



Qualitative analysis of barley lipids through the ethanol production process
by Irene Susan Eidet

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:

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Granola was made with DDG and consumer panelist acceptance was determined. Granola formulations included plain DDG, defatted DDG and barley. A commercial granola was used for control. Panelists were unable to detect a significant difference between granola types and these results suggested that DDG can be successfully incorporated into a food product such as granola.

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Bozeman, Montana

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APPROVAL

of a thesis submitted by

Irene Susan Eidet

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

March 8, 1984
Date

Jacquelyn W. Polka
Chairperson, Graduate Committee

Approved for the Major Department

March 8, 1984
Date

Margaret A. Buggé
Head, Major Department

Approved for the College of Graduate Studies

March 16, 1984
Date

Henry F. Parsons
Graduate Dean

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ABSTRACT

Distiller's dried grains (DDG) is a by-product of ethanol production and is a good source of protein and fiber. Research to date indicates that it could be a nutritious addition to food products but there is an inherent problem with a characteristic off-flavor. It was hypothesized that this flavor problem was due to the degradation of fatty acids during processing. This study traced fatty acid composition of palmitic, stearic, oleic, linoleic, and linolenic acids throughout the processing of ethanol from whole barley to DDG by use of gas chromatography (GC). Results indicated triacylglyceride (TAG) and mono-diacylglyceride (MAG-DAG) bands were being degraded by nonselective acid hydrolysis and the remaining fatty acids of the TAG band were remarkably stable throughout ethanol production. The free fatty acid (FFA) band showed a slight increase in saturated fatty acids while the unsaturated fatty acids decreased. The decrease in unsaturated fatty acids was probably due to oxidation of the double bonds. It was determined by GC that ethyl esters were formed and these were not present in the parent barley.

Granola was made with DDG and consumer panelist acceptance was determined. Granola formulations included plain DDG, defatted DDG and barley. A commercial granola was used for control. Panelists were unable to detect a significant difference between granola types and these results suggested that DDG can be successfully incorporated into a food product such as granola.

CHAPTER 1

INTRODUCTION

Distillers' dried grains (DDG) and brewers' spent grains (BSG) are major by-products from commercial ethanol production and the brewing/distilling industry. Ethanol production from grain has become more widespread, and it is predicted that this industry will expand considerably as the demand for alternate fuels continues (Hunt, 1981). In many regions of the United States, ethanol is made from corn. In the dry, cool northern great plains, however, barley is a major carbohydrate crop and represents an alternative feedstock. The current interest in gasohol, which is a mixture of gasoline and alcohol as a fuel source (Ranhotra et al., 1982), and continued availability of alcoholic beverages produced by breweries in the United States provide voluminous quantities of DDG and BSG. In 1976, 750,000 tons of spent grains were produced (Pomeranz et al., 1976).

Spent grains are nutritionally beneficial even though the carbohydrate has been removed in the fermentation process. The remaining nutrients are concentrated and consist mainly of protein, fiber and some lipid. These by-products contain a 2.5- to 3-fold level increase in certain nutrients compared to the original barley before processing (National Research Council, 1981). Increased concern for dietary fiber in human diets has been emphasized in recent years. It has been suggested that fiber plays a role in the prevention of certain

diseases such as diverticulosis, hemorrhoids, arteriosclerosis, varicose veins and appendicitis. However, while the link between dietary fiber intake and cancer of the colon is attractive, it is as yet unproven (Spiller and Kay, 1980).

Spent grains are presently used primarily as an animal feed (Hunt, 1969). For example, barley DDG has been successfully incorporated into swine diets at levels up to 15 percent, as replacement for soybean meal (Newman and Gras, 1983). It would be much more profitable if the by-product could also be incorporated as an ingredient in human food products (Hunt, 1969).

The main advantages of adding spent grains to food products for human consumption are twofold: (1) increased protein in diet and (2) increased fiber in diet.

Previous research with food products and DDG incorporation has shown that there are three main problems inherent to DDG: color, baking qualities and flavor. (Prentice and D'Appolonia, 1977; Prentice, 1978; Kissell and Prentice, 1979; Dawson et al., 1983). Research suggests that further treatment or alteration of spent grains is required for acceptability in baked products. Preliminary baking tests in these laboratories indicate that DDG are most successfully incorporated in dark colored, highly flavored products such as pumpkin bread. Even in products such as these, the hedonic scores fall at levels of incorporation above five percent (Eidét et al., 1983). Dawson et al. (1983) described the off-flavors as being sharp, bitter and soapy, with a distinct bitter or metallic aftertaste. Further research showed that

when DDG were defatted and added to standard oatmeal cookies, hedonic scores increased, indicating better acceptability.

In summary, researchers have had some success with camouflaging the DDG flavor problem but it seems that DDG could be better utilized in human food if the flavor problem was eliminated.

CHAPTER 2

REVIEW OF LITERATURE

Spent grain is currently being utilized as an animal feed supplement. The spent grain market demand is based on nutritive value per dollar costs as compared to other competitive animal feeds such as soybeans or barley. Townsley (1979) stated that if spent grain can serve as a source of high nutritive value in animal feeds it could also be utilized in human diets once considerable quantities of non-digestible fiber are removed. Spent grain is also used at levels of 45-90 kilograms per metric ton of compost in order to increase the nutritive value of the compost for mushroom growing (Townsley, 1979).

The Industrial Products Division of Anheuser-Busch is marketing a flour made with spent grains (a mixture of rice and barley malt) called Malto-Rice which is used as a natural addition to their line of bakery ingredients (Anonymous, 1979). Anheuser-Busch suggests that Malto-Rice is an ideal ingredient in such foods as specialty breads, breakfast cereals, snacks, meat products, breading and butters (Anonymous, 1979).

Nutritional Characteristics of DDG

Protein content (N x 6.25) of DDG ranges from 23 to 35 percent depending on the type of grain utilized (Ranhotra et al., 1982; Pomeranz et al., 1976; Prentice and D'Appolonia, 1977; Finley and Hanamoto, 1980). Processing of DDG may affect the protein quality. Ranhotra

et al. (1982) reported that the "true" protein content for DDG ranged from 16 percent to 21 percent in five samples they tested and suggested that the nonprotein nitrogenous compounds (most likely from yeast) contributed substantially to the increased total nitrogen content. The protein efficiency ratio (PER) in DDG samples was found to be less than satisfactory. Fiber content has been shown to range from 29 to 77 percent, dependent upon the method of analysis (Pomeranz et al., 1976; Prentice and D'Appolonia, 1977; Finley and Hanamoto, 1980; Ranhotra et al., 1982). Pomeranz et al. (1976) compared the vitamin and mineral composition of spent grains and wheat bran. Spent grain contained fewer total mineral components (ash) and vitamins than did wheat bran. The author stated that the highly soluble minerals and water soluble vitamins such as potassium and vitamin B components were destroyed during processing. However, Ranhotra et al. (1982) found spent grains to have greater amounts of B vitamins than Pomeranz (1976). Ranhotra et al. (1982) also reported that all the starch in spent grains from breweries appeared not to have been utilized during fermentation. They found values for "available carbohydrates" (mainly starches) of up to 25 percent. Ranhotra et al. (1982) ether extracted fat from spent grain and found that fat levels ranged from 6.3 to 11.5 percent.

DDG in Baked Products

Various researchers have found that low levels of DDG can be successfully incorporated into baked products. In general, bread volume decreased as percent of DDG increased (Prentice and D'Appolonia, 1977; Dreese and Hoseney, 1982; Finley and Hanamoto, 1980; Pomeranz et al.,

1976). Prentice and D'Appolonia (1977) stated that at five, ten and 15 percent levels of DDG substitution loaf volume decreased by 0, 11, and 17 percent. Loaf color darkened as DDG amounts increased. Organoleptic evaluation by consumer panelists indicated that ten percent substitution of flour with BSG was probably the upper limit of substitution (Prentice and D'Appolonia, 1977). At the ten percent substitution level, the protein content of flour and bread crumb was increased by ten percent. Similarly, crude fiber and acid-detergent fiber were doubled in the ten percent BSG flour (Anonymous, 1979).

Chemically leavened (quick) breads and muffins have been tested because they are moist with a compact crumb structure and their formulations often incorporate high levels of flavoring agents and color (Eidet et al., 1983; Prentice, 1978). Eidet et al. (1983) found that more than five percent replacement of flour by DDG was not acceptable by untrained panelists due to a bitter aftertaste. Prentice (1978) found that finely milled spent grain could replace up to 15 percent of the flour in muffins that had a pronounced flavoring component.

