# Oxidative Stability of Menhaden/Soybean Oil Blends

by

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(ABSTRACT)

With the approval of menhaden oil pending, the food industry is trying to incorporate marine oils high in omega-3 fatty acids into food systems. The main problem obstructing its use as a food ingredient is fishy/painty flavors which occur with low levels of oxidation. The purpose of this study was to follow the formation of volatile compounds in menhaden/soybean oil blends and to correlate total volatiles with sensory odor scores. Specially Processed Menhaden Oil (SPMO) was supplied by Zapata Haynie Corporation (Reedville, VA). Blends of 0%, 10%, 20%, and 100% menhaden oil were stored for 15 weeks at 22 C, in the dark, with air in the headspace. Peroxide value (PV) was measured. The amounts of pentane and total volatiles produced were measured using the Shimadzu static headspace attachment for the Shimadzu GC-9A capillary gas chromatograph. Total volatiles also were followed using direct injection volatile analysis. Retention times for

selected volatiles were compared with those of known standards. Sensory analysis was completed using a modified version of the AOCS oil odor/flavor scorecard, with a panel of 12 trained judges. There was a significant increase in PV for each blend over the 15 week period ($p<0.05$). Pentane and total volatiles for the 0%, 10%, and 20% oils increased toward the end of the study but not significantly. Odor intensity scores did not increase over the 15 week period for any of the oils. The fifteen week study period may not have been long enough for sufficient development of volatiles in the 0%, 10% and 20% oils. The inclusion of the 100% menhaden oil altered the perceptions of the sensory panel since it had a much stronger fishy/painty odor. This caused the differences in the other oils to be over shadowed and poor correlations between sensory evaluation scores and PV and volatiles were obtained. Conditions responsible for the development of off-flavors in menhaden/soybean oil blends need further study before the commercial use of marine oils in food products is feasible.

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# CHAPTER 1

## INTRODUCTION

Although marine oil makes up only 2% of the world's supply of fats and oils, it is an important source of fat in many countries. Recent nutritional claims linking n-3 fatty acids to a reduction in the incidence of heart disease have sparked interest in using marine oil as a food additive. Marine oils already are being used in Europe, South America, and Japan for making frying fats, salad oils, table margarines, low-calorie spreads and industrial margarines and shortenings (Bimbo, 1987). Economically fish oils are cheaper to produce than the more popular oils which include soybean, rapeseed, coconut, and palm oil (Bimbo 1987). In 1986, the National Fish Meal and Oil Association (NFMOA) filed a petition with the United States Food and Drug Administration (FDA) to have menhaden oil and partially-hydrogenated menhaden oil placed on the generally recognized as safe (GRAS) list for use as a direct food additive (Federal Register, 1986). As of January 1990, only the hydrogenated and partially hydrogenated oils have received FDA approval.

One of the main problems which limits the use of menhaden oil as a food additive is flavor reversion which is

caused by fatty acid oxidation (Bimbo, 1987). The purpose of this study is to identify and follow the development of prominent volatiles in menhaden-soybean oil blends as oxidation occurs. Specific objectives are as follows:

1. To determine the oxidative stability of four menhaden-soybean oil blends using peroxide value, capillary gas chromatographic (GC) analysis of headspace volatiles, and descriptive sensory analysis.

2. To identify and quantify the major compounds that contribute to formation of off-odors from the GC headspace results.

3. To compare and contrast a static headspace analysis method with direct injection volatile analysis.

4. To correlate peroxide value, total volatiles, and individual volatiles with sensory odor scores.

# CHAPTER 2

## REVIEW OF LITERATURE

Menhaden oil represents 98% of the U.S. marine oil production (U.S. National Marine Fisheries, 1985). Menhaden are small, oily, herring-like fish which are found in large bays along the Atlantic and Gulf coasts (Bimbo, 1987). Their primary food source is algae and therefore they live near the surface of the water and can be spotted easily from the air. After a school of menhaden is spotted, the fishing vessels are directed toward them. The fish are caught in purse nets which are then partially retrieved. The ship comes alongside the catch, and pumps the fish and sea water aboard. After the water is disposed of, the fish are dropped into large refrigerated holds until processing takes place (Bimbo, 1987).

The processing techniques involved in production of edible marine oils are much like those used in the production of other fats and oils. The fish normally are processed by the wet reduction method, in which the principal operations are cooking, pressing, separation of the oil and water emulsion with the recovery of the oil (Bimbo, 1987). The oil is winterized to remove low melting

point triacylglycerides; degummed to remove phosphates and proteins; neutralized to remove free fatty acids; bleached to remove pigments, oxidation products, trace metals, and soaps; and deodorized to remove volatile compounds which include some pigments, chlorinated pesticides, and polychlorinated biphenyls (PCBs) (Bimbo, 1986). Marine oils are similar to other food fats and oils in that they are mainly a mixture of triacylglycerides of various long-chained fatty acids. Table 1 summarizes the fatty acid content of several edible oils. Marine oils are characterized by their higher proportion of highly polyunsaturated fatty acids which include five or six double bonds, with the position of the first double bond numbered from the terminal methyl group (Lands and Bimbo, 1983).

## Oxidative Rancidity in Oils

Lipids often become rancid due to autoxidation. This oxidative rancidity is a major cause of food deterioration. Oxidative breakdown products which lead to the development of off flavors and off odors, can decrease the nutritional quality of food and some of these products may be toxic. Since low energy thresholds are involved in these reactions, lowering the temperature does not greatly decrease the rate of oxidation. Exclusion of oxygen from the product is not

Table 1. Fatty Acid Composition of Several Edible Oils

| | % OF FATTY ACID | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SM | M | S | P | L | C | SS | R | O |
| 14:0 | 7 | 9 | - | - | 1 | - | - | - | - |
| 16:0 | 15 | 20 | 11 | 10 | 24 | 11 | 6 | 5 | 12 |
| 18:0 | * | 4 | 4 | 2 | 13 | 2 | 4 | 2 | 2 |
| 20:0 | - | - | - | 1 | 1 | - | - | 1 | - |
| 16:1 | 10 | 13 | - | - | 3 | - | - | 1 | 1 |
| 18:1 | 15 | 16 | 23 | 46 | 41 | 25 | 22 | 53 | 72 |
| 20:1 | 3 | 2 | - | 1 | - | - | - | 1 | 1 |
| 22:1 | 2 | 1 | - | - | - | - | - | - | - |
| 18:2 | 2 | 2 | 51 | 31 | 10 | 57 | 66 | 22 | 8 |
| 18:3 | * | 1 | 7 | - | 1 | 1 | - | 11 | 1 |
| 20:5 | 17 | 13 | - | - | - | - | - | - | - |
| 22:6 | 10 | 8 | - | - | - | - | - | - | - |

a SM, specially processed menhaden; M, menhaden; P, peanut; L, lard; C, corn; R, rapeseed; O, olive; S, soybean; SS, sunflower.

* data not availible

Source; Adapted from Bimbo, 1987.

always feasible or possible (Gray, 1978).

The principal lipids involved in oxidation of foods are those containing unsaturated fatty acids (Labuza, 1971). Menhaden oils are high in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have been shown to undergo autoxidation 5.2 and 8.5 times faster respectively than linolenate (Cho et al., 1987). This reaction proceeds via free radical mechanisms which can be characterized by: high yield of hydroperoxides, catalyzed by light, metal, moisture, temperature, and a relatively long induction period when starting with a pure substrate (Nawar, 1985).

The oxidation of fatty acids progresses geometrically, and if not controlled by antioxidants or enzyme systems, proceeds through a series of free-radical chain mechanisms which include initiation, propagation, and termination (Gray, 1978). In the first step initiation, some kind of catalytic substance must be present to lower the activation energy so the formation of free radicals can occur. Propagation then occurs when sufficient free radicals have formed. A chain reaction is propagated when the hydrogen atom alpha to the double bond is removed. Oxygen is then added at these places along the double bond forming peroxy radicals. These peroxy radicals then remove protons from additional alpha methyl groups yielding hydroperoxides.

Hydroperoxides are very unstable compounds which quickly decompose into aldehydes and ketones. Termination is the final stage and occurs when two free radicals join together and form a non-free radical. These products of oxidation are often thought to cause or be the cause of off-flavors in foods. Since menhaden oil contains relatively high percentages of polyunsaturated n-3 fatty acids, it is very susceptible to these types of oxidation reactions.

Antioxidants often are added to oils to delay the onset or slow the rate of oxidation. Substances which are used as antioxidants should have readily removable protons with the remaining free radicals capable of abstracting a proton from another molecule of an unsaturated substrate. The free protons are then available to terminate any free radicals formed.

The effectiveness of an antioxidant is related to many factors including, activation energy, rate constants, oxidation reduction potential, and solubility properties. In bulk oils there is a small lipid-gas interface or small surface-volume ratio so an antioxidant which concentrates itself at the surface is found to work best. Tertiary butylhydroquinone (TBHQ) is a common antioxidant which functions best at the lipid-gas interface in crude and refined polyunsaturated oils (Nawar, 1985).

Antioxidants are distributed widely in nature. Tocopherols are the principal antioxidants found in vegetable oils. A high proportion of tocopherols in crude vegetable oil survive the processing steps and provide adequate protection from oxidation (Nawar, 1985).

## Nutritional Attributes of Marine Oils

For many years the Greenland Eskimos have eaten a diet consisting mainly of fish, seal, and whale meat. Although this high fat, high protein, low fruit and vegetable diet would normally be associated with an increased occurrence of heart disease, researchers have found little evidence of this (Bang and Dyerberg, 1972).

Protection against heart disease by n-3 fatty acids may involve three mechanisms. First, n-3 fatty acids seem to decrease the effectiveness of platelets making the blood more fluid and less likely to clot. Secondly, n-3 fatty acids appear to lower blood triacylglycerides levels, decreasing the risk of atherosclerosis which is a build-up of fatty deposits in the arteries. Finally, fish or fish oil in the diet may favorably change the balance of fats and lipids in the blood (Pigott and Tucker, 1987).

In 1960 researchers in the Netherlands began a study of heart disease and diet. They took dietary histories from

852 middle-aged men and followed the health of these men for 20 years. Seventy-eight of these men who ate little or no fish died of heart disease. But the men who ate as much as one or two servings of flounder, cod, or herring a week were only half as likely to die from coronary heart disease as those who ate no fish. This finding was independent of age, body fat, physical activity, and energy intake and indicates that the consumption of fish containing n-3 fatty acids is good for the heart (Kromhout et al., 1985).

A study conducted in 1979 by Dyerberg and Bang supports the claim that n-3 fatty acids affect platelet aggregation. They investigated hemostatic function and platelet polyunsaturated fatty acids in Greenland Eskimos' and in an equal number of sex matched Danish controls. Platelet lipid analysis revealed that a high consumption of n-3 polyunsaturated fatty acids such as DHA and EPA in the diet increased the concentration of n-3 fatty acids in the platelets. The higher concentration of n-3 fatty acids in the Eskimos' blood, resulted in an increased bleeding time due to a reduction of platelet aggregation. EPA in the platelets may be converted by the vascular wall tissue to an anti-aggregatory prostacyclin. Previous <u>in vitro</u> evidence supports that EPA has an antithrombotic effect because it is only a relatively poor substrate for the production of

thromboxane when compared to arachidonic acid (AA)(Needleman et al., 1979). Hamazaki et al. (1989) compared epidemiological studies carried out in Japanese fishing and farming villages. Intakes of EPA, DHA, and AA were significantly higher in the fishing village. The increased EPA consumption directly related to a higher concentration of prostaglandin $I_3$ which was measured by its urinary metabolite prostaglandin $I_3$-M. This supports the conclusion of Needleman and coworkers (1979) that EPA is converted by the vascular wall tissue to prostacyclin. This research also supports the conclusions made by Dyerburg et al. (1978), that EPA does not induce platelet aggregation because of the formation of thromboxane $A_3$ a prostaglandin which does not have platelet aggregating properties. The increased levels of EPA and the decreased level of AA lead to an antithrombotic state in which active $PGI_3$ and non-active thromboxane $A_3$ are formed. Therefore it is possible that dietary enrichment with EPA will protect against thrombosis. The effect of marine oil supplements on cholesterol metabolism and thrombosis has been researched with varying results. Rats were fed diets high in linolenic acid or high in EPA/DHA supplemented with or without cholesterol. The researchers determined that feeding n-3 fatty acids lowered plasma cholesterol levels when

cholesterol was not supplemented and decreased AA content of the plasma and tissue when it was supplemented. The n-3 fatty acids also appeared to prevent the accumulation of cholesterol in the plasma and tissue lipids under a high dietary load of cholesterol (Garg et al., 1989). A similar study conducted by Campos et al. (1989) found n-3 supplementation in hypercholesterolemic rabbits had no beneficial effects on plasma lipid levels. However, it was noted that the magnitude of hypercholesterolemia induced in the animals may have overwhelmed any potentially positive effects the marine oil supplementation may have had.

A study utilizing 52 normal healthy volunteers investigated the effects of differing amounts of n-3 fatty acids on blood lipid composition. At dose levels of 1.4, 2.3, 4.1, and 8.2 g n-3 fatty acids per day, the researchers found a shift in the fatty acid composition of blood lipids in favor of n-3 fatty acids at the expense of n-6 and n-9 acids. In the group consuming 8.2 g per day a significant decrease in serum triacylglyceride and very low density lipoprotein levels was also found. No decrease or increase in cholesterol or high density lipoproteins was observed (Bronsgeest-Schoute et al., 1981). Previous studies have shown that total serum cholesterol and high density lipoproteins do change as a result of n-3 fatty acid intake

(Lossonczy et al., 1978).

The effects of clinically modest doses of n-3 fatty acids on lipids, lipoproteins, and apolipoproteins were investigated using eight hypertriglyceridemic subjects. At levels of n-3 fatty acids as low as 4.6 g per day, potent and substantial reductions in plasma triacylglyceride levels and decreases in total HDL-cholesterol concentrations were observed. These effects were accompanied by an elevation of LDL-cholesterol and apoprotein levels. These findings suggest that potentially beneficial hypotriglyceridemic effects of tolerable levels of fish oils may be limited by the lack of favorable changes in the levels of LDL-cholesterol and LDL apolipoproteins (Deck and Raduck, 1989).

## Measurement of Oxidative Rancidity

Researchers are interested in the extent of deterioration caused by different processing techniques and the effects of antioxidants on the stability of products. Many methods are used to measure the acceptability of a product. Sensory evaluation is considered to be the premier method, but it is expensive and lacks reproducibility. Various physical and chemical procedures have been developed to measure oxidation. Some of these tests are peroxide value, free fatty acids, anisidine value, and by the

measurement of volatile compounds by gas chromatograph. However, all these procedures lack the acuity of the human senses (Warner and Frankel, 1985).

Hydroperoxides are primary products of lipid oxidation and therefore should be a good indicator of lipid oxidation. However, peroxides are unstable intermediate compounds in the formation of carbonyl and hydroxy compounds. Peroxide content is usually expressed in milliequivalents of oxygen per kilogram of fat. The iodometric method for measuring peroxide formation is recommended by The American Oil Chemists' Society (AOCS) and is based on the measurement of the iodine produced from potassium iodide by the oxygen present in the oil. According to Mehlenbacher (1960), the two main sources or error in this method are the absorption of iodine at unsaturated bonds of the fatty material and the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated. The second is called the oxygen error and often leads to high values in the peroxide determination (Kokatnur and Jelling, 1941).

