Quantitative tracing of barley lipids through the industrial ethanol production process
by Kari Renee Dawson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Home Economics
Montana State University
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Abstract:
Dried distiller's grain (DDG) is an inexpensive fiber and protein source, that stems from the ethanol production process. Utilization of DDG for a human food source has been limited because of the off flavor associated with DDG. Lipids are a known flavor carrier and taste culprit. By thin layer chromatography (TLC) this study quantitatively traced the DDG lipids and their changes through the industrial ethanol production process. A consumer taste panel was run with the following unleavened products: DDG granola, defatted DDG granola, barley granola, and a commercially available granola. This taste test was conducted to determine if the lipids were the flavor problem or if the by-products of the lipid leavening agent reaction were the source of the off flavor. Thin layer chromatography showed that the percentage of free fatty acids increased and the percentage of triglycerides decreased during the ethanol production process. Methyl and/or ethyl esters were formed; these were not present in the parent barley. Statistically, the untrained taste panel could not differentiate between the four granolas. This study concluded that the lipids undergo substantial changes during processing. These changes are not the cause of objectionable flavor based on the consumer taste test. The off flavor may result from the lipid leavening agent interaction but that interaction was not a part of this study. Thus the DDG, as far as flavor is concerned, is a suitable nutrition enhancer in unleavened products.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1983

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Date Dec. 28, 1983

The days are not so different
But the years tell me I have changed.
Where?
A moment...
A process...
I discovered,
But I also searched,
Never stepping into the same river twice.
Sharing...
But not always.
Believing,
Sometimes.
I remember emptiness...
But not the change.

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Vita

Kari Renee Dawson, the daughter of Anna E. Dawson and Dr. Dolphus R. Dawson was born December 14, 1957 in Minot, North Dakota. She graduated from Augusta High School in 1976 and in 1981 received a B.S. in Home Economics from Montana State University. From 1981 until present she has been attending Montana State University where she has been completing the requirements for a Master of Science degree in Home Economics, with a specialization in nutrition, under the direction of Dr. Jacquelynn O'Palka.

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Abstract

Dried distiller's grain (DDG) is an inexpensive fiber and protein source, that stems from the ethanol production process. Utilization of DDG for a human food source has been limited because of the off flavor associated with DDG. Lipids are a known flavor carrier and taste culprit. By thin layer chromatography (TLC) this study quantitatively traced the DDG lipids and their changes through the industrial ethanol production process. A consumer taste panel was run with the following unleavened products: DDG granola, defatted DDG granola, barley granola, and a commercially available granola. This taste test was conducted to determine if the lipids were the flavor problem or if the by-products of the lipid leavening agent reaction were the source of the off flavor. Thin layer chromatography showed that the percentage of free fatty acids increased and the percentage of triglycerides decreased during the ethanol production process. Methyl and/or ethyl esters were formed; these were not present in the parent barley. Statistically, the untrained taste panel could not differentiate between the four granolas. This study concluded that the lipids undergo substantial changes during processing. These changes are not the cause of objectionable flavor based on the consumer taste test. The off flavor may result from the lipid leavening agent interaction but that interaction was not a part of this study. Thus the DDG, as far as flavor is concerned, is a suitable nutrition enhancer in unleavened products.

INTRODUCTION

Dried distiller's grain (DDG) stems from the two major sources of ethanol production, the brewing/distilling industry and industrial ethanol production. The brewing/distilling industry is one of the largest producers of DDG. In 1976 more than 750,000 tons were produced by breweries (Pomeranz et al., 1976). The DDG from the brewing/distilling industry is usually a mixture of several grains, depending on the nature of the product, and the desired flavor of the product. The second largest producer of DDG is industrial ethanol production, which in 1978-79 produced 496,000 tons of DDG (Hunt, 1981). Industrial ethanol production does not concern itself with the flavor aspect of DDG but rather the maximum production of high proof ethanol, suitable for use as fuel. Industrial ethanol production differs in comparison to the brewer's/distiller's process in several ways:

1) The variety of grain(s) and their proportions.
2) The sanitation standards.
3) The temperatures and times of the process.

Dried distiller's grain has three distinct attributes, which have stimulated interest in using DDG as a human food source:

1) DDG has high levels of protein, 25-35% (Pomeranz et al., 1976; Prentice et al., 1977; Finley et al., 1980).

2) DDG has high levels of all types of fiber, 29-77%, depending on method of analysis, (Pomeranz et al., 1976; Prentice et al., 1977; Finley et al., 1980; Ranhotra et al., 1982).

3) DDG is relatively inexpensive in comparison to other human food sources.

The northwest region of the United States is the primary barley producing region. The hardiness and short growth period required renders barley suitable to the climate in the Northwest. Montana in 1983 produced approximately 15% of the nation's barley (Montana Crop and Livestock Reporter, 1983). The current primary uses for barley are the brewing industry and livestock feed. The industrial ethanol plants in Montana are a good prospect for increasing the market demand for barley. Currently, the barley DDG from these plants is being used as an inexpensive livestock feed. If DDG could be rendered suitable for human consumption and a market developed, the net worth of the DDG could be increased. This increase in market value would make barley ethanol production a more profitable business venture, thus increasing the demand for barley and its monetary worth to farmers. The ultimate end results would be a more cost competitive product "gasohol" for the ethanol producers, increased barley markets for farmers and a viable food source for consumers.

The incorporation of DDG into baked products has not been without drawbacks. Due to the fermentation process's consumption of

the grain's carbohydrates, it is considered "spent". The lack of carbohydrates causes a reduction in baking qualities (Eidet et al., 1983; Prentice and D'Appolonia 1978; and Finley et al., 1980). The baked products showed an increase in roughness of texture as well as a decrease in volume. The color of the baked products was darkened considerably, and in taste tests they were rated significantly lower than control products. This is not to say that the products were deemed unacceptable, but the 15% level (by weight of flour) was found to be an upper limit to its acceptance (Prentice et al., 1977). Previous work by Dawson et al., 1983 showed that the lipids may be a major source of the objectionable flavor in the barley DDG. Thin layer chromatography (TLC) showed that a significant lipid degradation that occurred somewhere between the whole parent barley and the end product, dried barley DDG. Defatting the barley DDG helped remedy the problem but further work is warranted to identify where the lipid degradation occurred.

In view of the nutritional attributes of DDG, as well as the economic possibilities for farmers and ethanol producers, it would be highly advantageous to develop a more acceptable DDG. The color and lack of baking qualities may be avoided by using DDG in products that do not derive their quality from volume. Because the flavor problem cannot be suitably camouflaged, it must be the first of the problems to be addressed.

REVIEW OF LITERATURE

Dried Distiller's Grain

Dried distiller's grain has been used in relatively few products, mainly baked goods such as cookies, breads and muffins. In all these products the DDG was added as a percent of flour, thus requiring that the DDG be milled. It should also be noted that most of the starting material from which the DDG was derived contain very little if any barley. The content of distiller's grain varies with the availability of the feedstock while brewer's grain is mainly barley with the various adjuncts such as wheat, rice, and other cereal grains added.

Prentice (1978) successfully used 15% DDG in a wide array of muffins. The nut, prune, pumpkin, blueberry and bran muffins were tested for acceptance at the 5, 10, and 15% DDG replacement levels. All were deemed acceptable by untrained panelists, but it was noted that at the 15% replacement level, there was a pronounced off flavor. In an investigation of bread containing heat-treated DDG (45, 100, or 150°C) it was found that the loaf volume was inversely correlated with the levels of DDG added (Prentice and D'Appolonia, 1977). At the 10% level, the crude fiber and acid detergent fiber levels were doubled in comparison to the control. However, consumer taste panels found an undesirable flavor in all the breads

containing 15% DDG. An undesirable flavor was also noted without regard to replacement levels in all the breads that contained DDG that had received the additional heat treatments of 100 or 150°C. Kissell and Prentice, (1979) found that with the use of soy lecithin and heat treated DDG they were able to double the crumb protein and increase the dietary fiber by five times without significantly degrading the physical properties of the dough or its baking qualities. However, the organoleptic limits were 20% replacement with 45°C dried DDG. Prentice et al. (1978 and 1979), explored the addition of DDG to sugar, chocolate chip, oatmeal and raisin cookies. The organoloeptic quality was lowered but still acceptable at 15% and lower replacement levels. Soy lecithin improved the spreadability of these DDG cookies by approximately 1.0 cm.

Tsen et al. (1982) found that both the spice and chocolate chip cookies that contained 15% DDG were acceptable to untrained panelist (ages 11-12). On a scale of 1 to 6 (1 excellent, 6 poor) the 99 panelists rated the chocolate chip cookies at 2.41 and the spice at 3.05. An increased darkness in color was noted on all cookies containing DDG.

A comparision of white wheat bran to DDG by Pomeranz et al. (1976), found that DDG absorbed 2-5% more water (at the 15% level) than the wheat bran. At the 15% level the DDG also had considerably lower volume ratings (100 to 150 cc. lower). The crumb grain was deemed unacceptable at levels over 10% replacement in the DDG bread,

in comparison to the wheat bran where the limit of replacement was 15%.

Brewer's spent grain, from Molson's Western Breweries, Canada, was successfully incorporated into thirty-seven products by Townsley (1979). The levels of incorporation were not given, but most of the products involved one or more of the following: rye flour, molasses, cinnamon, ginger, cloves or allspice. Townsley also noted that the protein from this DDG was more nutritionally valuable than that of the original barley grain. This may be due in part to the adjuncts that are added in the brewing process and to germination of barley during malting which enhances the nutritional quality of the grain.

Eidet et al. (1983) found that various quick breads containing DDG at the 5% level were indistinguishable from the control products. Higher levels were rated lower by untrained panelists. The volume of the bread was improved by the addition of soy lecithin at 1% of flour level. Darker coloration resulted from higher levels, (greater than 5%), of DDG incorporation.

When using the bran fraction of dried distiller's grain in bread, Dreese and Hoseney (1982) found that water absorption and loaf volume were inversely related. The loaf weight increased 11-16 grams (16%) and the volume decreased 170-190 cc. in comparison to the control. Incorporation of additional shortening helped overcome these problems. Mixograms of the bread containing the DDG bran fraction showed little resistance to the mixer for long periods of

time, and when resistance developed it was maintained for only a short period.

In a comparison of the crude fiber analysis of the spent grain fractions from two different breweries, Finley and Hanamoto (1980) found that the fiber ranged from 3.8% to 26.5%. The neutral detergent fiber ranged from 10.6 to 77.1%. Incorporation of any of these fractions significantly lowered the bread volumes, as well as the scores for break, shred, grain and texture while increasing the moisture percentage. Six and twelve percent usage produced a darker bread color than that of the control. The six percent level was deemed acceptable in appearance, texture and grain.

Ranhotra et al. (1982) compared five different DDG samples for protein efficiency ratio, amino acid content, mineral and vitamin content. The maximum amount of barley in any one of these samples was 12%. The sample with 12% barley had the second highest protein efficiency ratio and the highest protein rating (N x 6.25). This same sample had the highest levels of cadmium, zinc, iron, niacin, and folic acid in comparison to the other DDG samples.

Dawson et al. (1983) explored the effects of defatting on 100% barley DDG. Untrained panelists preferred the oatmeal cookies with 15% defatted DDG over the cookies with 15% full fatted DDG. Panelists could not differentiate between the defatted DDG cookies and the oatmeal control cookies. Alkaline bleaching was tried to improve the DDG color, but the taste panelists commented on the bitter astringent taste in the cookies containing 15% bleached DDG.

Defatting after bleaching improved the product but it was still rated significantly lower than the plain defatted DDG cookie. Thus the authors felt that the lipids in DDG may contribute significantly to the flavor problem associated with DDG .

Barley and Barley Lipids

Barley composition, like other cereal grains, is dependent on two aspects, the environment and the genetic system. The protein and carbohydrate levels are greatly influenced by the environmental factors. The lipid content is generally recognized as being genetic, and varies with the cultivar of barley. Thus, the nutrient levels that are reported are generally specific to that barley and its growing environment. Breweries by choice use lower protein level barley because the protein and carbohydrate levels are inversely related. The high protein barleys are used mainly for animal feed and ethanol production. The use of barley in human food has mainly been limited to pearled barley in soups and small amounts of barley flour in commercial baked goods. Moore (1980) explored the use of barley as a snack food in various forms and found it generally very acceptable. The puffed or crunchy snacks were tested at the 1979 Institute of Food Technologist meeting. Testing of barley croutons, crunchies and breading is currently being done by Minnesota Grain Pearling Company.