Numerous studies have been reported in which distillers' grains or brewers' grains were incorporated into bakery products. Sugar cookies were prepared with 15 percent brewers' spent grain, increasing the dietary fiber threefold, with no loss in acceptability (Prentice et al., 1978). Kissell and Prentice (1979) also reported a 55 percent increase in protein, and a 90 percent increase in lysine in cookies incorporating 20 percent brewers' spent grain. These products were within the organoleptic limitations established for taste and texture. Spent grains decreased the diameter of the cookie and quality of appearance.

The addition of soy lecithin helped remedy diameter and appearance. Cookies with greater than a 20 percent BSG supplementation had an undesirable brown color. Tsen et al. (1982) suggested that DDG flour would be an appropriate supplement for dark colored cookies. Studies were conducted to evaluate sensory acceptability of bar, spice, and chocolate chip cookies made from wheat flour supplemented with 15 percent DDG flour (Tsen et al., 1982), and all were acceptable.

Dawson et al. (1983) defatted DDG before adding to oatmeal cookies at 15 percent flour replacement levels. A consumer panel found control oatmeal cookies and defatted DDG oatmeal cookies to be equally acceptable. These researchers suggested that the off-flavor problem may be related to lipid rancidity but further work is needed.

Barley Nutritional Quality

Barleys differ greatly in morphological, physiological and chemical characteristics due to genotype and environment and the interactions between the two. Barleys analyzed at the Montana Agriculture Experiment Station (MAES) show this variability (Table 1) (Newman, 1983).

Protein content varies inversely with the amount of starch in barley (Briggs, 1978). The starch polysaccharide is entirely alpha-glucan that is predominantly amylopectin in which the alpha 1,4 linked D-glucofuranose chains, are branched through alpha 1,6 linkages and straight chain amylose containing D-glucofuranose units linked alpha 1,4 (Briggs, 1978). Beta-glucan has also been extracted from barley and consists of beta-D-glucofuranose residues linked at the 1,3 position or 1,4 position forming an unbranched chain (Briggs, 1978). The

Table 1. Minimum, maximum and average selected nutrient composition of barleys observed at the Montana station, 90% dry matter.^a

Item, % ^b	Minimum Observed	Maximum Observed	Average
Protein, %	8.9	21.7	11.5
Crude fiber, %	1.7	8.9	4.6
Ether extract, %	1.6	4.4	1.9
Lysine, %	.26	.78	.36
Methionine, %	.10	.32	.19
Cystine, %	.18	.39	.23
Threonine, %	.31	.62	.40
Tryptophan, %	.13	.44	.21
Phosphorous, %	.26	.61	.33
Kernel wt., mg	30.0	51.0	46.0

^aNewman, 1983.

^bExpressed as a percentage or part of the whole kernel.

beta-glucan component in barley varies from 1.5 to 8.0 percent (Bourne and Pierce, 1970; Fox, 1981). About one-half of the beta-glucans in barley can be extracted with water without destroying the cell wall structure (Aastrup, 1979).

Covered barleys generally have a crude fiber content that ranges from four to eight percent averaging about six percent (90 percent dry matter) while hullless barleys containing the same moisture average two percent or less (National Research Council, 1971 and 1979). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Southgate, 1977) are more precise methods for expressing the true fiber measurements of the plant or seed cell wall. Total cell wall contents represent the NDF whereas ADF is a measure of the cell wall contents less cellulose

(Southgate, 1977). Fifty covered barleys were analyzed from Montana, North Dakota, Oregon, and Washington representing 20 varieties that average 5.2 percent crude fiber while containing 13.5 and 5.5 percent NDF and ADF, respectively (Newman, 1983). In view of these analyses, ADF more closely resembles crude fiber than NDF. Covered barleys contain approximately 8.0 percent cellulose (Newman, 1983).

The ash content of average barley ranges from 2.0 to 2.7 percent (National Research Council, 1971 and 1979). The predominant mineral constituents of barley ash are potassium and phosphorus with lesser amounts of chlorine, magnesium, sulfur, sodium and calcium. Lesser concentrations of iron, zinc, copper, manganese and selenium also occur in the barley kernel ash. Barley is an excellent source of the water soluble B-complex vitamins of thiamin (B-1), pyridoxine (B-6), riboflavin (B-2) and pantothenic acid. The only fat soluble vitamin in barley is Vitamin E which occurs in the barley germ (National Research Council, 1971 and 1979).

Barley Lipids

Lipids are nutritionally important because they contain two and one-fourth times the energy per weight of carbohydrates and proteins and add flavor (Price and Parsons, 1975). The lipids of barley account for 2.0 to 3.6 percent of the total dry weight of the grain (Banasik and Gilles, 1966; Price and Parsons, 1974). However, a level of 4.1 percent oil was reported by Munck (1975), in the high lysine mutant Riso 1508. The amount of lipid obtained was highly variable and depended on the method used for extraction.

The average values for glycolipids, phospholipids and neutral lipids were 9, 20, and 71 percent (Price and Parsons, 1974). Lipids were determined by use of preparative thin layer chromatography (TLC) as described by Price and Parsons (1974). The neutral lipid class consists of a complex group of compounds containing free fatty acids (FFA), glycerides, free sterols, and sterol esters (Price and Parsons, 1980).

The fatty acid composition and lipid content of different varieties of barley are shown in Table 2. Fatty acid composition of barley was determined by gas liquid chromatography (GLC) (Fedak and De La Roche, 1977; Price and Parsons, 1974). Soybean lipid has a fatty acid composition similar to barley lipid and was included for comparison. In general, linoleic acid was present in highest proportions (53.6 to 58.5 percent) followed by palmitic (19.0 to 28.4 percent), oleic (9.2 to 16.0 percent), linolenic (4.5 to 7.1 percent) and stearic (0.6 to 2.1 percent (Price and Parsons, 1974; Fedak and De La Roche, 1977).

Baikov et al. (1979) reported that there is intensive degradation and oxidation of barley lipids when drying grain at 55°C. They determined barley lipid composition by analytical TLC and GLC. Triacylglyceride and sterol ester content decreased whereas the content of FFA and sterols increased. These researchers found that the drying temperature had no effect on the content of saturated fatty acids but it did decrease the content of unsaturated fatty acids. The decrease in unsaturated fatty acids may be indicative of fatty acid oxidation leading to oxidative rancidity during processing. As Lillard (1978) stated, the initial substrate in lipid oxidation (autoxidation) is almost always unsaturated lipids which are quite plentiful in barley.

Table 2. Lipid content and fatty acid composition of soybean and barley.

Material	% lipid	Fatty acid composition (% by weight)					
		14:0 ¹	16:0	18:0	18:1	18:2	18:3
Soybean ^a	3.0	.3	12.4	4.2	23.3	51.9	7.9
Barleys:							
Firlbecks III ^b	3.0	1.10	27.9	2.1	9.2	54.5	5.2
Zephyr ^b	3.0	1.20	28.4	1.2	9.9	54.8	4.5
Bernberger ^c	3.0		20.7	0.8	12.4	58.5	6.9
Compana ^c	2.7		19.0	1.0	15.5	58.2	5.4
Donecky ^c	2.7		19.5	0.6	16.0	57.2	6.1
Fergus ^c	2.5		22.1	1.2	12.2	56.7	7.1
Marton Vasari ^c	2.6		23.6	0.7	15.7	53.6	5.9

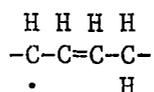
^aHonig et al., 1969.

^bPrice and Parsons, 1974.

^cFedak and De La Roche, 1977.

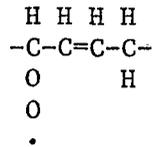
¹Myristic 14:0, Palmitic 16:0, Stearic 18:0, Oleic 18:1, Linoleic 18:2, Linolenic 18:3.

The development of oxidative rancidity in fats involves the simultaneous taking up of oxygen by unsaturated fatty acid components. Oxidation of fat is frequently referred to as autoxidation because the rate of oxidation increases as the reaction proceeds. Oxidation proceeds through a free-radical chain reaction mechanism involving three stages (Campbell, et al., 1979). (1) Initiation, formation of free radicals by removal of a hydrogen atom from a carbon adjacent to a double bond carbon:



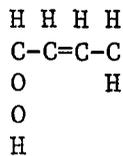
Free radical

(2) Propagation, the addition of molecular oxygen to give an activated peroxide:



Activated peroxide

The activated peroxide is very reactive and relatively little energy is required for removal of hydrogen from another carbon adjacent to a double bond in another chain. (3) Termination, the formation of a hydroperoxide on the initial chain:



Hydroperoxide

Secondary reaction products include short-chained aldehydes, ketones, hydroxyl compounds and other substances evidently formed through decomposition and further oxidation of the hydroperoxides (Schultz, 1962). Several of these products are of low molecular weight and appear to contribute to off-flavors and odors (Bennion, 1972).

