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Relationship of free fatty acids and acid degree values to lipolytic flavor of milk

Susan E. Duncan

To the Graduate Council:

I am submitting herewith a dissertation written by Susan E. Duncan entitled "Relationship of free fatty acids and acid degree values to lipolytic flavor of milk." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

H. O. Jaynes, Major Professor

We have read this dissertation and recommend its acceptance:

Genevieve Christen, M. P. Penfield, P. M. Davidson, B. Bell

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Benny R. Bell

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

RELATIONSHIP OF FREE FATTY ACIDS AND ACID DEGREE VALUES TO LIPOLYTIC FLAVOR OF MILK

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Susan E. Duncan

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DEDICATION

This dissertation is dedicated to my husband, Robert B. Duncan Jr., for his love, encouragement, and support.

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ABSTRACT

Free fatty acids were studied in relation to acid degree value (ADV) and rancid flavor (RF) in milk and ADV evaluated as a method for measurement of RF. Selected fatty acids (FA) were blended in milk at .25 μmol/ml milk with emulsifier. Recovery of FA was <.63 (C_4-C_8), 1.95-2.33 (C_{10}-C_{16}), 3.24 ($C_{18:0}$), and 4.12 μmol/g fat ($C_{18:1}$). Significant differences ($p<.05$) in recovery were found among all groups of FA. Recovery of 5 concentrations (.20, .25, .35, .45, .55 μmol/ml milk) did not differ ($p>.05$) for C_4 (<.51 μmol/g fat), was proportional to added concentration of C_{10} with significant differences between low and high concentrations (1.94-4.64 μmol/g fat), and was significantly higher with increasing concentration of $C_{18:1}$ (3.91-11.08 μmol/g fat). Fatty acid partitioning into skim (C_4) or fat (C_{10}, $C_{18:1}$), as separated by ADV procedure, was demonstrated by gas chromatography. Detection thresholds for C_4, $C_{18:1}$, and C_{10} were .20, .55, and >.55 μmol/ml milk.

A 6-member trained flavor panel scored milk samples collected from east Tennessee farms and laboratory-prepared rancid samples (LPRS) for RF by magnitude estimation. Correlation coefficients between rancidity scores (farm samples and LPRS) and standard methods ADV were .13 ($p=.16$) and .17 ($p=.002$). Mean rancidity scores were

not significantly different for samples divided into 5 groups based on ADV (<1.26, 1.26-1.75, 1.76-2.30, 2.31-2.75, >2.75) but LPRS were given higher rancidity scores. A consumer panel detected a difference between farm milk with low ADV (1.57), and high values (5.37, 7.67 meq/100 g fat) using paired comparison method. There was an increase in ADV and rancidity scores with storage (12 days at 4°C).

Correlations between concentration of FA and ADV were >.82 (p=.0001) for all major FA in farm milk but, for LPRS, the correlation was moderate (r=.53, p=.07) for total shorter chain (C_4-C_{10}) FA and low (r=.23, p=.47) for total longer chain (C_{12}-$C_{18:1}$) FA. C_4, C_{14}, $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ concentration significantly increased among ADV groups. Correlations between RF and FA concentrations were low (r<.45) for both farm and LPRS. Total FA concentrations were not significantly different among "slightly", "moderately" "very rancid" or "unpalatable" samples.

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CHAPTER I

INTRODUCTION

One of the major objectives of the dairy processing industry is to produce a fluid milk product that is desired by the consumer. Decrease in per capita consumption of fluid milk is of concern (6) and methods of improving milk quality and increasing consumer desire for the product are needed. Several factors may be influencing consumer demand for fluid milk but the flavor of milk and its storage life are of great importance. Current chemical methods for monitoring milk flavor and predicting its shelf-life may not be adequate for determining the sensorial response to milk flavor by consumers.

Bandler (6) found a direct correlation between milk flavor quality and level of consumption of fluid milk. When milk flavors were consistently poor, consumption level of milk decreased by 30%. Off-flavors, which were responsible for this decrease in consumption rate, were created during production and/or processing and included such flavors as feed, cooked, rancid, and oxidized among others. Quality control measures (evaluation of automatic cleaning of equipment, chemical methods of testing for off-flavors) were not satisfactory for minimizing these flavors. Barnard and

Moir (8) found 421 of 2,346 market milk samples (24%) had a poor flavor based on sensory examination and 356 of the 421 poor samples, 15% of all samples, were rancid. Consumption of milk may be increased if the flavor of milk is better controlled.

An individual trained to detect rancid flavor may have a lower threshold for rancid flavors than an untrained individual. A quality control technician trained to perceive rancid flavor may be able to detect the flavor at an early stage of processing. A chemical method (acid degree value (ADV)) is used for detection of rancid flavor. Raw milk with an ADV above 1.0 is considered rancid or on its way to becoming rancid and it is recommended that this milk should be discarded (52). Bandler (6) reported an average ADV of .9 from farm milk samples in New York State in 1978. Barnard and Moir (8) found that 50% of 2,346 market milk samples tested had an ADV greater than 1.0. If an ADV of 1.0 was used for screening samples for rancidity, 35% of the 2,346 samples tested would be rejected but would not have a rancid flavor. This could be an expensive waste of good milk if the ADV is not a reliable predictor of rancid flavors.

It is important that rancid flavors or the possibility of their development is detected as early as possible in processing. Acid degree value is the current quality

control method used for that purpose. The overall objective of this research was to determine if ADV, as currently described in *Standard Method for the Examination of Dairy Products* (50) is an adequate tool for measuring rancid flavor. Relationship of ADV and concentration of free fatty acids to sensory perception of lipolytic flavor, as perceived by a trained sensory panel, was explored by measuring recovery of specific fatty acids added to milk by ADV (Chapter III), determining the correlation between ADV and rancid flavor for fresh and stored milk (Chapter IV), and determining the relationship of free fatty acids, as measured by gas chromatography, to ADV and rancid flavor (Chapter V).

CHAPTER II

REVIEW OF LITERATURE

I. LIPOLYSIS IN MILK

Lipolytic flavor (rancid flavor) in milk is caused by enzymatic hydrolysis of the triacylglycerol molecule resulting in release of free fatty acids (FFA), mono- and diglycerides, and glycerol. The liberated FFA with 4-12 carbons may contribute an unpleasant odor and flavor to milk, even at low concentrations. Rancid flavor must be differentiated from oxidative rancidity which is attributed to the oxidation of unsaturated fatty acids and is characterized by a distinctly different off-flavor note. The lipolytic flavor which results from hydrolysis may cause a decrease in shelf-life and consumer acceptance of milk due to the sharp, unclean, astringent flavor and lingering, unpleasant aftertaste. A strong odor is also present when the off-flavor is intense (6).

Analytical methods for lipolysis should be useful in confirming the presence or predicting the development of rancid flavor. The most common technique used to measure concentration of total FFA in milk is acid degree value (ADV), a measure of the milliequivalents of FFA/100 g fat.

The most frequently used method for measurement of ADV is the Bureau of Dairy Industries (BDI) method, as listed in *Standard Methods for the Examination of Dairy Products* (50). In this procedure, a 35-ml sample of milk is pipetted into a Babcock test bottle to which 10 ml of BDI reagent is added to de-emulsify the fat. The sample is heated (100°C) for 20 min. and centrifuged to aid in fat separation. Aqueous methyl alcohol is added to define the fat-water interface. After tempering the sample (57+3°C) for 5 min., the fat is transferred to an Erlenmeyer flask and weighed. Fat solvent (1 part n-propanol: 4 parts petroleum ether) is used to dissolve the fat and the sample is titrated to a phenolphthalein endpoint with .02N methanolic KOH. Advantages of this method over other chemical methods used for detection of lipolysis are quantitative determination of FFA concentration and reproducible results (55). An ADV greater than 1.5 meq/100 g fat, as determined by the BDI method, is reportedly undesirable since the flavor threshold for detection of rancidity was reported as 1.5 (55) (Table 1). The determination of an ADV at which rancid flavor is perceived was based on laboratory-prepared rancid milk samples (LPRS), not samples collected directly from cow, farm, or processing, by 2 expert judges (55).

Table 1. Standard ADV descriptors[1].

ADV	Descriptors
< .4	Normal
.7 - 1.1	Borderline (indefinite)
1.2	Slightly lipolyzed
> 1.5	Unsatisfactory (extremely lipolyzed)

[1] (50).

The ADV method does not quantify individual FFA which are present. Therefore, it is not possible to determine which FFA are contributing to the ADV which is important because only some fatty acids (C_4-C_{12}) have been associated with rancid flavor (51). Short-chain FFA (C_4-C_8), which are partitioned in the fat and skim portions of milk, are not completely detected by the ADV (15). It is therefore difficult to relate ADV to sensory perception of rancid flavor since milk with high concentration of short-chain FFA may have a low ADV but a pronounced rancid flavor. Conversely, milk with a high ADV, due to a high proportion of hydrophobic FFA (C_{14}-C_{20}), may not receive criticisms of rancidity by sensory panelists (48).

The milk fat globule is normally protected from the action of lipase by a membrane composed of protein and

phospholipids. Adverse physical conditions (i.e., foaming, pumping, and freezing) may weaken the membrane, permitting lipolytic activity and resulting in an increased concentration of FFA (6). The milk fat globule membrane (MFGM) loses integrity after cold storage, becoming less resistant to lipolysis (16). Ice crystals will form around the fat globule in frozen milk and can rupture the membrane. On thawing, the triglycerides are liberated from the damaged globules, permitting the enzyme access to the substrate. Under conditions of homogenization, a new membrane composed primarily of casein particles as well as whey proteins and MFGM material, is formed around the smaller fat droplet. This new membrane is less structured and more easily permeable than the natural MFGM. Milk lipase is naturally associated with the casein (20) and homogenization brings the enzyme and fat into close proximity, allowing lipolysis to begin. It is for this reason that milk should be pasteurized prior to homogenization (16).

In addition to flavor problems, lipolytic changes in milk may have other adverse effects on milk quality and functionality (34). Quality may be affected in several ways; "free", destabilized fat may be lost due to adherence to the walls of containers, pipelines, or other surface areas; skimming efficiency may be decreased and residual

FFA content of skimmed fresh milk increased; and increased fat content in buttermilk obtained by churning can cause fat losses. These quality changes can have a direct effect on the functionality of milk products. For example, whipped cream may have reduced volume or whipping capacity (34) due to high concentration of FFA or formation of mono- or diglycerides during hydrolysis which have antifoaming abilities (16). Solubility, wettability, and flow characteristics of dry milk powder are also reduced (34).