Several papers have been published comparing barley to other grains in many different aspects. In a selected trace element

comparison of barley to oats, Matthews and Douglass (1978) found that barley and oats had similar amounts of iron and zinc (4.36 and 3.28 respectively), but that oats had almost three times the amount of magnesium and somewhat higher levels of copper in comparison to barley. The lipid content of oats averaged five to eight percent, with predominantly free fatty acids and triglycerides. When investigating seven different cereal grains (oats, barley, corn, triticale, rye, sorghum and wheat), Price and Parsons (1975) found that `Chief" oats was 6.6% lipid, with 72.9% of that being neutral lipids. The triglycerides were a major fraction as were the free fatty acids. `Pilar' barley, in this study had 3.2% total lipid. The neutral fraction was 78.2%, with the triglycerides being the largest portion. Corn was 5.8% lipid and 91.9% of this was neutral. Triticale and wheat consisted of 2.4% lipid, with the neutral fractions equaling 66.9%. Rye and sorghum were similar, 3.6% and 3.3% respectively, with neutral lipids representing 72.9% and 71.0% of the total fraction.

Barley lipid content is usually 2-3% of the total dry weight, (Banasik and Gilles, 1966), and the largest portion of this is triglycerides, (73.3 - 79.1%). After the malting process was completed, the triglyceride fraction decreased significantly (14.6-16.6% loss). Small increases were noted in the mono and diglyceride fractions. Bhatty and Rossnagel (1980) compared two barleys, Riso 1508 and "normal" barley. The Riso 1508 lipid content was 5.3% of dry weight with 79.9% neutral lipids. The normal barley, Bonanza,

had a total lipid content of 3.0%; the neutral fraction of this was 65.2%. It was also noted that the Riso contained 2.5 times the amount of lipoxygenase as compared to the Bonanza. While the increased lipids may be desired for livestock feed, the grain's storage qualities would be decreased by the increased lipoxygenase.

The lipid content of twenty-one different strains of barley was determined by Fedak and DeLaRoche (1977). None of these strains exceeded 3.1% lipid. The environment was deemed to have a significant effect on the lipid levels, in that the South Dakota grown CI 12116 barley had 3.9% lipid, while the Ottawa grown sample of the same strain had 3.1% lipid. Other barley strains also showed some variation but to a much lesser degree. An earlier study by Parsons and Price (1974) found that CI 12116 had 4.6% lipid content. In their search for barleys with higher lipids, eight were selected for analysis. Their total neutral lipids ranged from 65.8 to78.1% with an average of 70.7%. The triglyceride fraction of the neutral lipids was in excess of 50%. The main thrust of this paper was to find barleys that might be of use to geneticists and plant breeders so as to increase the lipid levels of barley.

In a 1980 publication, Price and Parsons compared the neutral lipids of `Kearney' and `Pilar' barley. By preparative TLC it was determined that the `Kearney' barley neutral fraction consisted of 16.0% diglycerides, 9.0% free sterols, 8.7% free fatty acids, 52.3% triglycerides, 10.1% sterol esters and hydrocarbons, and 3.5% unknown. Using the same methods for `Pilar' barley it was determined

that the neutral fraction consisted of 15.3% diglycerides, 10.1% free sterols, 9.2% free fatty acids, 51.4% triglyerides, 10.0% sterol esters and hydrocarbons, and 3.2% unknown. When comparing six cultivated barleys, Price and Parsons (1974) found little variation in the total lipid composition, 3.1 to 3.5%. The neutral fraction ranged from 69.1 to 74.6% of the total lipids; within the neutral fraction it was estimated that the triglycerides composed over 50%.

The percent lipid in 23 modern husked European barleys was without much variation, from 2.2% to 2.8%. Welch (1978) compared these values to the percent oil in twenty-seven other barley genotypes, which varied from 1.9 to 4.1% oil. He concluded that the high-yielding European varieties had not decreased in oil content when compared to the obsolete European varieties.

Welch positively correlated the higher the protein quality in the barley with the higher the oil content. However, the oil content was not correlated with the malting grade. One of the barley strains that had the high lipid content was Riso 1508; this was an expected result as increased embryo size is correlated with increased lipid content. Price and Parsons (1979) found that the embryonic axis contained about 17.9% of the lipid while the bran/endosperm and hull contained 77.1 and 5.0%. These results were for `Pilar Hulless' barley which had a total lipid content of 3.2%. `James Hulless' oats had 7.2% lipid; of this 7.2% was in the embryo, 84.7% in the bran/endosperm, and 8.1% was in the hull.

Barley Lipid Degradation

Barley lipids, like other oils are subject to degradation when exposed to heat and enzymes. Baikov et al. (1977) found that when barley was heated to 35°C, only a small amount of change was noted in the free fatty acids and triglyceride region. When the grain was heated over 40°C, very large changes occurred. Triglycerides decreased and free fatty acids increased. This was related to the hydrolytic process of the enzyme lipase and its increased activity at higher temperature. When the grain was dried at 45-50°C, the free fatty acid content and triglyceride percent were very close to the initial percentages. However, drying the grain at 55°C caused an increase in free fatty acids and a decrease in the triglycerides, which was correlated with the degradation and oxidation of grain lipids.

Rinke (1964) reported that the optimum pH for lipolytic enzymes in barley was 7.5 and the optimum temperature was 45-48°C. The exposure to moisture decreases the enzyme's ability to survive. Dry heat however has little effect within the 45-48°C range. When the moisture is over 40% the lipase activity is inhibited. Rinke also reported the pH of solution in which the barley fats may be hydrolyzed has a significant effect on the end products. Alkaline hydrolysis will result in the formation of fatty acid salts or soap while acid hydrolysis yields free fatty acids.

Dawson et al. (1983) reported that alkaline (pH 13.0) bleached

barley DDG had a free fatty acid level of 18.75%, mono and diglycerides of 39.1% and triglycerides of 42.19%. The lipids from barley DDG yielded 78.13% free fatty acids, 16.15% mono and diglycerides and 5.72% triglycerides. These results indicate that the alkaline treatment results in free fatty acids forming products such as discussed by Rinke. Whole `Kearney' barley is reported as 8.7% free fatty acids, 16.0% mono and diglycerides, and 52.3% triglycerides.

The usage of DDG in foodstuff has been limited by two major problems, reduced baking qualities and taste. Lipids and their degradation has been correlated with decreased taste scores in many products. The lipids may not be the sole source of flavor problems, but could account for a major portion of the off flavors reported. The methods for reduction of the lipid off flavor are one, remove the lipid and two, change the processing so that the degradation does not take place. When further research is done on DDG, barley, and barley lipids, it is hoped that the new information gained will help solve the problems associated with the use of DDG and barley DDG in foodstuffs.

PURPOSE

The purpose of this study was to determine the point or points in the ethanol production process where the degradation of the neutral lipids takes place. In a pilot study, comparing the end product DDG to the parent barley, data showed changes in the relative amounts of the lipid fractions. Fractions showing significant changes were the monoglycerides, diglycerides and free fatty acids. This study quantitatively monitored these fractions throughout the entire ethanol production process. This monitoring allowed for a quantitative comparison of the lipids in the various stages of processing and the parent barley. Finally, this study determined the impact of the DDG on an unleavened food product by comparing consumer taste panel acceptance of granola containing defatted DDG or fullfatted DDG against an industrially prepared standard granola and a barley granola.

METHODS

Lipid Analysis

Piroline barley, a two row malting and feed barley, is the barley that was used for lipid analysis. The sampling was done at Alcotech, an industrial ethanol production plant in Ringling, MT. Sampling of the barley and DDG took place at nine separate stages during processing. Two separate batches were sampled. The sampling stages are listed below and remained the same throughout both batches (Figure 1 for flow chart).

1) The whole barley.
2) Barley after milling.
3) After the addition of barley to 130^{0}F water.
4) After the grain, water and enzyme slurry was brought up to 180^{0}F.
5) After the above mixture had been cooled to 90^{0}F, but before the yeast preculture had been added.
6) After 47 hours of fermentation.
7) After the DDG had gone through the stripper distillation column.
8) After the centrifugation process but just prior to drying.
9) Immediately after drying.

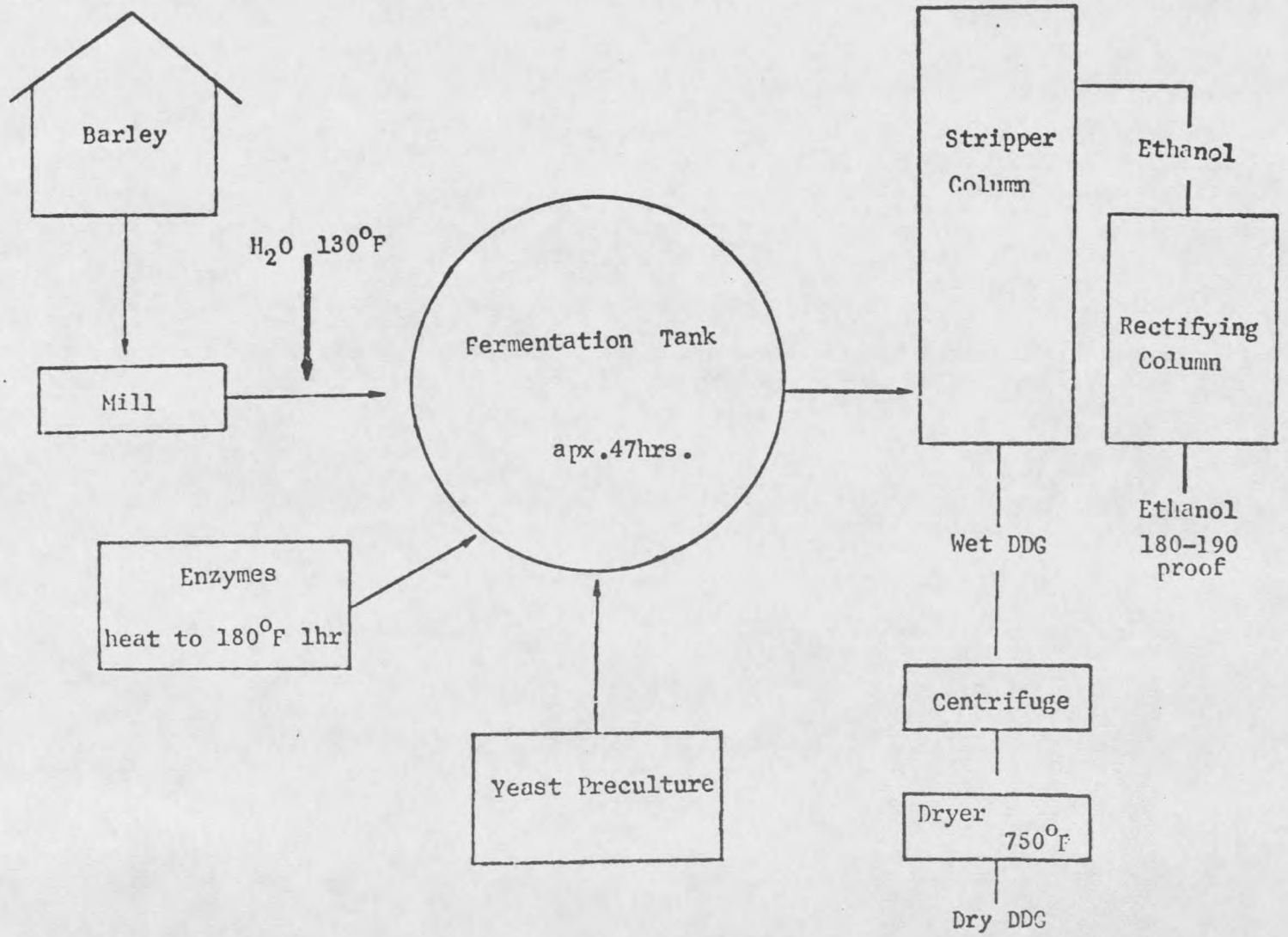


Figure 1. Flow chart of ethanol production plant.