Lipid oxidation may be easily influenced by several different factors. Schultz (1962) found that catalysts such as copper (Cu), iron (Fe), and cobalt (Co), seemed to increase the rate of formation of free radicals. Energy in the form of heat and light also accelerates the development of rancidity in fats. Dugan (1976) reported that lipolytic rancidity due to thermal stress usually creates less of a flavor problem

than oxidative rancidity. Lipolytic rancidity develops off-flavors only in those fats which contain short-chain fatty acids (less than C_{12}).

Soybean oil has an off-flavor that was misnamed "soybean flavor reversion." After more precise analytical tools were available, the relationship of oxidation and off-flavor was confirmed. After this breakthrough, industry started to blanket oils with inert gas at critical high temperatures to prevent oxidative rancidity. Soybean oil has gone from a minor edible oil to a major edible oil proudly labeled on premium products (Dutton, 1978).

Maga and Johnson (1972) reported that lipid changes occur throughout processing and storage of soy products. They found that 12 percent lipids remained in defatted soy flakes. Free fatty acids were found in relatively high concentrations but the majority of the lipids were triacylglycerides. Fatty acid analysis by gas liquid chromatography showed that with increased processing the level of unsaturated fatty acids was reduced. Prolonged room-temperature storage resulted in decreased unsaturated fatty acid levels.

Justification of Research

The literature contains no references to date on the lipid composition of industrial ethanol barley DDG. Preliminary research at MSU showed a definite change in DDG lipids from barley lipids. Both analytical and preparatory TLC plates showed little, if any, FFA in parent barley lipids. Dawson et al. (1983) reported that gas chromatographic analysis indicated that barley DDG lipid contained increased amounts of FFA and reduced amounts of unsaturated fatty acids

in both the FFA and triacylglycerol fractions compared to literature values for barley. These increased amounts of FFA could be partially responsible for the off-flavors noted in baked products containing barley DDG (Dawson et al., 1983). Further work at MSU confirmed the findings of Dawson et al. (1983). Preliminary fatty acid analysis values are shown in Table 3 compared with values reported by Fedak and De La Roche (1977). Chloroform extracted DDG lipid showed a twofold increase in palmitic acid, threefold increase in stearic acid, 12 percent decrease in oleic acid, 43 percent decrease in linoleic acid and a 66 percent decrease in linolenic acid. These changes in lipid composition may be due to degradation of the lipid as a result of the extensive processing. The decrease in three unsaturated fatty acids, oleic, linoleic and linolenic, may be indicative of fatty acid oxidation leading to oxidative rancidity during processing. The existence of a rancid flavor does not indicate whether the fat has undergone hydrolysis or oxidation or some of each (Campbell et al., 1979).

Table 3. Fatty acid composition of DDG and barley.

Material	Fatty acid composition (% by weight)				
	16:0	18:0	18:1	18:2	18:3
DDG ^a	50.6	5.2	12.5	30.3	1.4
Barley ^a	27.2	1.8	14.2	52.8	4.1
Barley ^b	21.5	0.9	14.9	55.9	5.7

^aPreliminary data MSU, 1983 (Barley Hector).

^bFedak and De La Roche, 1977 (Barleys; Bernberger, Compana, Donecky, Fergus, Marton Vasari).

Objectives

The objectives of this study were to (1) qualitatively analyze the fatty acid content of the barley DDG neutral lipid fractions (mono, di, triacylglycerides and free fatty acids) at nine stages throughout ethanol production by gas liquid chromatography, and (2) compare consumer taste panel acceptance of granola made with defatted DDG, full fatted DDG and ground barley to industrially prepared standard granola.

CHAPTER 3

MATERIALS AND METHODS

Sampling at Ethanol Plant

Sampling took place at the Alcotech ethanol plant in Ringling, Montana. Figure 1 is a flow chart of the processing plant. Approximately two liter samples were taken from the center of each tank at nine stages of the processing. Sample points are indicated on the chart 1-9. They included:

1. Whole barley (0 hours)
2. Barley after milling (1 hour)
3. After barley was added to 59°C water (8 hours)
4. After barley, water and enzyme slurry was brought up to 84°C (12 hours)
5. After barley mash had been cooled to 35°C and before the yeast preculture was added (24 hours)
6. After 47 hours of fermentation (71 hours)
7. After the DDG had gone through distillation (83 hours)
8. After centrifugation but just before drying (83 hours and 10 minutes)
9. After drying (83 hours and 20 minutes)

Sampling of the total process from starting parent barley variety 'Pirolina' to ending DDG was done twice. Each sample was mixed with reagent grade hexane as soon as the sample was taken to prevent any enzymatic reactions from taking place. Data for each sample included temperature, pH, time, location and researcher comments.

Lipid Extractions

Hexane was removed from each sample by vacuum filtration through Whatman GFC glass fiber filters and vacuum evaporation. Lipids were

Ethanol Production Plant

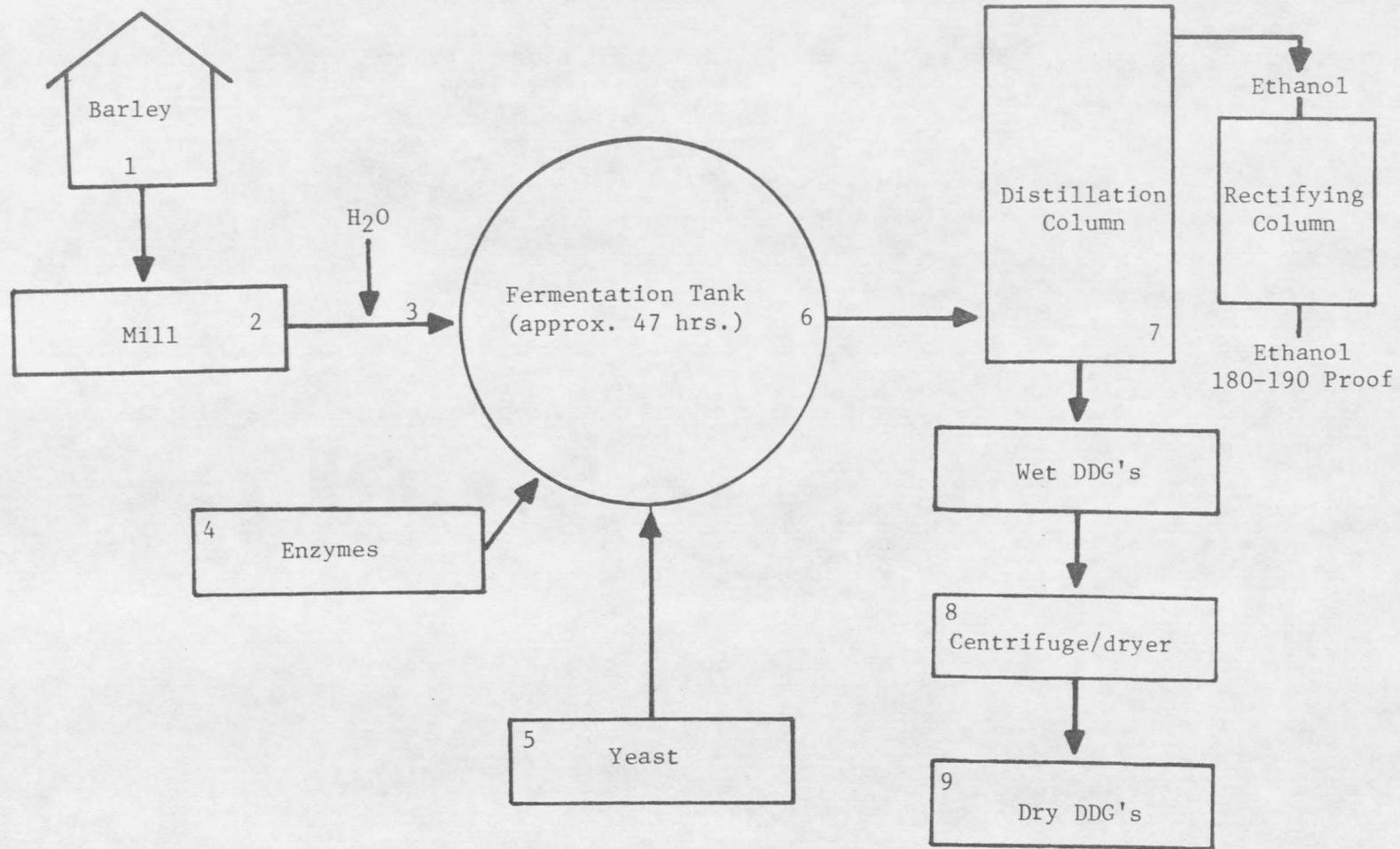


Figure 1. Flow chart of the ethanol production process showing the nine sampling points.

transferred to a tared glass vial. The collection flask was rinsed three times with additional hexane and added to the vial. The hexane was evaporated under a nitrogen stream until all traces of solvent were removed. The vial and lipid were then weighed and frozen until further analyses were completed.