Concentration of FFA in milk has risen over the past several decades (37). Lipolysis problems arose when new methods of milking and milk handling, such as pipeline systems and bulk collection and refrigerated storage, were initiated on the farm (29). Kirst (34) classified three areas, physiological (cow factors), physical (mechanical and thermal factors), and biochemical (enzymatic factors), as having an effect on post-secretory formation of FFA. A small amount of FFA is normally present in fresh raw milk because of incomplete esterification but, due to the effects of these three factors, it has been estimated that FFA content in raw milk has doubled worldwide, primarily because of physical factors (37).

There are two types of lipolysis, induced and spontaneous, which are related to the three causes of increased FFA. Induced lipolysis is caused by mechanical

agitation and turbulence (stirring, pumping, foaming) or thermal effects like heating and cooling or freezing and thawing (physical factors). Under these conditions, raw milk is subjected to treatments which cause disruption of MFGM and permit enzyme access to the fat substrate (34). Induced lipolysis may occur at any time during production, storage, and processing up to the time of heat treatment or pasteurization when the naturally inherent milk lipase is inactivated (6).

Spontaneous lipolysis occurs in the absence of agitation or thermal effects (29). It has been suggested that elevated blood-derived constituents in milk may prevent complete formation of, or cause damage to, MFGM in the udder. Physiological factors of the cow may lead to spontaneous lipolysis. Late lactation, poor nutritional status, mastitis, and low milk volume (which decreases in the latter part of lactation) have all been associated with lipolysis (20). Milk formed under these conditions appears to be more susceptible to temperature fluctuations, resulting in increased FFA concentrations when the milk is cooled, rewarmed, and then recooled (thermal effects) as could easily occur during the storage period on the farm and during transport. Other factors or conditions associated with spontaneous lipolysis include season, breed, and heredity of the cow (16). There is some

question as to the delineation between induced and spontaneous rancidity and it is quite possible that they may occur simultaneously (3).

II. MECHANISM OF LIPOLYSIS

Fresh raw milk contains .2 to 1.0 mmol FFA/100 g. The FFA present in fresh milk are not formed by hydrolysis of the triglyceride (57). Under optimum conditions of milk handling, the concentration of FFA should remain constant since the ester linkages between glycerol and aliphatic fatty acids are stable through the pH range of dairy products. Therefore, the primary production of FFA from triglycerides takes place due to the enzyme, lipase (11).

There are two sources of lipase in milk. Milk lipoprotein lipase (LPL), E.C. 3.1.1.34 (57), is primarily responsible for any lipolysis which occurs prior to heat treatment or pasteurization. The second type of lipase is produced by psychrotrophic bacteria. Under the conditions of cold milk storage, psychrotrophic bacteria may proliferate and produce lipolytic and proteolytic enzymes. While bacteria are inactivated by heat, some of these enzymes are heat-resistant and may survive pasteurization temperatures of 72°C for 15 to 20 seconds (19). Some may even survive UHT sterilization.

Milk lipoprotein lipase (MW=50,000) has an optimum temperature for activity of 37°C and pH optimum of 8.5 (57). Divalent cations such as Ca^{2+} stimulate lipase action but long-chain FFA, products of lipolytic activity, are inhibitory. The enzyme exists as a noncovalently linked dimer under physiological conditions. Downey and Andrews (20) reported that 70% of milk LPL was bound to micellar casein by ionic and hydrophobic bonding, reducing the concentration of free enzyme available for activity. The remainder was associated in a soluble casein-enzyme complex in milk serum (26). The enzyme is more stable when bound to casein (57). The physiological role for milk LPL is in uptake of lipids from blood into the mammary gland. It has no reported function in milk itself and its presence is probably due to leakage from tissue rather than secretion into milk (42).

Lipase has the potential of causing considerable damage to milk quality. Good quality raw milk has less than 1 μmol FFA/ml even after 24 hours of storage at 4°C. However, 1 lipase molecule may produce 3,000 molecules of fatty acid/second (57) or 1 μmol FFA/minute (42) under optimum conditions (pH=8.8, 37°C) (57). If optimum activity remained constant, milk could be rancid within 10 seconds (57).

The enzyme is active only at the fat-water interface in milk. Under conditions of abuse described earlier, the enzyme may come in physical contact with the fat substrate (12). The initial rate of activity is proportional to the susceptible fat surface area. Enzyme activity is highly correlated ($r=.8$, $p<.001$) with the cream fraction (1). Activity stabilizes due to product inhibition, however (57). The inhibition effect of FFA is attributed to the accumulation of FFA at the fat globule interface and the failure of the enzyme to de-adsorb from the interface preventing further activity of the enzyme and slowing and inhibiting lipolysis.

The milk environment (pH, ionic composition) is not optimal for enzyme activity and therefore also decreases the activity of lipase. The concentration of free enzyme available for activity is diminished due to association with casein micelles (57). Proteins, such as proteose-peptone and some apoproteins, inhibit enzyme attack. The physical barrier of the MFGM around the substrate acts as a deterent to the enzyme which then cannot penetrate the membrane unless it is damaged (57). Randolph and Erwin (48) suggested that increased lipase activity and increased ADV may be related to a decreased concentration of phospholipids because the concentration of phospholipids in

the MFGM may not be sufficient to provide adequate protection to the fat globule.

The inhibitory effect of proteins and MFGM can be overcome with sufficient quantities of a yet unidentified cofactor and lipoprotein activator (29). Milk LPL activity is stimulated by lipoproteins of serum which are secreted or leaked from the blood into milk, especially under conditions of mastitis (29). The balance between inhibitors and activators is tenuous but inhibitors have the greater influence. The cofactor is needed to bring the enzyme molecule into contact with substrate when substrate is present as or covered with lipoproteins. Temperature affects partitioning of the enzyme, by virtue of its association with casein, between fat globules and plasma. These normal physical and biochemical phenomena inhibit lipase activity, thus limiting lipolysis and FFA accumulation in excess of flavor thresholds (19).

The action of the enzyme on the triglyceride molecule results in the accumulation of FFA, mono- and diglycerides, and glycerol. Milk LPL preferentially hydrolyzes fatty acids from the 1(3)-position on the glycerol molecule. The main reaction sequence is triacylglycerol to 1,2(2,3)-diacylglycerol to 2-monoacylglycerol to 1(3)-monoacylglycerol and then to glycerol (41). The short-chain FFA are primarily located on the third position of

the glycerol molecule. Ninety-seven percent of butyric acid (C_4), 84% of caproic (C_6), and 45% of caprylic acid (C_8) are located on position 3 (57). The FFA released by milk LPL (in mole %) are 13.9% butyric, 2.1% caproic, 1.8% caprylic, 3% capric (C_{10}), and 2.7% lauric (C_{12}) (40). Over 20 mole % of C_{16}, $C_{18:1}$, and $C_{18:2}$ each are released by enzyme action (40).

The second type of lipase, that from psychrotrophic bacteria, may be of greater importance in pasteurized milk than milk LPL because some enzyme activity may remain after pasteurization. Raw milk of good microbial quality (<5 x 10^3 colony-forming units (CFU)/ml) or reasonable quality (< 10^5 CFU/ml) would probably develop lipolysis from milk lipase during cold storage rather than from lipase due to psychrotrophic bacteria (19). However, when psychrotrophic bacteria counts exceed 10^6 CFU/ml, microbial enzymes may be sufficient to cause lipolytic off-flavors (19).

Andersson et al. (4) measured the effect of active psychrotrophic lipase on acidity and flavor changes in cold-stored UHT sterilized milk. Cell-free lipase was isolated from *Pseudomonas fluorescens* and added to pasteurized cows' milk at 188 and 564 enzyme units/ml prior to sterilization. The concentrations used corresponded to those previously found in cold-stored raw milk. An alternative ADV (not BDI method) was obtained by diluting

100 ml milk with 200 ml water and titrating with .1 M NaOH to a phenolphthalein endpoint. Acid degree value was calculated as ml NaOH x 10 required to neutralize milk. Sensory analyses were completed using a triangle test method with a balanced design to determine if there were perceptual flavor differences. All 6 possible combinations of presentation among samples (milk samples with high enzyme activity, low enzyme activity, and reference) appeared equally often. Nine to 13 judges experienced in food evaluation and chosen for their ability to discriminate among milk samples were used in the sensory analyses. Lipase activity decreased after UHT sterilization to approximately half of the initial activity. The acid value increased more rapidly in milk samples with added lipase than in controls. Milk samples with ADV of 20 or greater were considered rancid. Rancid flavors were found in milk samples with high enzyme activity stored at 8°C after 5-8 days of storage. Twelve to 14 days at 8°C were required before rancid flavors were evident in milk with low enzyme activity level, and rancid flavors were observed after 22 days for reference samples. There were significant differences in flavor between each enzyme- treated milk sample and reference sample during the indicated time intervals. It was suggested that rancid flavor was due to lipolysis, based on a correlation between

increase in ADV and off-flavor development, but no statistical evidence of correlation was provided.

III. CHEMISTRY OF LIPOLYTIC FLAVOR

Although normal concentrations of FFA in fresh milk are less than 1% of the total milk fat, they are important for the normal flavor of milk (39). The flavor potency of the fatty acid has a strong bearing on the development and sensory perception of rancidity. The fatty acid may exist in any of three forms: protonated (undissociated), charged (dissociated), and water-insoluble combinations of anion with cations such as Ca^{2+} or Mg^{2+} (salts) (11). At the normal pH of milk, most shorter chain fatty acids (fewer than 14 carbons) in the aqueous phase exist as salts in a ratio of 60:1 with the acid form (33).

The equilibria of distribution of FFA between aqueous and fat phases in dairy products is influenced by several variables including polarity of the given acid, pH of the aqueous phase, temperature, and materials adsorbed at the fat-water interface. At low temperatures, when fat exists primarily as a solid, it is doubtful that fatty acid equilibrium between aqueous and fat phases would occur (11). As the pH decreases, more fatty acid partitions into the fat phase. This influence of pH on partitioning of

fatty acids is largest for those of short-chain length (57). The lipolyzed flavor is also more easily detected as the pH of the medium in which the fatty acid is found decreases (56). The protonated fatty acid has increased odor as compared to the dissociated anion. Bills et al. (11) observed this difference by acidifying rancid milk and neutral solutions of sodium butyrate to a pH of 4.9, near the pK_a of butyric acid (C_4). Kintner and Day (33) reported that a drop in the pH to 1.3-3.0 was needed to enable 98% recovery of FFA, including the short-chain (C_4-C_8) fatty acids, from laboratory-prepared rancid pasteurized, homogenized milk, FFA were recovered by lyophilization of milk, acidification of lyophilized milk powder to allow protonation of all salts of non-esterified fatty acids, and extraction with ethyl ether. FFA then were methylated and analyzed by gas chromatography. In addition to pH and temperature, physical distribution of the FFA in the milk system (serum, membrane, fat globule) should also be considered (11). During the separation of fat, 90% of FFA will associate with the cream phase and 10% will stay with skimmed milk (5). Twenty-five percent of those associated with the fat are found in the fat globule membrane (33). Lipase activity occurs at the fat-water interface and this permits the hydrolyzed acids to equilibrate between the aqueous phase and the interface, if

not the interior of the solid fat (11). Shorter chain fatty acids (12 or fewer carbons) are primarily associated with the milk plasma and are found predominantly in the dissociated form (57). Milk fat with higher concentration of short-chain or unsaturated fatty acids than normally found in milk is more sensitive to lipolytic changes (34).