During the fermentation process the samples were collected by lowering a one gallon plastic container into the second port hole on top of the 50,000 gallon fermentation tank. This port hole is located approximately 10 feet in from the front end of the tank. The end with the gauges was arbitrarily assigned as the front of the tank. Temperatures and pHs were taken and recorded on the information sheet (Figure 2). The samples were then immersed in reagent grade hexane (Baker Chemicals).

In the laboratory each sample was gravitationally filtered through Whatman GF/A glass fiber filter paper. The lipid hexane mixture was rotoevaporated in a 40°C water bath to remove the excess hexane. Samples three, four and six had to be reimmersed in solvent in order to obtain enough lipid extract. In these three samples it appeared that the water lipid mixture was an emulsion and more solvent was neccessary to achieve complete extraction. The resultant lipid hexane slurries that were obtained from all the samples were then placed in individual 4 dram vials, purged with nitrogen gas, and frozen until analyzed.

Analytical thin layer chromatography (TLC) of the whole lipids and neutral lipid standards (Nu-Check Prep standards, Elysian, Minn.) were run concurrently on silica TLC plates (Baker Chemicals), as described by Walsh et al. (1967). The plates were developed in TLC chambers of 85/15/1 (hexane/anhydrous ether/acetic acid, v/v/v). After allowing the solvent to evaporate from the TLC plate, it was sprayed lightly with a chromerge solution and charred for

SAMPLE NUMBER_____

Rep 1 Rep 2

Location of sample_____

Tank number_____

Time of sample_____

Date of sample_____

Temperature of sample_____

pH of sample_____(digital readout)

pH of sample_____(pH paper)

Comments and conditions:

SIGNATURE_____

Figure 2. Sample information sheet.

visualization.

Quantitative preparative TLC plates were run in triplicate for each of the samples. These TLC plates were developed in the same TLC chamber system as the analytical plates. Visualization was obtained by spraying with 0.5% rhodamine 6G in 50% ethanol and exposed to ultra violet (U.V.) light. While under the U.V. light, the 5 fraction bands were marked with a scriber. Each band was thoroughly scraped from the glass plate with a razor blade. Seven cm., 60o conical funnels were plugged with glass wool and thinly covered with 100-200 mesh silica gel. The fractions were added to the these funnels and the lipid on the silica gel was extracted with anhydrous ether. The ether was removed by rotoevaporation in a tared 50 ml. round bottom flask. After obtaining the weight of the fraction, the lipid was rinsed from the flask with reagent grade hexane and stored under a nitrogen gas atmostphere in an airtight vial. Percent recovery for each sample was determined by:

$$\frac{\text{Total amount of lipid recovered}}{\text{Total amount of lipid spotted}} \times 100$$

Relative percent of each fraction band was determined by:

$$\frac{\text{Total amount of fraction band recovered}}{\text{Total amount of lipid recovered}} \times 100$$

Analytical TLC plates were run on each of sample's fractions to determine the purity of each fraction band. Band three of all samples, except samples 1, 2, 4 (both reps) and sample 8 rep 2, had to be replated in order to obtain a clean triglyceride fraction band. After the first preparative TLC plate, the triglyceride band had several other components, free fatty acids, methyl and/or ethyl esters. The third fraction band was respotted on analytical Baker silica plates and treated the same as the preparative TLC plates. Total percent recovery and fraction percentages were determined as described earlier. Corrections for the quantatitive fractions were calculated on a percent basis. The clean fractions for each sample were then spotted on analytical TLC plates, along with the Nu-Check Prep standards, to determine purity.

Taste Panel

Granola was used as the sample product for two reasons. First it is an unleavened product, which allowed the researcher to determine whether the lipid or the products from the lipid/leavening agent interaction caused the off flavor. Secondly, granola's ease of preparation and serveability lend it to large scale consumer taste panel evaluation.

Fullfatted or defatted DDG or Piroline barley was added at the 7.5% of total weight level. The formulation for all the granolas is shown in Table 1. The rolled oats were placed in a 9" x 13" glass pan and baked for 10 minutes in a preheated 350°F oven. Sesame seeds were then added and stirred thoroughly. The soy lecithin was suspended in the oil, and this was combined with the brown sugar, honey and vanilla. These ingredients were then thoroughly mixed with the dry ingredients and placed in the preheated oven to bake. The granola was baked 15 minutes and stirred every 5 minutes to insure even baking. Upon completion of baking, the mixture was allowed to cool undisturbed in the pan before removal and being broken up into small chunks.

A commercial product, Nature Valley granola, was run concurrently in the taste test with the barley granola, fullfat DDG granola and the defatted DDG granola. On October 22, 1983 at the Main Mall in Bozeman, MT., the granola consumer taste panel was conducted. Half ounce servings of granola were placed in one ounce plastic cups and served buffet style on coded trays. The coded hedonic questionaire was placed on the tray next to the granola, (Figure 3). Consumer taste panelists were asked to taste and rate each granola. The completed coded questionnaire was then placed in the coded can. Tepid double distilled water was provided.

Table 1. Granola formula.

Ingredients	Granola	Barley Granola	DDG Granola	Defatted DDG Granola
Oats g.	216	174	174	174
Sesame Seeds g.	28	28	28	28
Brown Sugar g.	70	70	70	70
Honey g.	84	84	84	84
Oil ml.	75	75	75	75
Vanilla ml.	2.5	2.5	2.5	2.5
Soy Lecithin g.	5	5	5	5
Ground Barley g.	-	42	-	-
Fullfat DDG g.	-	-	42	-
Defatted DDG g.	-	-	-	42

Figure 3. Taste panel hedonic questionnaire.

Questionnaire

Please answer the following questions. Product no. 

1. ____male ____female

2. age ____

Taste the sample and check the face that best expresses your feelings about the product.



Statistical analysis of taste panel data

Improperly completed hedonic scales were not included in the sample population. Data were analyzed using the chi square test, from the MSUSTAT program, and the effect of age and sex was determined.

Other statistical analysis was the one way analysis of variance and the least significant difference (LSD) test as described in Snedecor and Cochran, 1980.

RESULTS AND DISCUSSION

Lipid Results

The quantitative changes in the lipid profile, of barley to DDG, are generally reflective of the processing steps. The whole Piroline barley is represented by number one on the bargraph, (Figure 4). The composition of Piroline is somewhat similar to that reported for other barley varieties in the literature. `Kearney' and `Pilar' barley as discussed by Price and Parsons, (1980) had the following composition.

Table 2. Lipid composition of Kearney, Pilar and Piroline barley.

	Diglycerides	Free Sterols	Free Fatty Acids	Triglycerides	Sterol Esters	Unknown
Kearney	16.0%	9.0%	8.7%	52.3%	10.1%	3.5%
Pilar	15.3%	10.1%	9.2%	51.4%	10.0%	3.2%
Piroline	7.3% (mono & di)	---	5.5%	73.2%	13.0%	---

This sample of Piroline barley differed somewhat from the barleys discussed by Price and Parsons (1980). However barley type and growing conditions can alter lipid composition (Fedak and DeLaRoche, 1977).

The processing stages in the production of industrial ethanol

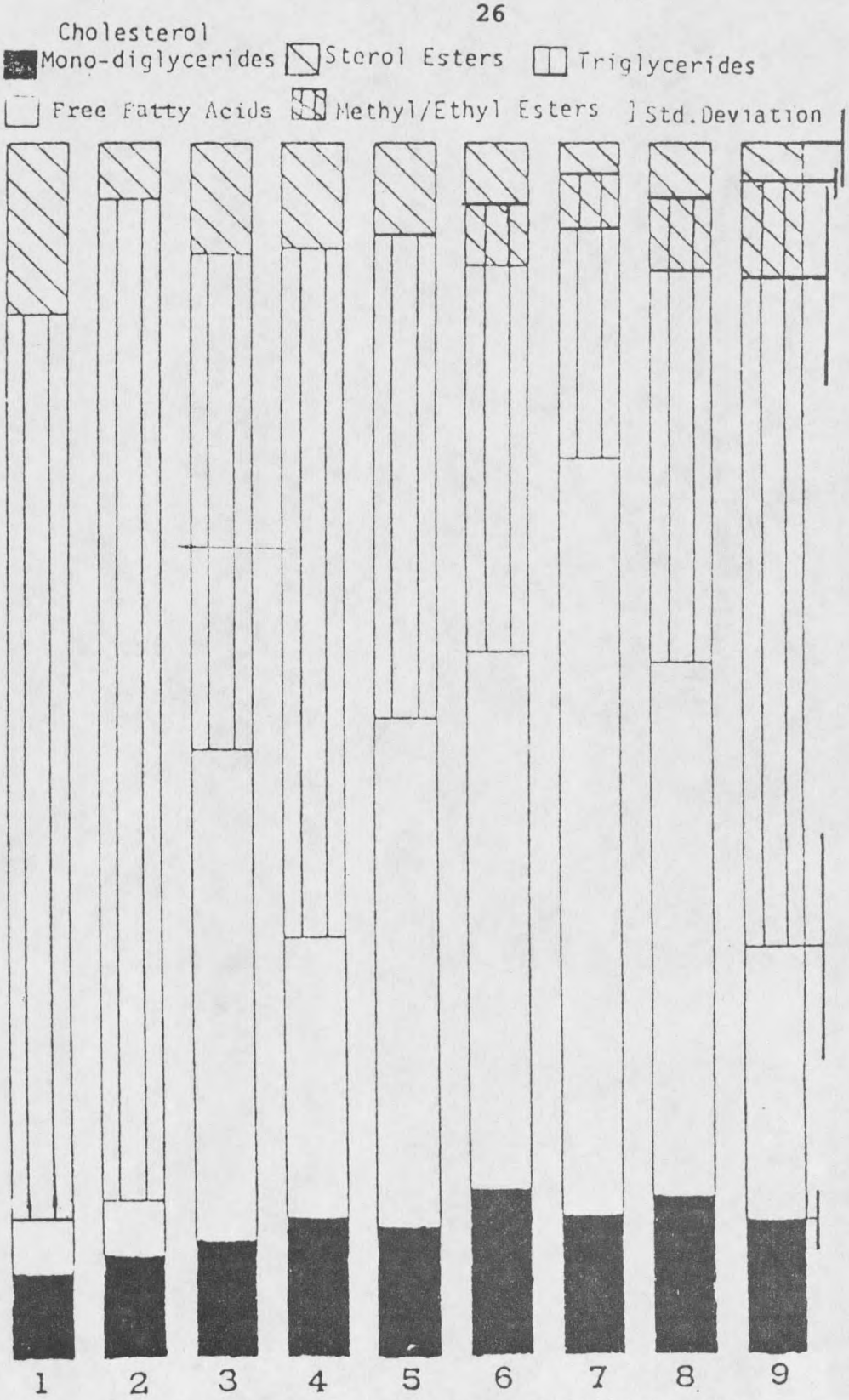


Figure 4. Quantitative percentages of lipid fractions..

vary greatly. Table 3 (appendix) contains the sample information data that were collected at each of the processing steps. The temperature was influenced by the processing methods. With the assistance of heat exchangers, the temperature was increased to 83 - 84°C as shown in sample four. This was to activate the enzymes. The next step in processing is to cool the `brew' down so that the yeast may be added (sample 6). The distillation process (sample 7) causes an increase in temperature. There was a tremendous increase in the temperature during the drying process (between samples 8 and 9). Due to the drying drum configuration, the DDG could not be sampled during drying.