## Analysis of Headspace Volatiles

In the past 20 years, various procedures have been developed for the analysis of headspace volatiles using gas chromatography (GC). By definition, gas chromatographic

eadspace analysis is an indirect method for the determination of volatile compounds in a liquid or solid. Headspace chromatography makes use of the equilibrium between the volatile components of the liquid sample and the surrounding gas phase in a sealed container. Aliquots of the gas phase are removed for GC analysis.

There are three GC procedures used to analyze volatiles from oil samples. These are: direct injection, dynamic headspace, and static headspace.

The earliest work evaluating the flavor of vegetable oils using gas liquid chromatography (GLC) was reported by Scholz and Ptak (1966). They reported that an overall correlation could be made between ppm pentane obtained by GLC and flavor scores based on a ranking system. This was the first reported use of direct injection as a method for analyzing volatiles. Evans et al. (1969) later published a revised method using direct GC procedures to measure the thermal release of pentane as an indication of the quality of oil. In 1971 Dupuy et al. developed a simplified direct GC technique to analyze for total volatiles in vegetable oils. Since then, many researchers have revised this method, including Williams and Wille (1976), and Waltking and Zmachinski (1977). These studies have refined direct GC analysis by improving its speed. The direct injection

technique involves the introduction of a small oil sample (2 microliters) onto glass wool positioned in the glass liner of the GC injector, which is set at 180 C (Warner and Frankel, 1985). At this temperature, volatile compounds are produced from the oil sample. These volatile components are then eluted onto a capillary column. The GC oven is held for 5 minutes at -65 C with the injector in the splitless mode. The temperature then is increased to 270 C at a rate of 5 C per minute. After separation is complete, the glass liner must be removed and replaced with a clean liner containing fresh glass wool. After each run, the injector should be held at 250 C for a period of 30 min to condition it before the next sample can be introduced (Snyder et al., 1988).

The second method, dynamic headspace, is also known as purge and trap analysis or gas sparging (stripping). Calling this procedure headspace analysis is not completely accurate because no equilibrium is reached between the vapor phase and the liquid sample. This procedure uses a much larger sample size. Oil samples of 5 g are heated to 180 C and purged with the inert gas helium for 15 min in a concentrator (Selke and Frankel, 1987). Volatiles are collected on a trap containing a porous polymer adsorbent and then thermally desorbed at 220 C onto a capillary

column. This is done with the GC in the splitless mode for a 5 min period. The GC oven is held at -65 C during this period and then raised to 270 C at 5 C per min. During the GC run, the trap also is heated to 270 C and flushed with helium to remove any remaining residual compounds. Between each GC run, a blank with a clean sample tube must be used to clean the column (Snyder et al., 1988).

The final method is called static headspace. This is true headspace analysis by definition since it depends on the exisitence of an equilibrium between the two phases. The use of a headspace sampler attached to the GC is required in this method. A sample of 0.5 g is placed in a clean 10 ml vial. The vial is sealed and heated to 180 C in the headspace magazine for 20 min (Snyder et al., 1985). The headspace sampler is then pressurized for one minute after which the volatiles are automatically transferred onto the capillary column. The GC oven is held for 10 min at 0 C and programmed to increase to 250 C at 5 C per min (Snyder et al., 1988).

These three methods of volatile analysis were compared using soybean oil (Snyder et al., 1988). Samples were refined, bleached, and deodorized. Each was held at room temperature, in the dark, under oxygen, and oxidized to certain peroxide values before analysis. Oil oxidized to a

peroxide value of 9.5 produced chromatographs containing similar compounds. These compounds were present in different concentrations in each of the three methods. The GC profiles were different because of the different collection methods. Primary compounds found in each were pentane, propanal, hexanal, 2,4-decadienal, and 2,4-heptadienal. Higher concentrations of carbonyl compounds were found when the direct injection method was used. These include pentanal and isomers of decadienal and heptadienal. High temperatures act on the small amount of sample in the injector causing thermal decomposition of the volatile precursors. A similar study using the direct injection method to evaluate flavor stability of soybean oil also was completed (Warner and Frankel, 1985). These researchers found pentane and 2,4-decadienal to be the best measures of deteriorative changes. These results also correlate with those reported by St. Angelo et al. (1980), for soybean oil exposed to light and with results reported by Jackson and Giacherio (1977), for soybean oils aged at room temperature under normal fluorescent lighting.

With the dynamic headspace method, relatively less pentane and high quantities of heptadienals and decadienals were detected in the volatiles (Snyder et al., 1988). In this method, the oil sample is boiled and the volatiles are

collected in a trap. Use of this technique can cause loss of low molecular weight compounds during transfer from trap to column, therefore concentrating other components such as heptadienal, decadienal and hexanal. Soybean oil volatiles were analyzed using different sampling temperatures by Selke and Frankel (1987). These researchers reported that dynamic headspace most closely resembled sensory testing if the samples were heated to temperatures between 60 and 90 C prior to GC analysis. At these temperatures the volatile products of linolenate and linoleate hydroperoxides, 2,4-heptadienal and 2,4-decadienal, were easily detected. At temperatures above 90 C, thermal breakdown occurs and increased volatiles are released. Since dynamic headspace is sensitive enough to detect volatiles in the temperature range of 60 to 90 C, it is a useful technique to use when comparing flavor scores of oils done by sensory panels.

Static headspace analysis of soybean oil concentrates compounds of low molecular weight or of low boiling volatile compounds. Higher concentrations of pentane and propanal were found in the volatiles when compared to other methods (Snyder et al., 1988). The concentrations of these volatiles were much larger when compared to relative amounts of heptadienal and decadienal.

Because of the higher vapor pressure of these lower

molecular weight compounds, they appear in the highest concentrations in the equilibrium mixture as it is eluted on the GC column (Ioffe and Vitenberg, 1984). A study on the effect of static headspace analysis on the volatile profiles of vegetable oils found large concentrations of propane, pentane, and hexanal in soybean oil (Snyder et al., 1985). These results are consistent with the previous studies on soybean oils (Frankel, 1982; Frankel et al., 1981).

These researchers show, the method used can play a crucial role in the concentrations and types of volatiles obtained during analysis, although most of the minor components found in the volatiles are apparently not affected by the method of analysis.

Advantages and disadvantages of GC analysis of oils are fairly apparent. Direct injection is suitable for routine analysis, however, an extra step is needed to clean the injector liner between samples. It also requires high temperatures, usually around 150 C. This causes thermal breakdown of the flavor precursors and alteration of the volatile profiles. Static headspace is the clear method of choice for routine analysis because it is rapid; up to 60 samples per day can be analyzed. No cleaning is required between samples. However, several disadvantages accompany this method. As in direct injection, high temperatures

around 150 C are required. The chemist is limited to one sample per vial unless successive approximations are made. Large volumes of glassware must also be handled if routine work is to be done. Dynamic headspace has two clear advantages over the other two methods. First, analysis can be completed at lower temperatures, as low as 60 C, reducing the chance of further oxidation of the sample. This method also allows for the volatiles to be concentrated revealing more minor components which could have flavor significance. Along with these advantages come numerous disadvantages. Dynamic headspace is more expensive, requires more equipment, and is slower (8-10 samples per day) than both direct injection and static headspace. It is slower because the sample tube and the trapping column are difficult to clean. Often, the whole system must be purged to remove volatile residues. There are also several disadvantages in the procedure including: artifacts due to impurities present in the stripping gas, large amounts water and water vapor passing into the trap, and adsorption of the less volatile components into the drying filters (Snyder et al., 1988; McNally and Grob, 1985).

Of the three methods examined here, direct injection is the least preferred method for the analysis of soybean oil volatiles. Since such small amounts of sample are injected

and then heated to high temperatures, the accuracy of the analysis is in question. Researchers cannot be certain that by injecting the sample and creating volatiles, normal conditions are being simulated. Static and dynamic headspace analysis procedures are much more acceptable for analysis of soybean oil volatiles (Synder et al., 1988).

The dynamic headspace method is more time consuming and more expensive than static headspace due to its complexity. Therefore, static headspace is generally preferred for routine analysis of soybean oil. A combination of these two methods is necessary to analyze and evaluate volatiles. This will ensure that all volatiles from lipid oxidation, both at tasting temperatures and volatiles produced during analysis will be recorded.

## Sensory Analysis

Sensory evaluation usually is used to determine flavor quality and stability of foods. This method of analysis is expensive, tedious, time-consuming, variable among panel members, and not always available. Sensory panels fall into one of two groups: affective (preference or acceptance) evaluation or analyticical (discrimitive or descriptive) evaluation. The quantitative results of a trained analytical panel often are used to evaluate new types of

products and to maintain quality control (Mounts and Warner, 1980).

Organoleptic evaluation has been used to judge the objectionable flavors or odors produced during the oxidation reaction (Gray, 1978). Robards et al. (1988) concluded that sensory evaluation is the best evaluator of rancidity because it measures what the consumer actually perceives, where as chemical or physical analyses only indirectly measure the composite sensory attributes of foods.

Variance among panel members is the largest cause of experimental error in sensory analysis. To prevent this a panel must be well trained to identify and distinguish odors or flavors of the test product. Panelists are usually screened by using a series of triangle tests to determine their abilities to differentiate among samples (White and Miller, 1988; Mounts and Warner, 1980). Further training sessions are needed using the test product to develop agreement among panelists as to the meaning of descriptors and sample scores (White and Miller, 1988).

Flavor scores calculated from sensory data have been correlated with chemical and physical tests in an effort to find an inexpensive method for predicting sensory panel scores. Gray (1978) concluded that no chemical method correlated well with the changes in organoleptic quality of

oxidizing lipids. Robards et al. (1988), as well as White and Miller (1988), stated that PV was not fully able to predict flavor scores of oil because it is not as sensitive as the human senses. This is because PV measures only hydroperoxide formation in oil and not other flavor contributing compounds such as phospholipids, tocopherols, chlorophylls, and cartenoids (Moultan et al., 1985). Gray (1978) found that hydrogenated oils analyzed for PV using the official AOAC method did correlate well with sensory analysis scores. This was supported by the claim of Kahl and Hildebrandt (1983) that PV often correlates well with data obtained with trained sensory panels.

Early attempts to correlate volatile analysis and flavor scores were unsuccessful because the GC methods were limited and not very sensitive (Sjostrom, 1968). More recent attempts have met with better success.

Schoz and Ptak (1966) developed a GC procedure to measure the amount of pentane present in an oil sample. They related this to sensory scores and reported that an oil containing <50 ppm of pentane was not rancid, 50-300 ppm of pentane was very slighty rancid, and 300-600 ppm pentane was slightly rancid. The researchers found a linear relationship between the concentration of pentane and the storage time of cottonseed oil, although no statistical

analysis was done to support this. Evans et al. (1969) found the quantity of pentane in an oil had an inverse relationship with sensory scores and a direct relationship with peroxide values.

In 1977 Dupuy et al. improved a direct injection method with a mass spectrophotometer which gave more accurate results. They found that pentane, t,t-2,4-decadienal, and total volatiles could be successfully correlated with sensory scores to produce an estimated flavor score. Dupuy also reported that the correlation coefficients for volatile compounds and light exposure times were better than those for sensory scores and light exposure times. This suggests that flavor scores obtained with the GC method are as good as those determined by sensory panels. Sano and Yamazaki (1976) also determined that 2,4-decadienals could be used as a method of determining oxidation flavor scores in oil. Warner (1983) was able to predict the flavor stability of soybean oils by measuring the induction periods based on GC volatile compounds.

Flavors in oils can be produced nonoxidatively during oil production and by the use of contaminated equipment during oil production. These compounds cause oil to have sour or bitter flavors. Nonoxidative flavors, along with nonvolatile compounds, contribute to the overall flavor of

an oil which makes correlation of flavor scores and any one chemical or physical measurement difficult (Warner, 1985).

## Flavor Stability in Oils

Flavor reversion is a problem unique to oils containing linolenate and other highly unsaturated fatty acids. Off-flavors described as beany or grassy can develop at peroxide values as low as five meq/kg (Nawar, 1985). The main theory used to explain flavor reversion involves the presence of linolenic acid. The oxidation of linolenate produces the compound 2-n-pentylfuran, 3-cis and 2-trans hexanals. These compounds produce reversion flavors when added to oil at levels as low as two ppm (Smouse and Chang, 1967).

Non-oxidation compounds also have been shown to produce flavor reversion in oils. Oxidation compounds not removed from oil during deodorization such as phospholipids, and nonsaponifiable materials such as tocopherols and plant pigments may all lead to reverted flavors (Smouse, 1985). Mounts et al. (1978) concluded that antioxidants were very effective in stopping oxidation but not flavor reversion and therefore flavor reversion was a nonoxidative process. Many of the off-flavors in oil are caused by aldehydes formed during oxidation. These compounds have very low thresholds making them easy to detect. Frankel (1985) listed the major

volatiles present in rancid soybean oil (Table 2). Frankel states that the impact of volatile oxidation products on flavor depends not only on concentration of the compound but also on its threshold. Trans, cis-2,4 decadienal and t,c-2,4-heptadienal both greatly influence odor due to their extremely low thresholds.

Hsieh et al. (1989) studied compounds contributing to fishy flavors in menhaden oil. By using dynamic headspace procedures with a mass spedtrophotometer they were able to separate and identify the volatiles present in winterized, undeodorized oil. Table 3 contains the major volatiles thought to impact the oil odor and the characteristics they impart. The researchers concluded that short chained saturated and unsaturated aldehydes and ketones contributed greasy, oily and oxidized characteristics to the oil, while short-chained carboxylic
acids gave objectionable sweaty odors. Although they found normal alkanes as major volatiles these were found to be without significant odor. Karahadian and Lindsay (1989b) characterized odors of menhaden and cod liver fish oils with similar results when using dynamic headspace methods.

Table 2. Flavor Significance of Soybean Oil Volatiles

| Major Volatiles | Relative (%) | Threshold (ppm) | Relative Order Of Importance |
|---|---|---|---|
| t,t-2,4-decadienal | 33.7 | 0.10 | 2 |
| t,c,-2,4-decadienal | 17.9 | 0.02 | 1 |
| t,c-2,4-heptadienal | 11.1 | 0.04 | 3 |
| 2-heptenal | 5.6 | 0.20 | 8 |
| t, t-2,4 heptadienal | 4.5 | 0.10 | 7 |
| n-hexanal | 4.5 | 0.08 | 6 |
| n-pentane | 3.1 | 340.00 | 15 |
| n-butanal | 1.5 | 0.025 | 5 |
| 2-pentenal | 1.2 | 1.00 | 13 |
| 1-octen-3-ol | 0.9 | 0.0075 | 4 |
| n-pentanal | 0.7 | 0.07 | 10 |
| 2-hexenal | 0.7 | 0.60 | 13 |
| n-nonanal | 0.7 | 0.20 | 11 |
| n-heptanal | 0.6 | 0.055 | 9 |
| 1-penton-3-ol | 0.5 | 4.20 | 14 |
| 2-octenal | 0.5 | 0.15 | 12 |

Source: Frankle, 1985.

