panellist effect on sensory aroma attributes of cultured chinook salmon (5 panellists, 9 panel days)

Aroma attributes	F ratio								Mean square	
	RL <sup>a</sup>	SS <sup>b</sup>	PAN <sup>c</sup>	RL X SS	RL X PAN	SS X PAN	RL X SS X PAN	error		
Seaweed	7.325**	0.513	56.275***	0.718	1.294	0.394	1.166	3.124		
Boiled milk	0.000	0.126	22.451***	0.345	1.415	1.117	0.256	0.719		
Boiled potato	5.428*	0.566	13.067***	0.148	1.580	0.853	0.091	1.831		
Lemony	0.467	2.393	20.771***	0.467	0.815	0.826	0.393	0.922		
Sour	6.460*	1.475	16.738***	0.487	0.872	0.583	0.596	1.302		
Fishy	0.344	2.237	18.730***	1.552	2.994*	0.583	0.337	3.003		
Chickeny	3.174	1.656	25.072***	1.717	1.059	1.263	0.980	1.373		
Oily	0.293	0.321	17.482***	0.409	0.399	0.347	0.425	0.879		
Fresh	0.626	0.089	21.823***	0.197	2.437*	0.329	0.560	2.320		

\* p<0.05, \*\* p<0.01, \*\*\* p<0.001

<sup>a</sup> RL = ration level (75% and 100% of full ration)

<sup>b</sup> SS = swimming speed (0.5, 1.0, and 1.5 bl/s)

<sup>c</sup> PAN = panellists

**Table 17** Summarised ANOVA results of ration level, swimming speed and panellist effect on sensory flavour attributes of cultured chinook salmon (5 panellists, 9 panel days)

Flavour attributes	F ratio							Mean square error
	RL <sup>a</sup>	SS <sup>b</sup>	PAN <sup>c</sup>	RL X SS	RL X PAN	SS X PAN	RL X SS X PAN	
Flavour	0.962	1.535	55.939***	1.370	5.034**	12.138	0.944	1.634
Fishy	0.184	0.118	90.126***	0.116	0.368	1.959	0.686	2.300
Earthy	0.463	0.465	32.565***	0.181	0.564	0.564	0.506	0.850
Papery	0.341	0.172	22.615***	0.163	1.014	0.800	0.392	1.921
Bitter	0.165	0.279	10.905***	1.764	0.839	0.468	0.675	1.300
Sour	1.470	0.440	4.721**	0.914	0.629	0.742	0.280	1.224
Lemony	0.751	0.655	43.306***	0.829	0.238	0.486	0.615	0.568
Salty	2.745	0.122	61.585***	0.238	0.799	0.200	0.448	0.821
Spicy	0.911	0.787	42.922***	1.062	1.318	0.450	0.290	1.545
Brothy	7.430**	0.180	238.529***	0.497	3.565**	0.822	0.113	0.777

\* p<0.05, \*\* p<0.01, \*\*\* p<0.001

<sup>a</sup> RL = ration level (75% and 100% of full ration)

<sup>b</sup> SS = swimming speed (0.5, 1.0, and 1.5 bl/s)

<sup>c</sup> PAN = panellists



**Table 18** Summarised ANOVA results of ration level, swimming speed and panellist effect on sensory texture attributes of cultured chinook salmon (5 panellists, 9 panel days)

Texture attributes	F ratio							Mean square error
	RL	SS	PAN	RL <sup>a</sup> X SS <sup>b</sup>	RL X PAN <sup>c</sup>	SS X PAN	RL X SSX PAN	
Moistness	4.073*	1.791	29.924***	0.306	0.714	0.823	0.534	2.246
Powderiness	0.663	0.197	10.727***	0.249	0.713	0.621	0.257	3.560
Flakiness	0.340	0.081	36.403***	0.797	0.846	0.724	0.881	2.410
Firmness	9.363**	2.029	6.565***	0.189	1.172	0.253	0.844	3.147
Chewiness	0.347	0.807	19.140***	0.868	0.816	0.780	0.541	3.615
Cohesiveness	5.037*	0.058	14.226***	0.331	0.772	0.915	1.604	2.577
Adhesiveness	0.027	0.593	26.163***	0.388	0.648	0.548	1.190	2.296
Mushiness	13.851***	0.622	41.549***	2.757	2.539*	0.942	0.698	2.531
Overall	2.158	0.665	11.362***	0.373	3.717**	1.953*	0.794	1.702
Acceptability								

\* p<.05, \*\* p<.01, \*\*\* p<.001

<sup>a</sup> RL = ration level (75% and 100% of full ration)

<sup>b</sup> SS = swimming speed (0.5, 1.0, and 1.5 bl/s)

<sup>c</sup> PAN = panellists

#### **4.1.2.5 Z-transformation of sensory attributes significantly affected by the treatment**

A z-transformation to remove the panellist effect was performed on the eight attributes that were significantly affected by RL. With the panellist effect removed, analysis of variance revealed that "Boiled Potato" was no longer significantly affected by RL (Table 19). The F scores from several of the other attributes, including "Seaweedy," "Sour" (aroma), "Brothy," "Cohesiveness" and "Mushiness" also decreased while those of "Firmness" and "Moistness" were largely unchanged.

#### **4.1.2.6 PCA and PCS of sensory data**

PCA and then PCS were performed on the transformed sensory attributes. The factor score coefficients of the first 6 PC and the percentage of the total variance that they explain are listed in Table 20. These six PCs, cumulatively accounting for approximately 92% of the variation in the data, were fed into the PCS program to calculate slope and coefficient of determination ( $r^2$ ).

PCS proved to be an ideal analysis method. First, the use of PCS is most effective when the individual PC do not account for a large percentage of the variation (Vodvotz et al., 1993) or the size of the data matrix is fairly large (Furtula et al. 1994a). The sensory data in this experiment fit both of these criteria; the first PC only accounted for 40 percent of the variation and the data set was extremely large.

PCS also has the capacity to represent a larger number of PC scores for graphic illustration than PCA alone (Furtula et al. 1994a) as well as giving better group resolution (Vodovotz et al., 1993). When graphs were produced from different pairs of PCs (not included), no clumping or trends were observed. In these types of graphs portions of the data that may be important are overlooked

**Table 19** Summarised ANOVA results of ration level and swimming speed effect on standardised data<sup>a</sup> from significant sensory attributes of cultured chinook salmon (5 panellists; 9 panel days)

Sensory attributes	F ratio			Mean square error
	Ration level <sup>b</sup>	Swimming speed <sup>c</sup>	RL X SS	
Seaweed <sup>d</sup>	7.840**	0.548	0.833	0.871
Boiled Potato <sup>d</sup>	3.681	0.684	0.071	1.013
Sour <sup>d</sup>	5.823*	1.057	0.200	0.992
Brothy <sup>e</sup>	4.839*	0.187	0.836	0.998
Moisture <sup>f</sup>	4.235*	2.344	0.329	0.987
Firmness <sup>f</sup>	9.964**	2.334	0.121	0.968
Cohesiveness <sup>f</sup>	4.731*	0.113	0.140	1.003
Mushiness <sup>f</sup>	10.264**	0.728	2.077	0.965

\* p<.05, \*\* p<.01, \*\*\* p<.001

<sup>a</sup> Standardised data = panellist effect removed by z transformation of all scores within each panellist

<sup>b</sup> ration level = 75% and 100% of full ration

<sup>c</sup> swimming speed = 0.5, 1.0, and 1.5 bl/s

<sup>d</sup> aroma term

<sup>e</sup> flavour term

<sup>f</sup> texture term

**Table 20** Factor score coefficients of the first 6 principal components of the z-transformed sensory attribute scores of the RL X SS treated chinook salmon samples (5 panellists; 9 panel days)

Sensory attributes	Principal Components					
	1	2	3	4	5	6
Seaweediness	0.161	0.341	0.234	0.822	-0.252	0.524
Boiled potato	-0.199	0.266	0.251	-0.347	-0.748	0.307
Sour	0.128	-0.222	0.851	0.049	0.146	-0.458
Brothy	-0.069	0.551	0.306	-0.372	0.585	-0.016
Moistness	0.237	0.224	-0.270	-0.070	0.463	0.198
Firmness	-0.273	0.099	0.014	0.152	0.239	-0.183
Cohesiveness	-0.202	-0.311	0.176	0.121	0.474	1.018
Mushiness	0.236	-0.103	0.118	-0.639	-0.152	0.609
Variance explained						
Fraction	0.398	0.177	0.119	0.090	0.083	0.061
Cumulative	0.398	0.575	0.694	0.784	0.867	0.928

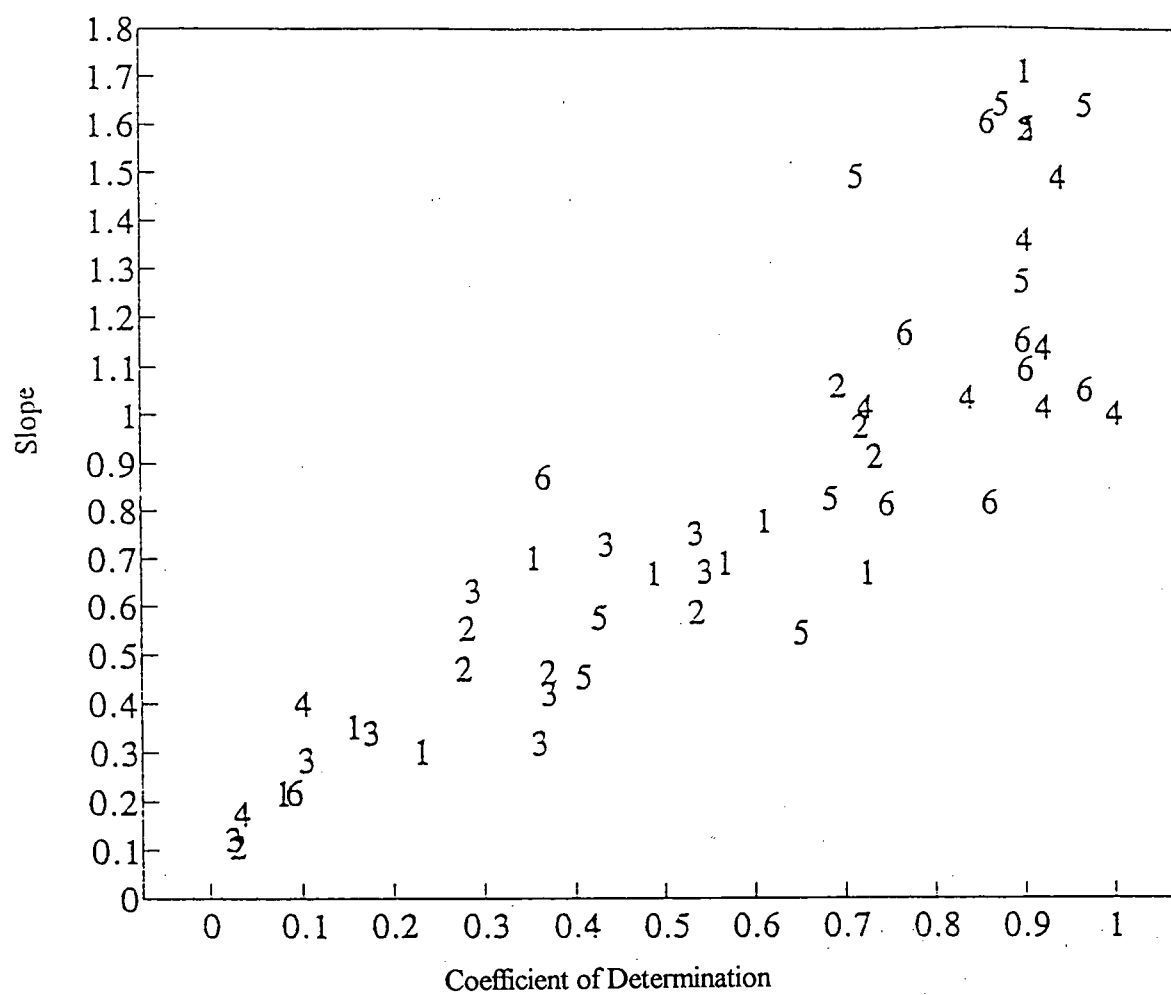
(Vodovotz et al., 1993). In flavour analysis this is critical because seemingly minor compounds often play critical roles in constituting characteristic flavour notes (Vodovotz et al., 1993).