Gas Chromatography

The fatty acid profile was determined using a Gas Liquid Chromatograph (GLC) Varian model 3700 (Varian Instruments, Walnut Creek, CA)¹ equipped with a Hewlett-Packard Model 3380 A electronic integrator (Hewlett-Packard, Avondale, PA). Peak identification was determined using retention time comparison with a known standard ester mixture, GLC reference standard (Hormel Institute). Peaks of interest were palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), and linolenic (C 18:3) acids.

Five bands representing mono- and diacylglycerides (MAG-DAG), free fatty acids (FFA), triacylglycerides (TAG), methyl/ethyl esters, and sterol esters were scraped from preparative plates and eluted from the silica with anhydrous ether. The ether was removed by rotoevaporation and the lipid was rinsed from the flask with reagent grade hexane and stored under a nitrogen gas atmosphere in an airtight vial at 0°C.

A sample of the lipid fraction was pipetted into a previously weighed vial, dried under nitrogen and reweighed. Pentadecanoic acid (Alltech Assoc.) (C₁₅), an internal standard, was added to toluene

¹Mention of firm names or products does not constitute endorsement by Montana State University over others of a similar nature.

(1 mg/ml). The dried lipid sample was diluted with the toluene/pentadecanoic acid mixture (1 mg lipid/100 microliters toluene mixture). The FFA were analyzed by gas chromatography (GC) as methyl esters. They were methylated by co-injection with 1 microliter Meth Prep I which converts free acids to methyl esters in the injector of the gas chromatograph (Applied Science).

Mono- and diacylglycerides and TAG were prepared for analysis using the same procedure as for FFA. In addition they were transesterified by adding Meth-Prep II (Applied Science) to an aliquot of each fraction. The reaction mixture was allowed to stand at room temperature for approximately 30 minutes before coinjecting with Meth Prep I. Three injections were made for each FFA and TAG sample. Mono and diacylglycerides were injected one time.

The methyl/ethyl esters (band 4) were prepared for GC injection by two different procedures. The first method used was to determine if ethyl esters were present. This was done by preparing each lipid sample in an internal standard and toluene mixture (1 mg lipid/100 microliters toluene mixture). The second method was the same procedure as MAG-DAG and TAG as described above, except the methyl/ethyl esters were not coinjected with Meth Prep I.

Fatty acid methyl esters were analyzed on a 2m x 5 mm ID glass column of ten percent DEGS on 80-100 mesh Chromosorb W-AW (Ultra Scientific, Hope, RI). Helium flow was set at 40 ml/min, injector temperature at 180°C, and detector temperature at 240°C. All samples were run isothermally at 185°C.

Internal normalization was used to calculate percentage of fatty acid composition. The quantity of individual components was expressed as percentage of the total quantity of the five fatty acids (palmitic, stearic, oleic, linoleic, linolenic) recorded on the chromatogram. There were two limitations of this method: (1) changes in the content in lipids of one component causes changes in the percent quantity of other acids and (2) it did not permit the determination of the absolute quantity of fatty acids and lipids.

Conventional analysis of variance (MSUSTAT) was done with bands 1, 2 and 3 for each fatty acid separately to detect changes over the nine sampling times. Conventional analysis of variance was performed on pH and temperature values as well (Snedecor and Cochran, 1980).

Granola Preparation

Granola was baked using the formulations listed in Table 4. Plain DDG, defatted DDG or 'Pirolin' barley was added to comprise 7.5 percent of the total weight. Rolled oats were placed in an ungreased 22.8 cm x 33 cm pan and baked for ten minutes. Sesame seeds were added to the oats and stirred thoroughly. Soy lecithin was added to oil before blending with brown sugar, honey and vanilla. The liquid ingredients were added to the oats and sesame seeds and stirred until thoroughly coated. The granola was baked at 163°C in conventional electric ovens for 15 minutes. The granola was removed from the oven and was left undisturbed in the pan to cool. After granola was completely cooled, it was broken into chunks and stored in plastic freezer bags until sensory evaluation.

Table 4. Granola formulations for consumer taste panels.

Ingredients	1 ^a	2	3	4
Oats	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	-	-
DDG (g)	-	-	42	-
Defatted DDG (g)	-	-	-	42

^aGranola types as follows: 1-Control; 2-Barley; 3-DDG; 4-Defatted DDG.

Sensory Testing

The granola consumer taste panel was run on October 22, 1983, at the Main Mall shopping center in Bozeman, Montana. Untrained panelists recorded their age and sex and were asked to rate each granola type in the series using a five point facial hedonic scale (Figure 2). The series consisted of Nature Valley Commercial Granola (control), DDG granola, defatted DDG granola, and barley granola. The granola was placed in one ounce plastic cups and served buffet style on coded trays. Room temperature drinking water was provided. Hedonic scales that were improperly completed were eliminated from the sample before statistical analysis.

Data were analyzed using the Chi Square test (MSUSTAT), and the effects of age and sex on granola preference was determined. Analysis of variance (MSUSTAT) was also performed (Snedecor and Cochran, 1980) to determine if type of granola, age, or sex affected granola rating.

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.

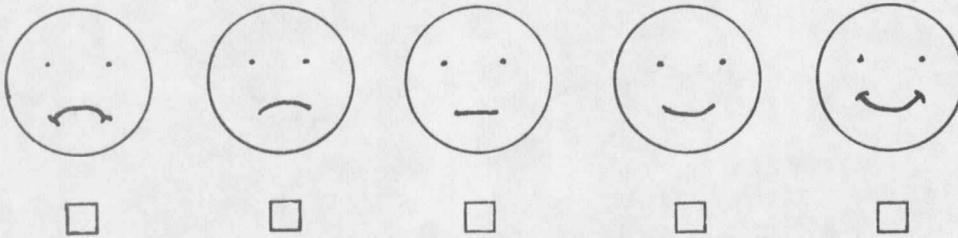


Figure 2. Taste panel hedonic questionnaire.

CHAPTER 4

RESULTS AND DISCUSSION

Sampling Temperature and pH

Average sample temperature varies with process and pH decreases throughout the nine stages of ethanol processing (Table 5). Analysis of variance of pH and temperature change indicated that temperature change was highly significant at $P < 0.01$ and pH decrease was significant at $P < 0.05$ (Tables 6 and 7). The length of time for ethanol production (approximately 83 hours) along with the highly significant temperature change and significantly increased acidic conditions were factors that would enhance acid hydrolysis of the lipids. As will be discussed later, these significant differences can account for much of the variation observed in FFA band fatty acid composition.

Table 5. Sample pH and temperature throughout nine stages of ethanol processing.

Sample	pH	Temperature (°C)
1	NA ¹	NA
2	NA	19
3	5.4	59
4	5.3	84
5	4.7	36
6	3.6	37
7	3.4	61
8	NA	45
9	NA	31
LSD (0.05)	1.4	4.1

¹NA - Not applicable.

Table 6. Analysis of variance of temperature throughout nine stages of ethanol processing.

Source of Variation	Degrees of Freedom	Mean Square	P-Value ¹
Replication	1	9.000	
Treatments	7	848.700	0.000**
Residual	7	3.000	

*,**Indicate significance at the 5% and 1% levels, respectively.

¹P-value is the probability of a greater F.

Table 7. Analysis of variance of pH throughout nine stages of ethanol processing.

Source of Variation	Degrees of Freedom	Mean Square	P-Value ¹
Replication	1	0.025	
Treatments	4	1.709	0.046*
Residual	4	0.250	

*,**Indicate significance at the 5% and 1% levels, respectively.

¹P-value is the probability of a greater F.

Tables 8, 9 and 10 present data characterizing the effects of ethanol processing on quality changes in the fractional fatty acid composition of 'Pirolina' barley to DDG. Samples 1-9 were taken from the ethanol production process starting with parent barley and ending with DDG and were based on relative percentage composition of palmitic, stearic, oleic, linoleic and linolenic acids.

Table 8. Triacylglyceride quality changes in relative percentage of fatty acid composition of barley throughout nine stages of ethanol processing.