Table 3. Chemicals Contributing to Off Flavors in Oils

| Chemical Name | Odor Characteristics |
|---|---|
| t-but-2-enal | painty |
| 2,4-decadienal | oxidized oil |
| decatrienal | burnt, fishy |
| c-4-heptanal | waxy green, grassy |
| 2,4-heptadienal | vegetable green |
| hexanal | oxidized, painty |
| t-2-hexanal | cut grass, green |
| hex-2-enal | sharp green, oily |
| t,c,-2,6-nonadienal | green |
| 1,5-octadien-3-one | green, grassy |

Source: Hsieh et al., 1989.

# CHAPTER 3

## MATERIALS AND METHODS

Specially Processed Menhaden Oil (SPMO) produced by Zapata Haynie Corporation (Reedville, Virginia) was blended with commercial soybean oil (Wesson Oil, Beatrice/Hunt Wesson, Fullerton, CA.) for the study. The SPMO is refined, bleached, steam deodorized, and preserved with 0.02% tertiary butylhydroquinone (TBHQ). This marine oil contains approximately 17% EPA and 10% DHA.

Soybean oil, menhaden oil, and two blends containing 10% and 20% menhaden oil by weight were analyzed during weeks 1, 3, 5, 7, 9, 11, 13, and 15, with each analysis completed in duplicate. Oil samples were divided in half and 60 ml was sealed in two 60 ml glass containers for each week. These samples were then stored at 22 C in the dark. Repetitions for each test were split evenly between each storage container.

### Peroxide Value:

Peroxide value (PV) measures the amount of hydroperoxides formed due to lipid oxidation (Appendix A). Potassium iodide and an acetic acid/chloroform solution (2:3

v/v) was added to 5 g of oil. The excess iodide was titrated with 0.01 N sodium thiosulfate using a 1% starch solution as an indicator (AOAC Official Methods of Analysis, 1984).

Gas Chromatography Analysis of Headspace:

Two different methods of headspace analysis of volatile compounds were compared, static headspace and direct injection analysis of volatiles.

With the static headspace analysis, 5 g of oil was placed in a glass headspace vial (Shimadzu No. 221-29083-91), capped with a teflon septum (Shimadzu No. 221-29084-91), and then sealed with an aluminum cap (Shimadzu No. 221-29468-91). The sample was then conditioned at a temperature of 145 C for 25 min in a Shimadzu headspace sampler model HSS-2A. Volatile analysis was done using a Shimadzu gas chromatograph model GC-9A with a SE-54 capillary column from AllTech, (Deerfield IL, 30 m x 0.32 mm inner diameter, 0.25 micrometer film, No. 19646).

After each sample was conditioned to 145 C, 400 microliters (split injection 25:1) of headspace was injected (syringe temperature 150 C) into the column. The initial column temperature was 40 C. The volatiles were held for 3 min at this temperature, then the column was heated to 200 C

at 5 C per min. The final temperature was not held.

The flow rate of the carrier gas (helium) was 20 cm/sec. The makeup gas consisted of helium, hydrogen (flow rate=40 ml/min), and air (flow rate=400 ml/min). The flow rate of this makeup gas through the flame ionization detector (FID, 4 x 10E-10 AFS) was 50 ml/min.

Two samples of air were run at the beginning of each day to clean and condition the column before use.

Standards of volatile compounds found in soybean and fish oils were used to identify the peaks by retention times. Pentane, t,t-2,4-decadienal, and t,t-2,4-heptadienal were obtained from Aldrich Chemical Supplies (Milwaukee, WI.), hexanal, 1-pentanol, and t-2-hexanal from Sigma Chemical Company (St. Louis, MO.) and c-3-hexen-1-al and t-2-tridecen-1-al from Bendoukian Research Inc (Danbury, CT.). Dodecane (Sigma Chemical Company, St. Louis, MO.) was added as an internal standard to quantify the results.

The direct injection GC analysis of volatiles was conducted using a Hewlett-Packard GC (model 5890A) with an external closed inlet device attachment. An Ultra 2 capillary column (crosslinked 5% Ph Me Silicone) was used from Hewlett-Packard (Kenner, LA, 50 m x 0.32 mm inner diameter, 0.52 micrometer film). Approximately 200 mg of oil was placed on glass wool in the ECID. The sample was

held at 140 C for 20 min while helium was passed though it eluting the volatiles onto the column. Volatiles were trapped on the column by placing the first few centimeters of the column in a dry ice acetone mixture. Column temperature was increased from an initial temperature of 30 C to 150 C at a rate of 2.5 C per min. The temperature was futher increased to 250 C at 5 C per min and then held for 30 min. The column was conditioned at 150 C for 20 min prior to each run.

The flow rate of the carrier gas helium, through the column was 1.36 ml/ min, the make-up gas (helium) 30 ml/min, air 240 ml/ min, and hydrogen 30 ml/ min.

Oil samples for the direct injection procedure were prepared by combining 2.5 g of oil from the duplicate bottles together with 0.4 g of the internal standard dodecane. The internal standard had to be reduced due to the higher recovery of volatiles with the direct injection method. One GC run was then completed for each of the four oil blends.

## Sensory Analysis

Sensory analysis was used to follow the formation of off odors in the oil samples. A panel of 12 members evaluated the samples during weeks 1, 3, 5, 7, 9, 11, 13,

and 15. Panelists were screened by using a series of six triangle tests to determine their individual ability to smell differences in the oil samples. Of the 16 volunteers, the 12 with the most correct answers to the triangle tests were chosen for further training. Fresh and rancid oils were provided as examples of the descriptors used on the score card. These examples were made available during the training sessions. The 12 panel members were trained using oil samples similar to those in the study. During four sessions each panelist smelled four samples of oil and rated each by using the score card. These training sessions were used to acquaint the panel members with the score card descriptors and with the procedures to be used in the sensory booth. All of the final panel participants were able to pick out bland, buttery, grassy, fishy, and painty odor when presented during training. Fresh soybean oil, butter, soybean oil with hexanal, fresh menhaden oil, and rancid menhaden oil (PV=125) were used as examples of bland, buttery, grassy, fishy, and painty, respectively.

All sensory testing was completed in the sensory analysis laboratory in the Human Nutrition and Foods department at Virginia Polytechnic Institute and State University. Samples were presented simultaneously in glass petri dishes (diameter 5 cm) coded with a random 3-digit

code. The placement order for samples was random for each panel member. Panel members randomly received samples from one set of sample storage bottles designated A or B. Each petri dish contained 5 ml of sample which was heated to 50 C for 30 minutes to develop volatiles. The score card (Appendix H) asked each panel member to describe the predominate odor characteristics and rate their intensities on a scale of 1 to 3 with 1 = weak, 2 = moderate, and 3 = strong. The overall intensity of the oil was also noted using a 15 cm line. A score of 1 indicated a bland oil and a score of 15 indicated an extreme odor intensity (adapted from Mounts and Warner, 1980).

Extra samples mixed at the beginning of the study were frozen at -10 C under argon until week 14. This oil was measured and stored as above and given to the odor panel to judge. This was done to simulate weeks one through five and check the consistency of the panel.

The data collected was analyzed with statistical analysis software (SAS) using analysis of variance, Duncan's multiple range analysis, and regression analysis. Correlation coefficients were calculated between each of the variables. All statistical analysis was completed at an alpha level of 0.05.

# CHAPTER 5

## RESULTS

This research project was designed to provide data on the stability of soybean oil, menhaden oil, and two blends containing 10% and 20% menhaden oil in 90% and 80% soybean oil, respectively. To assess the extent of the oils' oxidation three different experiments were run; peroxide value, capillary gas chromatographic analysis of volatiles, and descriptive sensory analysis of odor.

### Part 1: Peroxide Value

The initial oxidation products found in fats and oils are hydroperoxides. Peroxides were measured using the official AOAC method of analysis (1984). Mean peroxide values (PV) determined during each week of the study are presented in Tables 4-6. The peroxide values were affected by both week of storage and by level of menhaden oil. The PV of each different oil sample significantly increased over the 15 week period (Table 4). Table 5 shows that during the beginning of the study significant differences were found among each of the oils, but toward the end only the 100% menhaden oil was significantly different from the others.

Table 4. Peroxide Values for Oil Blends

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 0.10±0.00a | 0.40±0.00a | 0.60±0.00a | 1.15±0.07a |
| 3 | 0.80±0.00b | 0.95±0.07b | 1.25±1.07b | 4.90±0.16ab |
| 5 | 0.95±0.07bc | 1.00±0.00b | 1.55±0.07c | 6.68±0.28abc |
| 7 | 1.20±0.00d | 1.35±0.07c | 1.79±0.15c | 6.29±0.15abc |
| 9 | 1.00±0.00c | 1.35±0.06c | 1.65±0.07c | 7.78±0.31abc |
| 11 | 1.60±0.00e | 1.95±0.07e | 2.53±0.08d | 6.69±0.15abc |
| 13 | 1.54±0.06e | 1.70±0.14d | 2.34±0.06d | 11.19±7.36bc |
| 15 | 1.54±0.21e | 1.99±0.00e | 2.54±0.21d | 13.01±2.46c |

* Mean of 2 trials (meq/kg) ± SD.

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined by Duncan's multiple range analysis.

Table 5. Peroxide Values for Oil Blends

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 0.10±0.00a | 0.40±0.00b | 0.60±0.00c | 1.15±0.07d |
| 3 | 0.80±0.00a | 0.95±0.07a | 1.25±1.07b | 4.90±0.16c |
| 5 | 0.95±0.07a | 1.00±0.00a | 1.55±0.07b | 6.68±0.28c |
| 7 | 1.20±0.00a | 1.35±0.07a | 1.79±0.15b | 6.29±0.15c |
| 9 | 1.00±0.00a | 1.35±0.06ab | 1.65±0.07b | 7.78±0.31c |
| 11 | 1.60±0.00a | 1.95±0.07b | 2.53±0.08c | 6.69±0.15d |
| 13 | 1.54±0.06a | 1.70±0.14a | 2.34±0.06a | 11.19±7.36a |
| 15 | 1.54±0.21a | 1.99±0.00a | 2.54±0.21a | 13.01±2.46b |

* Mean of 2 trials (meq/kg) ± SD.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 6. Peroxide Values for Oil Blends

| Week | Level of Menhaden Oil 0% | 10% | 20% |
|---|---|---|---|
| 1 | 0.10+0.00a | 0.40+0.00b | 0.60+0.00c |
| 3 | 0.80+0.00a | 0.95+0.07a | 1.25+1.07b |
| 5 | 0.95+0.07a | 1.00+0.00a | 1.55+0.07b |
| 7 | 1.20+0.00a | 1.35+0.07a | 1.79+0.15b |
| 9 | 1.00+0.00a | 1.35+0.06b | 1.65+0.07c |
| 11 | 1.60+0.00a | 1.95+0.07b | 2.53+0.08c |
| 13 | 1.54+0.06a | 1.70+0.14a | 2.34+0.06b |
| 15 | 1.54+0.21a | 1.99+0.00a | 2.54+0.21b |

* Mean of 2 trials (meq/kg) + SD.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Since the menhaden oil sample had values much greater than the others, an analysis of variance was run without it. With the 100% menhaden oil removed from the analysis the 20% menhaden oil sample became significantly different from the other two samples during each week of the study (Table 6).

## Part 2: Capillary Gas Chromatographic Analysis of Volatile Compounds

Volatiles of the stored oils were measured using two different methods. First, static headspace analysis was run with a Shimadzu GC-9A. The static headspace method concentrated the low molecular weight or low boiling volatile compounds such as propanal and pentane (Snyder et al., 1988). The results depicted in Tables 7-9 show the mean total areas for the volatiles measured and Tables 10-12 show the mean areas for the compound pentane, which was present during each week of the study. Only the 100% oil showed a significant increase in total volatiles measured over the 15 weeks (Table 7). However the total volatiles did begin to increase toward the end of the study. Differences were found at the beginning of the study between the 0, 10% oils and the 20, 100% oils. However, there was no difference between the 0%, 10%, or 20% oils after week 5 when the 100% menhaden oil was also analyzed (Table 8).

Table 7. Total Area of Volatiles: Static Headspace Analysis

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 20.1a | 30.1a | 56.3a | 235.3a |
| 3 | 38.3c | 48.0a | 173.9a | 635.6ab |
| 5 | 36.0c | 43.4a | ---- | ---- |
| 7 | 16.6ab | 26.3a | 41.2a | 604.3ab |
| 9 | 28.3bc | 32.3a | 48.4a | 622.4ab |
| 11 | 11.0a | 26.6a | 45.2a | 384.1a |
| 13 | 32.6c | 40.1a | 70.2a | 577.0ab |
| 15 | 26.0bc | 41.8a | 53.7a | 1017.2b |

* Mean of 2 scores (x 1000)

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 8. Total Area of Volatiles: Static Headspace Analysis

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 20.1a | 30.1ab | 56.3b | 235.3c |
| 3 | 38.3a | 48.0a | 173.9b | 635.6c |
| 5 | 36.0a | 43.4a | ---- | ---- |
| 7 | 16.6a | 26.3a | 41.2a | 604.3b |
| 9 | 28.3a | 32.3a | 48.4a | 622.4b |
| 11 | 11.0a | 26.6a | 45.2a | 384.1b |
| 13 | 32.6a | 40.1a | 70.2a | 577.0b |
| 15 | 26.0a | 41.8a | 53.7a | 1017.2b |

* Mean of 2 scores (x 1000)

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 9. Total Area of Volatiles: Static Headspace Analysis

| Week | Level of Menhaden Oil 0% | 10% | 20% |
|---|---|---|---|
| 1 | 20.1a | 30.1a | 56.3a |
| 3 | 38.3a | 48.0a | 173.9a |
| 5 | 36.0a | 43.4a | ---- |
| 7 | 16.6a | 26.3ab | 41.2b |
| 9 | 28.3a | 32.3a | 48.4a |
| 11 | 11.0a | 26.6ab | 45.2b |
| 13 | 32.6a | 40.1a | 70.2b |
| 15 | 26.0a | 41.8ab | 53.7b |

* Mean of 2 scores (x 1000)

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

However, when the 100% was removed from the analysis there was a significant difference between the 0% and 20% blends toward the end of the study (Table 9).

Pentane, as identified by retention time, was the major volatile produced in each of the oil samples (Tables 10-12). Therefore, the resultant areas of pentane and the areas of total volatiles measured by static headspace were very similar. Formation of pentane varied little each week, only the 100% menhaden oil sample showed an overall significant difference during the 15 week trial period (Table 10). The oils were significantly different during week 1 of the study but, only the 100% menhaden oil sample was significantly different from the other samples during the remainder of the study (Table 11). When the 100% was removed from the analysis the 20% oil was determined to be different from the 0% sample for 5 of 8 weeks (Table 12). Figures 1-4 compare typical chromatograms during week one and week 13 for each of the four oil samples (week 15 could not be reanalyzed). The largest peaks have been identified as pentane and dodecane, the internal standard. The overall number of individual volatile compounds increased with time and percentage of menhaden oil.