The reference chosen for PCS was a slow SS, high RL (treatment 4) replicate from panellist 1. This treatment was singled out as the reference because, according to Kiessling et al. (1994b), this was the most cost effective of the six treatments. The particular replicate used for Figures 8-11 gave the best group resolution of those tested.

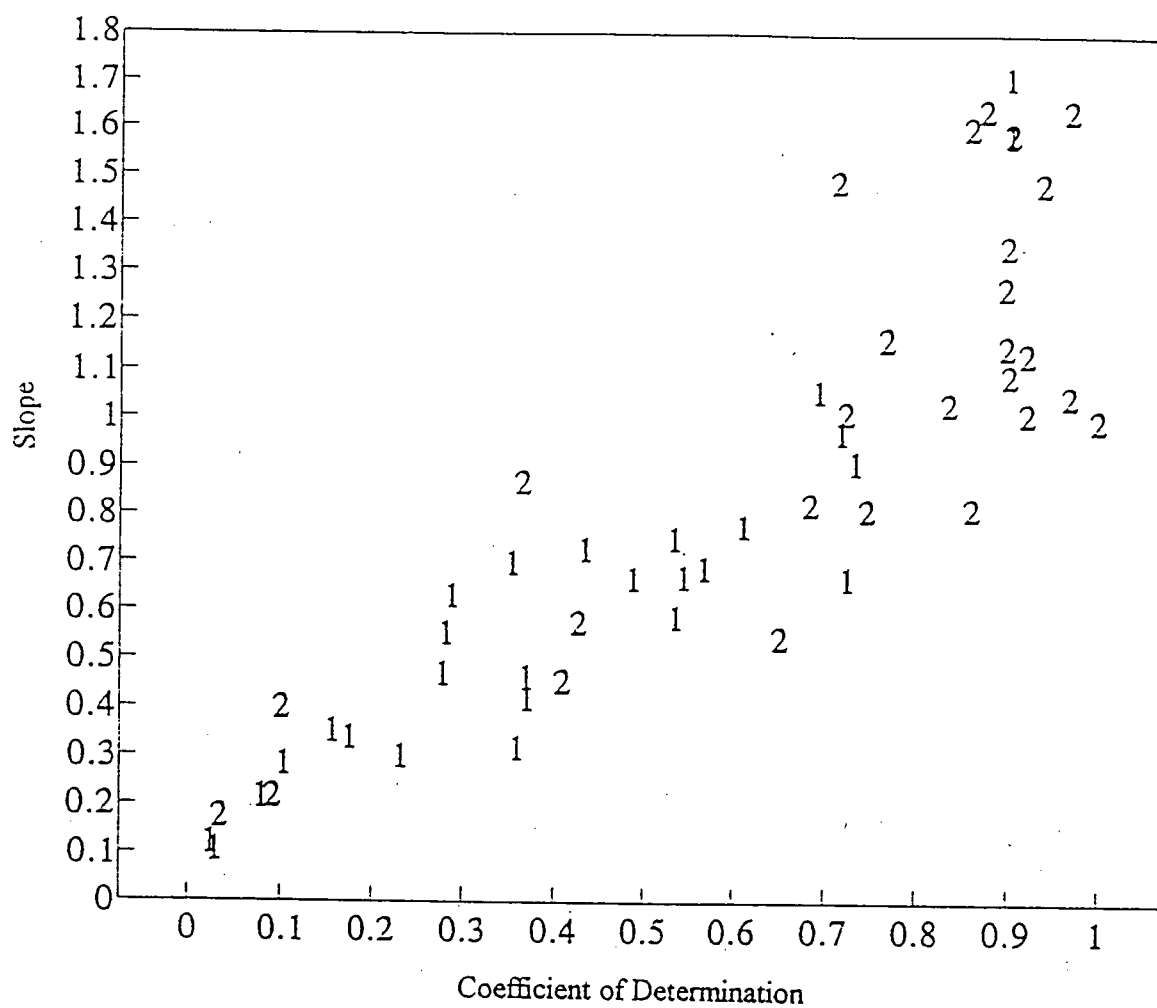
The PCS results were imported into a spreadsheet, where a series of graphs of  $r^2$  versus slope were constructed. The data points were labelled with either the treatment number, ration level number, swimming speed number or the panel day that they represented (Figures 8-11 respectively). These results served to reconfirm that ration level, and not swimming speed, was largely responsible for the effect on the sensory properties of the cooked salmon. In Figure 9, although a small degree of overlap is present, the two ration levels are largely in two separate groups. The lower RL was in the lower, left side of the graph, while the higher RL was primarily in the upper right portion of the graph. In Figure 10, where the data points are labelled with the SS level, the three swimming speeds appear randomly scattered indicating that SS did not affect the sensory properties of the salmon.

There was no appreciable evidence of an interaction effect observable from the treatment number graphs (Figure 8). Within the groups of treatments with the same RL, data from the three SS appear randomly distributed.

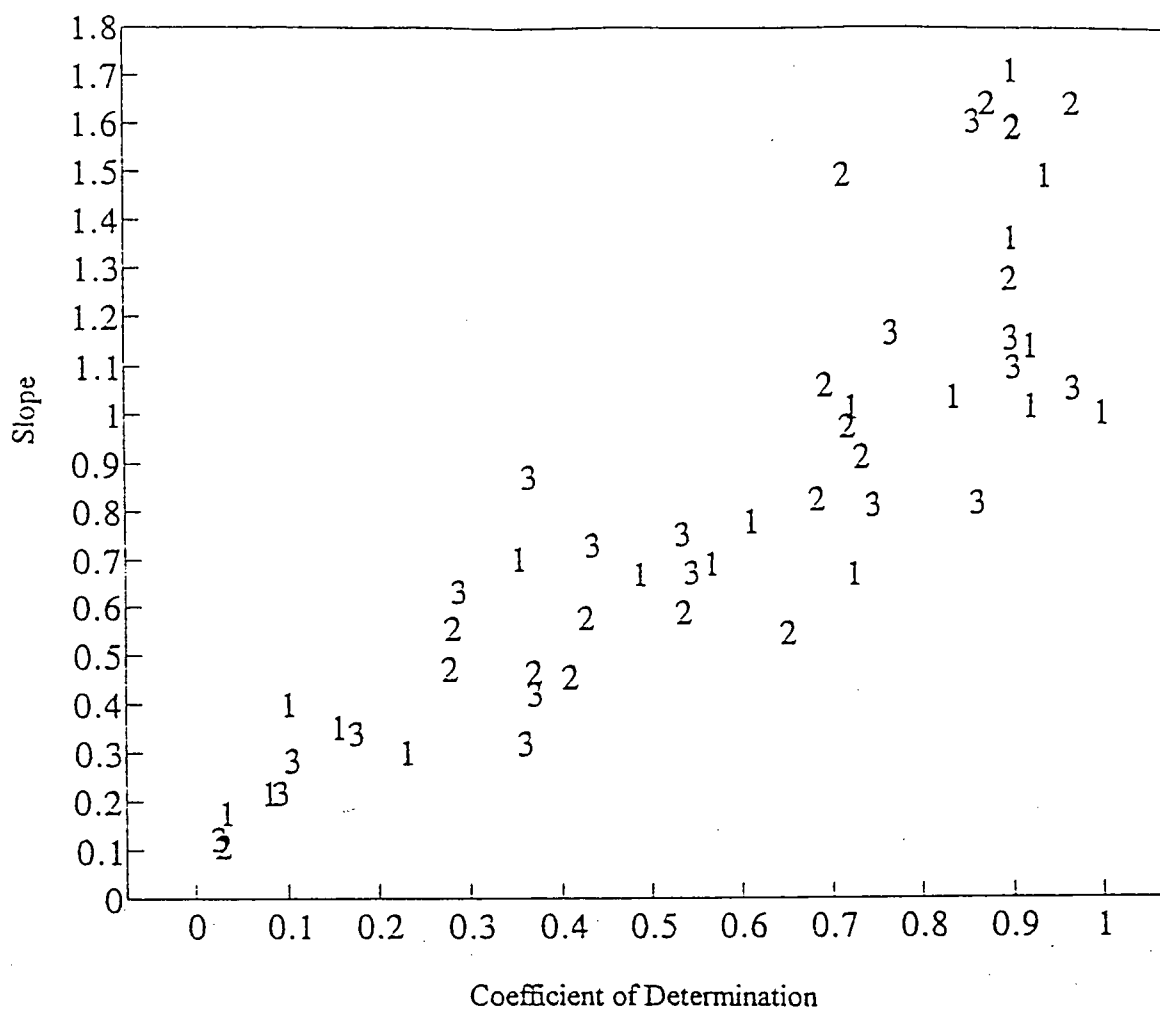
Some of the overlapping of the two RL in Figure 9 may have been due to fish to fish variability. Individual fillets were used in this experiment, and not samples compiled from several fish from the same treatment, as in all the other portions of this research. Fish to fish variation may be partially