Sample	Fatty Acid %				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
1 (whole grain)	18.2	2.5	19.2	55.3	4.8
2 (ground grain)	18.7	1.9	18.9	55.2	5.3
3 (grain & H ₂ O)	18.8	2.0	20.2	54.3	4.7
4 (H ₂ O, grain & enzymes)	18.5	2.6	18.8	55.7	4.4
5 (cooled grain slurry)	18.4	2.1	19.1	56.2	4.4
6 (end of fermentation)	18.4	2.9	19.3	55.2	4.2
7 (stripper column)	18.3	2.3	19.7	55.4	4.3
8 (after centrifugation)	18.8	2.1	19.7	54.7	4.7
9 (dried DDG)	19.1	1.9	19.8	53.5	5.6
LSD (0.05)	NS	NS	0.38	1.09	0.31

Table 9. Free fatty acid quality changes in relative percentage of fatty acid composition of barley throughout nine stages of ethanol processing.

Sample	Fatty Acid %				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
1 (whole grain)	25.7	3.8	9.5	51.5	9.5
2 (ground grain)	22.5	2.5	8.7	59.0	7.4
3 (grain & H ₂ O)	24.1	0.9	7.5	61.9	5.6
4 (H ₂ O, grain & enzymes)	25.7	1.6	6.7	60.6	5.3
5 (cooled grain slurry)	32.6	1.4	6.6	54.9	4.4
6 (end of fermentation)	30.4	3.2	7.7	55.0	3.8
7 (stripper column)	28.6	2.1	7.4	57.7	4.3
8 (after centrifugation)	32.3	3.2	7.2	53.8	3.5
9 (dried DDG)	30.9	4.0	9.3	52.2	3.7
LSD (0.05)	5.2	1.4	1.7	5.5	1.9

Table 10. Mono- and diacylglyceride quality changes in relative percentage of fatty acid composition of barley throughout nine stages of ethanol processing.

Sample	Fatty Acid %				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
1 (whole grain)	24.6	2.5	10.8	57.7	4.4
2 (ground grain)	22.7	2.1	11.1	59.8	4.0
3 (grain & H ₂ O)	20.8	1.7	15.2	58.5	3.8
4 (H ₂ O, grain & enzymes)	23.8	2.0	11.8	58.7	3.7
5 (cooled grain slurry)	22.9	1.5	12.8	59.3	3.6
6 (end of fermentation)	21.4	1.8	14.8	58.1	3.9
7 (stripper column)	21.0	1.8	15.0	58.3	4.0
8 (after centrifugation)	21.8	1.7	15.3	56.9	4.3
9 (dried DDG)	22.7	1.4	15.8	55.5	4.6
LSD (0.05)	NS	NS	2.5	NS	NS

The fatty acid composition of sample 1 (whole barley) in the MAG-DAG and TAG bands were similar to those found by Price and Parsons (1974) in whole lipids (Table 2). In the FFA and TAG bands sample 9 (DDG) fatty acid composition did not change as much as in similar work done by Dawson et al. (1983). Dawson et al. (1983), however, analyzed a different barley variety and this may account for the difference in lipid composition. Fedak and De La Roche (1977) reported that barley type and growing conditions could alter lipid composition.

Triacylglyceride Band

Little quality change occurred in the relative percentage of fatty acid composition of the TAG of barley throughout the nine stages of ethanol production (Table 8). For example, palmitic acid ranged from 18.2 to 19.1 percent, stearic acid from 1.9 to 2.9 percent, oleic acid from 18.0 to 20.2 percent, linoleic acid from 53.5 to 56.2 percent and linolenic acid from 4.2 to 5.6 percent. Analysis of variance results of the TAG band indicated that differences in saturated fatty acids were not significant (Table 11). Significant differences across sampling times were observed among unsaturated fatty acids but no consistent pattern was detected. Changes in TAG (band 3) over sampling times were judged to be small compared to changes observed in composition for FFA (band 2). The precision of the test instrument was such that the variability among samples was not significantly different. The changes in TAG did not vary more than + or - five percent which is the amount of testing error accepted for GC.

Table 11. Analysis of variance of fatty acid composition for the triacylglyceride band throughout nine stages of ethanol processing.

Source of Variation	Degrees of Freedom	Mean Squares				
		16:0	18:0	18:1	18:2	18:3
Replication	1	0.084	0.036	0.109	0.279	0.011
Treatments	8	0.169	0.250	0.445**	1.226*	0.464**
Residual	8	0.085	0.270	0.027	0.223	0.018

*,**Indicates significance at the 5% and 1% levels, respectively.

Prior to GC analyses, TLC quantitative analyses of lipids from barley to DDG (Dawson, 1983) showed that percentage FFA content increased and percentage TAG decreased while MAG-DAG remained stable during the ethanol production process (Figure 3). Qualitative GC fatty acid profile analyses, however, indicated that the TAG band percentages did not change appreciably throughout the ethanol process. Examination of quantitative and qualitative changes indicated that the TAG band probably underwent hydrolytic, nonselective destruction. Some of the TAG were being hydrolyzed to FFA and MAG-DAG. Figure 4 shows the remarkably stable fatty acid profile of TAG which indicates the process was destroying TAG by nonselective hydrolysis.

The ester linkages of barley lipids are subject to hydrolysis resulting from enzymes, thermal stress, or chemical action. These classic reactions are known as lipolysis, lipolytic rancidity, and hydrolytic rancidity (Dugan, 1976).

Enzymatic hydrolysis was probably not responsible for the hydrolysis of TAG because Rinke (1964) reported optimum pH and temperature for this reaction were 7.5 and 45-48°C, respectively. These

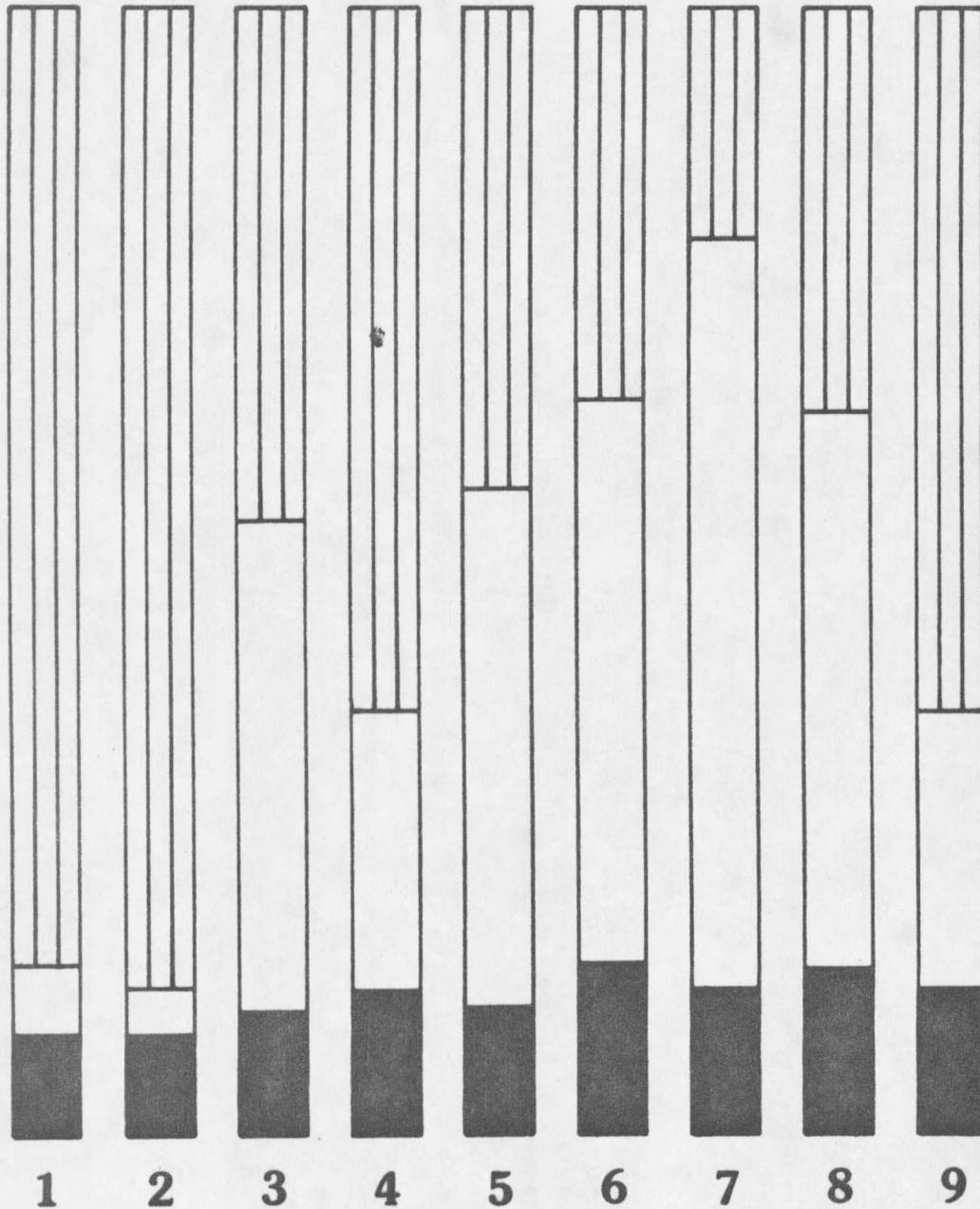
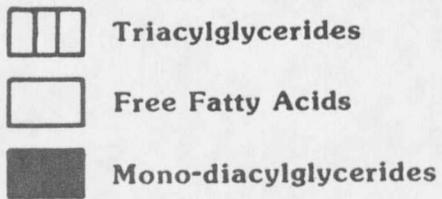


Figure 3. Quantitative percentages of lipid fractions in nine stages of ethanol processing (Derived from Dawson, 1983).