The second GC method was direct injection volatile analysis. Table 13 shows the total volatiles measured. A

Table 10. Pentane Area: Static Headspace Analysis

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 13.2bcd | 19.2ab | 34.0a | 149.0a |
| 3 | 28.4a | 35.9c | 47.3ab | 393.9ab |
| 5 | 26.4a | 31.4bc | 64.9b | 264.5a |
| 7 | 8.3cd | 16.8a | 25.7a | 368.1ab |
| 9 | 19.9ab | 20.7ab | 33.3a | 376.1ab |
| 11 | 5.5d | 17.7ab | 26.8a | 237.7a |
| 13 | 16.4bc | 22.6abc | 41.5ab | 327.7ab |
| 15 | 15.3bcd | 25.7abc | 33.4a | 611.1b |

* Mean of 2 scores (x 1000)

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 11. Pentane Area: Static Headspace Analysis

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 13.2a | 19.2a | 34.0b | 149.0c |
| 3 | 28.4a | 35.9a | 47.3a | 393.9b |
| 5 | 26.4a | 31.4a | 64.9a | 264.5b |
| 7 | 8.3a | 16.8a | 25.7a | 368.1b |
| 9 | 19.9a | 20.7a | 33.3a | 376.1b |
| 11 | 5.5a | 17.7a | 26.8a | 237.7b |
| 13 | 16.4a | 22.6a | 41.5a | 327.7b |
| 15 | 15.3a | 25.7a | 33.4a | 611.1b |

* Mean of 2 scores (x 1000)

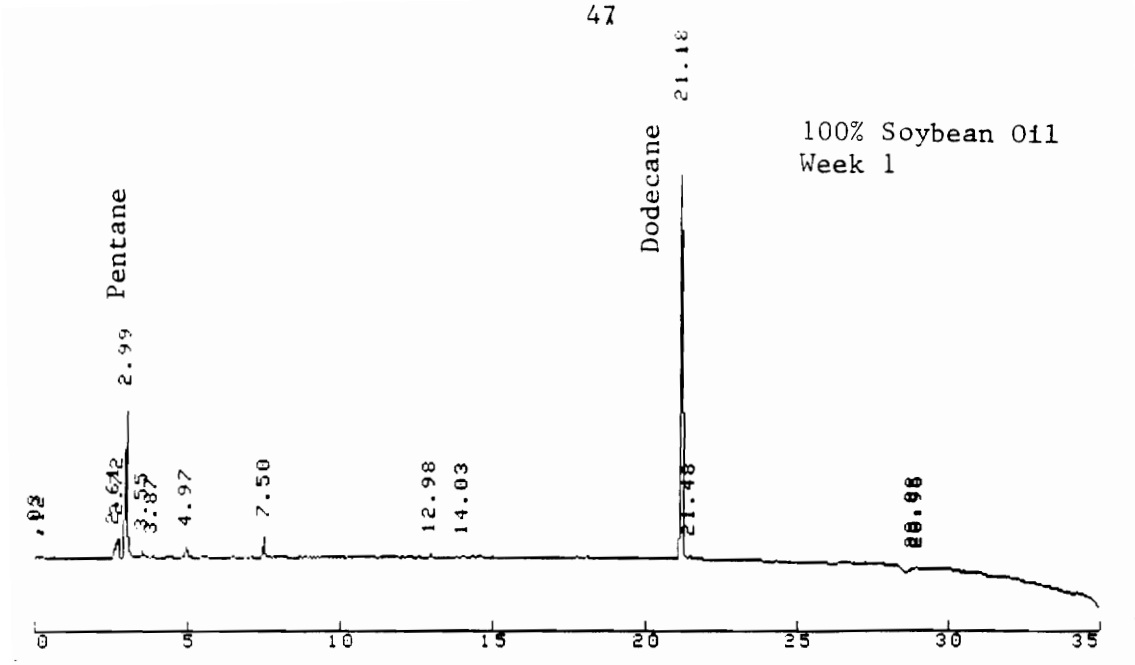
* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 12. Pentane Area: Static Headspace Analysis

| Week | Level of Menhaden Oil 0% | 10% | 20% |
|---|---|---|---|
| 1 | 13.2a | 19.2a | 34.0b |
| 3 | 28.4a | 35.9a | 47.3a |
| 5 | 26.4a | 31.4a | 64.9a |
| 7 | 8.3a | 16.8ab | 25.7b |
| 9 | 19.9a | 20.7a | 33.3a |
| 11 | 5.5a | 17.7b | 26.8c |
| 13 | 16.4a | 22.6a | 41.5b |
| 15 | 15.3a | 25.7ab | 33.4b |

* Mean of 2 scores (x 1000)

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.



