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

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.

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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.

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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 (Eidet 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-glucopyranose chains, are branched through alpha 1,6 linkages and straight chain amylose containing D-glucopyranose units linked alpha 1,4 (Briggs, 1978). Beta-glucan has also been extracted from barley and consists of beta-D-glucopyranose 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

[a]Newman, 1983.

[b]Expressed 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 hulless 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

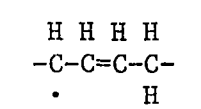
[a]Honig et al., 1969.

[b]Price and Parsons, 1974.

[c]Fedak and De La Roche, 1977.

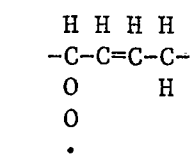
[1]Myristic 14:0, Palmitic 16:0, Stearic 18:0, Oleic 18:1, Linoleic 18:2, Linolenic 18:3.

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:



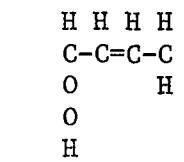
Free radical

(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.

	Fatty acid composition (% by weight)				
Material	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

[a]Preliminary data MSU, 1983 (Barley Hector).

[b]Fedak 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 'Piroline' 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