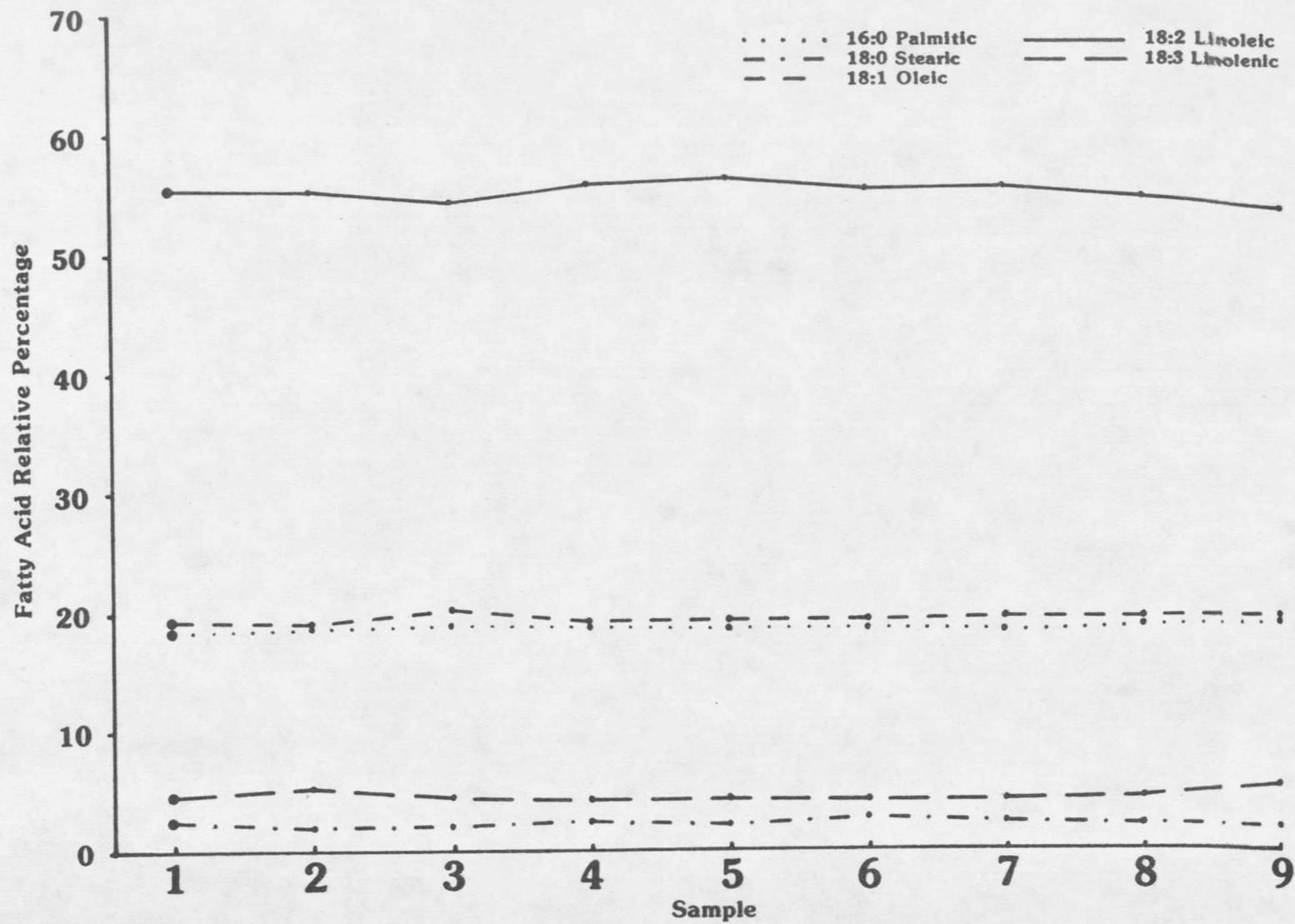
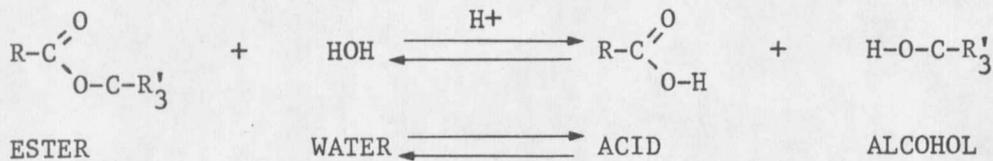


Figure 4. Relative percentage of fatty acids in the triacylglyceride band.

temperature and pH conditions, did not occur together at any time in processing. Dawson (1983) found no change between Sample 1 and 2 in FFA and TAG and concluded that enzymatic hydrolysis did not take place. Rinke (1964) also reported that exposure to moisture decreased the enzymes' ability to survive. When moisture was over 40%, the lipase activity was inhibited. Therefore, the possibility of enzymatic activity was decreased between stages 3 to 9.

The pH of the solution in which the barley fats are hydrolyzed has a significant effect on the end products (Rinke, 1964). Acid hydrolysis probably yielded FFA and the mechanism was initiated by breaking a carbon-oxygen bond to form an oxygen-hydrogen bond in the presence of an acid. The acid hydrolysis reaction may have occurred in the following way (Miller and Neuzil, 1979):



Highly acidic conditions throughout processing combined with large temperature changes created a positive environment for acid hydrolysis to take place. The endothermic activity and prolonged processing time allowed for more product as a result of increased reaction time.

Free Fatty Acid Band

Palmitic acid values ranged from 22.5 to 32.6 percent; stearic acid values ranged from 0.9 to 4.0 percent; oleic acid ranged from 6.6 to 9.5 percent; linoleic acid ranged from 51.5 to 61.9 percent; and linolenic

acid ranged from 3.5 to 9.5 percent (Table 9). Analysis of variance showed significant differences among sample means for all five fatty acids in the FFA band (Table 12). The fatty acid profile of the FFA band indicated only a slight increase in saturated fatty acids from the first step of processing through stage 9 (Table 9). Figure 5 showed the FFA profile did not remain as stable as did the TAG. The relative amount of palmitic acid remained the same until stage 5 when it increased significantly by six percentage points. Stage 5 was after the grain, water and enzyme slurry had been cooled from 83.5°C to 35.5°C and before yeast preculture had been added. The general trend for palmitic acid percentage was an increase becoming significantly greater in DDG than in the original barley sample. No apparent trend appeared for relative percentage values for stearic acid. The percentage of stearic acid in whole barley was not significantly different from that in DDG.

Table 12. Analysis of variance of fatty acid composition for the free fatty acid band throughout nine stages of ethanol processing.

Source of Variation	Degrees of Freedom	Mean Squares				
		16:0	18:0	18:1	18:2	18:3
Replication	1	32.53*	3.092*	1.908	56.04*	1.693
Treatments	8	27.81*	2.429**	2.287*	27.10*	7.988**
Residual	8	5.11	0.368	0.571	5.65	0.700

*,**Indicates significance at the 5% and 1% levels, respectively.