**Figure 8** PCS graph of significant sensory attributes, data points labelled with treatment numbers

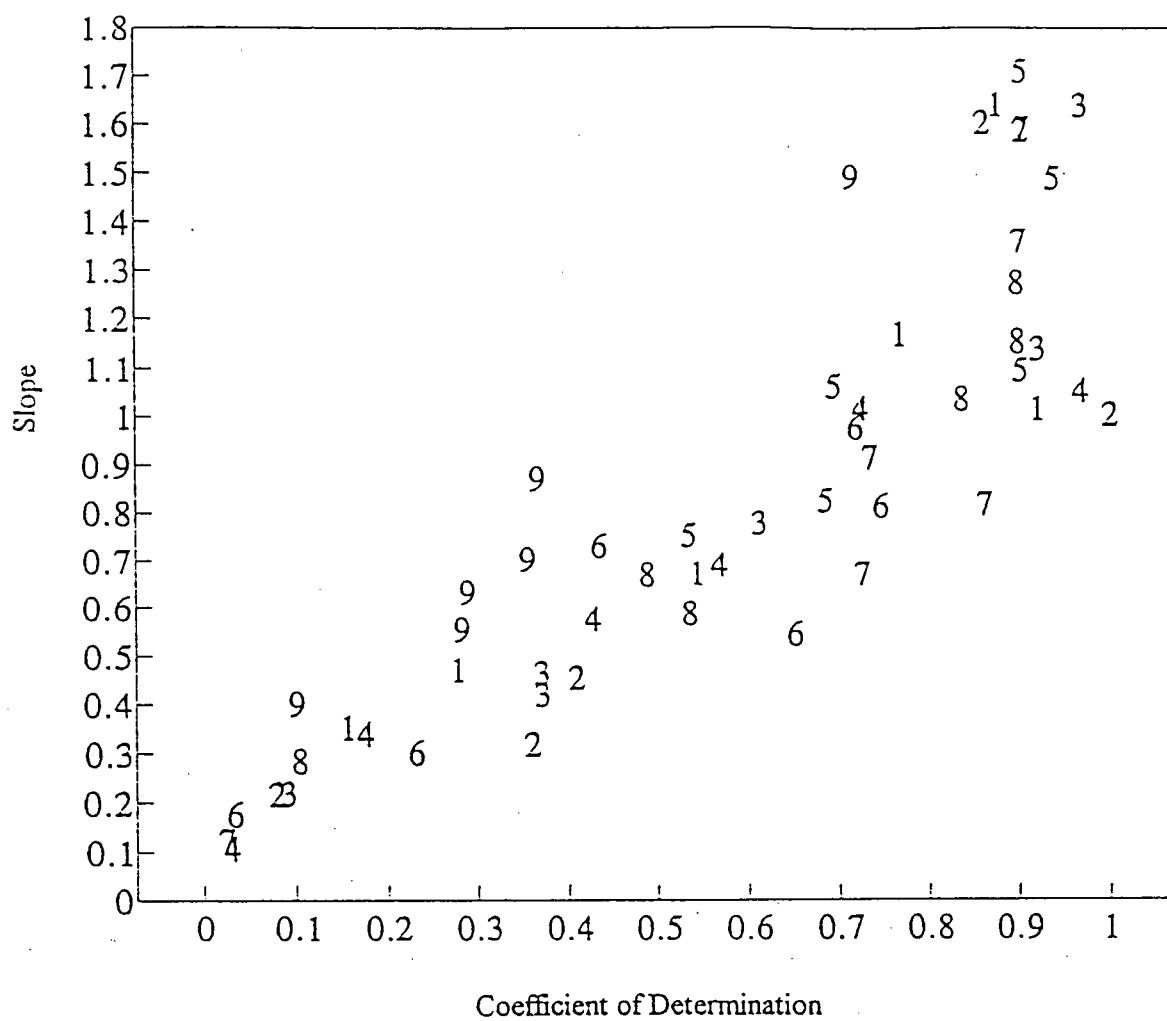


**Figure 9** PCS graph of significant sensory attributes, data points labelled with ration level numbers (1 = 75%; 2 = 100% ration level)



**Figure 10** PCS graph of significant sensory attributes, data points labelled with swimming speed numbers (1=0.5, 2=1.0, 3=1.5 bl/s)





**Figure 11** PCS graph of significant sensory attributes, data points labelled with panel day number

responsible for this lack of clear separation that is evident in the GC headspace data. Graphs using the panel day numbers (Fig. 11) showed no obvious day effect.

#### **4.1.2.7 Effect of SS on the sensory attributes**

From these results, it appears that changing swimming speed did not result in significant changes in the aroma, taste and texture of the cooked fish. This conclusion agrees with the results of Kiessling et al. (1994a,b). Kiessling et al. (1994b), measuring the proximate composition of the fish from this study, did not find that SS had had any effect on the composition of the chinook fillets (Kiessling et al. 1994b). Furthermore, Kiessling et al. (1994a) did not find any evidence of hypertrophy in the white muscle of the salmon due to exercise. Additionally, no evidence was found to indicate that SS affected the total muscle area. Also, SS did not influence the fibre size distribution.

Kiessling et al. (1994a), however, did find a significant increase in the amount of fibre hypertrophy in the red muscle that occurred as a result of the increase in SS. However, since the red muscle is only a fraction of the size of the white muscle tissue, it is unlikely that the hypertrophy of red muscle would have a large impact on the texture of the fish.

#### **4.1.2.8 Effect of RL on the sensory attributes**

RL significantly affected some aspects of the taste, aroma and texture of the treated fish samples. This is likely attributable to the significant difference in fat content between the two RL noted by Kiessling et al. (1994b). Fat content can affect the mouthfeel, aroma and taste of a food system. Van Gemert et al. (1987), working with smoked sausages, found that there was a strong positive linear relationship between the percentage of fat in the sausages and their odour intensity.

Fat can affect the mouthfeel qualities of food systems in several ways. First, it lubricates the food while it is being chewed. Additionally, fat also imparts an oily sensation in the mouth; this affects the surface tension and can cause changes in the viscosity of the product (Szczesniak, 1963).

#### **4.1.3 Instrumental analysis**

#### **4.1.4 GC headspace analysis**

As with the sensory data analyses, ANOVA, PCA and PCS were used to determine what, if any, effect the treatment had had on the headspace gases. Of the 71 GC peaks that consistently appeared, 27 were significant for either SS or RL (Table 21, Figure 12). Significant peaks were found throughout the graph, and included both large and small peaks. PCA was performed using the areas of the significant peaks. The factor score coefficients from the first seven PC and the percentage of total variance explained by each of these is presented in Table 22. The graphs of the PCS results, once again using a slow SS, high RL treatment for the reference and labelled with treatment number, ration level number and swimming speed number, (Figures 13-15 respectively) were similar to those produced from the sensory data (Figures 8-11). Again, there is a very clear group resolution of the data due to ration level (Figure 14).

The ration level PCS graphs of both the GC and sensory results showed very similar cluster patterns. In both, the data points fell into the same pattern; the lower ration level having a coefficient of determination between 0 and 0.8, with a range in slopes between 0 and 1. The higher ration level fell in the range of 0.7-1 coefficient of determination with a slope range of 0.8-2.0.

**Table 21** Peak labels, retention times and level of significance for ration level and swimming speed of GC peaks that consistently appeared in purge and trap GC headspace analysis of cultured, cooked chinook salmon

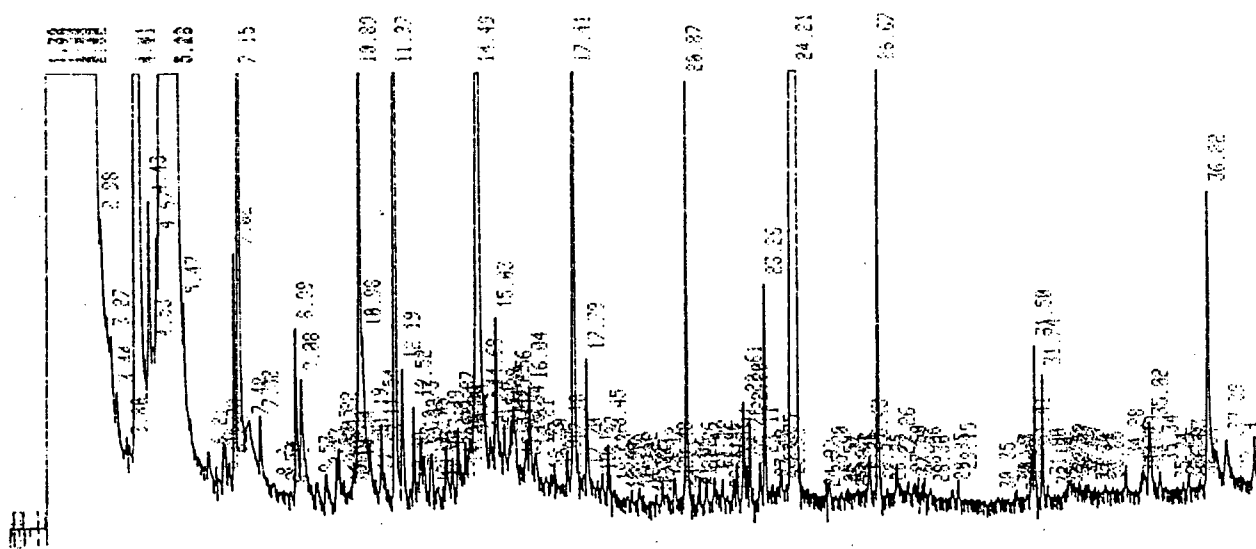
Retention time (minutes)	Peak labels	Significance		
		RL <sup>a</sup>	SS <sup>b</sup>	RL X SS
6.69	a	*		
7.08	b			
7.21	c	*		
7.82	d	**		
8.94	e	**		
9.1	f			
9.51	g			
9.84	h			
10.25	i			
10.4	j			
10.93	k	*		
10.97	l			
12.19	m			
12.52	n			
12.74	o			
13.04	p			
13.19	q	*		
13.49	r			
13.68	s	*		
13.87	t			
14.1	u	**		
14.5	v			
15.04	w	*		
15.28	x			*
15.29	y	*		
15.49	z	*		
15.57	aa	**		
15.96	ab	*		
16.05	ac			
16.21	ad	*		
16.68	ae			
17.19	af			
17.4	ag	*		
17.81	ah			
18.27	ai			
18.45	aj			
19.18	ak			
19.42	al			