Analytical methods for evaluation of fatty acids in dairy products do not distinguish between forms of acid (protonated, dissociated, or insoluble salt) and, therefore, cannot be related back to the flavor properties of each (11). An analytical method which provides information about the recovery and forms of FFA is needed. Sensory analysis is important with respect to quality changes in milk products due to lipolysis but cannot provide a defined measurement of FFA form or recovery. Sensory measurements are primarily needed for the impact of rancid flavor on the product (13). Analytical measurement and sensory response are both needed because the flavor significance of fatty acids in dairy products is not revealed by analytical laboratory procedures alone (11).

IV. SENSORY PERCEPTION OF LIPOLYZED FLAVOR

The sensory perception of rancid flavor is difficult to measure because of the many FFA involved in rancid

flavor. Purely lipolyzed flavor is dependent on the level of lipolysis, other possible off-flavors associated with the milk, and the sensitivity of human taste organs (13). The initial sensation of rancidity is attributed to the characteristic odor of rancid milk, resulting from volatile short-chain fatty acids formed from fat hydrolysis (12). The taste sensation may not be noticed immediately after sampling but awareness increases as the sample is manipulated toward the back of the mouth. The flavor sensations predominantly occur as an aftertaste and are described as "soapy", "bitter", or "unclean". An unpleasant aftertaste tends to linger after expectoration, fading gradually. The mouth interior may feel rough or astringent-like. Sensitive evaluators may find the flavor experience nauseating or revolting (12). The bitter flavor sometimes associated with rancidity may be due to protein degradation (9) or mono- and diglycerides (30).

Reported flavor thresholds for rancid flavor vary according to investigator. Most people perceive rancid flavor when the fat acidity is greater than 1.5 meq/100 g fat. This value is based on the ADV as determined on LPRS. Most methods do not measure total fatty acids however, and as much as one quarter to one third of the FFA are not recovered but remain in the plasma. Therefore, the true acidity at threshold of taste is higher (57). Untrained

panelists may have taste thresholds as high as 3.0 meq/100 g fat (13). Krukovsky and Herrington (36) reported a threshold value of .8 meq/100 g fat for recognition of lipolysis. This threshold point was based on values of two judges. Thomas et al. (55) reported a threshold ADV for lipolyzed flavor based on the BDI method as 1.2 to 2.0 meq/100 g fat. Kirst (34) reported that rancid defects were detected when FFA content was in excess of 4 meq/100 g fat or equivalent to 1.5 meq/liter of milk.

Flavor and distribution of the fatty acids in the milk system are of considerable importance in the intensity and perception of rancid flavor (11). A low concentration of one fatty acid may have a greater flavor impact than a high concentration of another. The shorter chain FFA, C_4-C_{12}, are the predominant contributors to detectable rancidity. Experimental results on threshold concentration are not consistent however, because factors such as solubility, pH, and distribution may influence detection. The lowest threshold level is for caprylic acid (C_8), at 10 ppm in H_2O (57). The threshold for C_4 in milk is 12.9 ppm. At such low levels, any hydrolysis may permit development of a perceptable rancid flavor (4). Patton (44) found higher flavor thresholds for C_8, C_{10}, and C_{12} in oil than in water but C_4 and C_6 had higher flavor thresholds in water than oil. The very short-chain FFA, formic, acetic, and

propionic acids, have not been found to contribute to lipolyzed flavor (35). The soapy flavor associated with rancidity has been reported to be due to C_{10} and C_{12} (34).

Flavor thresholds will vary dependent on sensitivity and training of the taster. A sensory panel may be used as a flavor-measuring instrument if properly trained and the performance properly evaluated. The sensory scores obtained from such a panel represent characteristics of the samples and are not dependent on extraneous factors, such as other flavor notes (14). Judges rate the intensity or strength of the rancid flavor and these scores can be statistically correlated with chemical analyses (i.e., ADV). In this manner, it is possible to determine the relationship between intensity of rancid flavor as measured by sensory analysis and the degree to which the chemical method reflects that intensity (43). The level of FFA at which lipolyzed flavor is detectable varies among judges, therefore a minimum of 3 trained judges is required (15).

The training of a sensory panel is an important step in calibrating this flavor-measuring instrument. Trained judges are better suited to the task because of the large number of flavors which may occur in milk. It is difficult for an untrained judge to detect rancid flavor (15). Some individuals have greater difficulty in detecting rancid off-flavors but may eventually learn through practice.

Others may not find the flavor unpleasant or may have relatively high thresholds for this off-flavor (12). Reference standards are helpful in characterizing or identifying rancid flavor, in determining intensity levels of the flavor, and for anchors for sensory scales (47).

The threshold level for rancid flavor criticism as related to the BDI method for ADV was found to be 1.3 meq/100 g fat by Speer et al. (54). They considered this value as the differentiating level between rancid and nonrancid milk. Variability was found from sample to sample, especially with different judges, indicating that not all milk samples with an ADV greater than or equal to 1.3 were rancid to all judges. No description of the panel training or number of judges used on the panel was included in the article.

Scanlan et al. (51) studied the influence of specific fatty acids in rancid flavor as detected by an 11-member panel utilizing a 7-point intensity scale (1=not rancid; 7=extreme). Rancid flavor was duplicated by adding mixtures of specific FFA to milk. The simulated flavor was close but not identical to actual rancid milk samples. They reported that lipolyzed flavor was due to FFA with 4-12 carbons but no single FFA was responsible and FFA of longer chain length (14 or more carbons) had little influence. Discrepancy between simulated flavor and actual

rancid milk flavor was attributed to unknown constituents or differences in distributions of FFA between the fat and aqueous phases. Al-Shabibi et al. (2) added 16 FFA to milk but determined that only C_6, C_8, C_{10}, and C_{12} produced the rancid flavor. Capric (C_{10}) and lauric (C_{12}) contributed the most characteristic rancid flavors. When C_4 was added to milk, the flavor resembled that of the FFA but was not the same as the flavor encountered in lipolyzed milk.

Reference standards for rancid flavor have been developed but may not capture the true note of rancid flavor as found in naturally occurring rancid milk. Reference standards have been made by adding certain fatty acids to milk to imitate the off-flavor (51) or by adding raw milk to homogenized, pasteurized milk and permitting adequate time for lipase in the raw milk to act on available substrate (7). Another alternative is to homogenize raw milk and permit adequate time for lipolysis to occur due to milk LPL. These last 2 methods require pasteurization of the sample prior to sensory evaluation. Scanlan et al. (51) used LPRS for comparison with milk with added fatty acids in determining which fatty acids were important in rancid flavor.

V. CHEMICAL AND SENSORY MEASUREMENT OF LIPOLYZED FLAVOR

As previously mentioned, the BDI method of ADV is frequently used as a measure of rancid flavor but is really a measure of the concentration of titratable FFA associated with the fat. The relationship between rancid flavor and ADV is conservative at best, because of the limitations of the BDI methodology. Deeth et al. (18) suggested that the BDI method was suitable for most milk but difficulties existed in the de-emulsification step when using late-lactation milk or homogenized milk. In addition, the test is not suitable for skim milk due to the low fat content. The ADV procedure does not account for selective solubility, molecular weight, and the flavor potency of individual FFA. It has been difficult to get good recovery of FFA, especially lower molecular weight, hydrophilic FFA, without extracting interfering lactates, citrates, and phospholipids (13). The long-chain, hydrophobic FFA are the major fatty acids detected in the BDI method and alternative ADV methods but rancidity is caused by fatty acids of short- and medium- chain length (C_4-C_{12}) released into the skim phase (13). Losses of 98, 93, and 16% of added C_4, C_6, and $C_{18:0}$, respectively, were found using the BDI method (45). This is one reason why a complete correlation between ADV and flavor is not possible (13).

The ADV may underestimate the true extent of lipolysis and flavor defects (18).

Earley and Hansen (22) used the BDI method of ADV for measuring rancid flavors in UHT milk. They found some milk samples had ADV as high as 3.0 but no rancid flavor criticisms were reported from their trained sensory panel of 5 judges. They concluded that all acids titrated in the ADV procedure do not contribute to rancid off-flavors in UHT milk. Rerkrai et al. (49) also studied the increase in ADV over time in UHT milk. Sensory evaluation by 7 trained panelists found no rancid flavor in milk samples with ADV of 1.25 to 1.36 meq/100 g fat, although an ADV of that value indicates that the milk should have a borderline or lipolyzed flavor based on the table of values associated with rancid flavor in *Standard Methods for the Examination of Dairy Products* (50).

Alternative analytical methods for routine determination of fatty acids as produced by lipolysis may be divided into 3 groups based on acidity of free fat as measured by churning (25) or detergent de-emulsification such as the BDI method (55), solvent extraction followed by alkali titration of an organic (ether) phase (31, 23, 45), or colorimetric methods (38, 32, 53). The solvent extraction method for measurement of FFA may result in extracting interfering lactates, citrates, and

phospholipids while recovering the short-chain (C_4-C_8) FFA (13). Fat acidity measurements are helpful in classifying products as "not rancid" or "rancid" but offer little information as a measurement of intensity of rancidity when compared with sensory evaluations (21).