Analysis of variance indicated that the unsaturated fatty acids (oleic, linoleic, and linolenic) of the FFA band varied significantly across sampling times (Table 13). Oleic acid, showed a decreasing trend until stage 5 when the percentage again increased to near its original level in whole barley. The initial decrease could possibly be explained

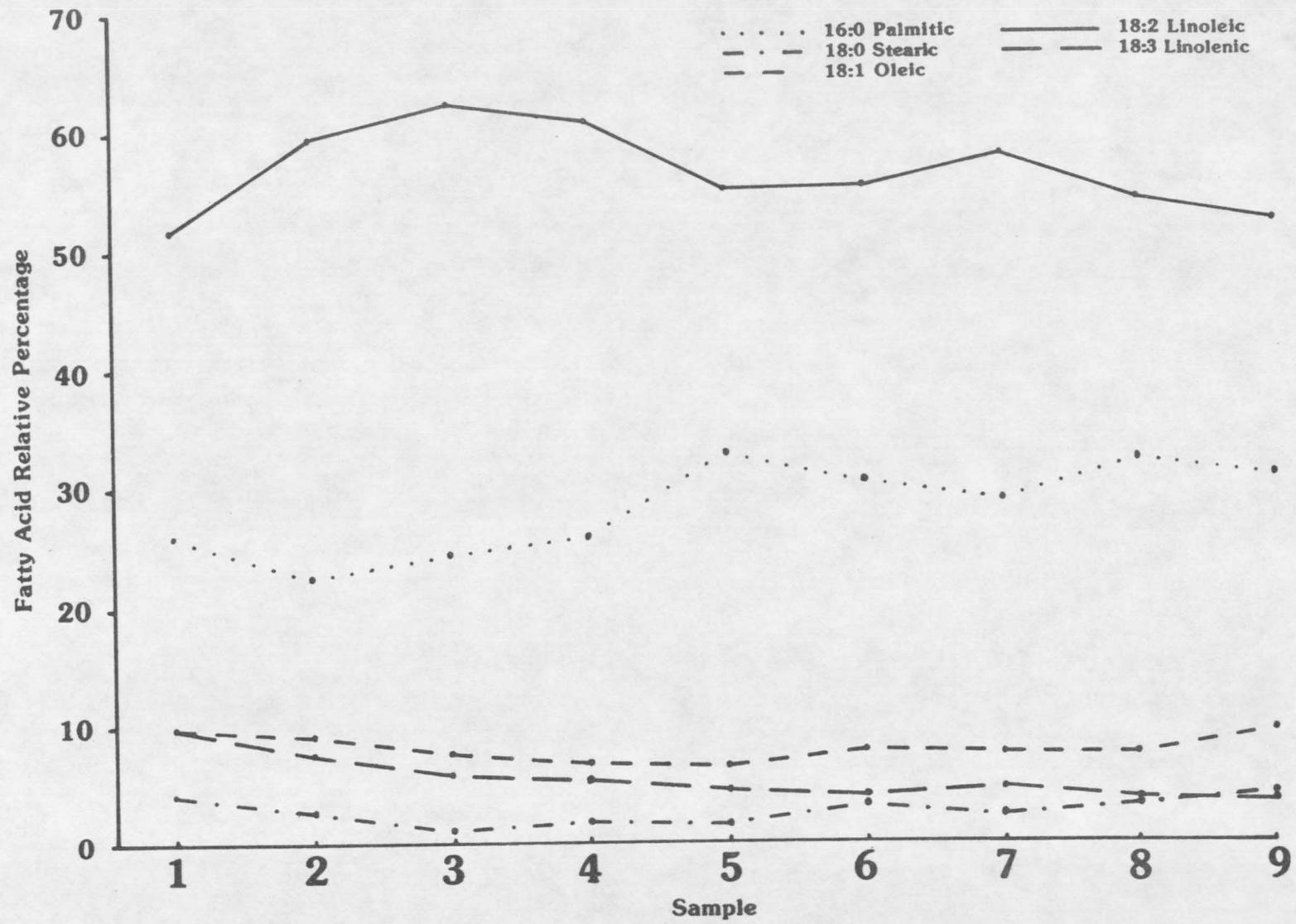


Figure 5. Relative percentage of fatty acids in the free fatty acid band.

Table 13. Analysis of variance of fatty acid composition for the mono- and diacylglyceride band throughout nine stages of ethanol processing.

Source of Variation	Degrees of Freedom	Mean Squares				
		16:0	18:0	18:1	18:2	18:3
Replication	1	91.30**	7.737**	0.513	6.31	11.65**
Treatments	8	3.31	0.225	7.855**	3.28	0.219
Residual	8	1.72	0.143	0.801	3.62	0.385

*,**Indicates significance at the 5% and 1% levels, respectively.

by oxidation of the double bond and the increase could have been due to acid hydrolysis of TAG to FFA. The percentage of linoleic acid showed a significant increase from stage 1 to stage 3 followed by a significant decrease from stage 3 to 5 and then remained unchanged to the end of the process. Linoleic acid showed an eight percent fluctuation throughout the nine steps of processing and back to the original barley fatty acid profile in step 9. The increase in percent from stages 1-3 in linoleic acid was accounted for by the simultaneous degradation of TAG to FFA which caused the percent of linoleic acid to increase to 60 percent, followed by a decrease probably due to oxidation. Linolenic acid gradually decreased from eight percent in whole barley (time 1) to four percent in DDG (time 9). This gradual decrease in linolenic acid was indicative of oxidation. Campbell (1979) reported that the more double bonds, the more susceptible a fatty acid is to oxidation. Oxidation proceeds through a free-radical chain reaction mechanism involving three stages: initiation, propagation, and termination as described in Review of Literature (Campbell, et al., 1979).

Secondary reaction products that are formed after termination include short-chained aldehydes, ketones, hydroxyl compounds and other

substances evidently formed through decomposition and further oxidation of the hydroperoxides (Dugan, 1976). Several of these products are of low molecular weight and appear to contribute to off-flavors and odors (Bennion, 1972).

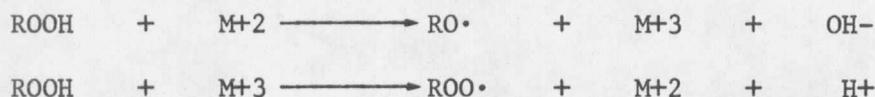
Dawson (1983) found organic impurities of low molecular weight in the ethanol by use of a vacuum gas meter spectrometer. These impurities could have been the breakdown products (short-chained aldehydes, ketones, carbonyls, esters) of FFA oxidation and TAG destruction and may have been released into the ethanol, DDG or air. Dawson (1983) also found 20 percent methanol in the ethanol which was indicative of unsanitary conditions at the ethanol plant resulting in microbial growth. Haymon and Acton (1978) discussed the complex nature of microbial reactions and lipids and concluded that flavor components came from oxidative reactions which were initiated after hydrolytic activity. The release of FFA was accompanied by an increase in total carbonyls and peroxide values.

Lipid oxidation is a major cause of food deterioration. Lipolytic rancidity (thermal stress) usually causes less of a flavor problem than oxidative rancidity since lipolytic rancidity develops off-flavors only in those fats which contain short-chain fatty acids (less than C₁₂) (Dugan, 1976).

Free fatty acids are unstable lipid components because they are not attached to the glycerol backbone. A number of reactions could possibly have occurred with the FFA component throughout processing. An overall decrease in unsaturated fatty acids occurred which would have caused an

apparent increase in percent saturated fatty acids. It is possible that oxidation occurred in the unsaturated fatty acids.

The iron in the fermentation tanks may have been a primary catalyst to initiate the oxidation reaction. Heavy metals (Lillard, 1978) may also have been effective as secondary catalysts of oxidation where they acted as electron donors to hydroperoxides to produce free radicals. The M+3 can be converted back to M+2 by reacting with hydroperoxides to form ROO radicals.



It is possible that the Vitamin E found in barley could have acted as an antioxidant during the first three stages of ethanol processing. Vitamin E is a natural antioxidant that reacts with oxygen in the air and prevents the oxidation of polyunsaturated fatty acids and the formation of peroxides in fats and therefore rancidity. Barley contains a small amount of Vitamin E which may have slowed the oxidation process in stages 1, 2, and 3. Vitamin E is destroyed by heat processing (Whitney and Cataldo, 1983) however, and may have been eliminated during stage 4 when temperatures in processing stages reached 84°C.

A study was done to determine the influence of lipids on yeast metabolism and fermentation using pure lipid compounds (Taylor et al., 1979). Taylor et al. (1979) found that TAG were inactive and spent grain lipids exerted their effect on fermentation through the synergistic action of free unsaturated fatty acids, sitosterol and phospholipids. This finding suggested a possible explanation for the

overall stability of the TAG that remain after hydrolysis of TAG to FFA and the decrease of the free unsaturated fatty acids in this study.

Mono- and Diacylglyceride Band

Mono- and diacylglyceride percentages indicated a pattern of breakdown where nonselective acid hydrolysis of TAG resulted in MAG-DAG and finally FFA because the percentages of MAG-DAG fell in between TAG and FFA (Table 10). Figure 6 showed that the MAG-DAG fatty acid profile varied slightly. Significant differences among means across sample stages were detected only for oleic acid where percent oleic acid generally increased from stage 1 to 6 and then stayed constant to stage 9 (Table 10). It appears that oleic acid of the MAG-DAG band was more stable to oxidation than linoleic acid or linolenic acid and was probably the reason for the steady increase of five percentage points in oleic acid.