Retention time (minutes)	Peak labels	Significance		
		RL <sup>a</sup>	SS <sup>b</sup>	RL X SS
20.12	am			
20.51	an	***	**	**
20.88	ao	*		
21.27	ap			
21.48	aq	*		
21.72	ar	*		
21.97	as			
22.32	at			
22.42	au			
22.62	av			
22.71	aw			
22.81	ax			
23.12	ay			
23.27	az	*		
25.21	ba			
26.41	bb	***		
26.68	bc	*		
27.27	bd	*		
27.61	be	*	*	*
28.07	bf			
29.16	bg			
30.94	bh			
31.42	bi			
31.51	bj			
31.75	bk			
31.91	bl			
33.14	bm	*	*	
35.03	bn			
35.37	bo			
36.23	bp			
36.58	bq			
36.84	br			
38.28	bs	*		

<sup>a</sup> RL = ration level (75% and 100% of full ration)

<sup>b</sup> SS = swimming speed (0.5, 1.0, and 1.5 bl/s)

<sup>c</sup> Level of significance \* p<.05, \*\* p<.01, \*\*\* p<.001



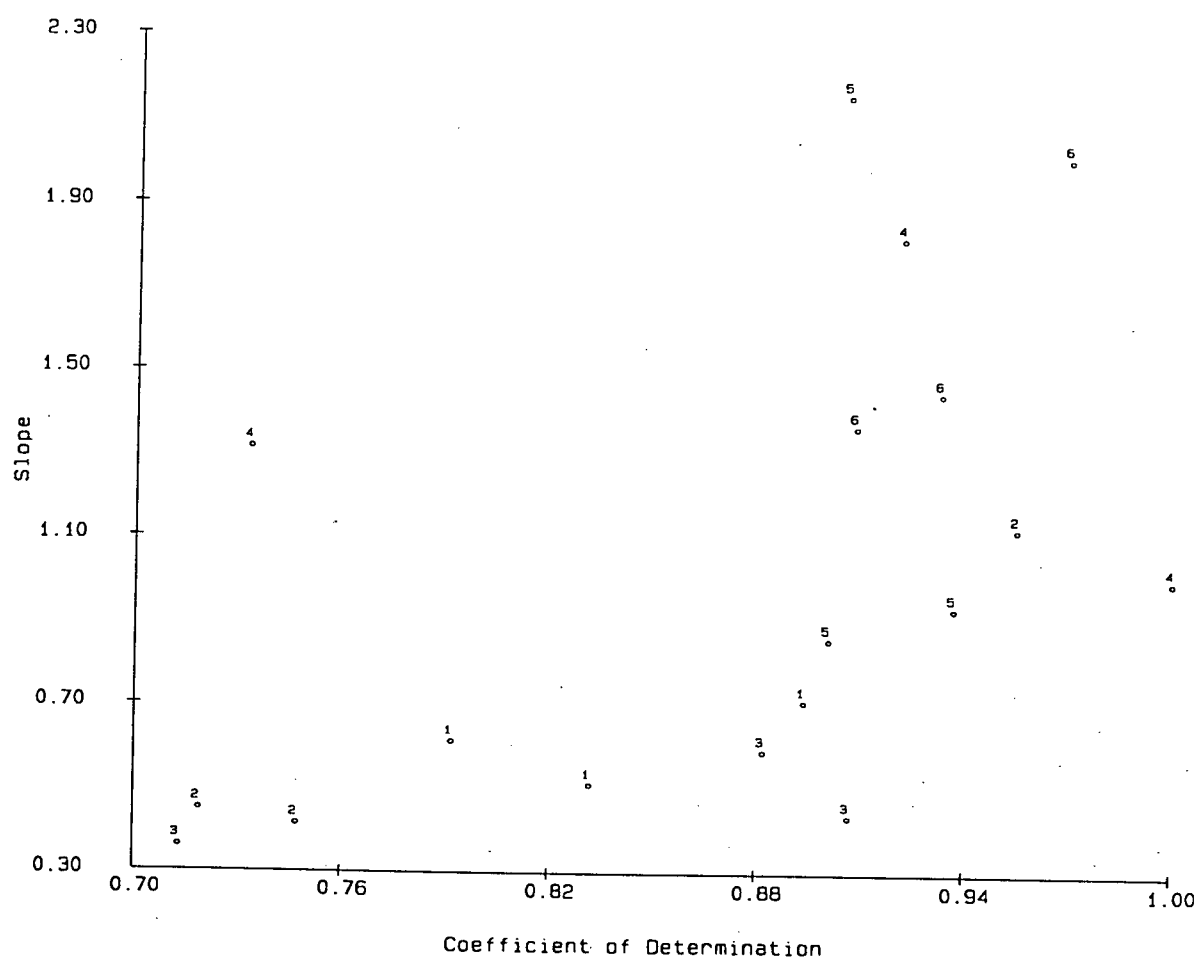
**Figure 12** Sample gas chromatogram from a purge and trap extract of cultured chinook salmon

**Table 22** Factor score coefficients of the first 7 principal components from GC headspace peaks significantly affected by either ration level<sup>a</sup> or swimming speed<sup>b</sup>

Peak Label	Principal Components						
	1	2	3	4	5	6	7
A	0.741	-0.547	-0.102	0.205	0.087	-0.173	-0.187
C	0.735	0.582	-0.147	0.008	-0.225	0.134	0.131
D	0.841	0.364	-0.011	0.107	0.054	-0.238	0.240
E	0.932	0.090	-0.286	-0.045	0.038	-0.029	0.016
K	0.734	0.591	-0.001	0.007	-0.200	-0.199	0.175
Q	0.468	0.587	-0.057	0.386	0.418	-0.181	0.011
S	0.847	-0.329	0.067	-0.117	0.119	-0.039	-0.253
U	0.774	0.183	-0.441	0.059	0.086	0.223	-0.140
W	0.748	-0.599	0.075	-0.157	0.055	-0.042	0.136
X	0.529	-0.489	0.115	-0.547	0.250	0.020	0.286
Y	0.682	-0.427	0.301	0.275	-0.188	0.033	0.288
Z	0.796	-0.423	0.047	0.215	0.163	-0.234	-0.196
AA	0.676	-0.543	0.139	0.416	0.031	0.024	0.079
AB	0.596	0.292	-0.681	0.016	0.131	0.247	0.054
AD	0.673	0.524	0.135	0.184	0.297	0.150	-0.030
AG	0.768	0.484	0.189	0.094	-0.276	-0.082	-0.116
AN	0.713	-0.257	-0.477	-0.232	-0.077	0.141	-0.087
AO	0.710	0.134	-0.291	-0.358	-0.178	-0.151	-0.221
AQ	0.845	-0.183	0.271	-0.026	-0.121	-0.155	-0.207
AR	0.922	0.042	0.095	-0.082	-0.113	0.016	0.004
AZ	0.782	0.421	0.163	-0.210	-0.099	-0.239	0.048
BB	0.861	0.021	-0.077	-0.123	0.125	0.391	-0.055
BC	0.644	-0.344	0.448	0.160	0.059	0.278	0.097
BD	0.841	-0.056	0.127	-0.238	0.113	-0.107	0.161
BE	0.412	-0.539	-0.463	0.264	-0.388	0.003	0.068
BM	0.161	0.296	0.863	-0.228	0.070	0.011	-0.108
BS	0.433	0.134	0.722	0.081	-0.202	0.355	-0.149
Variance explained							
Fraction	0.517	0.155	0.114	0.050	0.034	0.031	0.024
Cumulative	0.517	0.672	0.786	0.836	0.087	0.901	0.925

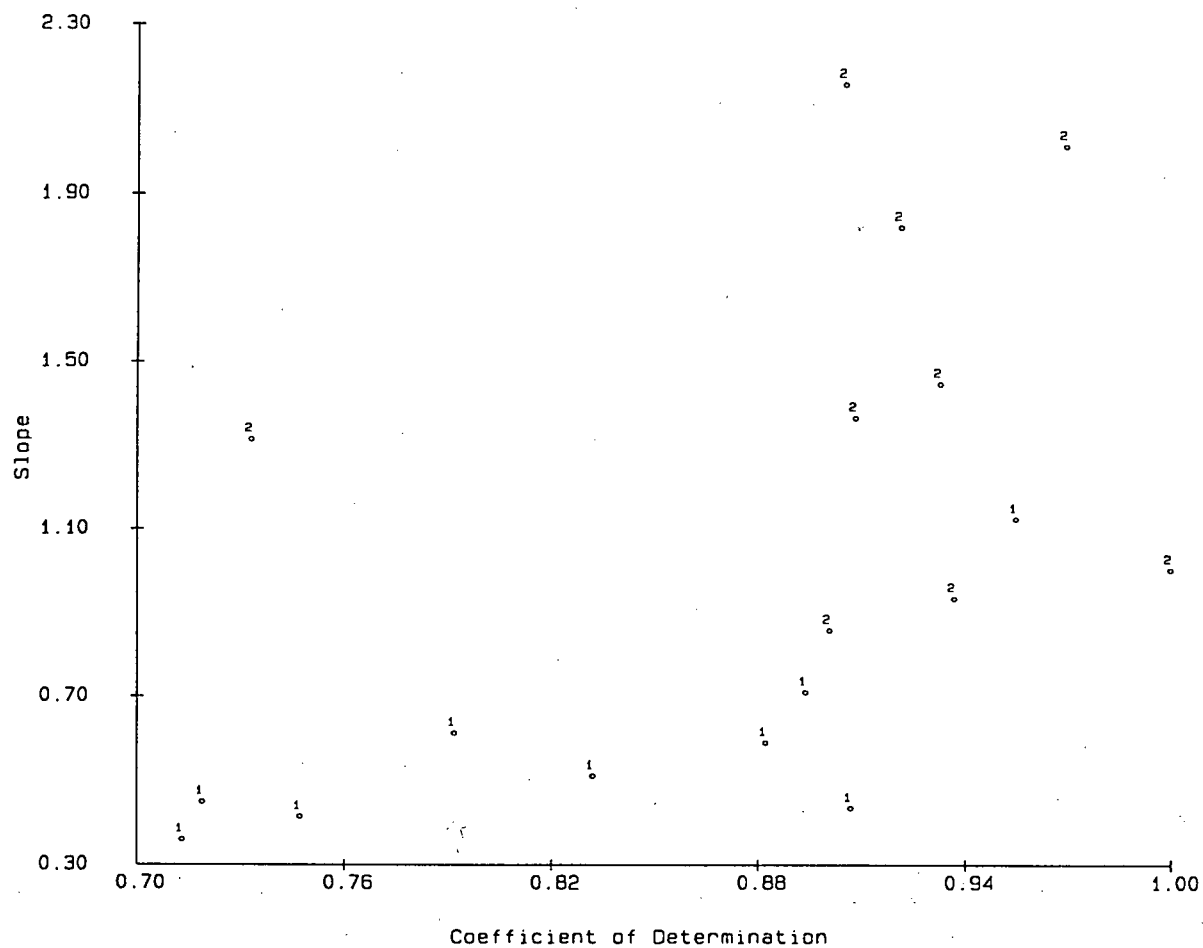
<sup>a</sup> ration level = 75% and 100% of full ration