An overview of the changes that occurred in the lipids can best be seen in Figure 4, 5 and 6. Figure 4 is representative of the averages of both reps. The TLCs of rep one and two (Figure 5 and 6) are general overviews of the whole lipids and their components. The occurrence of the free fatty acids in sample 3 and the methyl and/or ethyl esters in sample 6 is highly visible in the aboved mentioned TLCs. The first and last columns are NuCheck-Prep standard 18-4-A which contains, from the bottom up, cholesterol, oleic acid, triolein, methyl oleate and cholesterol oleate. The second and twelfth column contain NuCheck-Prep standard 18-1-A, which consist of, from the bottom up, monoolein, diolein, triolein and methyl oleate. The changes in the amounts of these components is illustrated in Figure 4. The numerical values that correspond to Figure 4 are listed in Table 8 (appendix). The data in Table 8 are

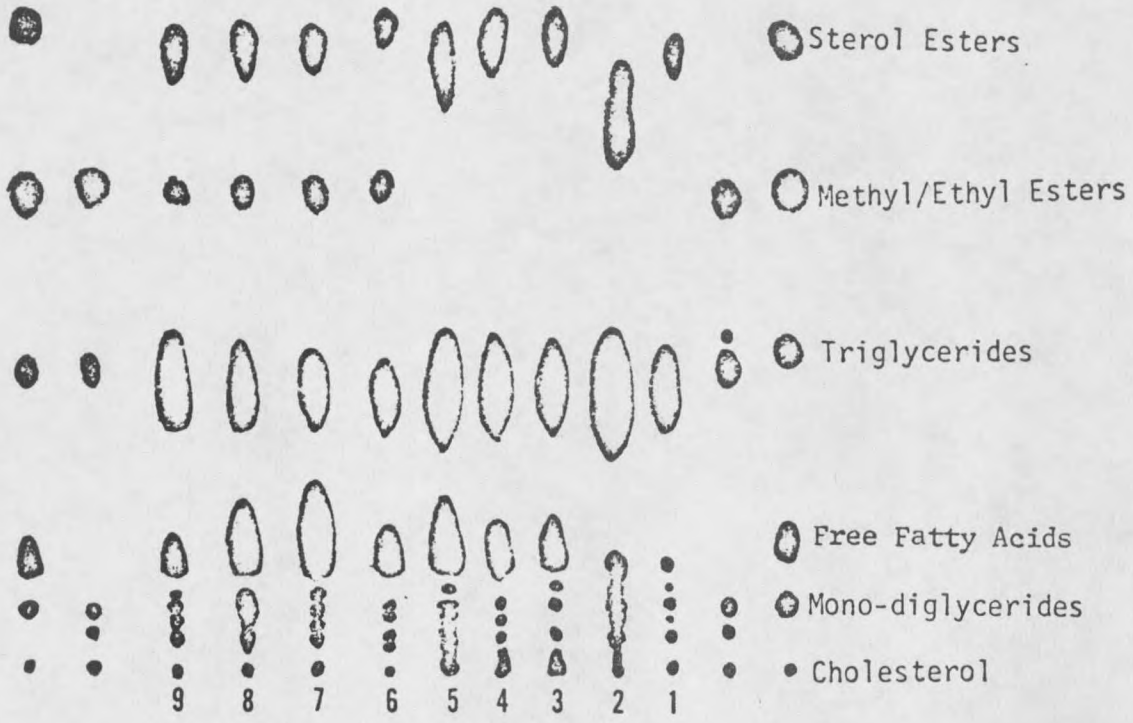


Figure 5. Analytical TLC of samples 1 through 9 rep 1.

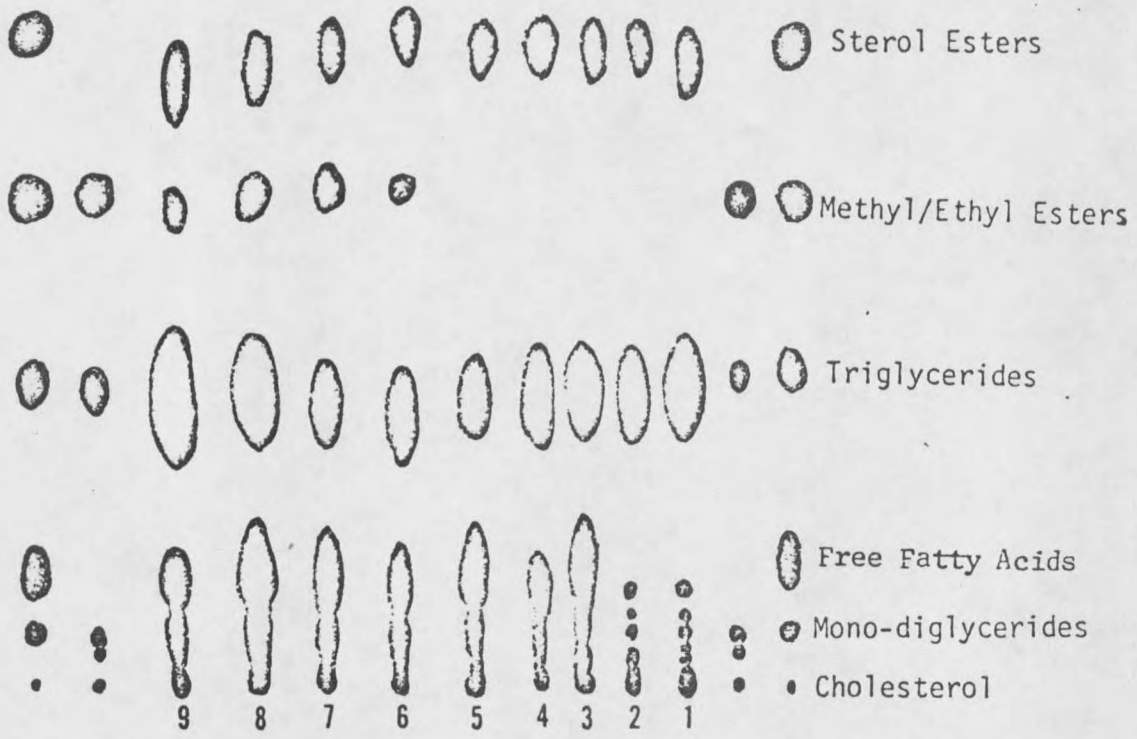


Figure 6. Analytical TLC of samples 1 through 9 rep 2.

drawn from the preparative TLC plates as described in the methods. Very little change occurred between sampling the whole barley and sample 2, the ground barley, (Figure 4). The lack of change in the triglyceride and free fatty acid regions could be due in part to the temperature, 19°C, which is well below the optimum for enzyme activity. The grain was ground very fine, (Table 7, appendix), which should allow for sufficient mixing of the lipids and enzymes. The grinding itself had no apparent effect on the lipids.

After the addition of the finely ground grain to the heated water, sample 3, lipid degradation occurred. The free fatty acid fraction went from 5.5% in sample one to 38.4% in sample three. The percent of triglycerides decreased drastically, from 73.2% to 41.9%. The other fractions, mono and diglycerides, and sterol esters remained relatively unchanged from those reported for previous processing steps. The water is one of several factors which could aid initiation of the lipid degradation. The heated water, 58 - 60°C, serves as a source of heat and a medium for the enzyme/lipid reactions. The water is also a readily available supply of O_2 for the initiation reaction. Water can supply up to 40 ml of O_2 / L of cold water and 30 ml of O_2 / L of boiling water. The reaction is started with unsaturated lipids (RH) and once started is considered auto catalytic. The following steps are a representation of the reactions common to lipids from Supran, M.K. (1978).

Initiation:

$$RH \longrightarrow R\cdot + H\cdot$$

$$RH + O_2 \longrightarrow ROO + H\cdot$$

Propagation:

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

Termination:

$$ROO\cdot + R\cdot \longrightarrow ROOR$$

$$R\cdot + R\cdot \longrightarrow R\text{-}R$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

The mild steel fermentation tanks are a good supply of transition metals which may be involved as a primary catalysts in oxidizing the lipids (Supran M.K. Ed., 1978, and Heaton F.W. et al., 1961). Supran proposed the generation of free radicals from the transition metals as follows. After the formation of the free

radicals, the reaction will continue with the propagation as shown earlier.

$$ROOH + M^{+2} \longrightarrow RO\cdot + M^{+3} + OH^{-}$$

$$ROOH + M^{+3} \longrightarrow ROO\cdot + M^{+2} + H^{+}$$

Samples 4 and 5 closely parallel sample 3. There is a marked decrease in sample 4's free fatty acid content; this may be due in part to the fact that fraction three of sample 4 contains some free fatty acids in rep 2. Sample 3 and 5 are somewhat cleaner by comparision.

Sample six showed the formation of the methyl and/or ethyl esters, (band four in Figure 4). The presence of methanol in the end product, ethanol, was determined by using a vacuum gas mass spectrometer, V.G.M.S.16MM (Figure 7). Methanol was present at somewhat less than 20 % of the total (Figure 8). The sample however had many other undetermined impurities (Figure 9). The standards in the TLC plates contained methyl esters, but by TLC analysis ethyl esters will appear in the same region. The significant reduction in the amount of sterol esters, samples 6 through 9, can best be explained by the formation of the methyl and/or ethyl esters. The following reaction is a plausible explanation, with R_1 being the fatty acid and R_2 being the sterol.

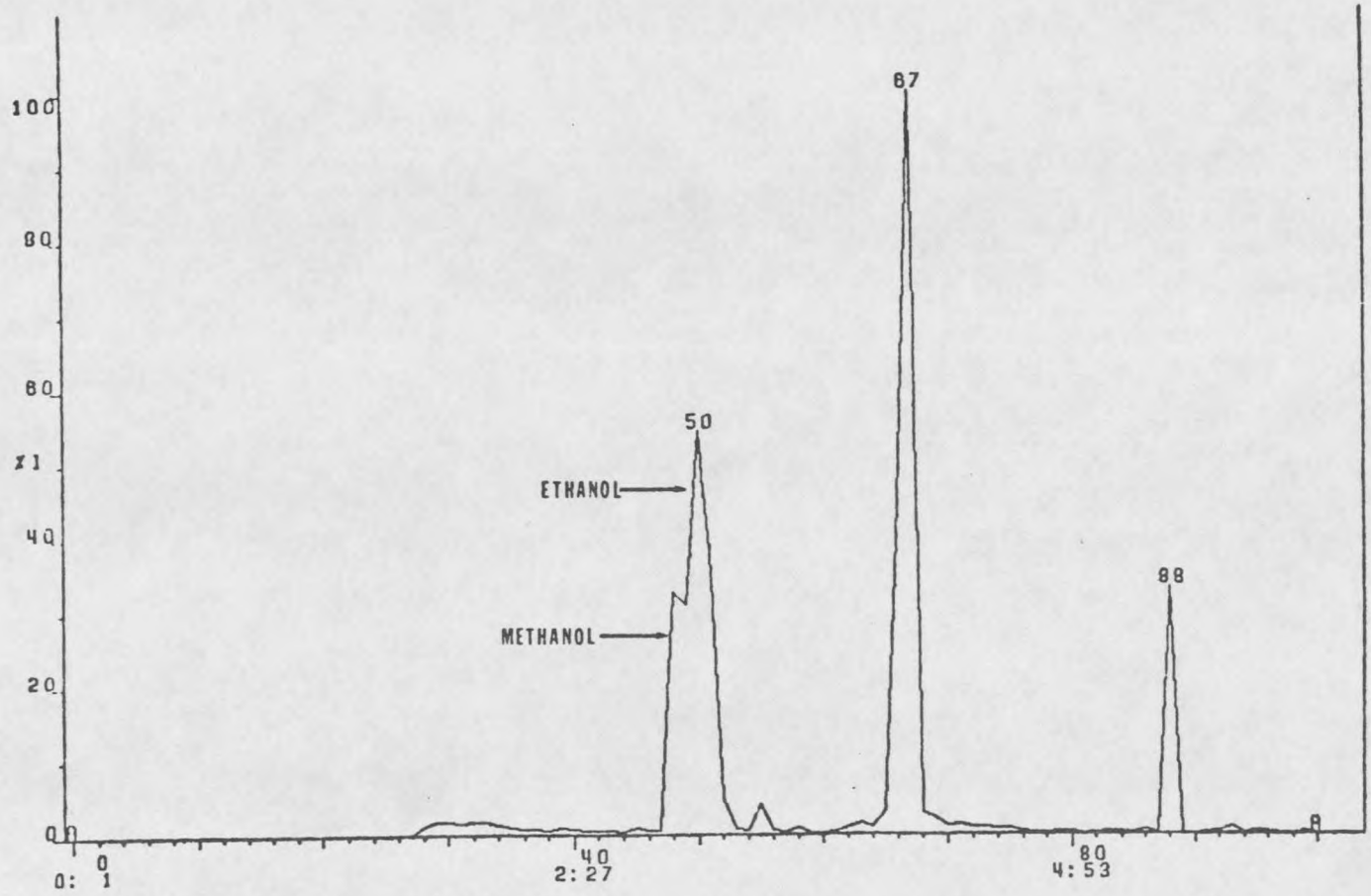


Figure 7. Mass spectrum of presence of methanol.