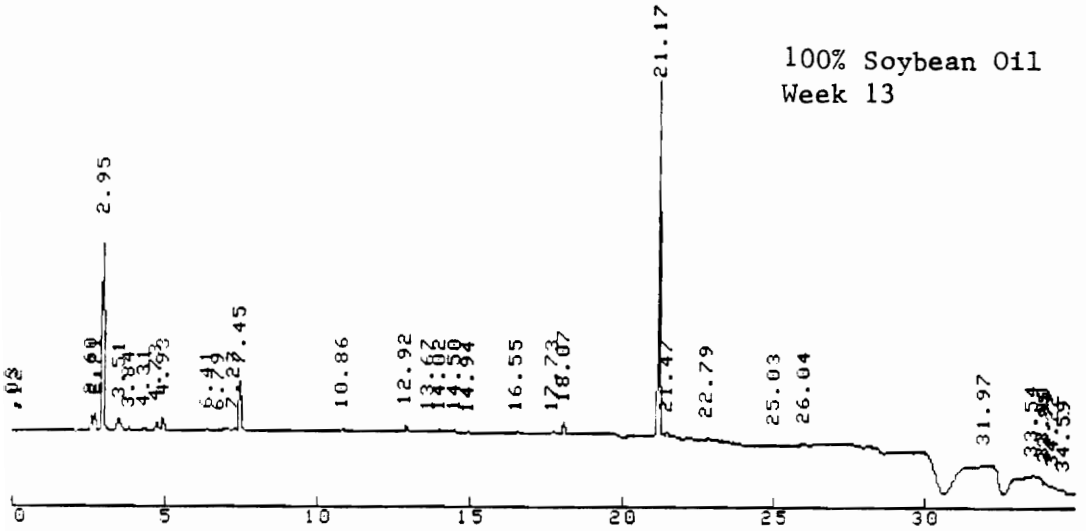




FIGURE 1: STATIC HEADSPACE ANALYSIS OF 100% SOYBEAN OIL COMPARING WEEKS 1 AND 13

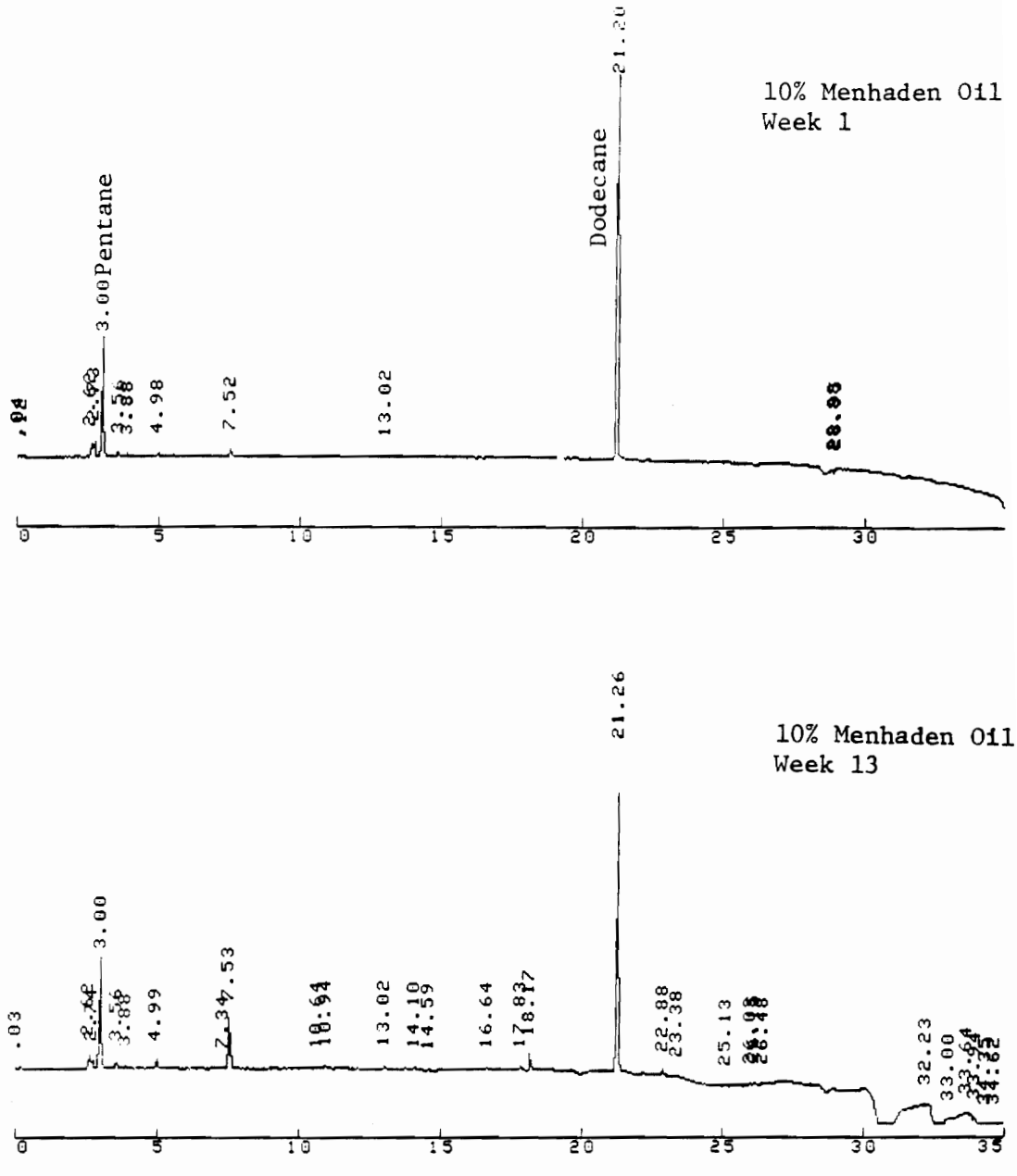



FIGURE 2: STATIC HEADSPACE ANALYSIS OF 10% MENHADEN OIL COMPARING WEEKS 1 AND 13

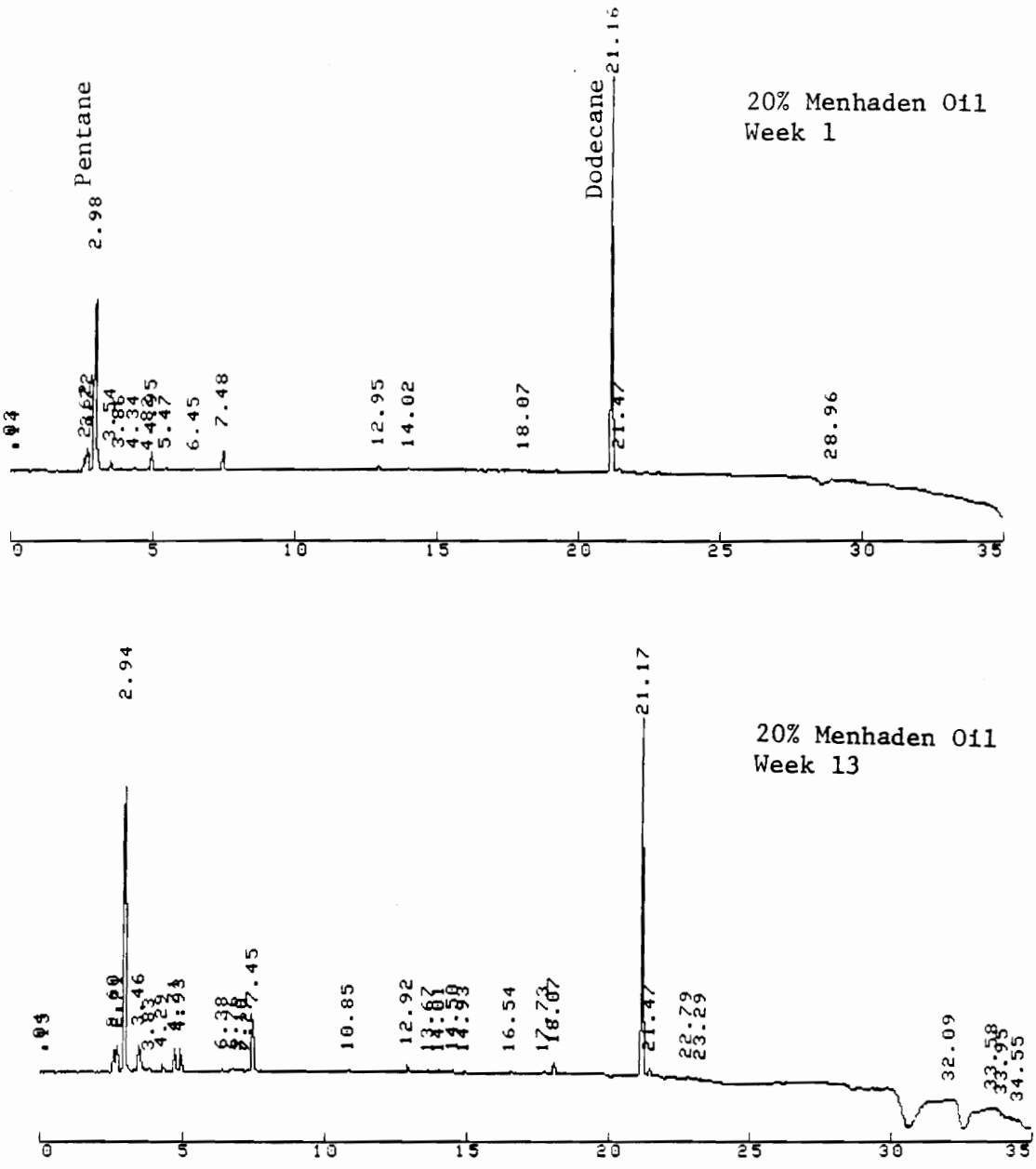



FIGURE 3: STATIC HEADSPACE ANALYSIS OF 20% MENHADEN OIL COMPARING WEEKS 1 AND 13

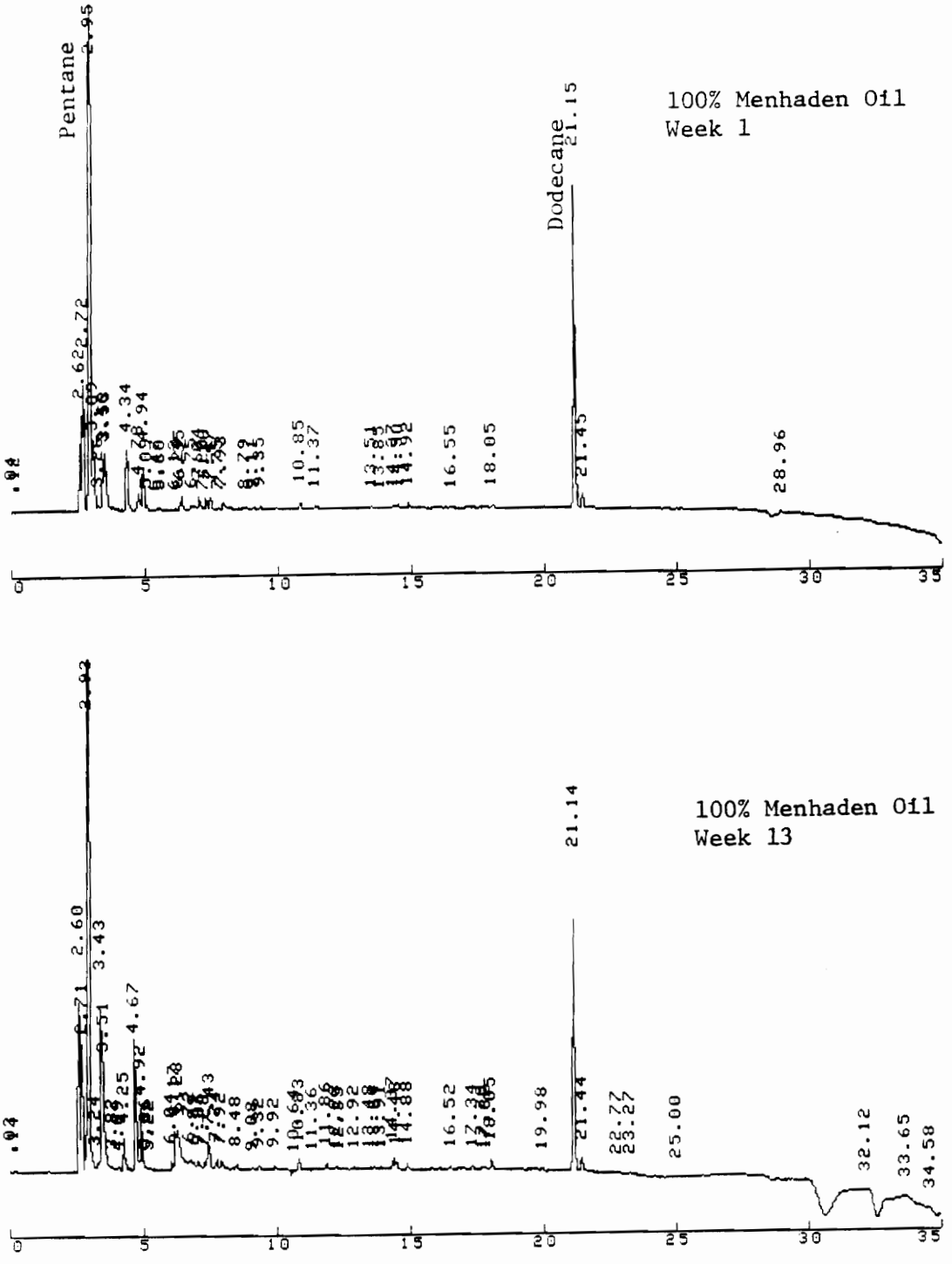



FIGURE 4: STATIC HEADSPACE ANALYSIS OF 100% MENHADEN OIL COMPARING WEEKS 1 AND 13

Table 13. Total Area: Volatile Analysis by Direct Injection

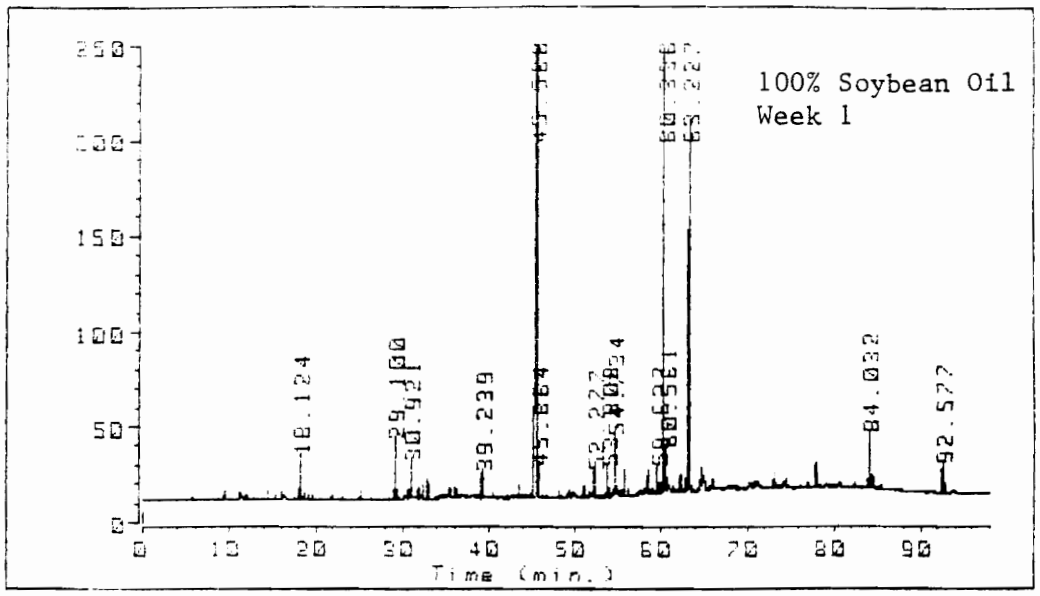
| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 121.6 | 29.1 | 43.3 | 93.3 |
| 3 | 159.6 | 479.7 | 492.7 | 427.9 |
| 5 | 188.5 | 698.7 | 227.6 | 870.0 |
| 7 | 250.3 | 779.0 | 379.4 | 391.8 |
| 9 | 330.2 | 333.0 | 205.5 | 806.4 |
| 11 | 249.9 | 117.4 | 75.5 | 36.5 |
| 13 | 801.2 | 97.1 | 347.1 | 508.2 |
| 15 | 231.8 | 627.1 | 246.7 | 856.1 |

* Total area x 1000, n=1

two to three fold increase in the total volatiles was observed between weeks 1 and 15. The development of individual volatile compounds could not be followed because there were too many peaks present to make a positive identification from retention times. A mass spectrophotometer would be needed to identify and quantify the individual volatile components in the case. Figures 5-9 compare typical chromatograms for weeks 1 and 15. A definite difference in the quantity and size of the individual peaks of each of the oil blends can be seen as time increases (Fig. 5-9). Also the number of volatile compounds present increases with a corresponding increase in menhaden oil.

## Part 3: Descriptive Sensory Analysis

Twelve trained panel members evaluated each oil blend during the fifteen week period for the following odor attributes; bland, buttery, grassy, fishy, and painty. Each characteristic was rated on a scale between one and three. Mean values are shown in Tables 14-23. Appendix B through Appendix F contain the individual panelist's scores for each week of the experiment. A score of one was equal to weak or indistinguishable, two was equal to moderate odor, and a three was equal to a strong odor present. Bland can be



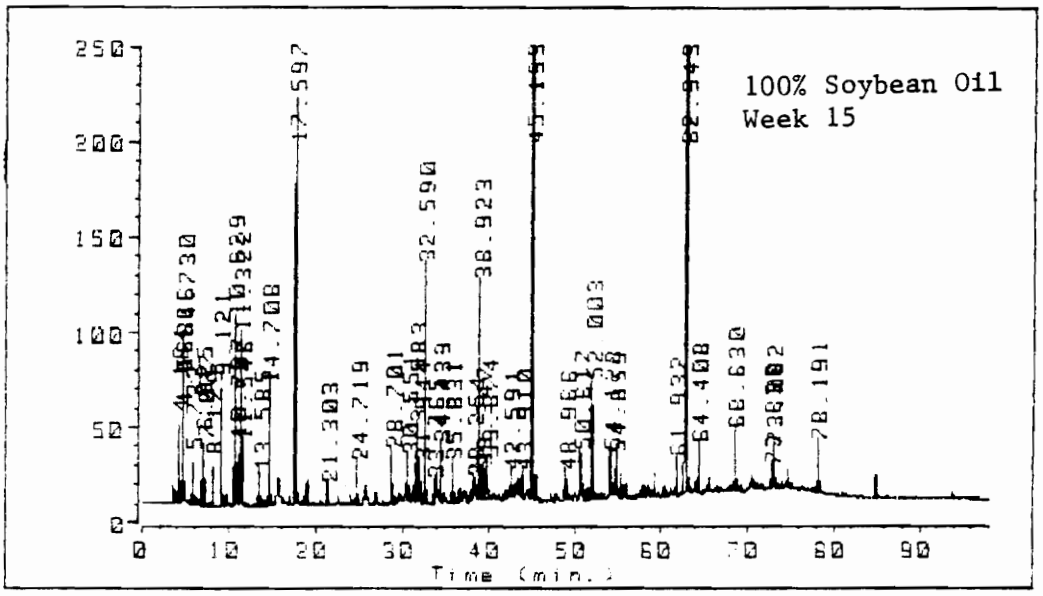
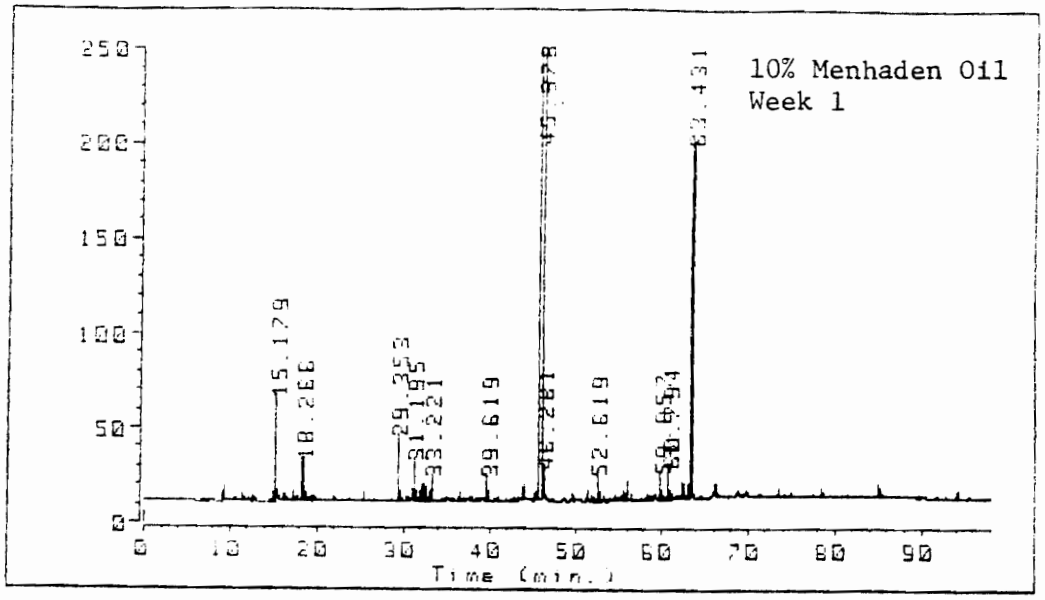




FIGURE 5: DIRECT INJECTION VOLATILE ANALYSIS OF 100% SOYBEAN OIL COMPARING WEEKS 1 AND 15



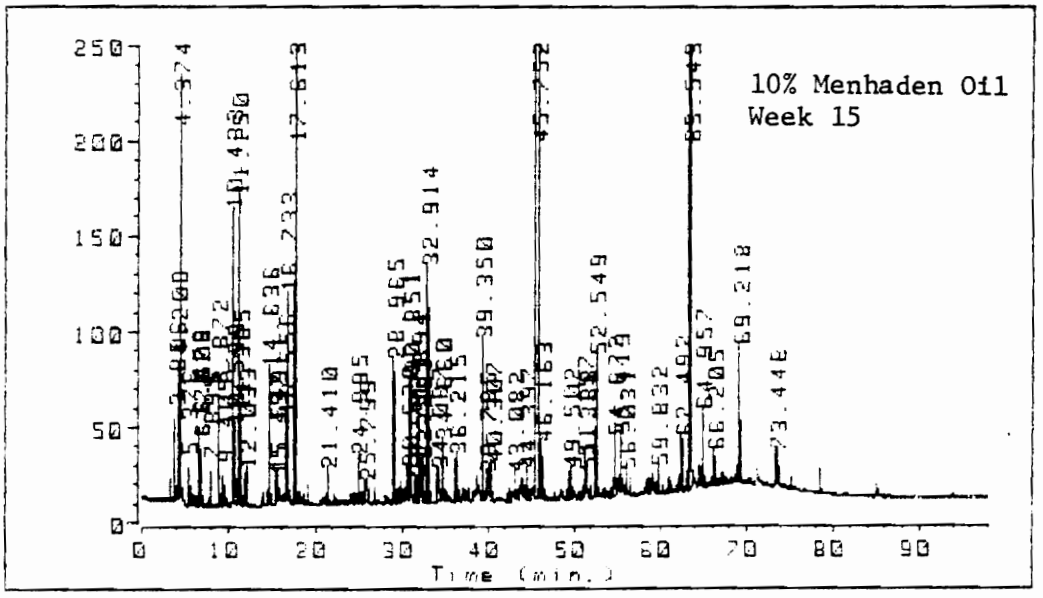
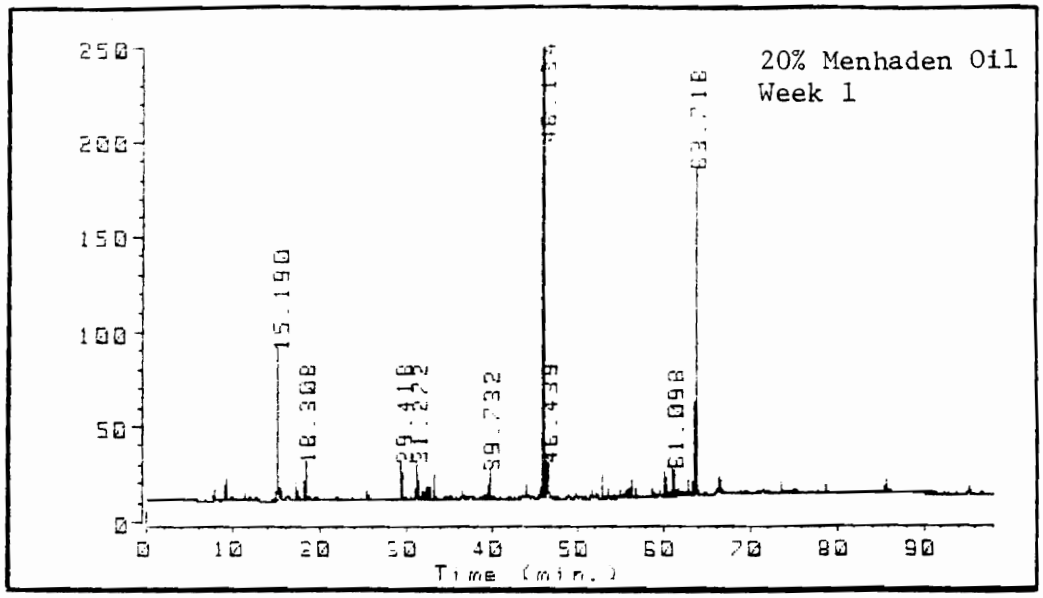