<sup>b</sup> swimming speed = 0.5, 1.0, and 1.5 bl/s

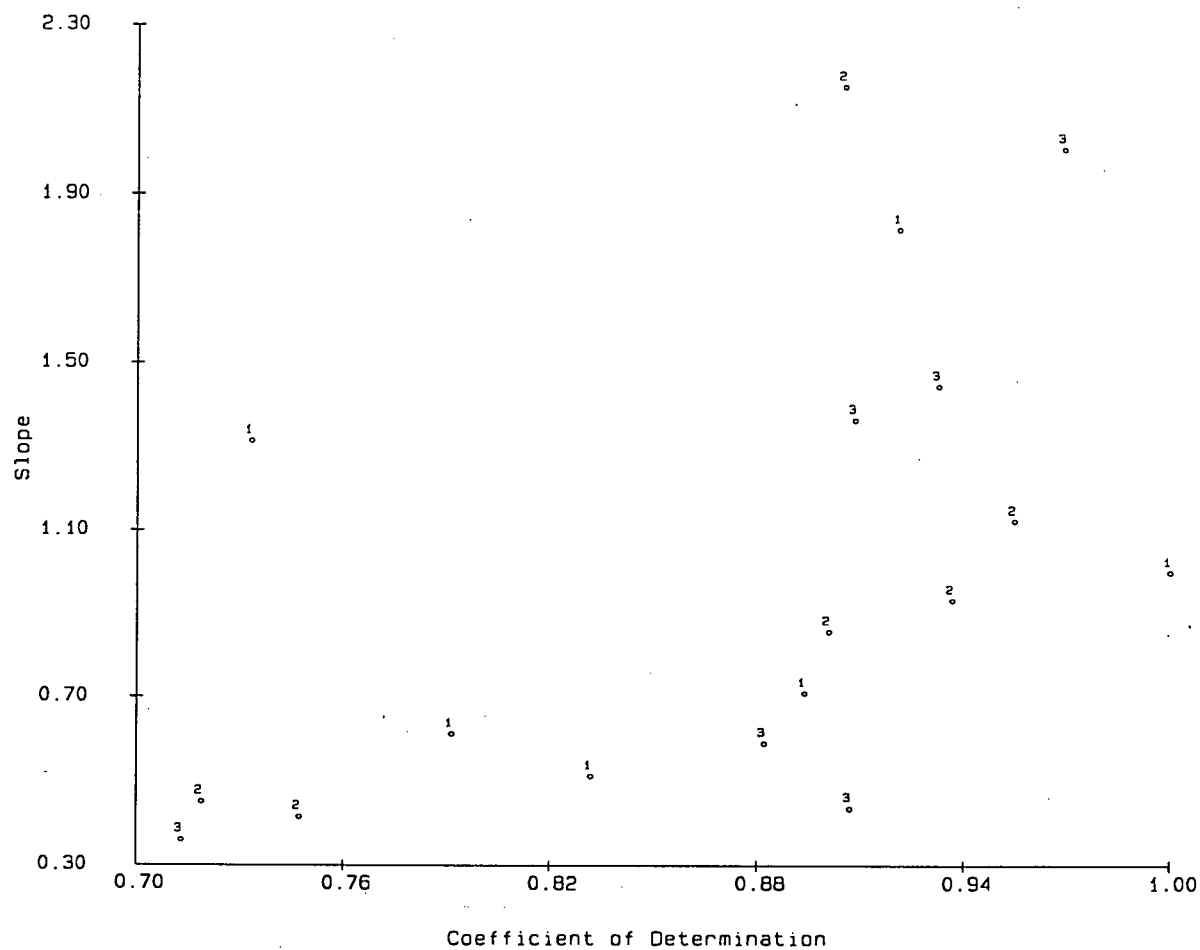


**Figure 13** PCS graph of significant GC peaks produced from a purge and trap headspace extraction of cooked, cultured chinook salmon, data points labelled with treatment numbers





**Figure 14** PCS graph of significant GC peaks produced from a purge and trap headspace extraction of cooked, cultured chinook salmon, data points labelled with ration level number (1 = 75%, 2 = 100% ration level)



**Figure 15** PCS graph of significant GC peaks produced from a purge and trap headspace extraction of cooked, cultured chinook salmon, data points labelled with swimming speed number (1 = 0.5, 2 = 1.0, 3 = 1.5 bl/s)

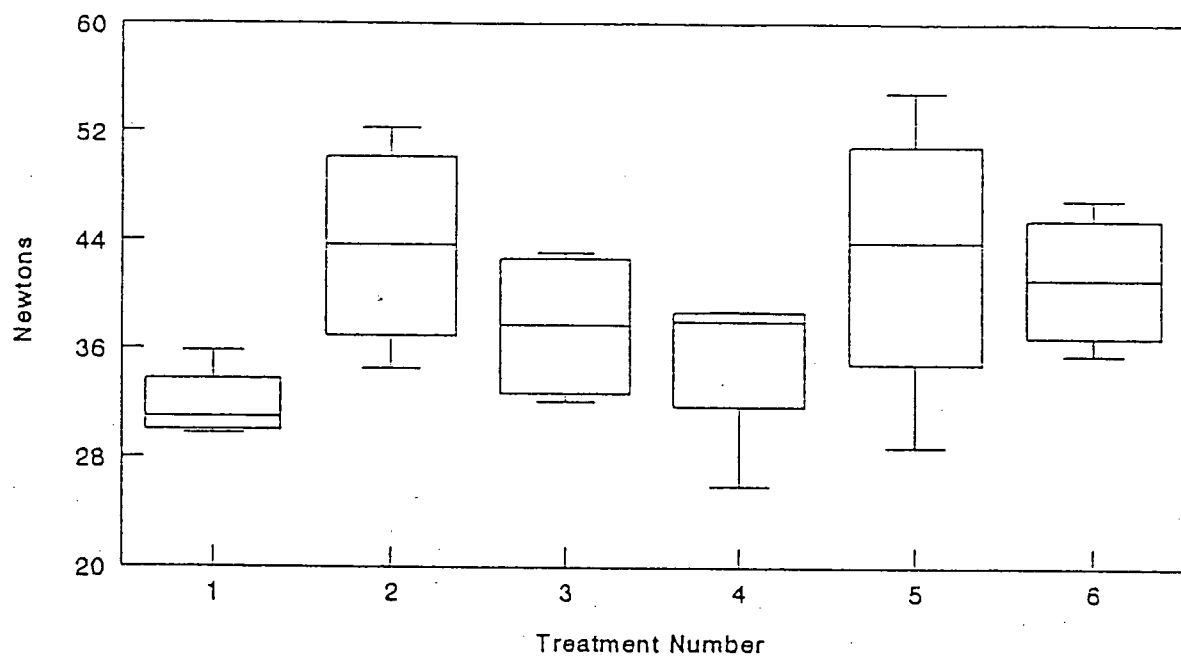
#### 4.1.5 Instron TPA analysis

Six TPA parameters were determined from the curves produced by the Instron Universal testing machine (Figures 16 - 21). Two factor ANOVAs, looking at the effect of RL, SS and the interaction of these treatments, were performed on all six parameters. The results of these analyses are presented in Table 23. Unlike the sensory texture results, SS and not RL was significant. Hardness 1, Firmness 1 and 2 were all significantly affected by SS ( $p < 0.05$ ).

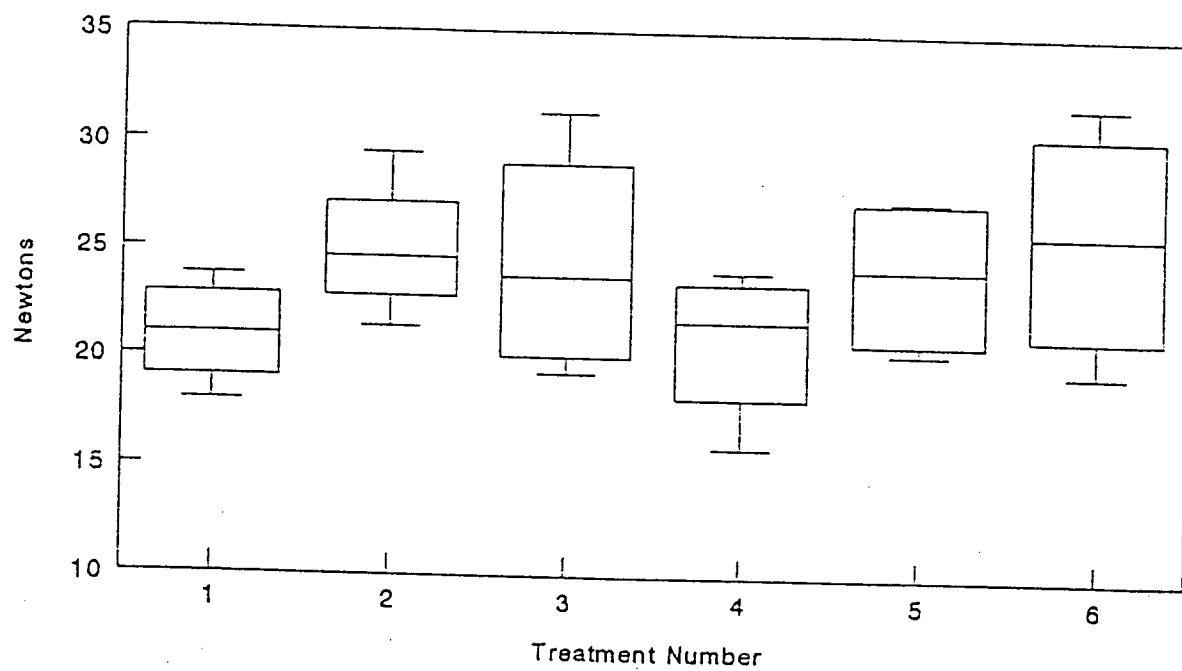
Dunajski (1979) did not feel that devices used for rheological testing of food were suitable to measure the texture of fish muscle. She felt that at best they may be applicable to raw, but not to cooked fish. She pointed out that when fish is eaten, the majority of the energy is used for mastication. As a result, the mechanical measurement should measure the resistance of the fibres to mechanical disintegration (Dunajski, 1979). In this experiment, only compression forces were measured. Since the force required to shear the fibres was not measured, this is likely not a true representation of the texture experienced by the panellists.