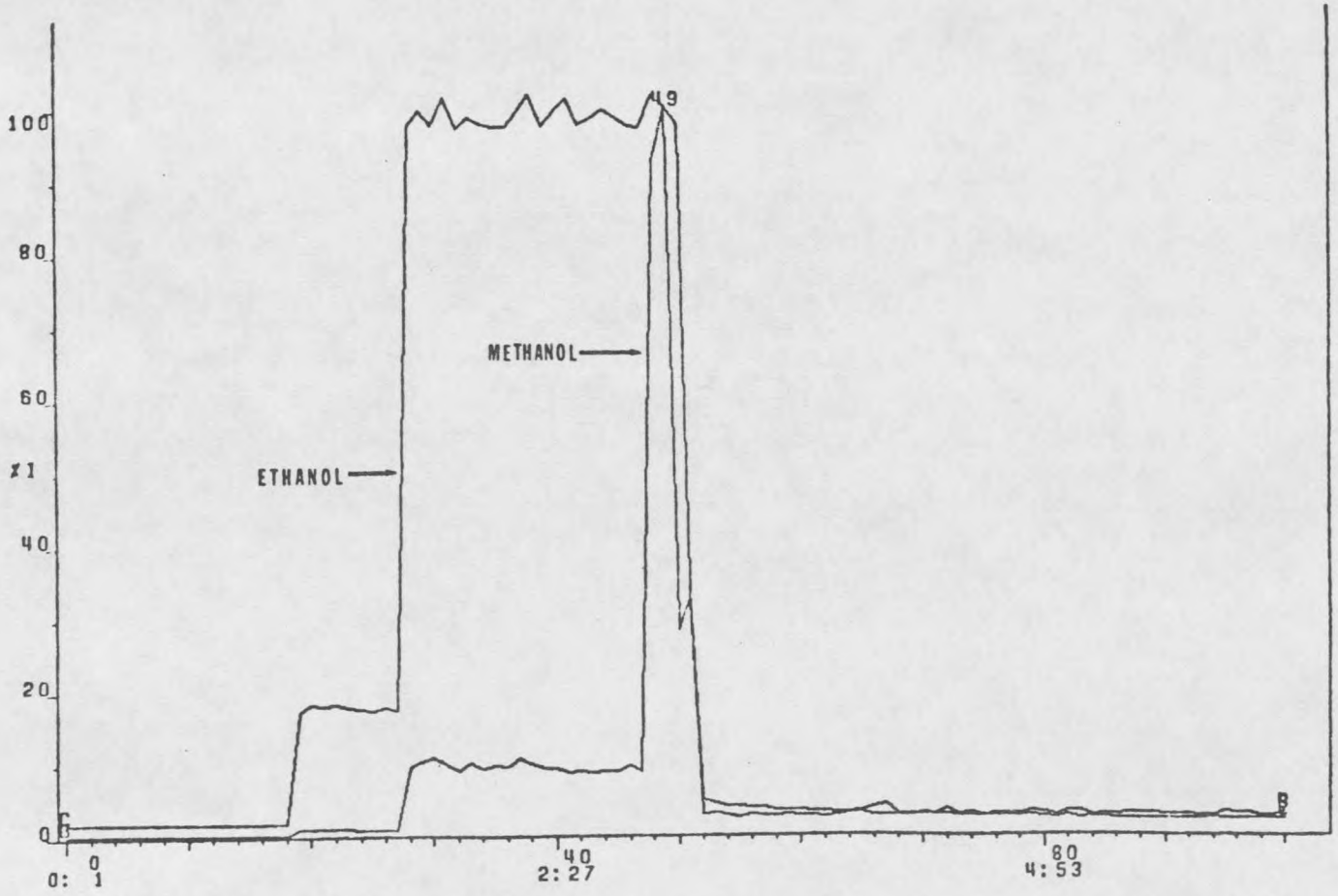


Figure 8. Mass spectrum of percentage of methanol.

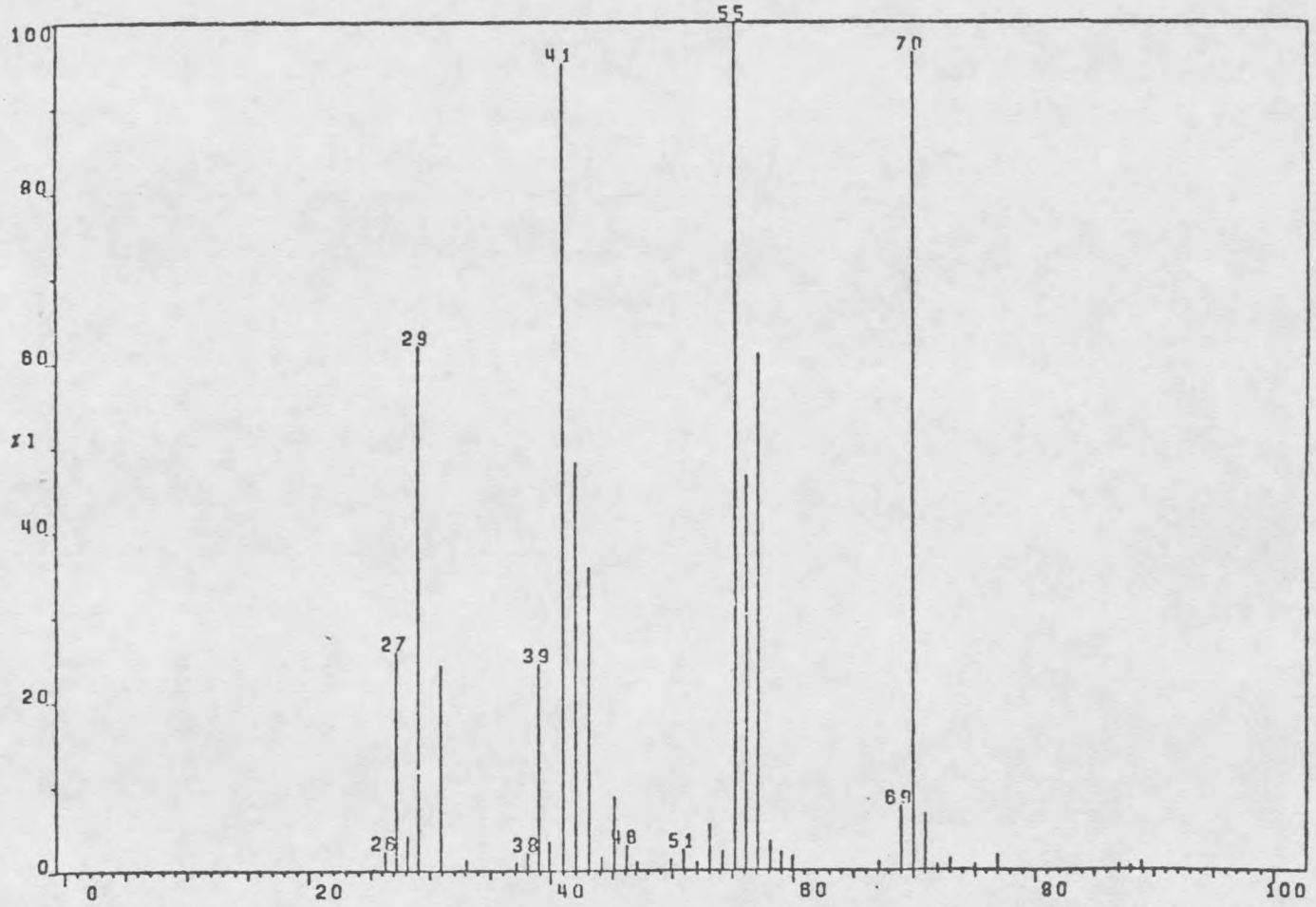
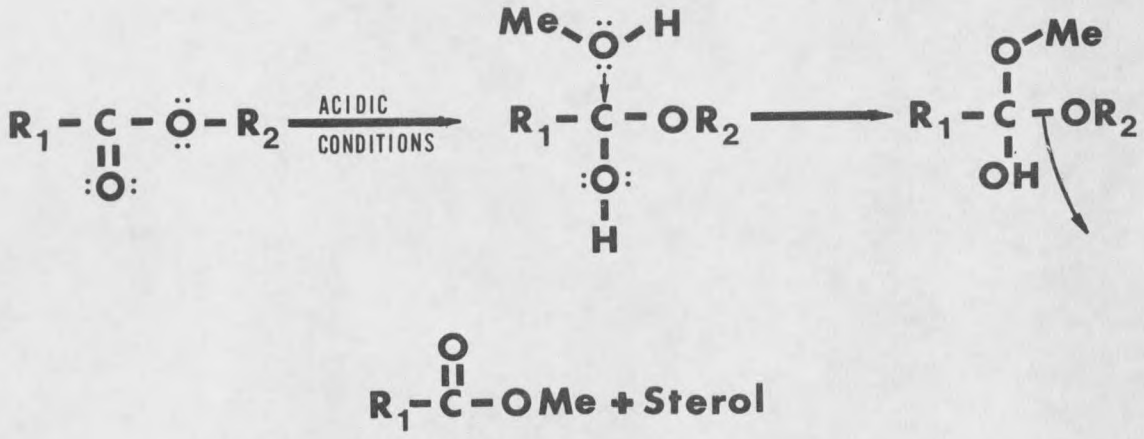


Figure 9. Mass spectrum of impurties in the ethanol.



The conditions of sample 6 were acidic, pH. 3.2 - 3.7. The sterol that was displaced would be most likely to remain in band one in the TLC fractions. This fraction band increased in sample 6 and remained fairly constant through sample 9.

Sample 7 was taken about one hour after the distillation process had started, to allow for the removal of the previous batch's DDG. Therefore, the DDG had not been subject to the heat of distillation for an extended period of time. The temperature increased 25o C over sample 6. Sample 7 was a measurement of the initial effect of the distillation conditions. The free fatty acids continued to increase while the triglycerides decreased. The stripper column is made of mild steel which may serve as a cataylst for the formation of free fatty acids. Sample 7 also showed some triglycerides in the free fatty acid fraction, which would inflate the percentage of free fatty acids.

Sample 8 showed a decrease in free fatty acids in comparison to sample 7, while the triglycerides increased. This sample is representative of the DDG that has gone through the whole distillation process and centrifugation. The DDG is damp to the touch but a majority of the liquid has been removed. The prolonged exposure to the high temperature of distillation may have promoted the decomposition of the free fatty acids to the secondary reaction products and shorter chain fatty acids. The shorter the fatty acid the more volatile the lipid. These may have been driven off in the distillation process. This may partially account for the decrease in the free fatty acids in sample 8.

Sample 9 appeared to be a continuation of the process that occurred in sample 8. The drying drum reaches temperatures in the area of 400°C which is very easily capable of volatilizing the free fatty acids as well as the secondary reaction products, (carbonyls, semialdehydes, lactones and esters). This is comparable to the formation of acrolein from glycerol in the home deep fat fryer, from over heating. The free fatty acids have lower smoke points than the triglycerides, which would explain their decrease. The contact time for the DDG in the rolling drum dryer is around 4 minutes, sufficient time for the decomposition of the free fatty acids. Thus, on a percent basis sample 9 shows an increase in the triglycerides, when it is most likely due not to the formation of triglycerides but the decomposition of the free fatty acids.

Taste panel results

Untrained consumer taste panels are one of the largest sources of variation and information in any project that uses them. Unlimited amounts of uncontrollable factors can enter into each panelist decision, and yet these factors will just as likely affect the consumer's decision on buying a product. Trained panelists help alleviate many of these discrepancies but it will always be the consumer who makes the final judgment.

The 163 untrained panelist in this testing ranged in age from 3 to 61 years of age. The average age was 21.8. Sixty-six males participated and 97 females participated in the panel. Analysis of variance on the taste panel data showed that age, sex and type of granola had no significant effect on the rating. Age however, was the closest with a P-value of .1116 (Table 3).

Table 3. Analysis of variance of granola taste panel.

Source	Df	F-value	P-value
1	3	1.339	.3218
2	1	.1606	.6980
1,2	3	1.4323	.2364
3	3	2.6561	.1116
1,3	9	2.3167	.1135
2,3	3	1.0742	.4089
Error 1,2,3	9		

* denotes significance at P < .05
1= type 2= sex 3= age

With no significant difference ($P < .05$) the granolas were rated as equal, with the following hedonic means.