FIGURE 6: DIRECT INJECTION VOLATILE ANALYSIS OF 10% MENHADEN OIL COMPARING WEEKS 1 AND 15



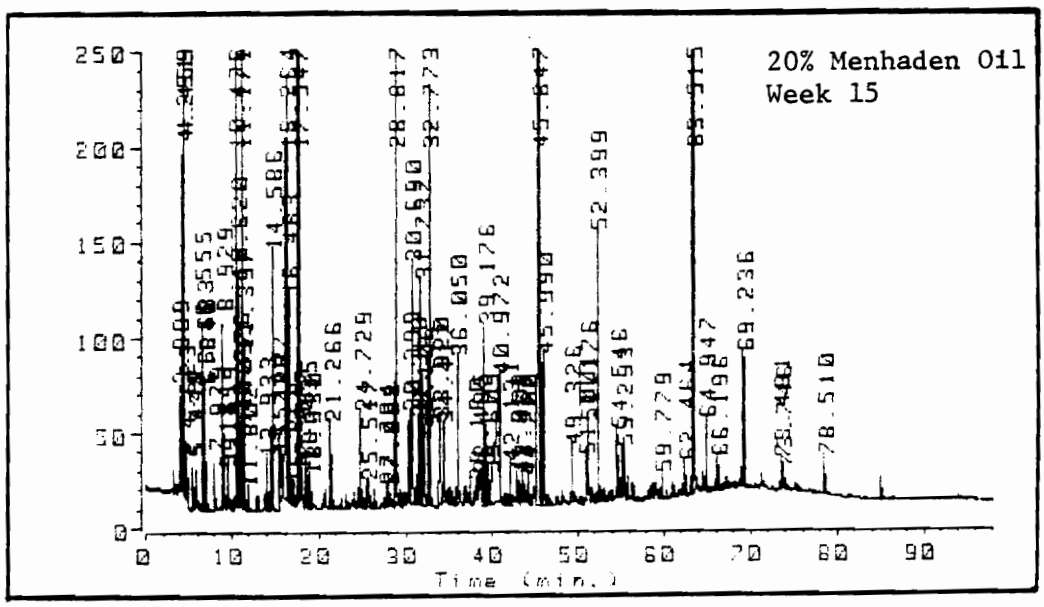
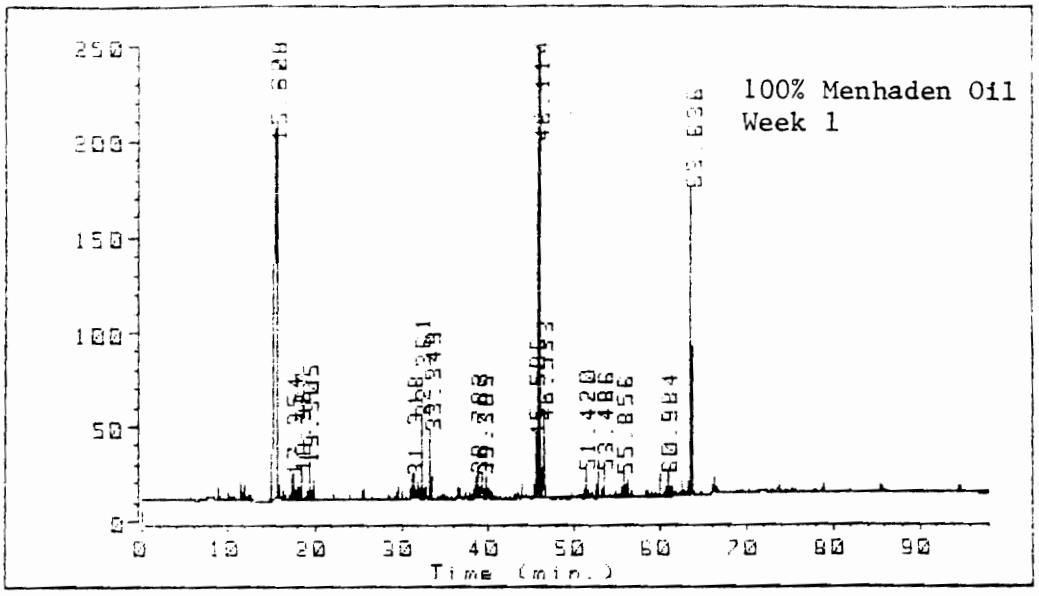




FIGURE 7: DIRECT INJECTION VOLATILE ANALYSIS OF 20% MENHADEN OIL COMPARING WEEKS 1 AND 15



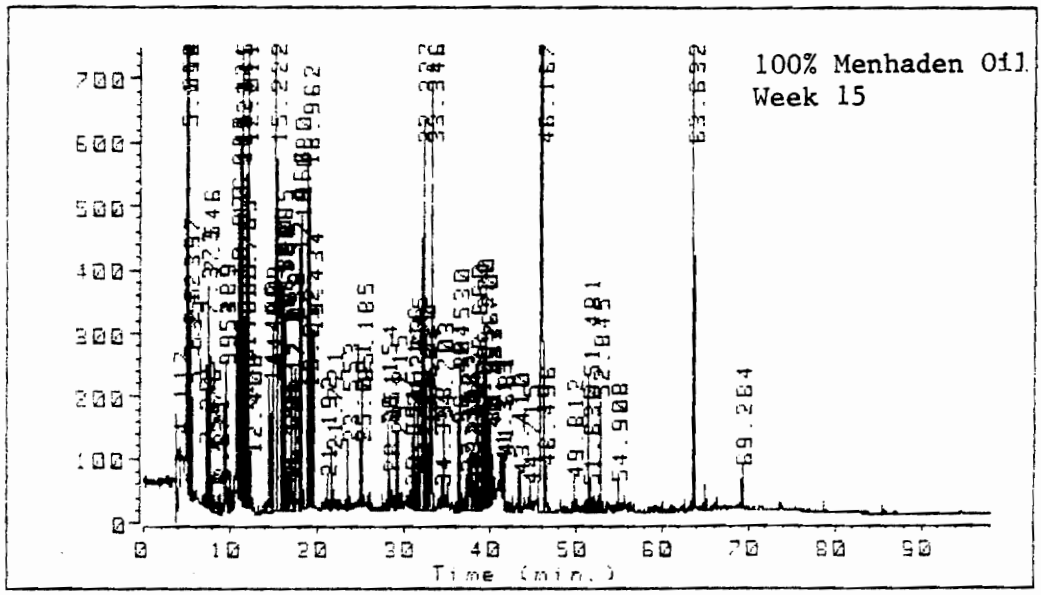




FIGURE 8: DIRECT INJECTION VOLATILE ANALYSIS OF 100% MENHADEN OIL COMPARING WEEKS 1 AND 15

Table 14. Sensory Evaluation Scores for Bland

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 2.41±0.90a | 1.92±0.90abc | 1.25±0.62ab | 1.00±0.00a |
| 3 | 2.67±0.65a | 2.33±0.89a | 1.83±0.84a | 1.08±0.29a |
| 5 | 1.92±1.00a | 1.50±0.79c | 1.33±0.65b | 1.00±0.00a |
| 7 | 2.17±0.94a | 2.25±0.97ab | 1.25±0.45ab | 1.00±0.00a |
| 9 | 1.33±0.78a | 1.58±0.79c | 1.58±0.79ab | 1.33±0.65a |
| 11 | 2.17±0.94a | 1.58±0.90c | 1.25±0.62ab | 1.00±0.00a |
| 13 | 1.67±0.89a | 1.75±0.87c | 1.33±0.65ab | 1.00±0.00a |
| 15 | 2.08±0.90a | 1.67±0.89bc | 1.50±0.90ab | 1.00±0.00a |

* Mean of bland score 1-3 ± SD; n=12.

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 15. Sensory Evaluation Scores for Bland

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 2.41±0.90a | 1.92±0.90a | 1.25±0.62b | 1.00±0.00b |
| 3 | 2.67±0.65a | 2.33±0.89ab | 1.83±0.84b | 1.08±0.29c |
| 5 | 1.92±1.00a | 1.50±0.79ab | 1.33±0.65b | 1.00±0.00b |
| 7 | 2.17±0.94a | 2.25±0.97a | 1.25±0.45a | 1.00±0.00a |
| 9 | 1.33±0.78a | 1.58±0.79a | 1.58±0.79a | 1.33±0.65a |
| 11 | 2.17±0.94a | 1.58±0.90b | 1.25±0.62bc | 1.00±0.00c |
| 13 | 1.67±0.89a | 1.75±0.87a | 1.33±0.65a | 1.00±0.00a |
| 15 | 2.08±0.90a | 1.67±0.89b | 1.50±0.90b | 1.00±0.00c |

* Mean of bland score 1-3 ± SD; n=12.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

described as no scent or a slightly oily odor. No differences were found among weeks for any of the samples (Table 14). The 0% and 10% samples were significantly different from the 20% and 100% samples during most of the study (Table 15). As expected the soybean oil had the highest bland score and the scores decreased with an increase in menhaden oil. Buttery odor (Tables 16-17) was more difficult to detect. As time increased there was a significant increase in the buttery smell of both the 0 and 10 % samples. The menhaden oil tended to mask any development of buttery odor in the 20 and 100% oils so no differences were found among weeks (Table 16). At the beginning of the study no differences were found among the different blends for buttery odor, but toward the end of the study the 0% began to exhibit a buttery smell while the others did not (Table 17). Grassy odors may also have been difficult for the panel to identify in these samples. Low scores were found during each week and for each blend of oil (Tables 18-19). There was no significant difference due to week or level of menhaden oil for the descriptor grassy. Fishy was described as the odor of fresh fish or fresh menhaden oil. The 0% sample received a mean score of about 1.00 throughout the study. The mean score tended to increase with an increase in menhaden oil. No significant

Table 16. Sensory Evaluation Scores for Buttery

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.24±0.45ab | 1.17±0.39a | 1.25±0.62a | 1.08±0.29a |
| 3 | 1.17±0.39a | 1.17±0.39a | 1.17±0.58a | 1.08±0.29a |
| 5 | 1.42±0.51ab | 1.50±0.52abc | 1.42±0.79a | 1.08±0.29a |
| 7 | 1.42±0.51ab | 1.25±0.62ab | 1.17±0.39a | 1.08±0.29a |
| 9 | 1.67±0.65bc | 1.67±0.89bc | 1.33±0.65a | 1.00±0.00a |
| 11 | 1.33±0.65ab | 1.67±0.65bc | 1.25±0.45a | 1.00±0.00a |
| 13 | 1.92±0.90c | 1.42±0.51abc | 1.25±0.45a | 1.00±0.00a |
| 15 | 1.67±0.89bc | 1.83±0.83c | 1.25±0.62a | 1.08±0.29a |

* Mean of buttery scores 1-3 ± SD; n=12.

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 17. Sensory Evaluation Scores for Buttery

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.24+0.45a | 1.17+0.39a | 1.25+0.62a | 1.08+0.29a |
| 3 | 1.17+0.39a | 1.17+0.39a | 1.17+0.58a | 1.08+0.29a |
| 5 | 1.42+0.51a | 1.50+0.52a | 1.42+0.79a | 1.08+0.29b |
| 7 | 1.42+0.51a | 1.25+0.62a | 1.17+0.39a | 1.08+0.29a |
| 9 | 1.67+0.65a | 1.67+0.89a | 1.33+0.65ab | 1.00+0.00b |
| 11 | 1.33+0.65a | 1.67+0.65a | 1.25+0.45a | 1.00+0.00a |
| 13 | 1.92+0.90a | 1.42+0.51ab | 1.25+0.45b | 1.00+0.00b |
| 15 | 1.67+0.89a | 1.83+0.83a | 1.25+0.62ab | 1.08+0.29b |

* Mean of buttery scores 1-3 + SD; n=12.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 18. Sensory Evaluation Scores for Grassy

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.00±0.00a | 1.33±0.65a | 1.08±0.29a | 1.17±0.39a |
| 3 | 1.00±0.00a | 1.25±0.45a | 1.42±0.67a | 1.42±0.79a |
| 5 | 1.33±0.65b | 1.08±0.29a | 1.17±0.58a | 1.25±0.45a |
| 7 | 1.08±0.29ab | 1.33±0.49a | 1.25±0.62a | 1.17±0.58a |
| 9 | 1.16±0.39ab | 1.17±0.39a | 1.33±0.65a | 1.50±0.67a |
| 11 | 1.17±0.39ab | 1.25±0.45a | 1.58±0.79a | 1.83±0.94a |
| 13 | 1.00±0.00a | 1.33±0.65a | 1.33±0.65a | 1.25±0.45a |
| 15 | 1.08±0.29a | 1.08±0.29a | 1.08±0.29a | 1.25±0.62a |

* Mean of grassy scores 1-3 ± SD; n=12.

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 19. Sensory Evaluation Scores for Grassy

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.00±0.00a | 1.33±0.65a | 1.08±0.29a | 1.17±0.39a |
| 3 | 1.00±0.00a | 1.25±0.45ab | 1.42±0.67b | 1.42±0.79b |
| 5 | 1.33±0.65a | 1.08±0.29a | 1.17±0.58a | 1.25±0.45a |
| 7 | 1.08±0.29a | 1.33±0.49a | 1.25±0.62a | 1.17±0.58a |
| 9 | 1.16±0.39a | 1.17±0.39a | 1.33±0.65a | 1.50±0.67a |
| 11 | 1.17±0.39a | 1.25±0.45a | 1.58±0.79a | 1.83±0.94a |
| 13 | 1.00±0.00a | 1.33±0.65ab | 1.33±0.65b | 1.25±0.45ab |
| 15 | 1.08±0.29a | 1.08±0.29a | 1.08±0.29a | 1.25±0.62a |

* Mean of grassy scores 1-3 ± SD; n=12.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 20. Sensory Evaluation Scores for Fishy

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.00±0.00a | 1.25±0.62a | 1.58±0.69a | 2.58±0.69a |
| 3 | 1.08±0.29a | 1.33±0.29a | 1.33±0.49a | 2.00±0.85a |
| 5 | 1.08±0.29a | 1.50±0.67a | 1.58±0.51a | 2.67±0.65a |
| 7 | 1.00±0.00a | 1.17±0.39a | 1.67±0.65a | 2.58±0.67a |
| 9 | 1.00±0.00a | 1.33±0.49a | 1.25±0.45a | 2.50±0.67a |
| 11 | 1.17±0.39a | 1.42±0.67a | 1.67±0.78a | 2.67±0.65a |
| 13 | 1.00±0.00a | 1.17±0.39a | 1.58±0.79a | 1.92±0.90a |
| 15 | 1.08±0.29a | 1.17±0.39a | 1.50±0.67a | 2.29±0.86a |

* Mean of fishy scores 1-3 ± SD; n=12.