It is also possible that the reason for the disagreement between the sensory and Instron results may be due to the way in which the samples were prepared. Samples for the sensory tests composed of slices of the fish placed side by side, gelled together into a composite sample upon cooking. For the Instron samples, once cooked and cooled to room temperature, the fish was flaked and formed into a cylinder. It is possible that these differences in sample temperature and structure contributed to the discrepancy in results.

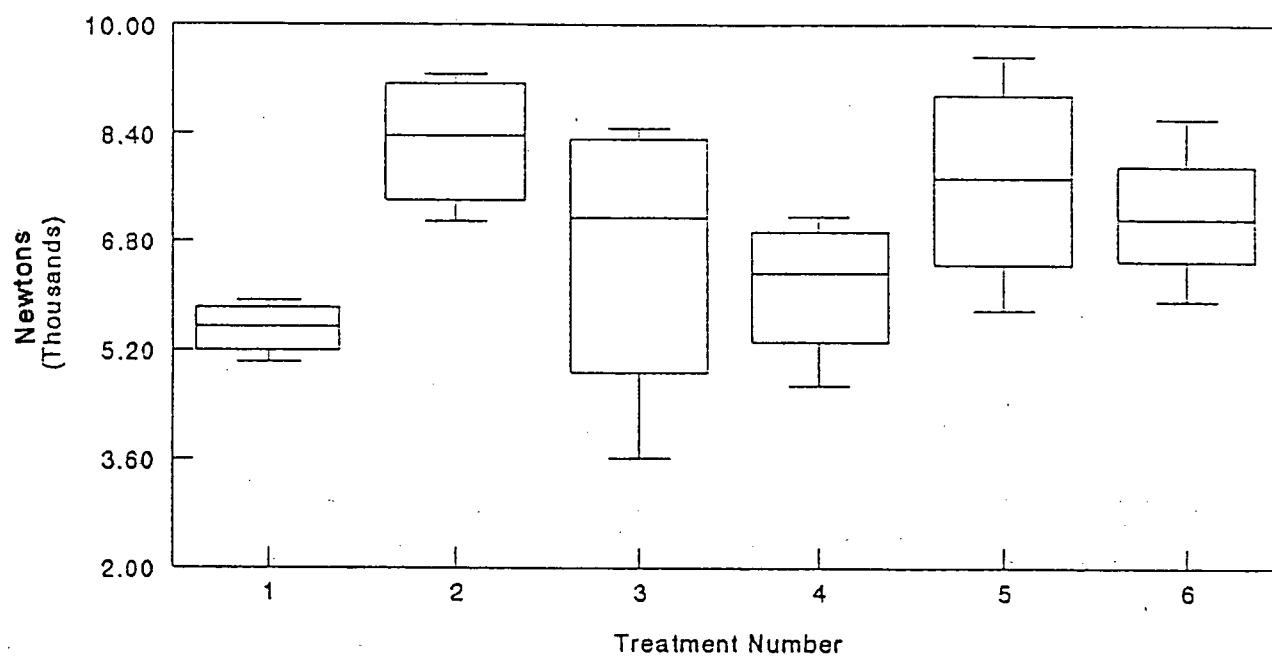
There were many difficulties producing consistent samples. The cylinders of fish would often fall apart and/or distort, prior to, or in the process of transportation to the Instron platform for testing. Additionally, the production of samples of consistent height proved to be nearly impossible. Following



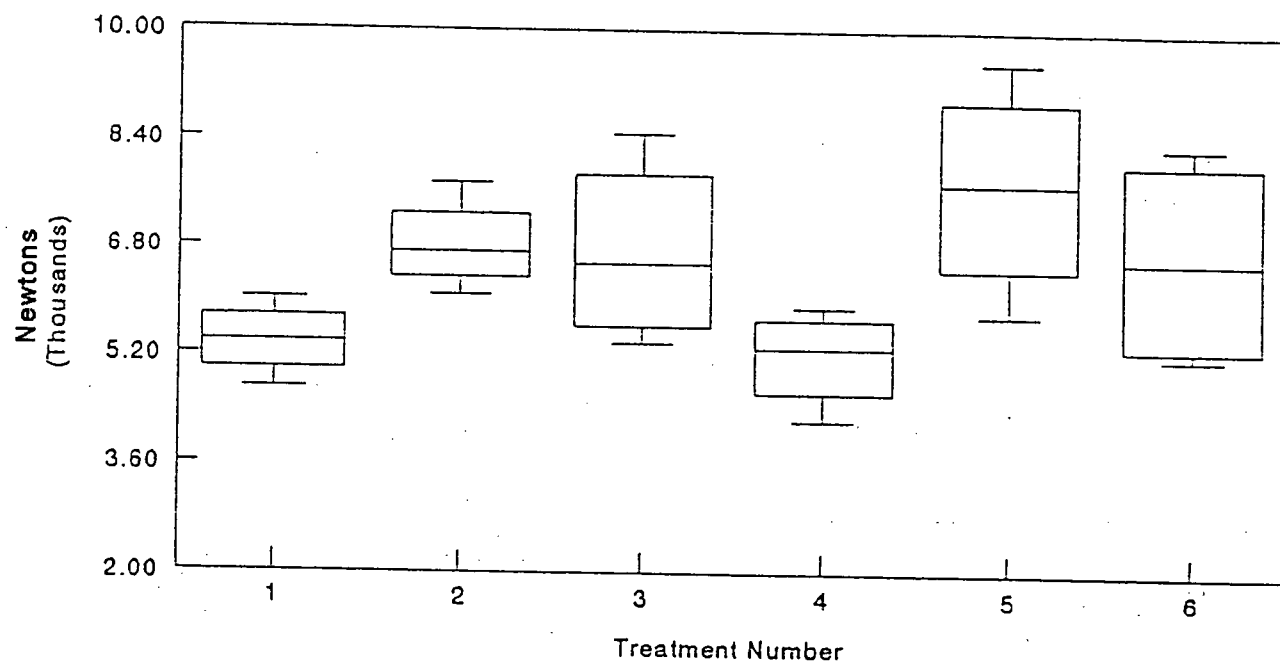
**Figure 16** Boxplot of the Instron TPA parameter Hardness 1 for cooked, cultured chinook salmon samples, results by treatment number



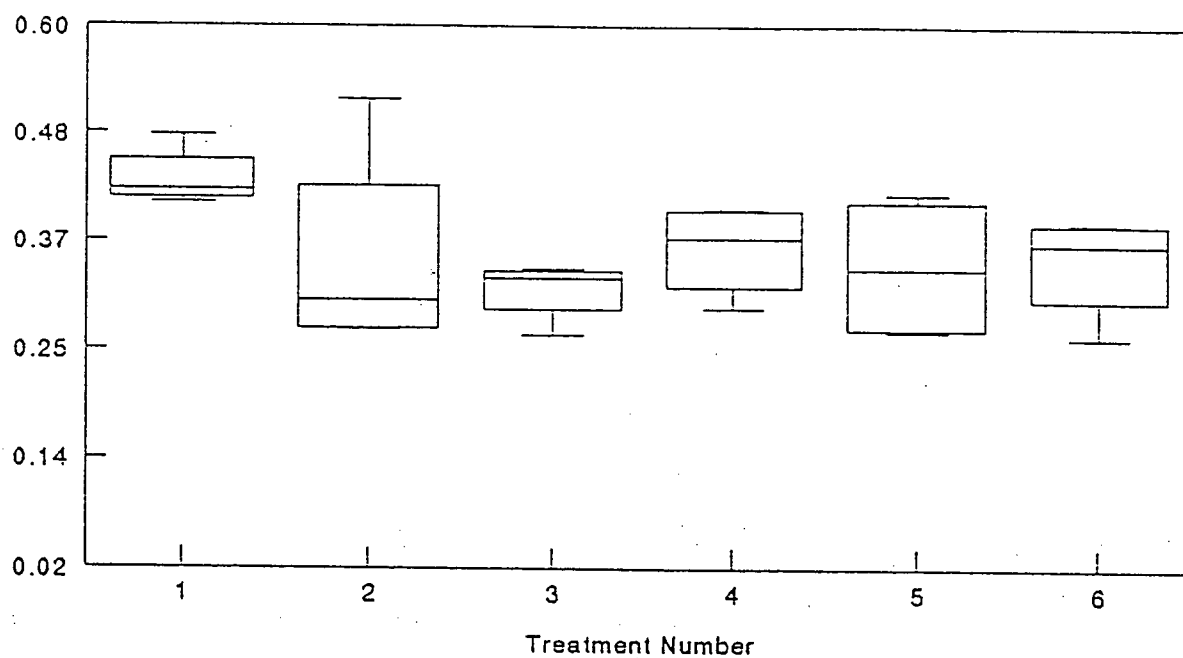
**Figure 17** Boxplot of the Instron TPA parameter Hardness 2 for cooked, cultured chinook salmon samples, results by treatment number



**Figure 18** Boxplot of the Instron TPA parameter Firmness 1 for cooked, cultured chinook salmon samples, results by treatment number

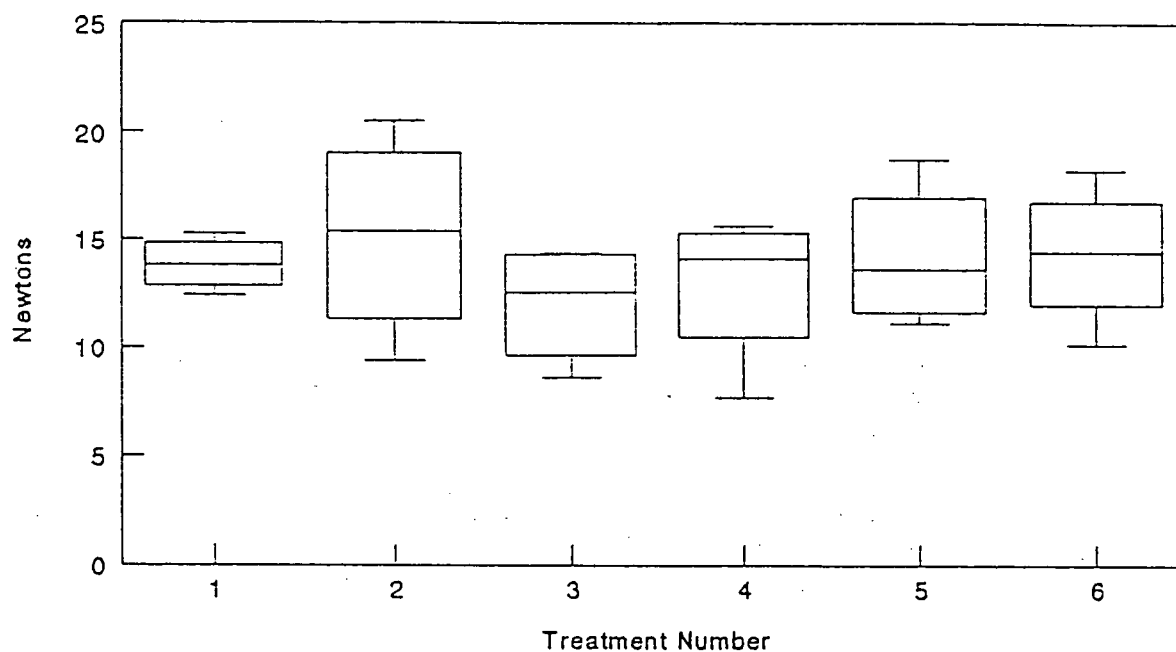


**Figure 19** Boxplot of the Instron TPA parameter Firmness 2 for cooked, cultured chinook salmon samples, results by treatment number