Sensory analyses in relation to these alternative ADV methods or solvent extraction methods are often related back to ADV. Shipe et al. (53) developed an alternative method called the copper soap method (CSM). FFA were converted to copper soaps, extracted, the copper reacted with a color reagent, and color measured spectrophotometrically. Six trained sensory panelists evaluated homogenized milk samples for intensity of rancid flavor on a structured 5-point scale (0=none; 4=strong) and the scores correlated with ADV and CSM. Correlations of .82 and .78 were found between CSM and flavor scores and ADV and flavor scores, respectively. The correlation between CSM and BDI was .88. Pillay et al. (46) found a correlation of .96 between ADV and the modified Frankel and Tarassuk (FT) method, an ether extraction of the fat from 10 ml milk and titrated with .025N alcoholic KOH to a phenolphthalein endpoint (23). The threshold level for the FT method, as determined by 14 consumer panelists was 4.5 meq/100 g fat in ether extract, which was equivalent to a ADV of 1.85 - 2.05 meq/100 g fat as determined by

regression. The relationship between alternative methods and ADV for measuring rancid flavor provides little information if, in fact, the ADV does not adequately measure rancid flavor.

Quantitation of individual FFA may be achieved with gas chromatography. Several methods have been devised which quantify FFA by employing silicic acid/KOH (24, 28, 58) or anion exchange resins (33, 10) as solid supports for the removal of fat. These methods are advantageous over the ADV method because they quantify FFA. However, hydrolysis of fat during the isolation procedure may cause overestimation of FFA. (13). Other disadvantages of these methods include loss of short-chain fatty acids in the water phase and tedious pretreatment of the samples. The method of Deeth et al. (17), which employs an alumina support, does not require extensive pretreatment of samples and loss of short-chain acids and hydrolysis of lipids is minimized. Reported recoveries using this method are greater than 92% for all major fatty acids (13). Ikins et al. (27) used the method of Deeth et al. (17) to determine the correlation between different fatty acid groups and ADV for cheddar cheese. The correlation between ADV and concentration of FFA determined with gas chromatography was .739 for total FFA, .561 for fatty acids of shorter chain length (C_4-C_{10}), .470 for C_4, and .750 for fatty acids with

longer carbon chains (C_{12}-C_{18}). Woo and Lindsay (58) used the gas chromatography method employing silicic acid/KOH to measure fatty acid concentrations in butter and related those to ADV and sensory analysis as completed by 35 trained panelists. The concentrations of individual FFA were better able to explain the variability in the coefficient of determination (R^2) in relation to sensory detection of rancidity than were the ADV data. Active consideration must be given to selecting routine, standard, and reference methods for detection of FFA levels in milk and dairy products and establishing statistical correlations with organoleptic assessment (19).

VI. THE PROBLEM AND THE PLAN

Acid degree value is frequently used to determine the presence or possible development of rancid flavor in milk. It is often used as a quality control criterion for discarding milk or directing it elsewhere, away from fluid milk distribution, so as to avoid consumer dissatisfaction with fluid milk due to rancid flavor. However, based on review of the literature, it appears that, while rancid flavors in fluid milk are a problem, ADV may not be an adequate method for measuring the flavor or assessing its

development. If the method is to be used in the dairy industry, its reliability and validity must be determined.

Research described herein involved assessment of the relationship between ADV and sensory detection of rancid flavor in milk collected from area farms and in laboratory prepared rancid milk, the influence of selected fatty acids on ADV, and the contribution of FFA present in farm milk samples and laboratory-prepared rancid milk samples to ADV and perception of rancid flavor. The sensory evaluation of rancid flavor included an extended study with a trained panel and a study of short duration with a consumer panel.

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CHAPTER III

SENSORY DETECTION AND RECOVERY BY ACID DEGREE VALUE OF SELECTED FATTY ACIDS IN MILK

I. ABSTRACT

The influence of selected short-, medium-, and long-chain fatty acids on acid degree values of pasteurized Vitamin D milk samples was determined to help understand the relationship between acid degree value and rancid flavor. Milk samples were blended with .25 μmol FFA/ml milk and acid degree value determined by standard method. Concentration of medium- (C_{10}-C_{16}) and long-chain ($C_{18:0}$-$C_{18:1}$) fatty acids recovered (1.95-2.33, 3.24-4.12 μmol FFA/g fat, respectively) was significantly higher ($p<.05$) than concentration recovered for short-chain (C_4-C_8) fatty acids (<.63 μmol/g fat). Five concentrations (.20, .25, .35, .45, .55 μmol FFA/ml milk) of C_4, C_{10}, $C_{18:1}$ were added to milk with an emulsifier. Recovery of C_4 was less than .51 μmol/g fat, regardless of concentration. Capric acid (C_{10}) had a significantly higher recovery at the .45 and .55 μmol/ml levels (3.64 and 4.86 μmol/g fat, respectively) when compared to the .20 μmol/ml concentration (1.94 μmol/g fat). There was a significant increase in recovery of $C_{18:1}$ at each increasing

concentration level (3.91, 5.44, 7.96, 8.81, 11.08 μmol/g fat, respectively). Detection threshold for C_4 by 6 trained panelists (2 replications) was .20 μmol/ml. Detection of $C_{18:1}$ occurred at .55 μmol/ml but C_{10} was not detected. Gas chromatography demonstrated partitioning of fatty acids into fat or skim (based on absence from fat). There was no significant difference in recovery of C_4, C_{10}, or $C_{18:1}$ from milk when added at .55 μmol/ml milk as determined by gas chromatography. Recovery of C_4 (.06 μmol/g fat) from fat separated by acid degree value method and analyzed by gas chromatography was significantly lower ($p<.05$) than recovery of C_{10} (7.44 μmol/g fat) or $C_{18:1}$ (14.83 μmol/g fat). Long- and medium-chain fatty acids have more influence on ADV than short-chain fatty acids because the hydrophobic nature of the carbon chain allows partitioning of medium- and long-chain fatty acids into the fat. Butyric acid is easily detected in milk by flavor evaluation but C_{10} and $C_{18:1}$ are not. Acid degree value is not measuring the short-chain fatty acids which contribute to the sensory detection of rancid flavor.

II. INTRODUCTION

Several laboratory analyses are used to measure milk quality prior to and during processing. One such analysis,

the acid degree value (ADV), has been used as an indicator of rancid flavor development in milk. This test is used as a measure of hydrolysis of the fatty acids from glycerides due to lipase which results in increased levels of free fatty acids (FFA). In the ADV method of the Bureau of Dairy Industries (BDI) procedure (14), milkfat is extracted by heat, detergent, and centrifugation and free fatty acids (FFA) in the fat are titrated. Milk with an ADV greater than 1.00 meq/100g fat is considered slightly rancid (14). Based on an ADV of greater than 1.0 meq/100 g fat, Barnard and Moir (3) identified 356 (15%) of 2,346 Vitamin D milk samples as rancid. This would indicate a need for concern about rancid flavor in milk.

Although ADV is used as an indication of rancid flavor development, it has some limitations (5). Bandler (2) stated that ADV alone was not a complete measure of rancid flavor. Randolph and Erwin (12) measured fatty acid concentration and ADV of normal and mastitic milk and found varying concentrations of fatty acids related to ADV. They suggested that milk with high levels of short-chain FFA but a low ADV could have a more rancid flavor than another with a high proportion of long-chain FFA and high ADV. No sensory work was completed to support this suggestion. Sensory evaluation of milk must also be conducted to confirm the presence of rancid flavors (2).

A strong relationship between ADV and rancid flavor has not been clearly established, so an ADV greater than 1.0 meq/100 g fat may not be a true indicator of rancid flavor development. Ultra-high temperature (UHT) pasteurized milk samples stored for 24 weeks at 25°C were evaluated for flavor by 7 sensory panelists trained to detect rancid flavors (7). Although the ADV ranged from 1.24 to 1.36 meq/100 g fat, no lipolyzed flavor was detected by panelists (13). Increase in ADV during storage indicated that lipase was active but rancid flavor had not developed. Rerkrai et al. (13) suggested that long-chain fatty acids were selectively hydrolyzed or that acidic compounds other than FFA were responsible for ADV. Similar results have been observed in other studies. Acid degree values as high as 3.0 meq/100 g fat did not elicit criticisms of rancidity by trained panel members tasting UHT pasteurized milk (7, 8). All acids titrated by ADV may not be involved in the rancid flavor of UHT milk (7,8).

The ADV procedure does not account for selective solubility, molecular weight, and flavor potency of individual FFA (18). In addition, it is difficult to obtain good recovery of FFA, especially shorter chain, hydrophilic fatty acids, without extracting interfering lactates, citrates, and phospholipids (4).

The ADV procedure does not account for composition of FFA in the fat portion which is extracted and titrated (10). The FFA associated with rancid flavor (C_4-C_{12}) are partially released during lipolysis of milk into the water phase due to their hydrophilic nature. Extraction of these fatty acids into fat by the ADV procedure is limited. Major fatty acids detected in the BDI method and many alternative ADV methods are C_{14}-C_{20} (4).

The objectives of this study were to determine the influence of selected FFA on ADV, to determine the phase, skim or fat, in which specific free fatty acids were found after separation by the ADV procedure, and to determine if selected free fatty acids may be detected in milk by experienced panelists.

III. MATERIALS AND METHODS

Acid Degree Values

A concentration equivalent to .25 μmol FFA/ml milk of fatty acid (C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, $C_{18:0}$, $C_{18:1}$) was added to 500 ml of pasteurized homogenized Vitamin D milk. Tween 80 was added at .02% and the sample homogenized for three minutes in a blender (Waring Products Division, New Hartford, CT). ADV was completed by standard methods (14) immediately after sample preparation. Analyses were

completed in duplicate and replicated 4 times. Recovery of FFA was determined by converting the ADV (meq/100 g fat) to μmol FFA/g fat and calculating the difference in concentration recovered between the spiked fatty acid sample and the control (milk and emulsifier).

Three fatty acids (C_4, C_{10}, and $C_{18:1}$) were selected to study the effect of concentration (.20, .25, .35, .45, .55 μmol FFA/ml milk) of short-, medium-, and long-chain fatty acids on ADV. Milk samples were prepared in a similar manner with 500 ml whole milk, .02% Tween 80, and selected fatty acid and a control sample with milk and emulsifier was prepared. Acid degree values were completed in duplicate on each sample and the experiment replicated 3 times. Recovery of FFA from the spiked sample compared to that of the control was determined as previously described.

Determination of Fatty Acid Partitioning

The partitioning of FFA into skim or fat phase in the ADV fat extraction procedure was determined using a fatty acid extraction and gas chromatographic procedure (6). Preparation of samples with C_4, C_{10}, and $C_{18:1}$ was the same as previously described. Presence of a greater concentration of fatty acid in fat as compared to a control (milk and emulsifier) indicated that the fatty acid was partitioned into fat during the ADV fat separation

procedure. If the fatty acid was not present in the fat then it was assumed to have remained in the skim phase.