* Means within a column with a different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 21. Sensory Evaluation Scores for Fishy

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.00±0.00a | 1.25±0.62a | 1.58±0.69a | 2.58±0.69b |
| 3 | 1.08±0.29a | 1.33±0.29a | 1.33±0.49a | 2.00±0.85b |
| 5 | 1.08±0.29a | 1.50±0.67a | 1.58±0.51b | 2.67±0.65c |
| 7 | 1.00±0.00a | 1.17±0.39a | 1.67±0.65ab | 2.58±0.67b |
| 9 | 1.00±0.00a | 1.33±0.49b | 1.25±0.45b | 2.50±0.67c |
| 11 | 1.17±0.39a | 1.42±0.67a | 1.67±0.78a | 2.67±0.65a |
| 13 | 1.00±0.00a | 1.17±0.39a | 1.58±0.79a | 1.92±0.90b |
| 15 | 1.08±0.29a | 1.17±0.39a | 1.50±0.67a | 2.29±0.86b |

* Mean of fishy scores 1-3 ± SD; n=12.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

difference was found by week in any of the four samples (Table 20). Only the 100% menhaden oil sample was judged consistently to be different from the other blends (Table 21). The mean scores for painty aroma were comparable with those of fishy. Again no differences among weeks was found (Table 22) and only the 100% menhaden oil was ever judged to be different from the other samples (Table 23).

Few differences ($p<0.05$) were found among the descriptors for several reasons. First, the scoring range of one to three was too small to enable the panel members to accurately describe the samples. Also by including the 100% menhaden oil which was significantly different from week 1 the differences between the other samples were decreased.

Tables 24 and 25 show the data for overall odor intensity. Panelists rated each oil for overall odor using a 15 cm hedonic scale. One indicted that the oil was bland and 14 indicted that the oil had an extreme odor (Appendix G). Scores remained relatively constant throughout the 15 week period (Table 24). The 100% oil started with an initial odor score of 9.54. This was significantly different from the other samples (Table 25). The strong fishy/painty odor of the 100% menhaden oil may have over shadowed the differences between the other oils.

Table 22. Sensory Evaluation Scores for Painty

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.00±0.00a | 1.00±0.00a | 1.50±0.67a | 1.25±0.45a |
| 3 | 1.00±0.00a | 1.08±0.29a | 1.08+0.29a | 1.67±0.98a |
| 5 | 1.08±0.29a | 1.42±0.79a | 1.67±0.39a | 1.58±0.79a |
| 7 | 1.00±0.00a | 1.00±0.00a | 1.50±0.67a | 1.58±0.67a |
| 9 | 1.00±0.00a | 1.08±0.29a | 1.25±0.45a | 1.75±0.86a |
| 11 | 1.00±0.00a | 1.00±0.00a | 1.25±0.45a | 1.67±0.89a |
| 13 | 1.08±0.29a | 1.17±0.39a | 1.08±0.29a | 1.75±0.87a |
| 15 | 1.17±0.58a | 1.17±0.39a | 1.42±0.67a | 1.92±1.00a |

* Mean of painty scores 1-3 ± SD; n=12.

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 23. Sensory Evaluation Scores for Painty

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.00±0.00a | 1.00±0.00a | 1.50±0.67ab | 1.25±0.45b |
| 3 | 1.00±0.00a | 1.08±0.29a | 1.08±0.29a | 1.67±0.98b |
| 5 | 1.08±0.29a | 1.42±0.79a | 1.67±0.39a | 1.58±0.79a |
| 7 | 1.00±0.00a | 1.00±0.00a | 1.50±0.67a | 1.58±0.67a |
| 9 | 1.00±0.00a | 1.08±0.29a | 1.25±0.45ab | 1.75±0.86b |
| 11 | 1.00±0.00a | 1.00±0.00a | 1.25±0.45a | 1.67±0.89b |
| 13 | 1.08±0.29a | 1.17±0.39a | 1.08±0.29a | 1.75±0.87b |
| 15 | 1.17±0.58a | 1.17±0.39a | 1.42±0.67a | 1.92±1.00a |

* Mean of painty scores 1-3 ± SD; n=12.

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 24. Sensory Evaluation Scores for Overall Odor Intesity

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.96±1.06a | 3.23±2.48a | 6.10±3.55a | 9.54±2.48a |
| 3 | 2.93±2.09ab | 3.98±2.74ab | 5.84±2.42a | 9.81±2.09a |
| 5 | 3.87±2.84b | 5.66±2.98b | 4.81±2.31a | 11.16±1.13a |
| 7 | 1.96±0.59a | 3.18±1.48a | 5.87±2.78a | 10.56±2.23a |
| 9 | 3.01±1.59ab | 3.37±1.63a | 4.99±2.35a | 11.03±1.88a |
| 11 | 2.84±1.86ab | 5.77±1.86b | 7.04±2.99a | 11.24±1.27a |
| 13 | 3.70±2.06b | 4.58±2.37ab | 6.97±2.95a | 11.52±1.49a |
| 15 | 3.44±2.12b | 4.83±2.76ab | 5.67±2.99a | 10.37±2.46a |

* Mean of 12 scores ± SD; 1=bland, 15=extreme

* Means within a column with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Table 25. Sensory Evaluation Scores for Overall Odor Intensity

| Week | Level of Menhaden Oil 0% | 10% | 20% | 100% |
|---|---|---|---|---|
| 1 | 1.96±1.06a | 3.23±2.48a | 6.10±3.55b | 9.54±2.48c |
| 3 | 2.93±2.09a | 3.98±2.74ab | 5.84±2.42b | 9.81±2.09c |
| 5 | 3.87±2.84a | 5.66±2.98b | 4.81±2.31ab | 11.16±1.13c |
| 7 | 1.96±0.59a | 3.18±1.48ab | 5.87±2.78b | 10.56±2.23c |
| 9 | 3.01±1.59a | 3.37±1.63a | 4.99±2.35a | 11.03±1.88b |
| 11 | 2.84±1.86a | 5.77±1.86a | 7.04±2.99a | 11.24±1.27b |
| 13 | 3.70±2.06a | 4.58±2.37ab | 6.97±2.95b | 11.52±1.49c |
| 15 | 3.44±2.12a | 4.83±2.76a | 5.67±2.99a | 10.37±2.46b |

* Mean of 12 scores ± SD; 1=bland, 15=extreme

* Means within a row with different letters are significantly different ($p < 0.05$). Differences determined with Duncan's multiple range analysis.

Correlation Coefficients

Peroxide value, total volatiles and the individual volatile pentane were correlated with the odor intensity scores of the sensory panel (Table 26). Each oil was correlated individually to eliminate any effect due to quantity of menhaden oil.

Table 26: Comparison of Overall Odor Intensity with Chemical and Physical Analysis

| | Level of Menhaden Oil | | | |
|---|---|---|---|---|
| | 0% | 10% | 20% | 100% |
| Peroxide Value | 0.39 (p<0.13) | 0.40 (p<0.12) | 0.17 (p<0.53) | 0.54 (p<0.03) |
| Total Volatiles - Static Headspace | 0.60 (p<0.01) | -0.11 (p<0.67) | -0.31 (p<0.28) | 0.12 (p<0.69) |
| Pentane - Static Headspace | 0.49 (p<0.06) | 0.25 (p<0.35) | -0.33 (p<0.40) | 0.04 (p<0.87) |

* Correlation coefficients determined using Pearson correlation procedures, n = 16

# CHAPTER 6

# DISCUSSION

This study was designed to accomplish four objectives, to determine the oxidative stability of menhaden-soybean oil blends, to identify and quantify volatile compounds responsible for off odors in these oils, to compare and contrast direct injection and static headspace capillary gas chromatography, and finally to correlate total volatiles and peroxide value with sensory evaluation scores.

## Peroxide Value

The oil samples were stored with air in the headspace to encourage oxidation during the study's 15 week period. Peroxide value was used as a general measure of oxidation in the oils. Robards et al. (1988) concluded that freshly refined oil should have a PV of less than 1. The soybean oil had a PV of 0.01 meg/kg indicating that there were no measurable hydroperoxides at the beginning of the study. The menhaden oil had been stored frozen for one month prior to the start of the study and had an initial PV of 1.15 which increased to 4.90 by week 3. Flavor deterioration of

soybean oil is difficult to measure because it occurs at low levels of oxidation, usually at a peroxide value of between 5 and 10 or below (Selke and Frankel, 1987). Only the 100% menhaden oil sample reached a peroxide value above 5 meq/kg this occurring by week five and increased to above 10 meq/kg by week 13. Peroxide formation resembles a bell shaped curve consisting of an initiation period followed by an increase then decrease in PV. These samples were stored with approximately 5 ml of air in the headspace. After increasing in value over the first 10 weeks, the PV of the 0%, 10% and 20% leveled off for the last 4 weeks indicating the available oxygen in the headspace may have been depleted. Peroxide values of oil blends stored in bottles with air in the headspace were reported by Schnepf et al. (1990). These researchers found a similar increase in PV with time, but no leveling off of PV occurred during the 20 week period. An increase in the amount of headspace and the continued opening of the storage containers would have provided more oxygen for the samples to use during oxidation. In this study, the 100% menhaden oil sample increased in PV until the end of the study but at a slower rate compared to Schnepf et al. (1990) which would be expected. The continued increase in PV observed in the 100% menhaden oil could be the result of the higher percentage of

EPA and DHA present in the sample.

Static Headspace Analysis

Static headspace volatile analysis relies on the pressurization of the sample vial prior to injection of the volatile compounds onto the column. This creates an equilibrium between the headspace and the sample allowing more volatiles to concentrate in the headspace. The Shimadzu GC-9A model used in this experiment does not pressurize the sample vial so heavier, higher molecular weight volatiles were not recovered. Static headspace is known to concentrate low molecular weight or low boiling point volatiles such as pentane and propanal (Snyder et al., 1988). Pentane was the major volatile recovered from each sample and was the major component of the total volatiles.

The isomers of decadienal and heptadienal have been associated with the breakdown of soybean and marine oils (Snyder et al., 1985; Karahadian and Lindsay, 1989a). These isomers were found in the static headspace analysis in this experiment in low concentrations after week 7 in the 100% menhaden oil samples and in the 20% menhaden oil samples after week 11. The decadienal isomers have detection threshold concentrations of between 20-320 ppb which indicate they may have an influential role in off-flavors of

oils (Meijboom, 1964). Decadienals have been noted to contribute fatty, fried, aldehyde-like aromas at higher concentrations, above 10 ppm and to suppress the aromas of green compounds through subthreshold interactions (Day et al., 1963). t,c-2,4-heptadienal and t,t-2,4-heptadienal also have been found in high concentrations in oxidizing fish oils (White and Hammond, 1983). At levels above 1 ppm these isomers were found to contribute grainy, straw-like flavors to soybean oils (Karahadian and Lindsay, 1989b). Although this static headspace system was unable to consistently recover these compounds, they were present and may have contributed to the general rise in painty, oxidized odors in the samples toward the end of the study.

Pentane often has been used as an indicator of oxidation even though it does not contribute significantly to flavor or odor changes in oils. Scholz and Patk (1966) determined that levels of 300-600 ppm indicated that soybean oil was slightly rancid. Levels of pentane in the 100% menhaden oil samples increased significantly by week 15, but no overall significant differences were found in the 20, 10, and 0% blends. Pentane is thought to be an oxidation product of linoleate which makes up 51% of the total fatty acids in soybean oil and only 2% of the total fatty acids in menhaden oil (Federal Register, 1986). The low

concentration of linoleic acid in the fish oils explains why the pentane concentration was lower than would be expected in the 100% menhaden oil which had a distinct fishy odor in beginning of the study and a strong painty odor at the end of the trial period. Spencer (1989) concluded that unsaturated fatty acids in menhaden/soybean oil blends were stable to oxidation when stored for 20 weeks under argon. The lack of production of pentane and low PV in both the soybean oil and the menhaden oil blends indicate that little oxidation of linoleic acid and other unsaturated fatty acids occurred during this 15 week period of time. Decatrienal isomers have been reported to contribute significantly to the fishy flavor of autoxidized linolenic acid and as being solely responsible for the fishy odor in strongly oxidized oils containing n-3 fatty acids (Badings, 1973).

Direct Injection Volatile Analysis

Direct injection volatile analysis of each of the oils was completed with limited success. The total volatiles were calculated from the internal standard added to each sample. The number and quantity of volatiles was highly variable from week to week. Snyder et al. (1988) found that pentane and the isomers of heptadienal and decadienal were the predominate volatiles found in aged soybean oil. When a

pentane standard was run with the external inlet device no corresponding peak was identified. It is believed that the pentane was too volatile and was not trapped in the column before the run. Heptadienal was identified by standard but too many peaks were present in the samples to be able to positively identify and quantify the amount present. It was not possible to identify the peak corresponding to decadienal by use of a standard. Even the addition of 20 microliters of decadienal to 5 ml of oil failed to produce an identifiable peak. The small sample required for use in direct injection analysis has been reported to cause thermal breakdown of the oil. Decadienal is a large molecular weight compound which could be susceptible to thermal breakdown especially at a temperature of 140 C for 20 min. If thermal breakdown did occur only a small residual amount of decadienal and its breakdown products would be recovered. The internal standard method of quantifying data relies on the assumption that an equal amount of the internal standard and the test compound are going to volatilize. This may not have been the case in this experiment.

## Sensory Evaluation

Descriptive sensory analysis of the four oils was completed using a panel of eleven students and one faculty

member. Inconclusive analysis of the data collected on the individual descriptors may have been the fault of the scoring system. A scale of 1-3 was not enough to properly separate the samples. The 100% menhaden oil was far more rancid smelling by the end of the study that it was impossible to rate it and the other oils on the same scale. The large difference between the 100% menhaden oil and the other three oils over shadowed the differences between the 0, 10, and 20% oils. The odor intensity scores provided a better indication of the condition of the oil samples. Slight increases in odor score was found in each of the oils. This increase is similar to the small increase in peroxide values found during the study further indicating that little autoxidation was taking place in the samples. Although the sensory panel was trained to distinguish the different descriptors used (bland, buttery, grassy, fishy, and painty) it is extremely difficult to pick out any individual odor when it is grouped with others. Perhaps a more highly trained panel which had more time to practice could detect greater differences among the oils. The use of a 10 or 15 point scale may have also been helpful in differentiating among samples.

Correlation Coefficients

Sensory evaluation scores for flavor and odor of oil have been correlated with physical and chemical tests with varying results. Researchers generally agree that PV is not fully capable of predicting flavor scores (Mounts et al., 1981). Correlations between odor intensity score and peroxide value ranged from 0.17 in the 20% menhaden oil to 0.54 in the 100% menhaden oil in this experiment. The results obtained here support the conclusion that PV does not correlate well with sensory evaluation scores.

A close correlation of sensory panel scores with total volatiles and with some individual volatile compounds has been reported by Dupuy et al., (1976) and by Warner, (1985). Total volatiles determined by static headspace and the odor score for the 100% soybean oil had the best correlation coefficient (0.60). All other oil blends showed poor correlations between total volatiles and odor scores. Poor correlations between pentane and sensory scores were also observed.

Small changes in odor scores and in total volatiles over the fifteen week period resulted in small correlation coefficients. If the samples had been allowed to age longer more significant differences may have been found as the level of oxidation increased. Also the incorporation of the

100% menhaden oil may have adversely affected the sensory panel decreasing their ability to judge differences in the other oils.