Barley granola	3.899	A
DDG granola	3.885	A
Nature Valley	3.821	A
Defatted DDG granola	3.651	A

The analysis of variance is statistically not as sound as some of the other tests, due to the fact that we can not assume normality. That is, each cell (age group in this study) is not represented equally. Therefore, goodness-of-fit statistics were run on the log-linear models. These are the chi square (X^2) and the likelihood of ratio test (G^2). "The statistics of X^2 and G^2 are asymptotically equivalent; that is, they are equivalent in very large samples when the null hypothesis is true" (Fienberg, 1981). The two can best be described as:

$$X^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

$$G^2 = 2 \sum (\text{Observed}) \log \left(\frac{\text{Observed}}{\text{Expected}} \right)$$

The 2 x 2 X^2 and G^2 analysis test were run on several factors, rating by type, rating by age, and rating by sex. Rating by type had

the only significant value (Table 4). However, rating by age was close to being significant.

Table 4. 2 x 2 analysis as compared to rating.

	P-values		
	Type	Sex	Age
X^2	.0275*	.2798	.0727
G^2	.0235*	.2680	.0661

* significant at P <.05

Therefore, a 3 x 2 test was run comparing rating by type by age. This test was nonsignificant at P < .05, thus age was deemed nonsignificant.

X^2 P-value .2389

G^2 P-value .2660

The results of the X^2 and G^2 test are statistically more sound than those of the analysis of variance. As a consequence it was determined that rating by type was the only factor that affected the results. Age was determined not statistically significant, at P < .05 but was at P < .10 . The distribution of the ratings could cause this. A granola could be either very much liked and very much

disliked, or consistently rated in the middle, and still have the same mean. Distribution may interact with age when all the cells are not evenly filled. For this reason the distribution of the granola ratings was determined (Table 5).

Table 5. Distribution of granola ratings.

	Above average	Average	Below average
DDG granola	57.5%	28.1%	14.4%
Defatted DDG granola	56.6%	24.6%	18.6%
Barley granola	66.6%	15.5%	15.5%
Nature Valley granola	69.1%	21.2%	9.7%

Though all the granolas were statistically considered equal, the distribution is not the same for all the granolas.

CONCLUSIONS

The lipids of Piroline barley undergo some very dramatic changes during the production of DDG. When these changes are compared to the parent barley, the most noted ones are in the free fatty acid region and the methyl and/or ethyl ester region. None of the processing steps is solely responsible for the changes but rather there is a cumulative effect of all the factors involved in each of the steps.

Earlier taste panel work involving barley DDG by Dawson et al. (1983) and Eidet et al. (1983) found a decreased taste score in DDG products. Dawson et al. (1983) found that lipid removal from DDG improved the scores by panelists. This study did not find a taste score difference between the DDG and defatted DDG granola. This evidence supports the hypothesis that the lower taste scores could be due to the lipid/leavening agent reaction by products. Granola is a nonleavened product so saponification is unlikely to take place, thus the higher ratings for the plain DDG granola.

If DDG is to be incorporated into human food in its present form, unleavened products would be choices, provided that the shelf life of the products is acceptable. Time stability studies would need to be conducted to accurately determine the effect of using fullfat DDG. But if the final product is to contain leavening agents, the DDG should be defatted. The choice to defat prior to processing the barley into DDG or after processing, is purely an

economic one. This too must be decided between the DDG producer and the food companies.

The results of this study indicate that the lipids change throughout the processing but when used in unleavened products these changes are not a factor in taste panel rating. In fact, the taste panel data supports the usage of barley, and barley DDG in the unleavened product granola. The closeness of the ratings for all the granolas shows that the products prepared for this study compare favorably with the commercially available product, Nature Valley granola.

* Mention of firm names or products does not constitute endorsement by Montana State University over others of a similar nature.

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APPENDIX

RAW DATA

Table 6. Sample information sheet data

Sample Number	Temperature of Sample	pH of Sample
1-Whole Grain	NA	NA
2-Ground Grain	NA	NA
3-Grain and H_2O	60-50°C	5.4-5.3
4-H_2O, Grain and Enzyme	84-83°C	4.9-5.7
5-Cooled Grain Slurry	33-38°C	5.2-4.2
6-End of Fermentation	36-37°C	3.5-3.7
7-Stripper Column	60-62°C	3.1-3.7
8-After Centrifugation	43-47°C	NA
9-Dried DDG	29-32°C	NA

Table 7. Sieve analysis.

SAMPLE: Barley

SUBMITTED BY: AlcoTech

SCREEN SIZE	GRAMS	%	SUGGESTED
12	4.20	4	3
16	15.42	15	8
20	21.18	21	36
30	24.12	24	20
40	26.27	26	14
60	6.34	6	12
Thru 60	2.60	3	7

Signed: Joan Conlin

Title: Research Director

Date: December 3, 1982

Table 8. Preparative TLC data.

Sample/rep Averages	% Band 1 Mono & Di glycerides	% Band 2 Free Fatty Acids	% Band 3 Triglycerides	% Band 4 Methyl/ Ethyl Esters	% Band 5 Sterol Esters	% Recovery
Average of 1 (whole grain)	7.3	5.5	73.2	0.0	13.9	76.2
Average of 2 (ground grain)	8.1	4.5	83.3	0.0	4.5	78.2
Average of 3 (grain & H_2O)	9.6	40.3	42.0	0.0	6.7	86.4
Average of 4 (H_2O, grain & enzymes)	11.7	22.8	57.3	0.0	8.2	68.6
Average of 5 (cooled grain slurry)	10.6	41.5	38.9	0.0	7.3	78.8
Average of 6 (end of fermentation)	13.8	44.5	31.5	7.5	7.2	72.2
Average of 7 (stripper column)	11.9	62.2	19.2	4.1	2.6	87.0
Average of 8 (after centrifugation)	12.9	44.6	32.4	6.2	4.7	88.8
Average of 9 (dried DDG)	11.5	22.1	55.7	7.7	3.1	86.2

Table 9. R_f values for analytical TLC of samples 1 through 9 rep 1.

Sample Number	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
1-Whole Grain	.04	.08	.23	—	.48
2-Ground Grain	.04	.08	.22	—	.49
3-Grain and H_2O	.04	.10	.23	—	.48
4-H_2O Grain & Enzyme	.03	.08	.22	—	.49
5-Cooled Grain Slurry	.04	.10	.22	—	.49
6-End of Fermentation	.03	.08	.21	.38	.47
7-Stripper Column	.03	.09	.22	.38	.49
8-After Centrifugation	.03	.09	.23	.38	.48
9-Dried DDG	.04	.08	.22	.36	.46
Standard 18-4A	.05	.09	.24	.37	.50
Standard 18-1A	.04	—	.23	.37	—

Table 10. R_f values for analytical TLC of samples 1 through 9 rep 2.

Sample Number	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
1-2 Whole Grain	.04	.08	.23	---	.48
2-Ground Grain	.04	.08	.22	---	.49
3-Grain and H_2O	.04	.10	.23	---	.48
4-H_2O, Grain & Enzyme	.03	.08	.22	---	.49
5-Cooled Grain Slurry	.04	.10	.22	---	.49
6-End of Fermentation	.03	.08	.21	.38	.47
7--Stripper Column	.03	.09	.22	.38	.49
8-After Centrifugation	.03	.09	.23	.38	.48
9-Dried DDG	.04	.08	.22	.36	.46
Standard 18-4A	.05	.09	.24	.37	.50
Standard 18-1A	.04	---	.23	.37	---

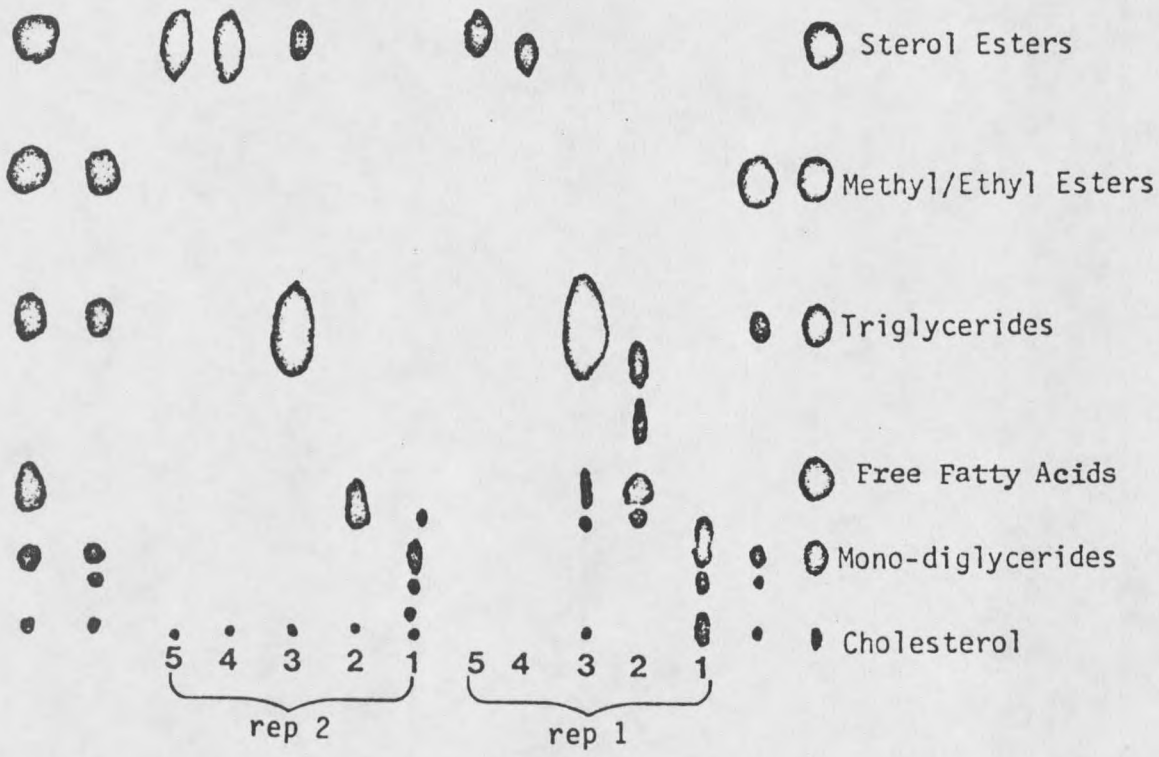


Figure 10. Analytical TLC of sample 1 rep 1 and 2.

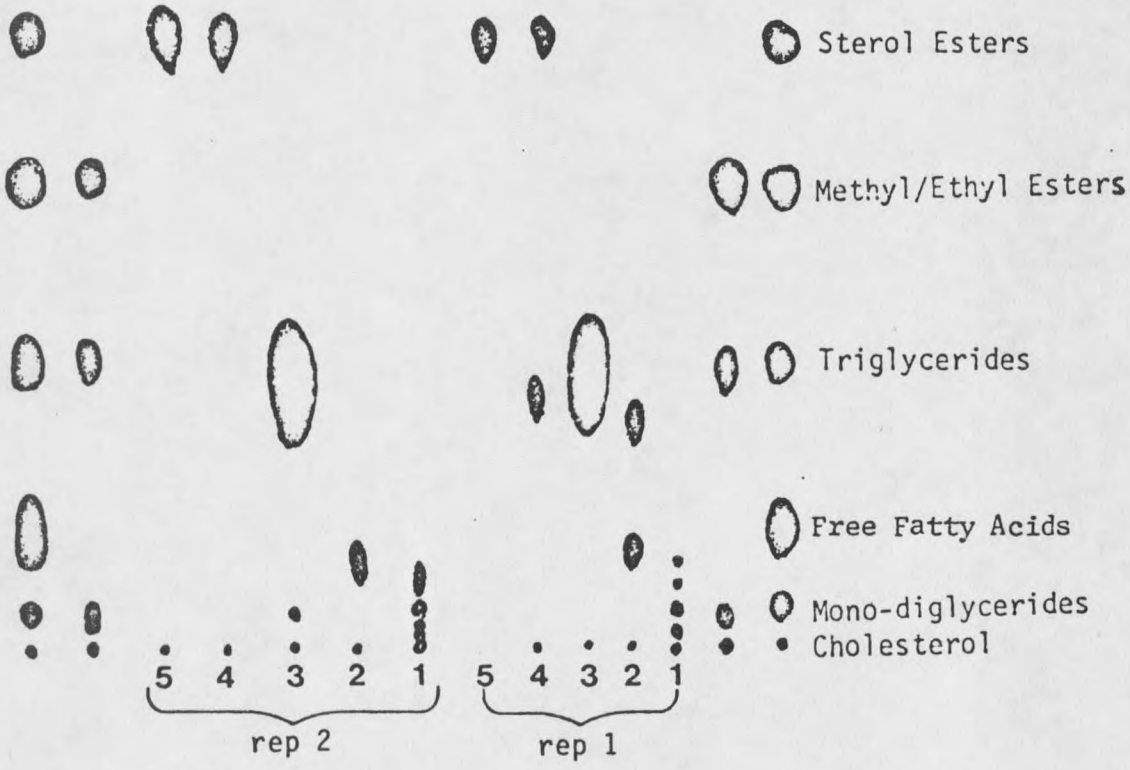


Figure 11. Analytical TLC of sample 2 rep 1 and 2.