Fatty Acid Extraction - Milk. The FFA were extracted from milk with cold HCl/diethyl ether containing C_5 as internal standard (6). Isolation of FFA was completed by extracting FFA from the lipid onto a small alumina column (12 cm x 5 mm i.d.) and removing triglycerides with hexane-diethyl ether. Adsorbed FFA were dried on alumina with vacuum and stored under nitrogen in a capped glass centrifuge tube at 4°C overnight. FFA were removed from alumina with diisopropyl ether containing 6% formic acid and the sample was centrifuged.

Fatty Acid Extraction - Fat. The ADV fat separation procedure was completed through the 57°C tempering bath step (14). Fatty acid extraction was completed by dissolving 1.0 g fat in 1:1 v/v hexane-diethyl ether (10 ml) containing .1 ml .2N H_2SO_4 and C_5 as an internal standard (6). The ether volume was added to the alumina column and isolation of FFA completed as described for milk samples.

Gas Chromatography

A 4-μl sample was injected directly into a Shimadzu Mini GC-2 equipped with 1 m x 3 mm glass column packed with 10% SP-216-PS on 100/120 Supelcoport (Supelco Inc.,

Bellefonte, PA) and a flame ionization detector, providing resolution of major short-, medium-, and long-chain FFA. A Shimadzu Chromatopak E-1A data printer and OmniScribe Recorder provided chromatograms, peak retention times and peak relative areas. The initial temperature was 110°C programmed to 195°C at 8°C/min. with a 25 minute duration. Carrier gas was nitrogen with a flow rate of 55 ml/minute.

Calculation of Fatty Acid Concentration

An increased area for spiked FFA (C_4, C_{10}, $C_{18:1}$) over that found in the control indicated the presence of the specific FFA in fat extracted by ADV. Concentration was calculated relative to the area of the C_5 internal standard, assuming complete recovery of the internal standard. Individual fatty acid standards were weighed and diluted with isopropyl ether to yield a concentration of .005 mol FFA/ml. Peak area and retention times for each fatty acid were determined under established GC conditions. Relative molar response (RMR) of each fatty acid was determined with respect to the internal standard (9). RMR was calculated by Equation 1. Grams of fatty acid were calculated (Equation 2) and concentration (μmol FFA/10 ml milk) was determined (Equation 3).

Equation 1:

$$RMR = \frac{\text{area/moles}_{f.a.}{}^{a}}{\text{area/moles}_{i.s.}{}^{b}}$$

[a] f.a. = fatty acid; [b] i.s. = internal standard.

Equation 2:

$$\text{Grams}_{f.a.} = \frac{\text{area}_{f.a.} \times \text{molecular weight}_{f.a.} \times \text{gram}_{i.s.}}{\text{area}_{i.s.} \times \text{molecular weight}_{i.s.} \times \text{RMR}_{f.a./i.s.}}$$

Equation 3:

$$\text{micromoles}_{f.a.} = \frac{\text{gram}_{f.a.} \times 1{,}000{,}000\ \mu\text{mol/}_{\text{mole}}}{\text{gram}_{f.a./\text{mole}}}$$

Sensory

Sensory evaluation of the contribution of specific FFA to rancidity was carried out with a 6-member experienced panel (5 female, 1 male). Panelists were trained for a minimum of 3 months to detect rancid and other off-flavors in milk. Preparation of homogenized milk samples with C_4, C_{10}, and $C_{18:1}$ was the same as ADV sample preparation. Triangle tests for each fatty acid were completed using milk homogenized with Tween 80 as the identical samples and presented at 14°C. Each sample was assigned a randomly chosen 3-digit code. Five sets of triangle tests were presented in increasing order of concentration of specific FFA (.20, .25, .35, .45, and .55 μmol/ml). The odd sample (spiked sample) was randomly assigned within each triangle test and panelists asked to identify the odd sample. Three

consecutive correct identifications of spiked samples were needed to determine the detection threshold for each FFA.

Statistical Analyses

General Linear Model (GLM) (SAS Institute, Cary, NC) was used to determine significant differences among recovery of specific fatty acids (C_4-$C_{18:1}$) and among different concentrations of each fatty acid (C_4, C_{10}, $C_{18:1}$). ANOVA was used to determine significant differences in recovery of C_4, C_{10}, and $C_{18:1}$ at each of 5 concentrations. Significant differences in recovery of C_4, C_{10}, and $C_{18:1}$ in milk and in fat as determined by fatty acid extraction and gas chromatography were determined using GLM. Duncan's multiple range test was used for mean separations when appropriate. Statistical differences for triangle tests were determined by using the appropriate statistical chart from Roessler et al. (15).

IV. RESULTS AND DISCUSSION

Recovery of specific fatty acids by the ADV is presented in Table 1[1]. There were significant differences ($p=.0001$) among groups of fatty acids with different chain

[1] All tables and figures may be found in the Appendixes.

lengths. No statistical difference was shown among short-chain fatty acids (C_4, C_6, C_8). Recoveries of fatty acids with 10 to 16 carbons were not significantly different but were significantly higher than recoveries of short-chain fatty acids. Stearic acid ($C_{18:0}$) was recovered at 3.24 μmol/g fat and was significantly higher than short- and medium-chain (C_{10}-C_{16}) fatty acids. Recovery of oleic acid ($C_{18:1}$), 4.12 μmol/g fat, was significantly greater than all other fatty acids. These results clearly demonstrate that the ADV procedure does not extract and measure all FFA equally. Short-chain FFA are partitioned to the skim milk phase (17) and are not completely detected by ADV determination.

Based on these results, the effect of increasing concentration of short-, medium-, and long-chain ($C_{18:0}$, $C_{18:1}$) fatty acids was evaluated using one free fatty acid (C_4, C_{10}, $C_{18:1}$) to represent each group. Recovery of each FFA due to increased concentration of specific FFA is presented in Table 2. There was no significant difference in recovery of C_4, regardless of concentration. Recovery was less than or equal to .51 μmol/g fat for all levels. Recovery of C_{10} was proportional to added concentration of fatty acid. Significant differences were observed between the lowest concentration level (.20 μmol/ml) with a recovery of 1.94 μmol/g fat, and the two highest

concentration levels (.45 and .55 μmol/ml) with recoveries of 3.64 and 4.86 μmol/g fat, respectively. There were significant differences among recoveries of $C_{18:1}$ at all added concentrations. The recovery was 3.91 μmol/g fat when .20 μmol/ml of $C_{18:1}$ were added and increased to 11.08 μmol/g fat when .55 μmol/ml were added. Therefore, short-chain fatty acid had no effect on ADV regardless of concentration. The medium-chain length fatty acid showed a moderate and gradual increase in ADV but the long-chain fatty acid showed a significant increase in ADV at each concentration level studied. Variability in recovery appeared to be greater for C_4 and C_{10} than for $C_{18:1}$. Oleic acid is apparently extracted more completely and with less variability by the ADV procedure than C_4 or C_{10}.

The hydrophilic-hydrophobic nature of the FFA is an important factor which must be considered when using the ADV as an indication of rancid flavor. Shorter chain fatty acids are much more soluble in water than medium-chain or longer chain fatty acids. Free fatty acid extraction and gas chromatographic separation demonstrated the partitioning of fatty acids into aqueous or fat phase. There were no significant differences in recovery of C_4 (3.26 μmol/10 ml), C_{10} (4.53 μmol/10 ml), and $C_{18:1}$ (3.25 μmol/10 ml) added at .55 μmol/ml of milk as measured by the gas chromatographic method (Table 3), indicating that the

GC procedure could adequately measure the recovery of each fatty acid and establish its presence in the fatty acid spiked sample. However, there were differences in recoveries of fatty acids from fat separated by ADV (Table 4). A low concentration (.06 μmol/g fat) of C_4 was extracted by the ADV fat separation method but most C_4 apparently remained in the skim milk phase based on the limited concentration present in fat extracted by the ADV procedure. The concentration of C_{10} recovered from fat was 7.44 μmol/g fat which was much higher than that of C_4. Recovery of $C_{18:1}$ was 14.83 μmol/g fat above the control and was greater than that of C_{10}. Therefore, medium- and long-chain FFA are more completely recovered and measured by the ADV extraction procedure. The variability in recovery of C_4 from fat was small (S.D.=.10) but the standard deviation for C_{10} (1.61) and $C_{18:1}$ (6.50) indicated greater variability in recovery from fat. The ADV procedure will extract those fatty acids which are more hydrophobic but those which are hydrophilic will be incompletely extracted and so contribute little to the titration. Table 5 provides the relative solubilities for fatty acids which were tested (17). Shorter chain fatty acids associated with rancid flavor (C_4-C_8) are more water soluble and are not extracted by the fat separation method in the ADV procedure. Capric acid (C_{10}) and C_{12} are

relatively more hydrophobic than C_4 yet are not completely extracted by the ADV procedure. Hydrophobic, long-chain fatty acids contribute the most to ADV.

The gas chromatographic procedure was used to demonstrate partitioning of the FFA in the ADV procedure because it has been reported to have 92% or greater recovery of FFA, regardless of chain length (6). Recovery of short- chain FFA in the fatty acid extraction procedure used for gas chromatography (6) is aided by reducing the pH of milk with HCl. Short-chain FFA, as calcium or magnesium salts or in anion form at the normal pH of milk, are protonated at the lower pH and become associated with the fat phase. The FFA were extracted with fat in the ether extraction. There is no step in the ADV procedure in which the pH is lowered.

Sensory results demonstrated differences in flavor perceptibility of short-, medium-, and long-chain fatty acids. Figure 1 illustrates detection thresholds for C_4, C_{10}, and $C_{18:1}$. Columns represent the sum of correct responses for two replications. A total of 11 responses were possible. Six panelists completed the first replication and 5 panelists completed the second. Seven correct responses of 11 judgments were needed to determine that the odd sample was significantly different from the like (control) samples (15). Seven correct responses were

obtained at the lowest concentration, .20 μmol/ml milk, for C_4. Therefore, the C_4 sample was significantly different from control samples at even the lowest concentration. It is obvious that C_4 is readily detected by sensory perception and its importance in rancid flavor has been demonstrated by Scanlan et al. (16). In contrast to sensory results, the ADV did not increase when C_4 was added to the milk.