# CHAPTER 6

## CONCLUSIONS

Several general conclusions can be drawn from this study. Of the four oil samples stored in the dark, at room temperature with air in the headspace, all showed signs of autoxidation during the 15 week period. Significant increases ($p<0.05$) in peroxide value were found at each level of menhaden oil. Significant increases in the quantity of pentane and of total volatiles recovered was found in only the 100% menhaden oil. Significant increases in volatile formation did not occur in the other samples due to the short storage time. Sensory evaluation scores for overall odor intensity also showed little change during the study. This may have been influenced by the presence of the 100% menhaden oil sample which had a much stronger odor than the other samples even at week 1.

The only individual volatile that was identified was pentane. The static headspace analysis procedure concentrated the low molecular weigh compounds such as pentane and propanol. Isomers of decadienal and heptadienal which are heavier molecular weight compounds were detected only in the 20 and 100% menhaden oil samples at the end of

the study when strong off-odors were reported by the sensory panel.

The direct injection volatile analysis procedure did not produce consistent results. This may have been caused by too long of a conditioning period or by too much sample being placed on the glass wool. Since there were so many peaks and so much noise on the chromatograms identification of compounds by retention time was impossible.

The fact that little oxidation was occurring is supported by the poor correlations between odor scores and peroxide and GC data. Since little change was noted in the samples between weeks 1 and 15 there was little correlation in the data.

The descriptive sensory analysis proved to be of little use except to gain an overall idea of the oils scent. The small 1-3 scale did not provide the panel with the flexibility to judge the differences between the oils.

Suggestions for improving the study would include, lengthening the study period or increasing the storage temperature so autoxidation would occur to a greater extent. The 100% menhaden oil should not be included in the study because it tend to overshadow smaller differences in the other oils. A range of blends including 5, 10, 15, and 20% menhaden oil samples may give better correlations between

sensory, physical and chemical tests. In order to follow the development of individual volatile compounds in the oils, a mass spectrophotometer must be used in conjunction with the GC to consistently and accurately identify the volatiles.

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## APPENDIX A

### Peroxide Value Determination

1. Prepare acetic-acid chloroform solution. 3 volumes acetic-acid to 2 volumes chloroform.

2. Prepare saturated potassium iodide (KI) solution by adding excess KI to freshly boiled water until saturated. Excess solid KI should remain in solution after cooling.

3. Weigh to within 0.05 g, 5.00 g of sample into a 250 ml erlenmyer flask. Add 30 ml HOAc- $CHCl_3$ solution and mix. Add 0.5 ml saturated KI solution. Let stand for one minute with occasional shaking.

4. Add 30 ml of water, mix. Slowly titrate with sodium thiosulfate ($Na_2S_2O_3$) with vigorous shaking until most of the yellow color is gone. Add 0.5 ml 1% starch solution. Continue titrating until the blue color just disappears.

5. Calculation of peroxide value;

$$PV \text{ (meq peroxide/kg sample)} = \frac{S \times N \times 1000}{g \text{ sample}}$$

S= ml $Na_2S_2O_3$ (blank corrected) used to titrate
N= Normality of $Na_2S_2O_3$ solution

Appendix B

Sensory Evaluation Scores for Bland

| Judge | Week 1 | | | | Week 3 | | | | Week 5 | | | | Week 7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 |
| 2 | 3.0 | 3.0 | 1.0 | 1.0 | 3.0 | 3.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 1.0 | 1.0 |
| 3 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 |
| 4 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 6 | 3.0 | 3.0 | 3.0 | 1.0 | 3.0 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 3.0 | 1.0 | 3.0 | 3.0 | 1.0 | 1.0 |
| 7 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 |
| 8 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 3.0 | 2.0 | 3.0 | 3.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 |
| 9 | 3.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 10 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 |
| 11 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 |
| 12 | 3.0 | 3.0 | 2.0 | 1.0 | 3.0 | 3.0 | 2.0 | 1.0 | 3.0 | 2.0 | 2.0 | 1.0 | 3.0 | 3.0 | 2.0 | 1.0 |

| Judge | Week 9 | | | | Week 11 | | | | Week 13 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2 | 3.0 | 2.0 | 2.0 | 1.0 | 3.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 |
| 3 | 1.0 | 2.0 | 1.0 | 1.0 | 3.0 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 |
| 4 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 |
| 6 | 3.0 | 3.0 | 3.0 | 1.0 | 3.0 | 1.0 | 3.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 |
| 7 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 8 | 1.0 | 2.0 | 3.0 | 3.0 | 3.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 1.0 | 3.0 | 1.0 | 3.0 | 1.0 |
| 9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 |
| 10 | 1.0 | 3.0 | 2.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 11 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 |

Appendix C

Sensory Evaluation Scores for Buttery

| Judge | Week 1 | | | | Week 3 | | | | Week 5 | | | | Week 7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 4 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 5 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 |
| 6 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| 8 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 |
| 9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.0 | 2.0 | 2.0 | 1.0 | 2.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 11 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 |
| 12 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |

| Judge | Week 9 | | | | Week 11 | | | | Week 13 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 |
| 4 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 5 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 |
| 6 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 2.0 |
| 8 | 3.0 | 3.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 3.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 |
| 9 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 10 | 2.0 | 3.0 | 3.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 2.0 | 2.0 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 |
| 11 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 |
| 12 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 | 1.0 |

Appendix D

Sensory Evaluation Scores for Grassy

| Judge | Week 1 | | | | Week 3 | | | | Week 5 | | | | Week 7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 3.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 |
| 2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 |
| 4 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 |
| 6 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 |
| 8 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 12 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

| Judge | Week 9 | | | | Week 11 | | | | Week 13 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 3.0 | 3.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 3.0 |
| 2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 4 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 6 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| 7 | 1.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 8 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 |
| 12 | 2.0 | 1.0 | 3.0 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

Appendix E

Sensory Evaluation Scores for Fishy

| Judge | Week 1 | | | | Week 3 | | | | Week 5 | | | | Week 7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 2.0 | 2.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 |
| 2 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 2.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 |
| 3 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 4 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 |
| 5 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| 6 | 3.0 | 3.0 | 3.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| 7 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| 8 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 3.0 | 3.0 |
| 9 | 1.0 | 1.0 | 2.0 | 3.0 | 2.0 | 2.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 | 3.0 |
| 10 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 | 3.0 |
| 11 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 |

| Judge | Week 9 | | | | Week 11 | | | | Week 13 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 2.0 | 2.0 | 3.0 | 2.0 | 2.0 | 3.0 | 3.0 | 1.0 | 2.0 | 3.0 | 3.0 | 2.0 | 2.0 | 3.0 | 3.0 |
| 2 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.5 |
| 3 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 2.0 | 1.0 | 3.0 |
| 4 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 |
| 6 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| 7 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 8 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 9 | 1.0 | 2.0 | 2.0 | 3.0 | 2.0 | 2.0 | 2.0 | 3.0 | 3.0 | 2.0 | 3.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 |
| 10 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 3.0 | 3.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 2.0 |
| 11 | 1.0 | 2.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 |

Appendix F

Sensory Evaluation Scores for Painty

| Judge | Week 1 | | | | Week 3 | | | | Week 5 | | | | Week 7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 2.0 | 2.0 |
| 4 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 2.0 |
| 6 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 2.0 |
| 7 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 8 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 2.0 |
| 9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 3.0 |
| 11 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 |

| Judge | Week 9 | | | | Week 11 | | | | Week 13 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 3.0 | 2.0 | 2.0 | 3.0 |
| 2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 3.0 | 1.0 |
| 4 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 2.0 | 1.0 | 3.0 | 1.0 | 2.0 | 2.0 | 3.0 |
| 5 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 6 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 8 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 |
| 9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 11 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| 12 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 2.0 |

Appendix G

Sensory Evaluation for Overall Odor

| Judge | Week 1 | | | | Week 3 | | | | Week 5 | | | | Week 7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 1.4 | 2.7 | 11.9 | 12.7 | 6.4 | 1.5 | 5.1 | 8.6 | 10.0 | 7.0 | 8.5 | 12.2 | 2.5 | 1.5 | 6.3 | 12.6 |
| 2 | 2.2 | 1.3 | 5.0 | 8.3 | 1.5 | 1.2 | 5.1 | 9.9 | 2.0 | 6.4 | 5.3 | 9.7 | 1.7 | 2.0 | 6.7 | 11.7 |
| 3 | 1.6 | 3.5 | 6.4 | 10.3 | 3.9 | 3.8 | 6.1 | 12.2 | 2.8 | 4.9 | 8.6 | 12.4 | 1.8 | 3.0 | 6.5 | 11.6 |
| 4 | 3.0 | 1.0 | 4.4 | 6.5 | 1.1 | 5.0 | 8.2 | 10.7 | 9.3 | 2.4 | 4.3 | 12.6 | 2.5 | 6.2 | 11.4 | 13.4 |
| 5 | 4.7 | 2.7 | 8.1 | 10.4 | 1.4 | 5.8 | 2.7 | 5.8 | 3.8 | 5.2 | 2.5 | 10.7 | 2.0 | 4.0 | 5.3 | 10.8 |
| 6 | 2.5 | 2.5 | 2.5 | 4.1 | 3.0 | 3.0 | 4.5 | 9.1 | 3.9 | 1.8 | 1.8 | 9.0 | 1.8 | 3.4 | 2.7 | 8.2 |
| 7 | 1.0 | 1.0 | 1.0 | 4.1 | 2.9 | 2.0 | 5.0 | 9.6 | 3.5 | 3.3 | 2.1 | 11.4 | 1.0 | 3.0 | 4.7 | 6.9 |
| 8 | 2.0 | 9.9 | 12.9 | 11.6 | 2.4 | 9.8 | 11.2 | 12.8 | 2.5 | 12.2 | 4.1 | 12.2 | 2.3 | 4.5 | 10.5 | 12.7 |
| 9 | 1.0 | 1.0 | 4.7 | 8.1 | 7.6 | 8.2 | 7.6 | 12.8 | 3.6 | 7.9 | 4.3 | 10.5 | 1.0 | 3.5 | 3.5 | 11.5 |
| 10 | 1.3 | 5.4 | 3.9 | 9.8 | 2.1 | 2.8 | 4.4 | 9.4 | 1.5 | 3.3 | 5.0 | 10.6 | 3.0 | 4.3 | 6.0 | 10.2 |
| 11 | 1.2 | 4.3 | 7.7 | 12.5 | 1.2 | 1.8 | 2.8 | 6.1 | 1.2 | 8.7 | 7.4 | 11.7 | 1.8 | 1.3 | 2.5 | 6.6 |
| 12 | 1.6 | 2.5 | 4.7 | 12.5 | 1.6 | 2.8 | 7.4 | 9.4 | 2.3 | 4.8 | 3.8 | 10.9 | 2.1 | 1.4 | 4.3 | 10.5 |

| Judge | Week 9 | | | | Week 11 | | | | Week 13 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% | 0% | 10% | 20% | 100% |
| 1 | 3.0 | 2.0 | 4.5 | 11.5 | 8.5 | 9.3 | 11.4 | 12.5 | 3.1 | 6.3 | 5.0 | 12.2 | 5.1 | 7.1 | 10.6 | 12.6 |
| 2 | 1.8 | 3.8 | 3.2 | 8.9 | 2.7 | 3.4 | 9.5 | 10.2 | 2.3 | 1.4 | 8.0 | 10.3 | 1.7 | 1.7 | 1.7 | 5.8 |
| 3 | 2.6 | 5.0 | 7.3 | 12.1 | 2.6 | 4.2 | 8.4 | 10.4 | 3.1 | 2.4 | 5.6 | 13.1 | 7.4 | 8.5 | 10.5 | 12.0 |
| 4 | 1.1 | 1.3 | 8.8 | 13.8 | 1.7 | 7.3 | 6.1 | 13.3 | 4.1 | 9.0 | 11.1 | 13.6 | 3.3 | 7.6 | 7.0 | 13.6 |
| 5 | 4.7 | 2.4 | 7.1 | 10.5 | 2.1 | 4.0 | 7.3 | 11.0 | 7.0 | 2.9 | 8.9 | 11.6 | 2.9 | 4.2 | 7.4 | 11.3 |
| 6 | 2.6 | 2.6 | 2.6 | 9.5 | 2.4 | 4.0 | 2.4 | 8.7 | 2.4 | 6.7 | 4.9 | 9.9 | 3.0 | 3.0 | 3.0 | 9.7 |
| 7 | 1.5 | 5.0 | 2.1 | 6.7 | 2.6 | 6.6 | 4.3 | 11.0 | 2.7 | 3.8 | 8.5 | 10.8 | 1.5 | 3.6 | 5.2 | 12.0 |
| 8 | 6.3 | 1.8 | 2.6 | 12.1 | 2.9 | 5.5 | 7.3 | 11.8 | 8.2 | 2.8 | 5.0 | 11.2 | 2.2 | 9.4 | 3.8 | 5.7 |
| 9 | 2.5 | 3.8 | 5.1 | 11.4 | 2.1 | 4.0 | 10.3 | 12.0 | 2.6 | 8.0 | 11.1 | 13.7 | 2.5 | 1.0 | 8.0 | 10.8 |
| 10 | 1.8 | 3.3 | 4.3 | 11.6 | 3.3 | 7.7 | 10.0 | 11.9 | 5.1 | 3.8 | 3.1 | 11.2 | 2.8 | 5.0 | 4.0 | 11.2 |
| 11 | 5.2 | 6.9 | 8.4 | 12.2 | 1.4 | 6.6 | 3.6 | 10.0 | 2.2 | 4.0 | 9.7 | 11.8 | 7.5 | 4.8 | 4.1 | 9.5 |
| 12 | 3.0 | 2.5 | 3.9 | 12.1 | 1.8 | 6.6 | 3.9 | 12.1 | 1.6 | 3.9 | 2.7 | 8.8 | 1.4 | 2.1 | 2.7 | 10.2 |

APPENDIX H

SENSORY SCORECARD

ID_____

DATE_____

**ODOR EVALUATION**

| SAMPLE NUMBER: | _____ | _____ | _____ | _____ |
|---|---|---|---|---|
| **DESCRIPTIONS:** | | | | |
| Bland | ☐ | ☐ | ☐ | ☐ |
| Buttery | ☐ | ☐ | ☐ | ☐ |
| Grassy | ☐ | ☐ | ☐ | ☐ |
| Fishy | ☐ | ☐ | ☐ | ☐ |
| Painty | ☐ | ☐ | ☐ | ☐ |
| _____ | ☐ | ☐ | ☐ | ☐ |
| _____ | ☐ | ☐ | ☐ | ☐ |

Odor Intensity: 1 for weak, 2 for moderate, 3 for strong.

Based on overall intensity of odor, rate of each oil:

Bland Odor — Extreme Odor

Comments:

# VITA

Jon Carlat was born July 15, 1966 in Topeka, Kansas. Following graduation from James W. Robinson Seconardy School, he attended Virginia Polyechnic Institute & State University (VPI&SU) where he graduated with a Bachelor of Science degree in Human Nutrition and Foods with minors in Biology and Chemistry. He then completed a Masters of Science degree in the Foods option of Human Nutrition and Foods in 1990 at VPI&SU.