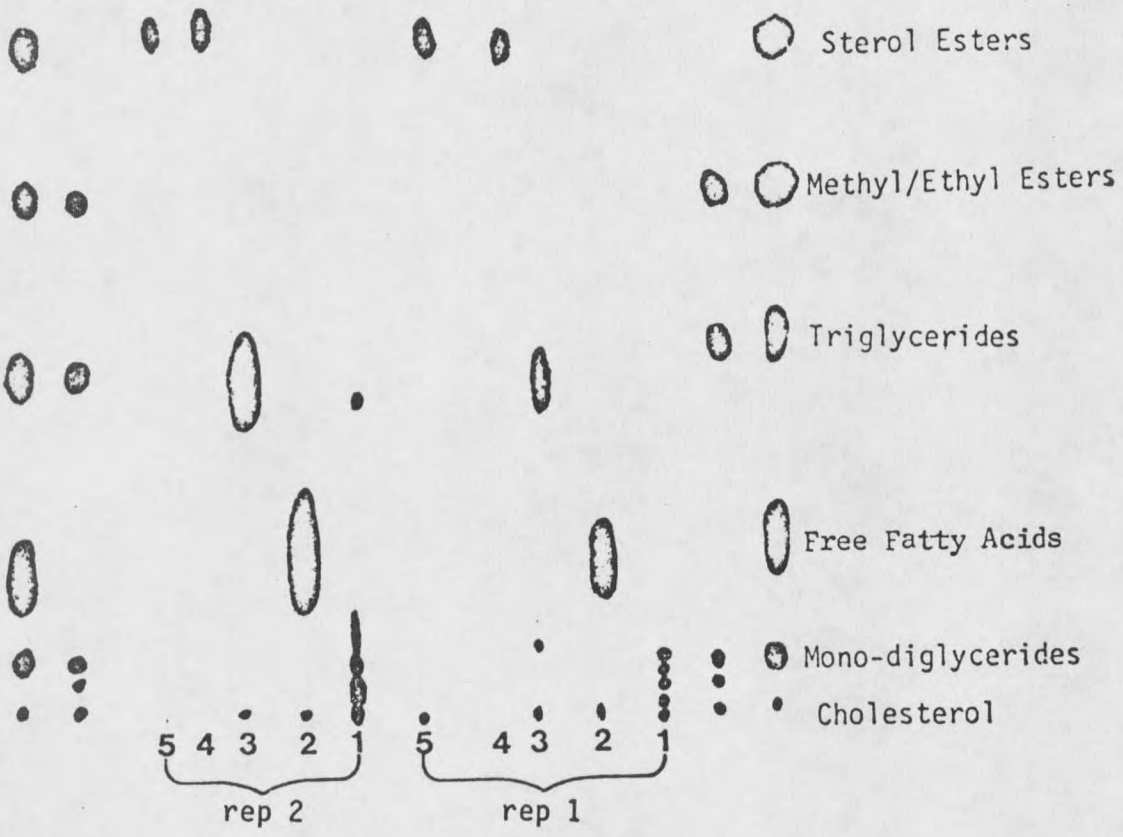


Figure 12. Analytical TLC of sample 3 rep 1 and 2.

Table 11. R_f values for analytical TLC of sample 1 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep.1 & 2	.06	.10	.23	---	.45
18-4A	.06	.11	.24	.35	.45
18-1A	.06	---	.24	.35	---

Table 12. R_f values for analytical TLC of sample 2 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.03	.07	.24	---	.47
18-4A	.03	.09	.22	.36	.47
18-1A	.03	---	.22	.36	---

Table 13. R_f values for analytical TLC of sample 3 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.04	.12	.26	---	.52
18-4A	.04	.12	.27	.40	.51
18-1A	.04	---	.27	.39	---

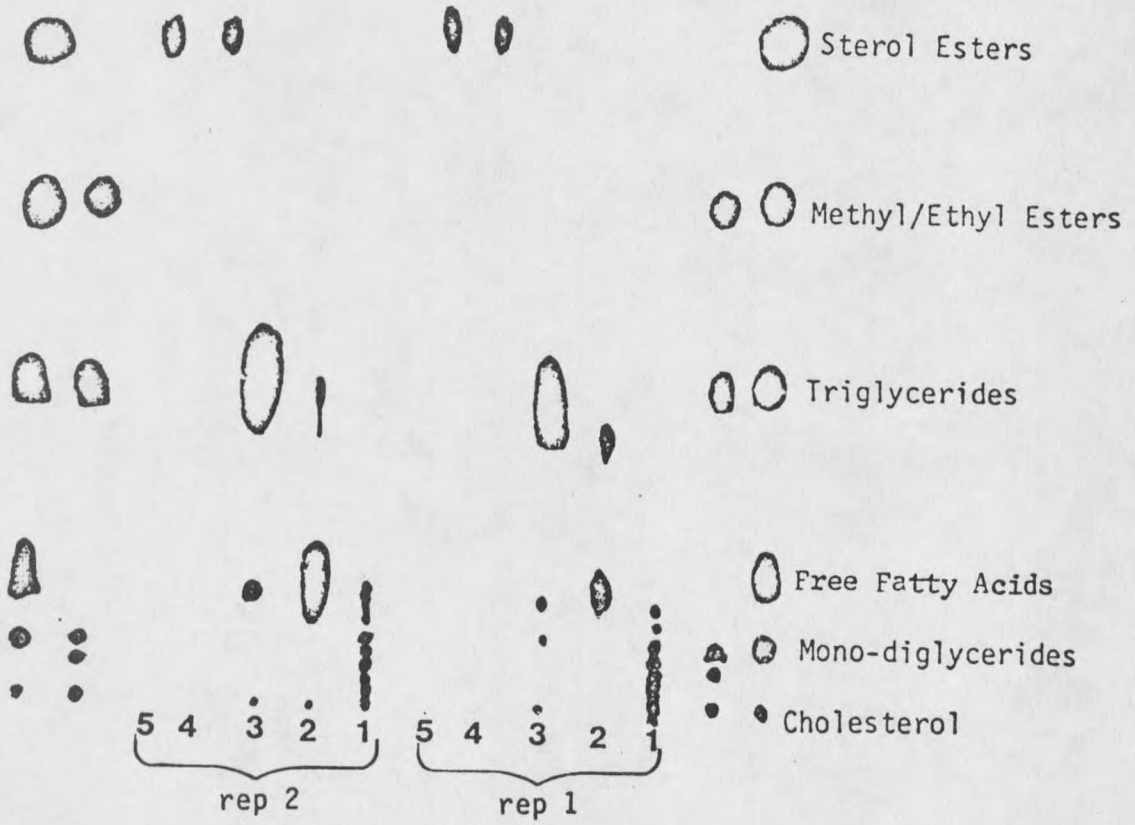


Figure 13. Analytical TLC of sample 4 rep 1 and 2.

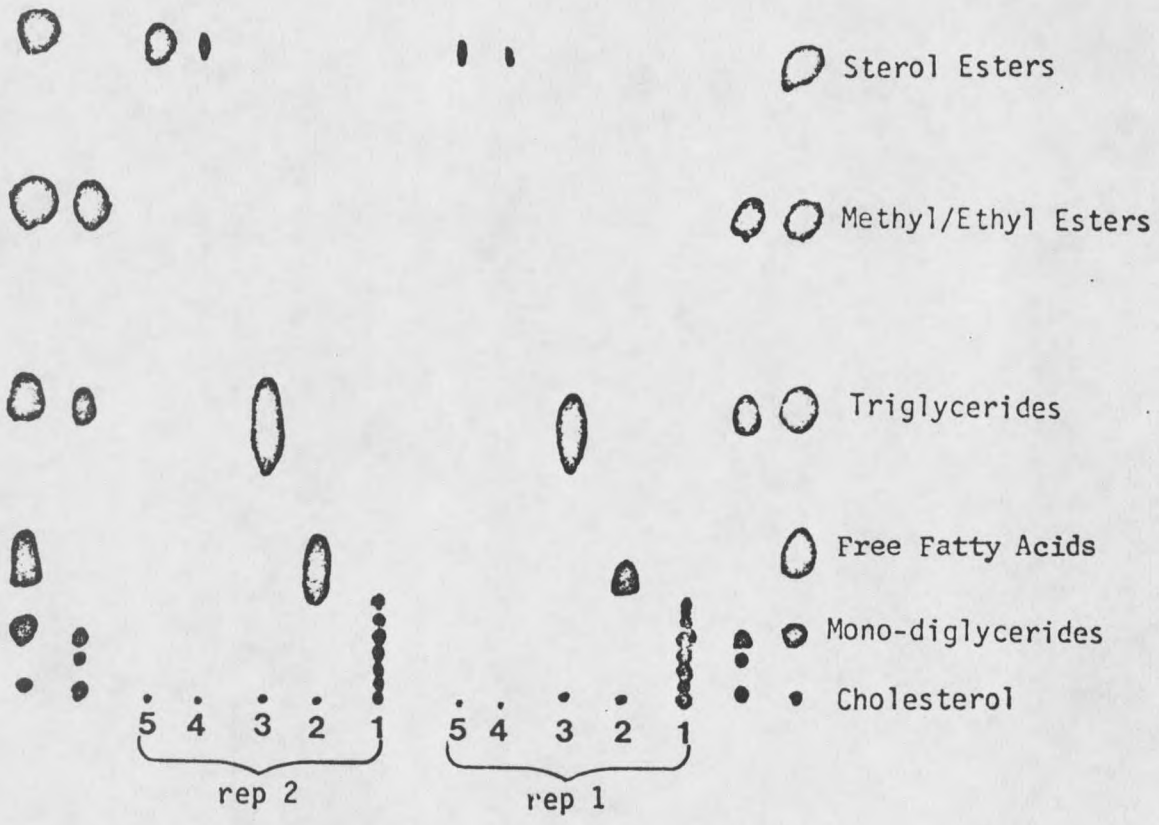


Figure 14. Analytical TLC of sample 5 rep 1 and 2.