Capric acid (C_{10}) is also reported to be important in rancid flavor (16) but panel members did not perceive it. Only 4 correct responses were obtained at the highest concentration, .55 μmol/ml. It cannot be concluded that the milk sample spiked with C_{10} was significantly different from the control in flavor yet the increase in ADV due to the medium-chain fatty acid is significantly greater than that due to the short-chain fatty acid. The contribution of C_{10} to rancid flavor perception has been demonstrated by Scanlan et al. (16). Perhaps C_{10} is important in rancid flavor when in combination with other FFA but does not contribute as much flavor independently. Patton (11) reported a higher threshold for capric acid in oil than in water. Butyric acid (C_4) and C_{10} have higher flavor thresholds in water than in oil. Caprylic acid (C_8) is reported to have the lowest threshold level for detection

at 10 ppm in water (17) and the reported threshold for butyric acid was 12.9 ppm in milk (1).

There were more correct responses for milk spiked with $C_{18:1}$ than were obtained for C_{10} spiked samples. Seven samples were correctly chosen at .55 μmol/ml milk, indicating that there was a significant difference between the spiked sample with $C_{18:1}$ and the control samples at this concentration. Oleic acid ($C_{18:1}$) must be added at concentrations greater than .45 μmol/ml before it becomes perceptible by taste in milk. Scanlan et al. (16) reported that long-chain fatty acids, including $C_{18:1}$, were not important contributors to rancid flavor.

The sensory results of this study indicate that C_4 has high flavor potential at low concentrations (.20 μmol/ml milk) but C_{10} was not perceptible at even the highest concentration (.55 μmol/ml). Oleic acid ($C_{18:1}$) was perceived at only the highest concentration. The recovery of $C_{18:1}$ when added to milk at .55 μmol/ml milk was 11.08 μmol/g fat (Table 2), much greater than that for C_4 (.40 μmol/g fat) or C_{10} (4.86 μmol/g fat). These findings indicate that the ADV procedure does not recover short- or medium-chain FFA (C_4 and C_{10}) which have been reported to be associated with rancid flavor (16) but does recover long- chain FFA ($C_{18:1}$) which has not been associated with rancidity (16).

V. CONCLUSIONS

The ADV procedure does not extract and measure all FFA equally. Those fatty acids associated with rancid flavor (C_4-C_{12}) (16) are incompletely extracted and contribute only slightly to the ADV. An ADV reflects the concentration of total fatty acids extracted into fat, primarily longer chain, hydrophobic fatty acids, which are not related to rancid flavor (16). Since ADV does not accurately measure the fatty acids important in rancid flavor, the relationship of ADV to rancid flavor is questionable and should be investigated further.

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APPENDIX A

Table 1. Recovery of added major fatty acids from milk by ADV.

Fatty acid	Recovery[1]
	(μmol FFA/g fat)
C_4	.63[a] ± .46
C_6	.49[a] ± .33
C_8	.55[a] ± .35
C_{10}	2.06[b] ± .34
C_{12}	2.23[b] ± .35
C_{14}	1.95[b] ± .97
C_{16}	2.33[b] ± .60
$C_{18:0}$	3.24[c] ± .66
$C_{18:1}$	4.12[d] ± .55

[1] Mean ± S.D., n=4. Fatty acids added to milk at .25 μmol/ml.

[a,b,c,d] Means with the same superscript are not significantly different; ($p>.05$).

Table 2. Recovery of added C_4, C_{10}, $C_{18:1}$ by the ADV procedure.

	Recovery[1]		
Fatty acid	C_4	C_{10}	$C_{18:1}$
(μmol FFA/ml milk)		(μmol FFA/g fat)	
.20	.18[a] ± .17	1.94[b] ± .42	3.91[e] ± .08
.25	.45[a] ± .64	2.60[bc] ± .23	5.44[f] ± .27
.35	.08[a] ± .11	3.15[bc] ± .94	7.96[g] ± .24
.45	.50[a] ± .28	3.64[cd] ± .21	8.81[h] ± .16
.55	.40[a] ± .57	4.86[d] ± .09	11.08[i] ± .27

[1] Mean ± S.D., n=3.
[a,b,c,d,e,f,g,h,i] Means with the same letter in row or column are not significantly different; ($p>.05$).

Table 3. Recovery of added C_4, C_{10}, $C_{18:1}$ from milk by gas chromatography.

Fatty acids	Recovery[1]
	(μmol FFA/10 ml milk)
C_4	3.26[a] ± .60
C_{10}	4.53[a] ± 1.00
$C_{18:1}$	3.25[a] ± .27

[1] Mean ± S.D., n=3. Fatty acids added to milk at .55 μmol/ml milk.
[a] Means with the same superscript are not significantly different; ($p>.05$).

Table 4. Recovery of added C_4, C_{10}, $C_{18:1}$ from fat (separated from milk by ADV) by gas chromatography.

Fatty acids	Recovery[1]
	(μmol FFA/g fat)
C_4	.06[a] ± .10
C_{10}	7.44[b] ± 1.61
$C_{18:1}$	14.83[b] ± 6.50

[1] Mean ± S.D., n=3. Fatty acids added to milk at .55 μmol/ml milk.
[a] Means with the same superscript are not significantly different; ($p>.05$).

Table 5. Relative solubilities of selected fatty acids in water[1].

Fatty acid	Solubility at 25°C (mg/100 ml H_2O)
C_4	miscible
C_6	970.00
C_8	73.00
C_{10}	6.00
C_{12}	0.55
C_{14}	0.18
C_{16}	0.08
$C_{18:0}$	0.04
$C_{18:1}$	-

[1] (17).

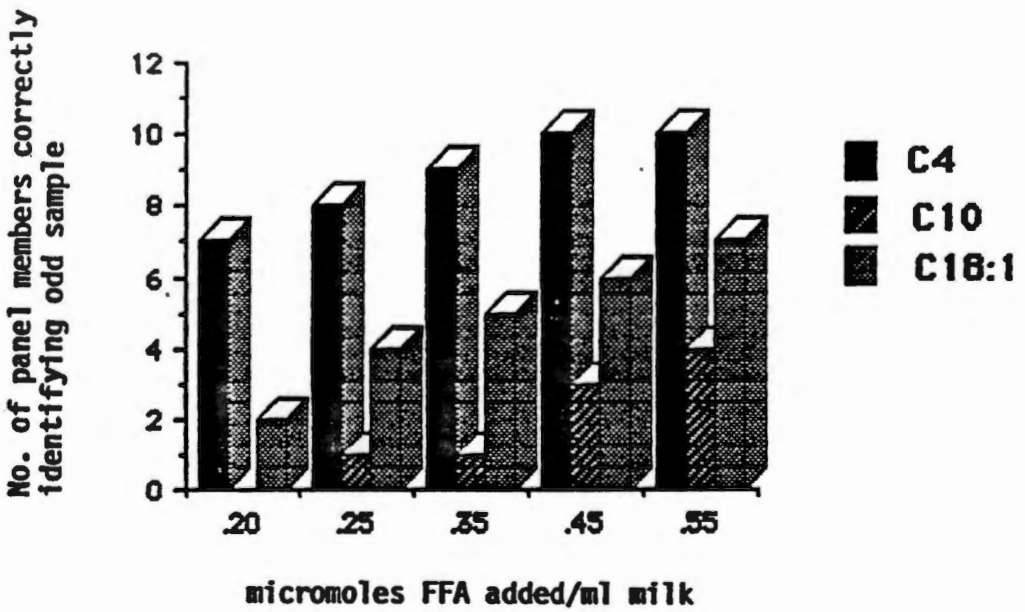

Figure 1. Detection thresholds (triangle tests) for milk samples with added butyric (C_4), capric (C_{10}), and oleic ($C_{18:1}$) acid by 6 experienced panelists in 2 replications.

APPENDIX B

ANOVA FOR FA AS MEASURED BY ADV

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	11	48.38056324	4.39823302
ERROR	23	7.65921428	0.33300932
CORRECTED TOTAL	34	56.03977752	

MODEL F = 13.21 PR > F = 0.0001

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.863325	28.9559	0.57706959	1.99292602

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	8	48.06512968	18.04	0.0001
REP	3	0.30707139	0.31	0.8198

ANOVA FOR INCREASE IN MICROMOLES RECOVERED DUE TO INCREASING CONCENTRATION OF FA (C_4, C_{10}, $C_{18:1}$)

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	14	329.91582617	23.56541615
ERROR	15	2.25287474	0.15019165
CORRECTED TOTAL	29	332.16870090	

MODEL F = 156.90 PR > F = 0.0001

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.993218	10.5693	0.38754567	3.66669626

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	256.08176467	852.52	0.0001
CONC	4	42.25699190	70.34	0.0001
FA*CONC	8	31.57706960	26.28	0.0001

ANOVA FOR INCREASE IN MICROMOLES RECOVERED DUE TO INCREASING CONCENTRATION OF C_4

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	4	0.27110599	0.06777650
ERROR	5	0.84597750	0.16919550
CORRECTED TOTAL	9	1.11708349	

MODEL F = 0.40 PR > F = 0.8019

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.242691	127.7182	0.41133381	0.32206369

SOURCE	DF	TYPE III SS	F VALUE	PR > F
CONC	4	0.27110599	0.40	0.8019

ANOVA FOR INCREASE IN MICROMOLES RECOVERED DUE TO INCREASING CONCENTRATION OF C_{10}

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	4	9.78023509	2.44505877
ERROR	5	1.17097399	0.23419480
CORRECTED TOTAL	9	10.95120909	

MODEL F = 10.44 PR > F = 0.0121

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.893074	14.9454	0.48393677	3.23802160

SOURCE	DF	TYPE III SS	F VALUE	PR > F
CONC	4	9.78023509	10.44	0.0121

ANOVA FOR INCREASE IN MICROMOLES RECOVERED DUE TO INCREASING CONCENTRATION OF $C_{18:1}$

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	4	63.78272042	15.94568010
ERROR	5	0.23592324	0.04718465
CORRECTED TOTAL	9	64.01864366	

MODEL F =	337.94	PR > F =	0.0001

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.996315	2.9196	0.21722028	7.44000350

SOURCE	DF	TYPE III SS	F VALUE	PR > F
CONC	4	63.78272042	337.94	0.0001

ANOVA FOR RECOVERY OF C_4, C_{10}, $C_{18:1}$ AT 0.20 MICROMOLES

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	13.90771783	6.95385891
ERROR	3	0.21289521	0.07096507
CORRECTED TOTAL	5	14.12061304	

MODEL F =	97.99	PR > F =	0.0019

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.984923	13.2592	0.26639270	2.00912029

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	13.90771783	97.99	0.0019

ANOVA FOR RECOVERY OF C_4, C_{10}, $C_{18:1}$ AT 0.25 MICROMOLES

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	25.02455699	12.51227850
ERROR	3	0.53113521	0.17704507
CORRECTED TOTAL	5	25.55569220	

MODEL F = 70.67 PR > F = 0.0030

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.979217	14.8665	0.42076724	2.83029913