Methyl/Ethyl Ester Band

Gas chromatography of methyl/ethyl esters showed the presence of ethyl esters in samples 6, 7, 8, and 9 of both replications 1 and 2. If methyl esters had been present they would have appeared at 16:0, 18:0, 18:1, 18:2, and 18:3 on the chromatogram. Ethyl esters were present and not methyl esters because ethyl esters appeared at 17:0, between 18:1 and 18:2, and between 18:2 and 18:3. To further determine if ethyl esters were present, the ethyl esters that appeared in samples 6, 7, 8, and 9 both replications 1 and 2 were methylated into methyl esters by Meth Prep II and appeared at 16:0, 18:0, 18:1, 18:2, and 18:3. Two samples were tested to see if further heating would complete the methyl

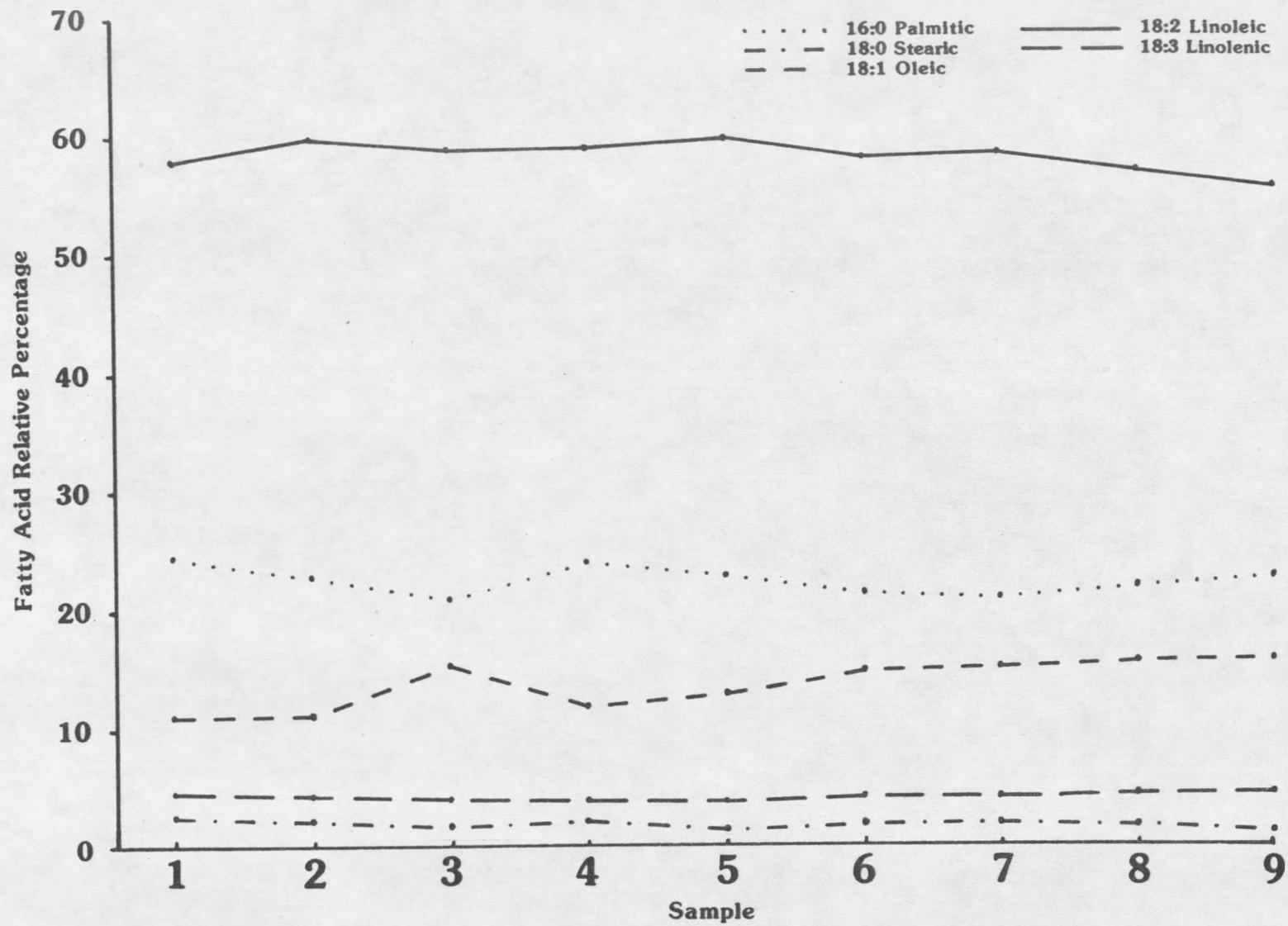


Figure 6. Relative percentage of fatty acids in the Mono-diacylglyceride band.

reaction. Approximately 50 percent of the ethyl esters were methylated to methyl esters but this amount remained constant before and after heating the sample.

Taste Panel

The four granola types were sampled by 163 untrained panelists (97 females and 66 males) whose age ranged from 3 to 61. The average taste panelist age was 22. Campbell et al. (1979) reported that at least 50 to 100 panelists should be included in consumer panels for acceptance-preference testing. The lack of experience of the consumer panel along with numerous uncontrolled variables increase the experimental error and therefore necessitates a large number of judges.

Chi Square analysis indicated that age and sex had no effect on rating by panelists but granola type did affect rating results (Table 14).

Table 14. Results of Chi-square analysis of granola rating x type, sex, and age interactions.

	<u>Probability of a larger Chi-square</u>		
	Type	Sex	Age
Rating	0.0275*	0.2798	0.0727

*,** Indicate significance at the 5% and 1% levels, respectively.

Analysis of variance showed that age, type of granola and sex had no significant effect on the consumer panelist rating. Age was approaching significance with a P-value of 0.112 (Table 15). The granola types were rated as equal with no significant difference (Table 16).

Table 15. Analysis of variance of granola taste panel.

Source of Variation	Degree of Freedom	Mean Square	P-Value ¹
Type	3	0.103	0.322
Sex	1	0.012	0.698
Type x sex	3	0.111	0.296
Age	3	0.205	0.112
Type x age	9	0.179	0.114
Sex x age	3	0.083	0.409
Error	9		
Type x sex x age			

¹P-value is the probability of a greater F.

Table 16. Consumer taste panel scores for granola with barley DDG.

Granola Type	Score ¹
Barley	3.9
DDG	3.9
Defatted DDG	3.7
Nature Valley	3.8
LSD (0.05)	NS

¹ 5 = Superior; 1 = Undesirable

These results suggested that plain DDG, defatted DDG, and barley could be successfully incorporated into the unleavened food product granola at 7.5 percent of the total weight. Previous research by Dawson (1983) suggested that the bitter, aftertaste of DDG resulted when free fatty acids were combined with leavening. The reaction that occurred may have been due to alkaline hydrolysis or saponification. The saponification reaction is the formation of a metallic salt of a fatty acid and is called a soap. The reaction involves treatment of FFA and/or glycerides with a base (Campbell et al., 1979). If leavening had

been incorporated in the granola formulations the defatted DDG and plain DDG may have been judged significantly different by the consumer panelists.

Conclusions

Both pH changes and temperature changes were significantly different, when measured across sampling times. This factor along with length of processing (approximately 83 hours) would enhance acid hydrolysis and oxidation. The fatty acid profile of the TAG band showed that TAG were destroyed by nonspecific hydrolysis. The remaining TAG were remarkably stable throughout ethanol production. This phenomenon occurred all across processing. The MAG-DAG results were similar to TAG. Free fatty acids showed the most variability with the overall trend being an increase in saturated fatty acids and a decrease in unsaturated fatty acids. Dawson (1983) found methyl or ethyl esters at Band 4 and this study showed that the esters formed were ethyl esters. Numerous variables were present that could possibly explain the hydrolytic and degradative changes that were observed for saturated and unsaturated fatty acids. They include: acid hydrolysis in relation to time, temperature and pH; oxidation of unsaturated fatty acids; reactions catalyzed by metals such as iron; microbial action; and utilization of unsaturated fatty acids by yeast during fermentation.

The work presented demonstrated that FFA changed throughout processing of barley DDG. These changes can result in the formation and accumulation of secondary products which can further contribute directly to DDG flavor. Previous work by Dawson et al. (1983) showed that taste

panel scores for defatted DDG cookies were higher than plain DDG cookies. The consumer taste panelists in this study were unable to distinguish between plain DDG and defatted DDG and indicated an overall acceptance for all granola types. This may be explained by the fact that granola contains no leavening and therefore saponification resulting in a bitter taste was unable to occur.

Further research in this area should include incorporation of leavening and DDG granola in a granola cookie, determination of how fatty acid degradation occurred by collection of end products of fatty acids and analyses of secondary products of oxidation in DDG and ethanol.

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