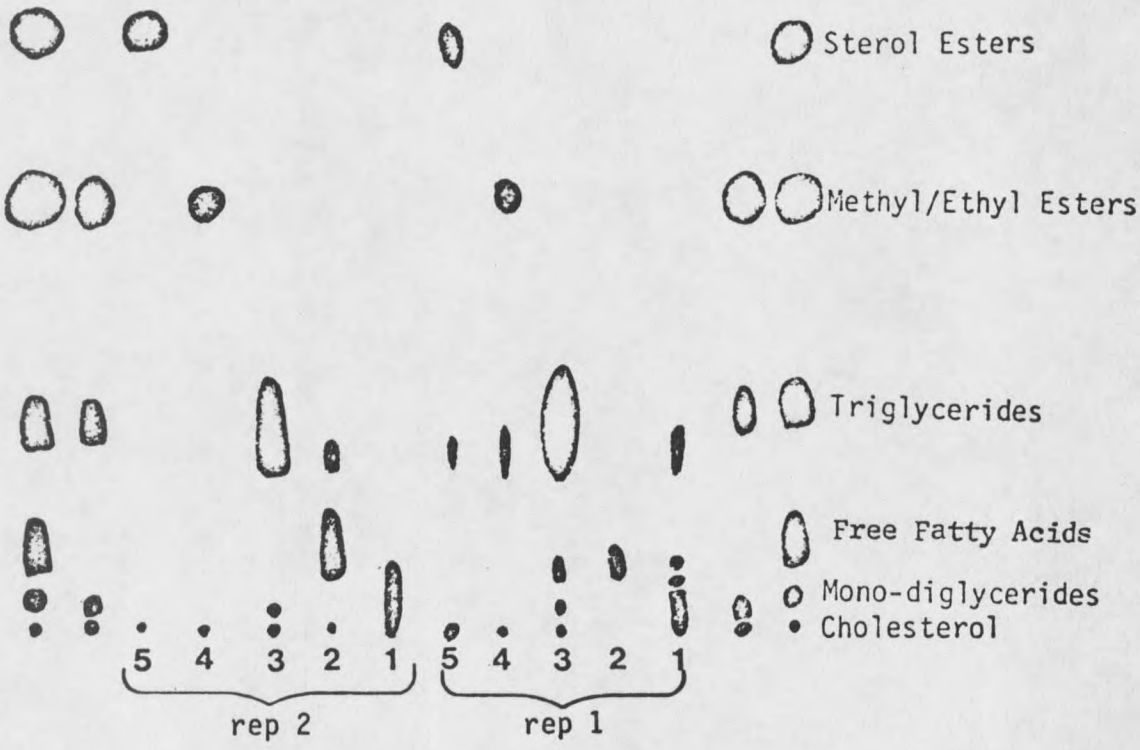


Figure 15. Analytical TLC of sample 6 rep 1 and 2.

Table 14. R_f values for analytical TLC of sample 4 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.03	.09	.24	---	.50
18-4A	.04	.10	.24	.38	.50
18-1A	.04	---	.24	.38	---

Table 15. R_f values for analytical TLC of sample 5 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.03	.10	.21	---	.49
18-4A	.05	.10	.22	.37	.49
18-1A	.04	---	.22	.37	---

Table 16. R_f values for analytical TLC of sample 6 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.03	.06	.15	.33	.45
18-4A	.03	.07	.17	.33	.46
18-1A	.02	---	.16	.33	---

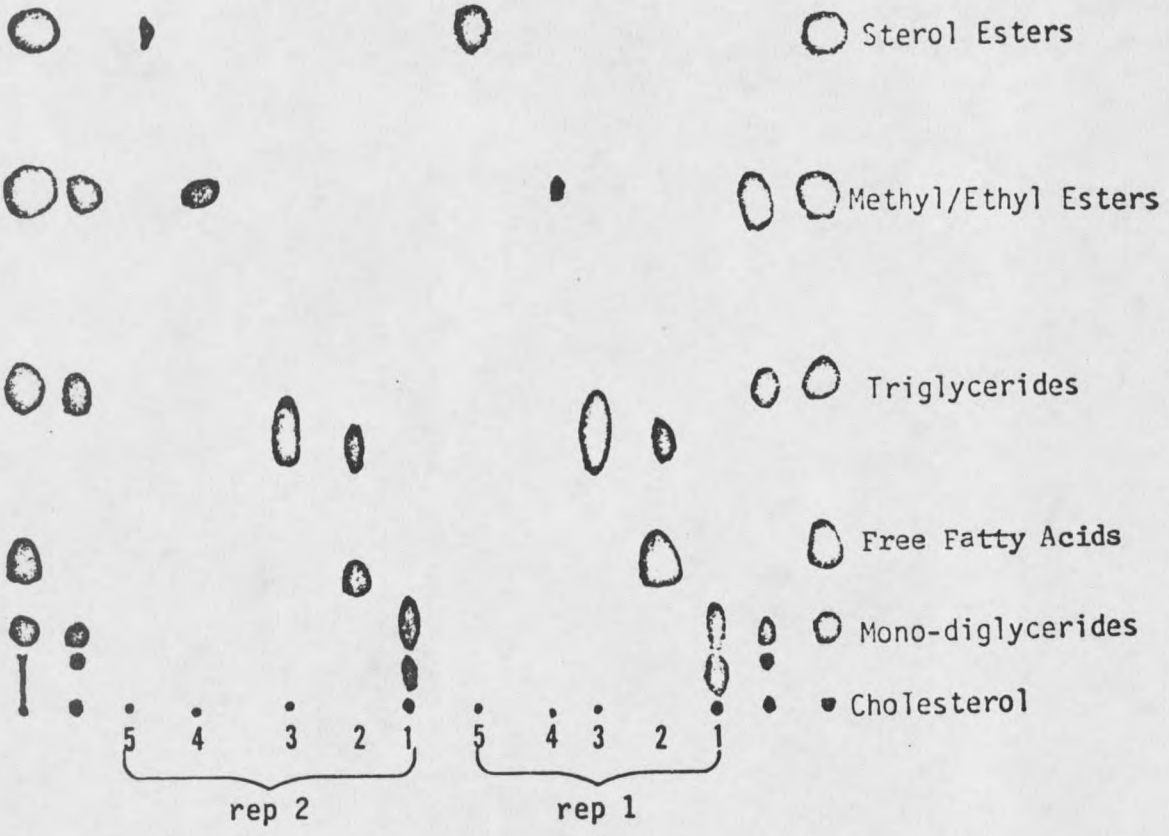


Figure 16. Analytical TLC of sample 7 rep 1 and 2.

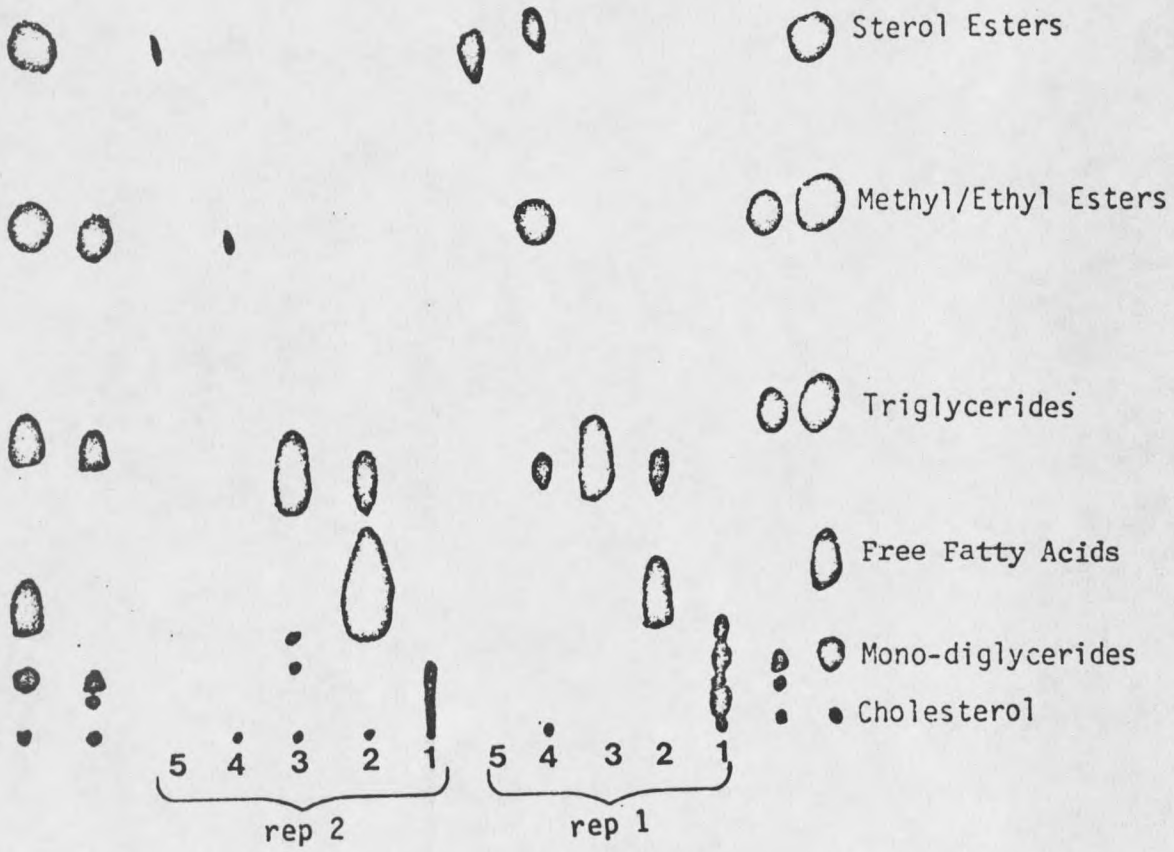


Figure 17. Analytical TLC of sample 8 rep 1 and 2.

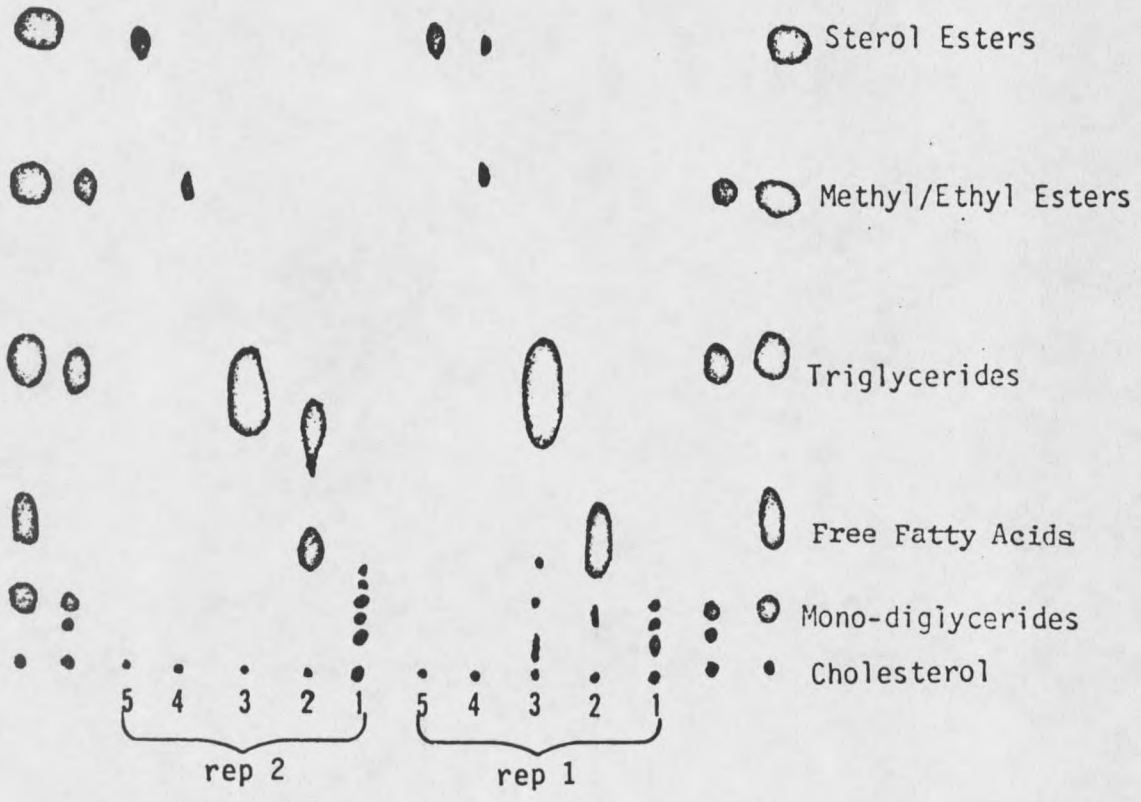


Figure 18. Analytical TLC of sample 9 rep 1 and 2.

Table 17. R_f values for analytical TLC of sample 7 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.04	.11	.21	.39	.51
18-4A	.06	.12	.24	.39	.51
18-1A	.05	---	.24	.39	---

Table 18. R_f values for analytical TLC of sample 8 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.03	.11	.21	.39	.53
18-4A	.05	.12	.24	.40	.53
18-1A	.04	---	.23	.39	---

Table 19. R_f values for analytical TLC of sample 9 rep 1 and 2.

	Mono-Diglycerides	Free Fatty Acids	Tri-Glycerides	Methyl Esters Ethyl	Sterol Esters
Average of Rep. 1 & 2	.04	.09	.21	.37	.48
18-4A	.05	.12	.23	.36	.47
18-1A	.04	---	.23	.36	---

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