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	25.02455699	70.67	0.0030

ANOVA FOR RECOVERY OF C_4, C_{10}, $C_{18:1}$ AT 0.35 MICROMOLES

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	63.11302821	31.55651410
ERROR	3	0.95979557	0.31993186
CORRECTED TOTAL	5	64.07282378	

MODEL F = 98.64 PR > F = 0.0018

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.985020	15.1675	0.56562519	3.72919406

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	63.11302821	98.64	0.0018

ANOVA FOR RECOVERY OF C_4, C_{10}, $C_{18:1}$ AT 0.45 MICROMOLES

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	70.48608790	35.24304395
ERROR	3	0.14749220	0.04916407
CORRECTED TOTAL	5	70.63358010	

MODEL F =	716.85	PR > F = 0.0001	
R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.997912	5.1343	0.22172971	4.31862027

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	70.48608790	716.85	0.0001

ANOVA FOR RECOVERY OF C_4, C_{10}, $C_{18:1}$ AT 0.55 MICROMOLES

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	115.12744334	57.56372167
ERROR	3	0.40155655	0.13385218
CORRECTED TOTAL	5	115.52899989	

MODEL F = 430.05 PR > F = 0.0002

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.996524	6.7176	0.36585815	5.44624756

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	115.12744334	430.05	0.0002

ANOVA FOR CONCENTRATION OF FATTY ACIDS IN MILK AS DETERMINED BY GC

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	2.14879518	1.07439759
ERROR	3	1.44443954	0.48147985
CORRECTED TOTAL	5	3.59323472	

MODEL F = 2.23 PR > F = 0.2549

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.598011	18.8356	0.69388749	3.68391340

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	2.14879518	2.23	0.2549

ANOVA FOR CONCENTRATION OF FATTY ACIDS IN FAT AS DETERMINED BY GC

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: MICROMOLES

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	2	327.55114729	163.77557365
ERROR	6	89.76993316	14.96165553
CORRECTED TOTAL	8	417.32108046	

MODEL F = 10.95 PR > F = 0.0100

R-SQUARE	C.V.	ROOT MSE	MICROMOLES MEAN
0.784890	51.9714	3.86802993	7.44260540

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FA	2	327.55114729	10.95	0.0100

SENSORY SCORECARD: MILK FLAVOR DISCRIMINATION TEST

You will be presented with 5 sets of 3 samples. Smell each set and identify which sample is different. If you cannot tell the different sample by smell, taste the sample. Circle the different sample. Please taste sets in order from 1-5.

1A	1B	1C
2A	2B	2C
3A	3B	3C
4A	4B	4C
5A	5B	5C

Thank you for completing these tests.

CHAPTER IV

RELATIONSHIP BETWEEN ACID DEGREE VALUE AND SENSORY PERCEPTION OF RANCID FLAVOR IN MILK

I. ABSTRACT

Fifty-nine milk samples from area (Maryville, TN) farms were homogenized and pasteurized prior to determination of acid degree value and sensory analyses. Samples were tested immediately after processing and after 12 days of storage at 4°C. Acid degree value was completed by standard methods (13). A trained panel (n=6) evaluated milk for rancid flavor by magnitude estimation. The correlation between acid degree value and rancidity scores was .13 (p=.16). There was no difference in rancidity scores across 5 sample groups based on acid degree value (<1.26, 1.26-1.75, 1.76-2.30, 2.31-2.75, >2.75 meq/100 g fat). Rancidity scores characterized the samples as "not rancid" to "very rancid" for samples in each acid degree value group. Laboratory-prepared rancid samples were given higher rancidity scores than milk samples with similar acid degree values collected from the farm. There was an increase in acid degree value due to processing and storage time as evaluated by paired *t*-test. The rancidity scores

increased with storage. A flavor difference was found between milk with low acid degree value (1.57 meq/100 g fat) and high acid degree value (5.37 and 7.67 meq/100 g fat) by a consumer panel (n=72) using a paired-comparison test. The trained panel scored the milk with acid degree value of 1.57 meq/100 g fat as "slightly rancid" and milk with high values (5.37, 7.67 meq/100 g fat) as "moderately rancid".

II. INTRODUCTION

The flavor of milk has a direct impact on its level of consumption (2). The bland, slightly sweet flavor associated with high quality milk may be easily displaced by off-flavors which must be avoided. Early detection of off-flavors or compounds responsible for off-flavors may provide opportunity for control of further development of these flavors or time to seek alternative uses for the fluid milk.

Rancid flavor in milk is frequently determined by acid degree value (ADV). This chemical method, as listed in *Standard Methods for the Examination of Dairy Products*, involves the de-emulsification and extraction of free fat by detergent, heat, centrifugation, and titration of the acids in a weighed portion of fat with .02 N alcoholic KOH

(13). It has been suggested that raw milk with an ADV of 1.0 meq/100 g fat or greater may be rancid or well on its way to becoming rancid and should be discarded (16). An ADV of 1.5 meq/100 g fat or greater is reported as "unsatisfactory (extremely lipolyzed)" (13). The relationship between ADV and rancid flavor intensity was initially evaluated by 2 expert judges using laboratory-prepared rancid samples (LPRS) (17).

Accuracy of ADV in predicting the sensory detection of rancidity is questionable. Many researchers have found farm milk samples with high ADV that did not taste rancid to trained panelists. Dunkley (6) reported a poor correlation between ADV and rancid flavor in cream. Two experienced judges were used to evaluate flavor. Dunkley found ADV to have little value in determining intensity of rancid flavor but could be useful in classifying samples as "not rancid" or "rancid". Early and Hansen (7) found UHT milk samples with ADV as high as 3.0 meq/100 g fat did not have a rancid flavor to trained panel members. Rerkrai et al. (12) reported that no lipolyzed flavor was detected in milk with ADV of 1.24 to 1.36 meq/100 g fat but there was an increase in ADV during storage, indicating that the hydrolytic mechanism was operative.

ADV is frequently used to determine rancidity with no concurrent sensory evaluation of milk samples. If ADV is

high, the milk sample may be discarded even though it may not have a detectable rancid flavor. This could be an expensive waste of good product and should be avoided. It is necessary to determine if ADV, as currently used, is an accurate predictor of rancid flavor development. The objectives of this research were to determine the effectiveness of ADV as presented in *Standard Methods for the Examination of Dairy Products* (13) as a quality control method for detecting rancid flavor in fresh and stored milk as perceived by sensory evaluation using a trained panel. A consumer panel also evaluated differences in milk flavor for samples with moderate and high ADV.

III. MATERIALS AND METHODS

Collection of Samples

Raw milk samples (2250 ml) were collected in sterile, amber glass bottles from bulk storage tanks on area (Maryville, TN) farms. Samples were stored in ice during transport to The University of Tennessee Food Technology and Science Department laboratory where they were stored at 4°C for a maximum of 36 hours until sample preparation and analyses could be completed. Fifty-nine samples were collected over a 9-month period; 3-6 different farm samples were collected at one time.

Sample Preparation

Samples were homogenized and pasteurized within 36 hours of collection. Samples were processed for 15 seconds at 72°C and a pressure of 2200 psi in a tubular indirect heat exchanger consisting of preheating coil, pasteurizing coil and cooling coil (18). The majority of samples were processed within 24 hours. Four 250-ml samples of homogenized, pasteurized milk from each farm sample were collected in sterile amber bottles, coded for identification of collection date and farm, and stored at 4°C for subsequent chemical, microbiological and sensory analyses.

Chemical and Microbiological Analyses

Standard plate count (SPC), coliform count (CC), psychrotrophic bacteria count (PBC), lipophilic bacteria count (LBC), and ADV were completed by standard methods (13) on all raw milk samples within 24 hours of sample collection for determination of raw milk quality. Somatic cell counts (SCC) were completed within 48 hours of sample collection. Samples for raw milk quality analyses were collected in sterile 250-ml amber bottles separate from the samples collected for pasteurization so as to avoid possibility of contamination.

One 250-ml sample of each pasteurized farm sample was used for SPC, CC, PBC, LBC, ADV, and phosphatase enzyme tests for adequate pasteurization within 24 hours of processing. The second sample was used for sensory evaluation within 48 hours of processing. The third and fourth samples were stored at 4°C for 12 days after processing. These samples then were used for chemical, microbiological, and sensory analyses as before. Standard methods were followed for all chemical and microbiological analyses (13).

Laboratory-prepared rancid samples were prepared prior to each sensory evaluation panel of farm-collected samples. Thirty-five milliliters of raw milk were added to 750 ml of purchased Vitamin D milk and incubated at 4°C for 24 to 36 hours. The milk was then batch pasteurized at 66°C for 3.5 minutes and cooled rapidly (3). Precautions were taken to avoid oxidative off-flavors by purchasing milk in pigmented containers and covering the milk during the incubation period to protect it from incident light. Raw milk used for preparation of LPRS was aliquoted in 35-ml portions into plastic bags and frozen at -20°C at onset of the study so the source of lipase would be the same for the duration of the study.

Collection and Preparation of Samples for Consumer Evaluation

Two farms were selected for samples for consumer evaluation. Farm A had consistently low ADV, SCC, CC, SPC, and PBC; Farm B had consistently high ADV, and moderate to high SCC, CC, SPC, and PBC. Eight thousand milliliters were collected as previously described from Farm A and 4000 ml were collected from Farm B. The sample from Farm A was divided into 2 equal volumes. The first sample of milk from Farm A and the milk from Farm B were pasteurized immediately after homogenization. The second sample from Farm A was homogenized but pasteurization was delayed for two hours, allowing time for lipolytic activity. This provided a sample with low ADV (Farm A), a sample with high ADV (Farm B), and a sample with an induced high ADV (Farm A, Induced) for consumer evaluation.

Sensory Evaluation

Trained Panel - Training Evaluation. Sensory evaluation was completed with a panel of 6 female graduate students and staff of the Food Technology and Science Department, The University of Tennessee, Knoxville. Panelists completed 6-10 months of training prior to data collection. Training consisted of familiarization of panel members with common off-flavors found in milk: oxidized,

cooked, feed, and rancid. Laboratory-prepared samples were used for the training period (3). Panelists were provided experience in detecting low levels of rancid flavor using LPRS diluted to different intensities with fresh milk. The multi-point external calibration method of magnitude estimation was chosen as the scaling method for data collection to reduce error due to inconsistent use of one scale by all panelists and to eliminate variability due to differences in rancid standards (10).