**Figure 20** Boxplot of the Instron TPA parameter Cohesiveness for cooked, cultured chinook salmon samples, results by treatment number





**Figure 21** Boxplot of the Instron TPA parameter Gumminess for cooked, cultured chinook salmon samples, results by treatment number

**Table 23** Summarised ANOVA results of ration level and swimming speed effect on Instron TPA parameters of cooked cultured chinook salmon

Sensory attributes	F ratio			Mean square error
	Ration level	Swimming speed	Ration level X Swimming speed	
Hardness1	0.533	3.888 <sup>*a</sup>	0.229	48.823
Hardness2	0.000	2.343	0.140	17.912
Firmness1	0.173	5.077 <sup>*</sup>	0.483	4.775
Firmness2	0.226	6.063 <sup>*</sup>	0.512	3.571
Cohesiveness	0.434	2.115	1.222	0.005
Gumminess	0.010	0.507	0.615	0.120

\* p<.05, \*\* p<.01, \*\*\* p<.001

<sup>a</sup> Tukey test results: swimming speeds 0.5 bl/s significantly different from 1.0 bl/s (p<0.05)

compression of the sample to the desired height and release of the plunger on the syringe, the sample would spring back.

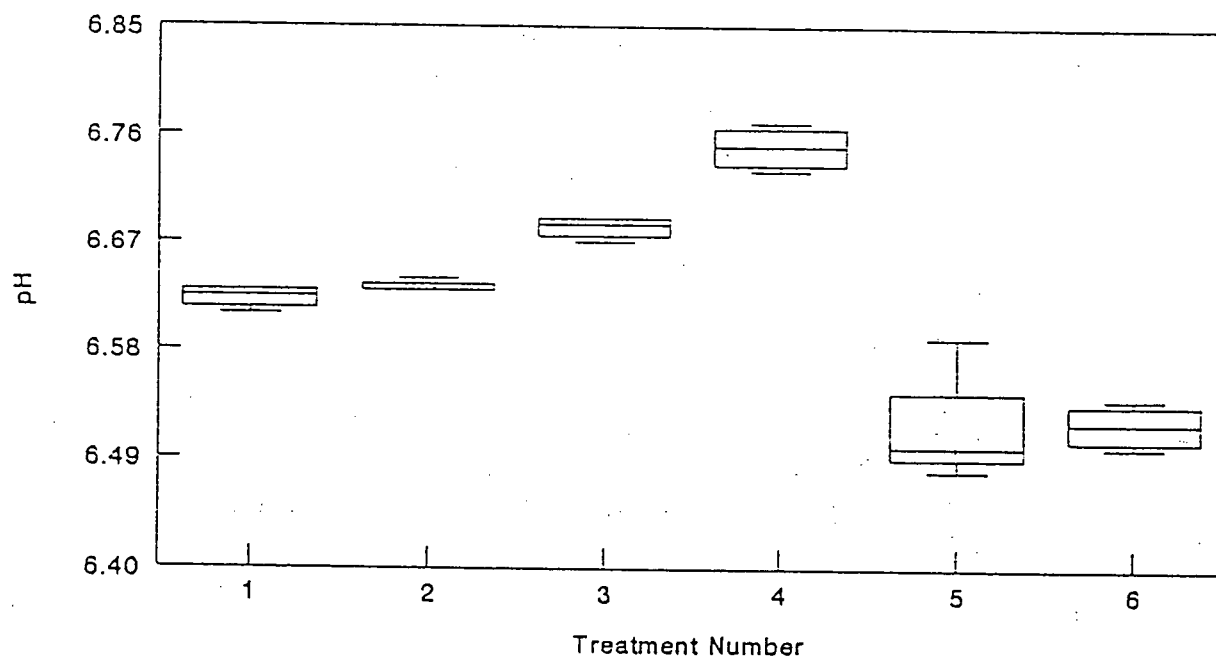
#### **4.1.6 pH analysis**

The post-mortem pH of fish, as with most other animals, is largely due to the degradation of glycogen to lactic acid via the Emden-Meyerhof-Parnas pathway. The ultimate post-mortem pH of most fish species usually falls in the range of 6.5 - 6.2 (Dunajski, 1979). The pH of most of the fish samples in this experiment was above this; the majority ranging between 6.5 - 6.7 (Figure 22).

Although the pH did not vary widely between treatments, significant differences were found (Table 24). Highly significant statistical differences ( $p < .001$ ) in the pH were found to have resulted from both RL and SS. The interaction between RL and SS also proved to be highly significant.

From the box plot showing the effect of the 6 treatment combinations on pH (Figure 22), some general trends become apparent. First, the fish that received the higher RL (treatments 4-6) generally had a lower pH when compared with the salmon fed the lower ration level. A small increase in the pH also occurred when the swimming speed was increased.

It is unfortunate that no data is available as to the glycogen content of the salmon used in this study. Kiessling et al. (1989b), in a study where fish were fed different ration levels, found an increase in the glycogen content of fish muscle when the fish ration level was increased in the range between 50% -100% RL. This appears to have also occurred in this experiment; there was a decrease in the post mortem pH of the fish at the higher ration levels that would be consistent with an increased glycogen content in the live fish prior to their being sacrificed.



**Figure 22** Boxplot by treatment number of the pH of the cooked chinook salmon samples

**Table 24** ANOVA table of ration level and swimming speed effect on the pH of cultured cooked chinook salmon

Source	DF	SS	MS	F	P
SS	2	0.054	0.027	49.189	0.000 <sup>a</sup>
RL	1	0.015	0.015	27.273	0.000
SS*RL	2	0.097	0.048	87.977	0.000
Error	18	0.010	0.001		

<sup>a</sup> Tukey test results: swimming speeds 0.5 bl/s significantly different from 1.0 bl/s ( $p < 0.001$ )

A slight increase in the pH that corresponds to an increase in the SS of the fish is also seen in Figure 22. There is no readily apparent explanation for this small increase in pH with the corresponding increase in swimming speed.

For an unknown reason, the replicates from treatment number 4 (SS = 0.5 bl/s, RL = 100%) were significantly higher than all but treatment number 3 (SS = 1.5 bl/s, RL = 75%). When the effects of SS and RL were looked at individually, the data from this treatment combination skewed the results.

It is worth noting that no similar deviations in the results are observed for this treatment in either the sensory, Instron or GC results.

#### **4.1.6.1 Comparison of pH and sensory analysis results**

According to Dunajski (1979) and Love (1988) the pH of the fish muscle is likely the most important factor affecting its rheological properties. With a drop in the pH, theoretically there should be an increase in the toughness of the fish. Thus, the drop in the pH of the cooked fish muscle that resulted when the RL was increased should have caused these well-fed fish to have a slightly firmer eating texture. In the sensory texture testing, however, although there was a significant difference in the texture of the fish due to RL, the opposite trend was observed. The fish reared on the 75% RL were significantly more firm and less mushy, despite their higher pH, than the fish reared on the 100% RL. Similarly, with a lower pH, it is expected that one would find the fish to be drier (Dunajski, 1979). Here too, the results were contrary to theory; in the sensory testing, the higher ration level fish samples were significantly more moist than those from their lower ration counterparts.

There are two factors that may have contributed to this contradiction of the popular theory regarding the relationship between pH and the texture of the fish. First, ignoring the treatment number

4 (RL=100% X. SS=0.5 bl/s) data, the change in pH is small, approximately 0.2 pH units. Second, the fish reared with the higher RL had a significantly higher fat content than those at the lower RL (Kiessling et al. 1994b). This higher fat content would have caused a higher degree of lubrication and an oily sensation in the mouth (Szczesniak, 1963). This would result in the sensory perception of a more moist, less tough or firm sample.

## 5. Conclusions

### 5.1 Sensory Analyses

RL and panellist effect significantly affected the sensory analysis portion of this experiment, while SS did not. Eight sensory attributes (three aroma, one flavour, and four texture) were significantly affected by ration level; no attributes were affected by swimming speed. The PCS graphs (Figures 8 and 9), using the PC from the seven significant sensory attributes, graphically depicted this phenomenon. The panellist effect was highly significant for all the attributes. After completing a z-transformation to remove the panellist effect, and a second ANOVA was performed, one aroma term "Boiled Potato" was no longer significant.

These results are consistent with those of Kiessling et. al. (1994b). Kiessling et al. (1994b) did not find that SS had any effect on the proximate composition of the fish. They, however, did observe that the fat content of the RL100 samples were significantly higher than those of the RL75 samples. This difference in fat content of the fish samples was likely responsible for the significant RL effect observed in the sensory analyses results. It could explain both changes in both the concentrations of flavour volatiles, affecting the aroma attributes "Seaweed" and "Sour," and flavour attribute "Brothy," and the mouthfeel of the samples affecting the perception of the texture attributes "Moisture," "Firmness," "Cohesiveness" and "Mushiness."

The statistical analyses of the sensory data also clearly demonstrated that the use of replacement panellists in QDA sensory analysis should be avoided. In this type of sensory analysis, despite training towards uniformity, each panellist develops their own unique style of grading the samples. As long as the panellist is consistent it is possible to remove the panellist effect during statistical analysis. Figures 2-7 illustrate that when the replacement panellist was used on panel days 7



and 9, that irregularities due to the substitution were evident in the data. When a replacement panellist is used on one or more panel sessions, as it was in this experiment, the removal of panellist effect is compromised.

## **5.2 GC headspace analyses**

Out of the 71 GC peaks that consistently appeared, 27 were found to be significant for either SS and/or RL (Table 21). An appreciable number of peaks were significantly affected by RL, while comparatively fewer were significant for SS. The PCS graph of these significant peaks clearly shows clear group resolution on the basis of RL (Figure 14), while no trend was observable when the PCS graph data points were labelled with either SS levels (Figure 15) or treatment numbers (Figure 13). These results compare favourably with both the sensory analysis portion of this experiment and the findings of Kiessling et. al (1994b).

## **5.3 Instron TPA**

The Instron TPA results did not follow the trend established in the other areas of this experiment where the RL significantly affected the sensory properties of cooked, cultured chinook salmon, and SS did not. Rather, the Instron TPA results showed a significant difference due to SS, while RL had no effect. It is possible that the compression method of texture measurement, employed in this experiment, measured changes in the cooked muscle that were not discernible by the panellists, and was unable to account for the textural changes due to the differences in fat content between the two ration levels. In the mastication of cooked fish, the majority of the energy is used for mastication. As a result, to get an accurate instrumental measurement of the texture of the fish, the mechanical

measurement should measure the resistance of the fibres to mechanical disintegration (Dunajski, 1979), which was not the case in this experiment. Other possible reasons for the discrepancy in results include: differences in method of sample preparation and extreme difficulty in sample preparation.

#### **5.4 pH**

The pH portion of this experiment was sensitive to both the changes in RL and SS, showing clear trends in both. There was a highly significant statistical difference found in SS, RL and the RL X. SS interaction.

In this experiment, the drop in muscle pH did not result in a firmer texture, but rather, the opposite trend was observed. In the sensory analysis portion of this study, as the pH dropped, the fish became more mushy and they were rated lower in firmness. This trend may be attributable to a corresponding increase in fat content.

The unexplainably high results of treatment 4 badly skewed the pH data. From the Tukey test results as well as the highly significant RL X. SS interaction, it became apparent that the significant difference due to SS was because of this aberration in the data and was not evidence for a true SS effect.

#### **5.5 Overall Conclusions**

This experiment demonstrated that although changing the ration level will have an effect on the sensory attributes of cooked chinook salmon muscle, the swimming speed of the fish does not. As a result, increasing the swimming speed of chinook salmon in a fish farming operation above that which is necessary for proper schooling, while decreasing the food conversion rate, is unlikely to result in any

appreciable difference in consumer acceptability. The significant effect due to ration level observed in the sensory and GC headspace analyses was likely attributable to the differences in fat content between the RL75 and RL100 samples.

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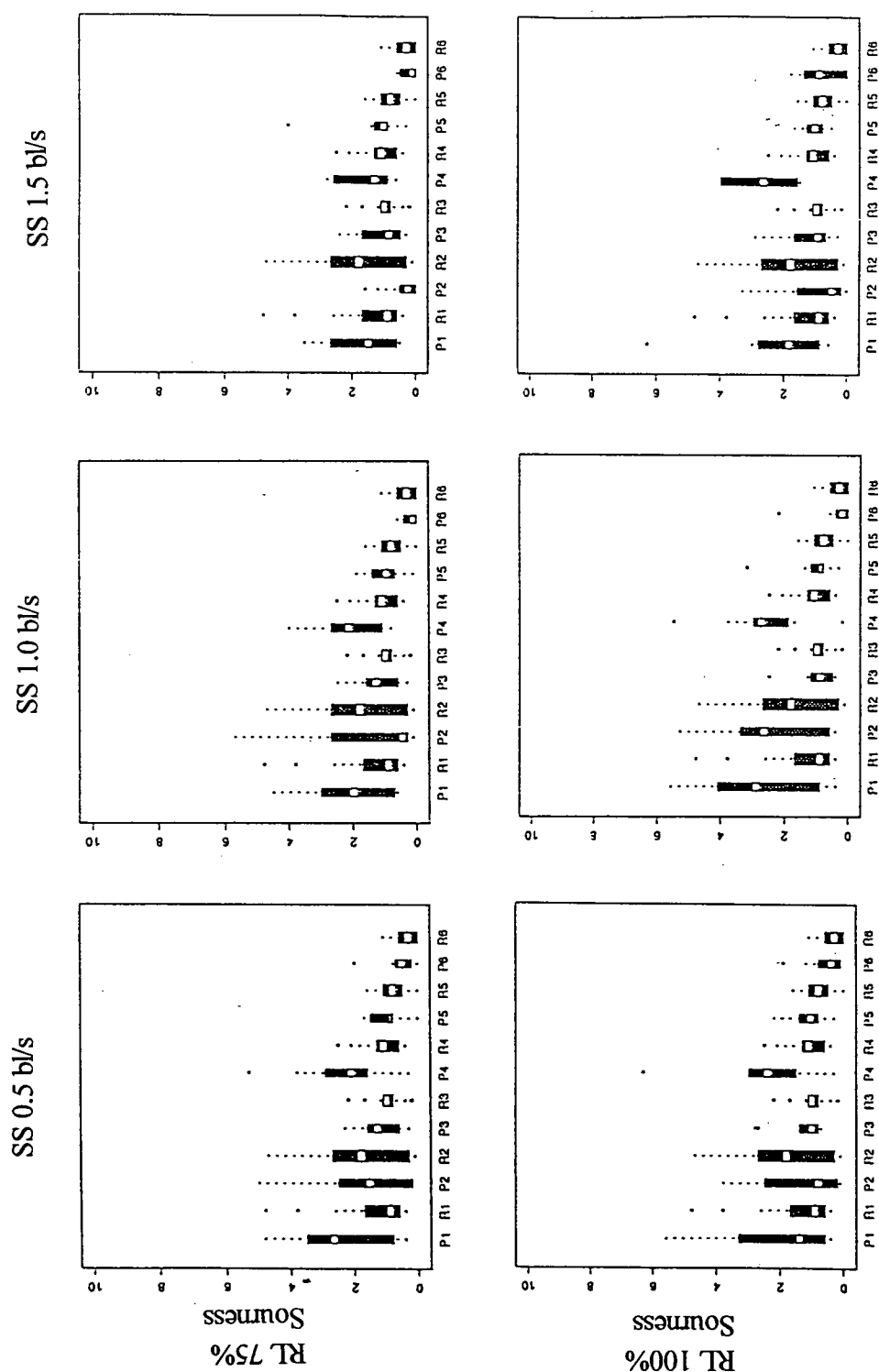


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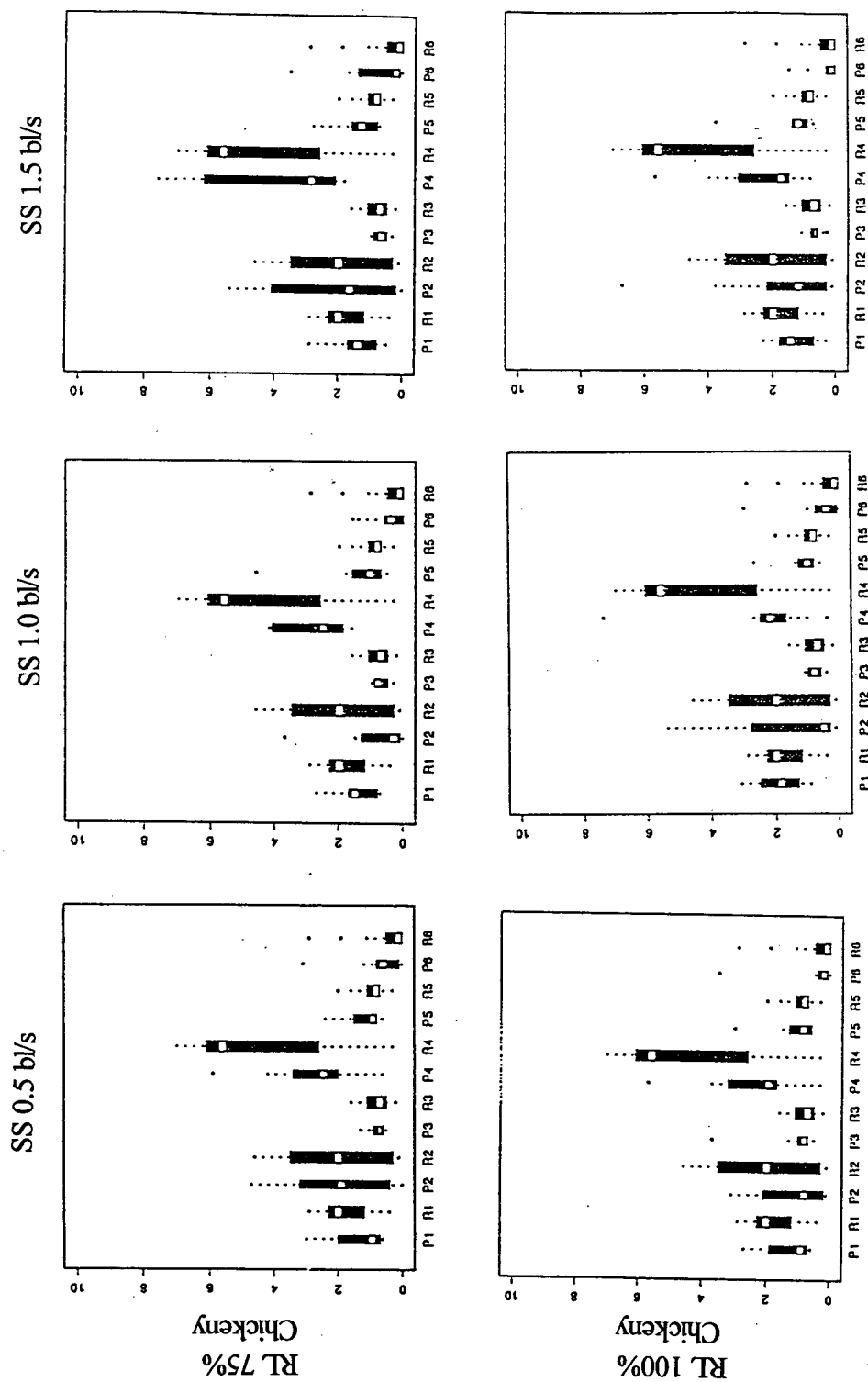
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## **Appendix A: Samples of sensory exploratory analysis boxplots**



**Figure A-1** Boxplots of the raw sensory data for "Sour" (aroma) including data from 10 panel sessions and 6 panelists. (P = treatment samples, R = reference samples, panelists labelled 1-6). The top three boxes represent RL 75% and the lower three boxes represent RL 100%.



**Figure A-2** Boxplots of the raw sensory data for “Chickeny” including data from 10 panel sessions and 6 panelists. (P = treatment samples, R = reference samples, panelists labelled 1-6). The top three boxes represent RL 75% and the lower three boxes represent RL 100%.