Evaluation of panelist training was completed immediately prior to data collection and after 33 farm samples were collected in addition to the ongoing evaluation using duplicate LPRS within each sample set. Laboratory-prepared rancid samples were prepared as previously described and diluted with fresh Vitamin D milk to different intensities of rancid flavor. ANOVA and General Linear Model (GLM) were used for statistical analyses of training with 2 replications in the initial evaluation and 3 replications for the second. Five dilutions (0 (fresh milk), 25, 65, 85, and 100% rancid) were used in the initial training evaluation panel. Four dilutions (0, 25, 75, and 100% rancid) were used in the second evaluation. Thirty-milliliter milk samples were presented in random order in amber glass bottles and coded with randomly chosen 3-digit numbers. Panelists could

taste the samples directly from the bottle or use plastic cups, selecting their own portion size from the 30-ml sample. Panelists waited 30 seconds between samples and rinsed with water between samples. Panelists were instructed to evaluate each sample before proceeding to the next. Evaluations were completed in individual booths. Sample temperature was 14°C. Panelists assigned numerical values to indicate intensity of rancid flavor in each sample, ignoring any other off-flavors in the milk. Zero represented the absence of rancid flavor. Ratio values were assigned to each sample to reflect rancid flavor intensity in relation to the first coded sample. Each panelist could develop their own intensity scale. Numerical values were estimated for concept scores for intensity levels of unpalatable, very rancid, moderately rancid, and slightly rancid, using the same scale the panelist used for the samples.

Magnitude scores were then normalized so comparisons among panelists could be made. A pivot number was determined by averaging the values given the four concept scores for each panelist for each calibrating scale. The pivot number for each panelist was divided into each sample magnitude estimate score and concept score to determine the normalized value for sample and concept score for each panelist. Logarithmic (base 10) transformation of the

normalized scores was completed to normalize the distribution since a small sample size (<200 samples) was evaluated (4). Means for log transformed scores across all panelists (log rancidity score) for each sample were used for statistical analyses.

Trained Panel - Sample Evaluation. Testing was completed on 3-6 farm samples per data collection period in the same manner as for the LPRS used for training evaluation. An identified LPRS was presented first so the panelist could orient the palate to the rancid taste. The samples for evaluation were then presented to each panelist in random order. Two randomly coded LPRS were included within the sample set for statistical evaluation of panelist performance.

Consumer Panel. Evaluation of milk samples for flavor was completed by 72 consumers, 18-70 years of age. Consumers were recruited from students, faculty, staff, and guests at the University Center of The University of Tennessee, Knoxville. Three sets of paired-comparison tests were presented to each panelist representing comparisons of milk with low ADV compared to milk with high ADV, milk with low ADV compared to milk with induced high ADV, and milk with high ADV compared to milk with induced high ADV. A balanced design was used. Panelists were asked to determine if there was a difference between the

samples and, if there was a difference, to describe it. Panelists were told only that the milk had undergone slightly different processing methods and the evaluation was to determine if processing had affected flavor. No mention of rancid flavor was made.

Milk samples for consumer evaluation were served at 14°C in plastic portion control cups with lids. Samples were identified with 3-digit codes. Water was available for rinsing between sets of pairs. Panelists were seated at a table for two and tables were separated from each other by dividers. All efforts were made to seat panelists at separate tables to avoid distraction.

The samples used for consumer evaluation were also presented to the trained panel and evaluated by magnitude estimation as previously described.

Statistical Analyses

General Linear Model and ANOVA (SAS Institute, Cary, NC) were used to determine significant differences in mean log rancidity score by ADV for farm milk samples and for panel training evaluation. Farm milk samples were placed into five groups depending on ADV at the time of sensory analysis. Samples in ADV Group 1 included those with ADV < 1.26; samples in Group 2 had ADV ranging from 1.26 to 1.75; Group 3 samples had ADV from 1.76 to 2.30; ADV for samples

in Group 4 were 2.31 to 2.75; and samples in Group 5 had ADV > 2.75. Means were separated with the Student Newman Keuls (SNK) multiple range test when appropriate. Paired *t*-tests were used to evaluate individual panelist performance through the study as well as to compare fresh and stored samples for mean log rancidity scores and ADV and comparison of raw and fresh samples for ADV. A correlation was determined for rancidity scores and ADV. Evaluation of consumer panel results was completed as described for 2-tailed paired-difference testing method by Roessler et al. (14).

IV. RESULTS AND DISCUSSION

Comparison of Raw, Fresh, and Stored Farm Samples

There were significant differences (p=.0001) in ADV between paired raw farm milk samples and freshly pasteurized samples and between fresh and stored farm milk samples indicating FFA concentration had increased, probably due to lipase activity (Table 1). Increases in ADV between paired raw and freshly pasteurized samples may be due to product abuse during homogenization and pasteurization which may increase opportunity for hydrolysis of the fat. Laboratory-scale equipment used for this research adequately completed the task but with

greater turbulence and incorporation of air might be observed in a commercial processing facility. These factors have been found to increase ADV (9). Most raw milk samples were of Grade A quality; 4 samples had SPC $>10^5$ CFU/ml and 2 samples (of those on which SCC were completed) had $>10^6$ somatic cells/ml.

Mean log rancidity scores for paired fresh and stored samples were significantly different (p=.0012) but actual difference in mean scores was small (Table 1). Mean log scores were interpreted as "slightly rancid" for both fresh and stored means. Mean log rancidity scores by ADV group are reported in Table 2.

Figure 1 illustrates the random scattering of log rancidity scores for fresh, stored and LPRS within appropriate ADV group. Fresh samples were primarily clustered in the region of low ADV (group 1, 2, and 3) with low to moderate log rancidity scores. The majority of stored farm samples were in the higher ADV groups with log rancidity scores ranging from low to moderate. The ADV for LPRS ranged from groups 2-5 but generally had higher log rancidity scores than fresh or stored farm samples. The correlation between ADV and log rancidity scores for fresh samples was low (r=.22, p=.09). There was a low negative correlation between ADV and log rancidity score for stored samples (r=-.12, p=.35). In contrast, the correlation

between ADV and log rancidity scores for LPRS was significant (p=.0023) but was still low (r=.17).

Evaluation by Trained Panelists

Means of log rancidity scores for farm milk samples were low across all ADV groups. The mean log rancidity scores for fresh and stored farm milk samples in 5 ADV groups are presented in Table 2. The relationship between mean log rancidity score and description of flavor may be determined from the calibration scale concept scores also included in Table 2. There were no significant differences among mean log scores for fresh farm samples or for stored farm samples even though the ADV range was very broad. Mean log rancidity scores for samples in each ADV group would be described as "slightly rancid" (.10) or "moderately rancid" (.18). The mean log score for the LPRS was .24 which would be described as "moderately rancid" (Table 2). The average ADV for LPRS was 2.61.

The mean log rancidity score for LPRS was higher than any mean log rancidity score for farm samples for any ADV group. The range of ADV for the LPRS would place them in ADV groups 3, 4, or 5. The range in log rancidity scores for LPRS was .16-.36, indicating "moderately rancid" to "unpalatable" rancid flavor (data not shown).

The range of log rancidity scores for farm samples within each ADV group ranged from "not at all rancid" to "very rancid", indicating that some farm samples in each ADV group had a noticeable rancid flavor to the trained panelists whereas other samples with similar ADV did not have a rancid flavor. Table 3 provides examples of samples with "moderately" to "very rancid" flavor by log rancidity scores compared with samples of similar ADV and less than "slightly rancid" log rancidity scores. One farm sample had an ADV of .76, indicating a "borderline (indefinite)" rancid flavor according to *Standard Methods for the Examination of Dairy Products* (Table 4) (13) but had a log rancidity score of .22, a "moderately rancid" score, based on the evaluation of the trained panel. Some samples with ADV as high as 3.54 meq/100 g fat had "not at all rancid" to "slightly rancid" flavor yet should be classified as "unsatisfactory (extremely lipolyzed)" (13).

Validation of Panel Performance

Panelists were able to assign similar scores to duplicate LPRS included in the sample set (Table 5). Correlations between ADV and log rancidity score for LPRS for most panelists were low and not significant (Table 5). One panelist had a significant moderate correlation ($r=.42$, $p=.003$) between log rancidity score and ADV. There were

some initial differences in individual panelist performance with respect to other panelists but these differences were not evident during the second training evaluation (Table 6). This evaluation demonstrated that the panel exhibited the characteristics of a fine-tuned instrument, each panelist replicating the performance of other panelists.

A panel that has undergone extensive training and performance evaluation provides more reliable perception of rancid flavor detection and intensity than an inexperienced panel. Trained judges are better suited to the task of rancid flavor evaluation due to the large number of flavors which may occur in milk. It is difficult for an untrained judge to be sure they are detecting the rancid flavor (5) but this error is reduced by practicing and learning the flavor. Some individuals have greater difficulty in detecting rancid off-flavors but may eventually learn through practice. Reference standards are helpful in characterizing the flavor of interest (11). Laboratory-prepared standards were used for this purpose. Variation still occurred in the LPRS, possibly because of variance in incubation time (24-36 h), decrease in lipase activity in frozen raw milk over time (9 months), or variation in concentration of FFA and lipase activity in the Vitamin D milk purchased for preparation of the standards. These

methods did provide a panel which was able to provide reliable and accurate scores for rancid flavor.

Consumer and Trained Panel Evaluation of Samples

Consumer panel results indicated that panelists were all able to detect a flavor difference between Farm B milk with high ADV and Farm A milk with low ADV, and between Farm A milk with low ADV and Farm A milk with induced high ADV but could not identify the difference as "rancid". A difference was found between samples from Farm A (low ADV) and Farm B (n=46) (Table 7). Forty-five of 72 responses were needed to find a significant difference ($p<.05$) (14). Therefore, a difference was noted between the two samples, although the flavor difference was not characterized as rancid by consumer panelists. A difference ($p<.05$) was also found between the Farm A sample with low ADV and the induced high ADV sample (n=46). There was no difference found between the Farm B sample with high ADV and the Farm A sample with induced high ADV (n=33). Comments associated with the Farm B milk were "stronger flavor, bitter, old tasting"; comments regarding the induced high ADV sample from Farm A included "stronger flavor, bitter, sour"; most comments with respect to the Farm A sample with low ADV were "sweeter".

Log rancidity scores from the trained panel indicated that there was a perceived difference in intensity of rancid flavor between the Farm A sample and the two samples with high ADV (Table 8). The Farm A sample had a low log rancidity score (.08), characterized as "slightly rancid". Log rancidity scores for the Farm B sample and the induced rancid sample from Farm A were .22 and .18, respectively, described as "moderately rancid" based on concept scores. The LPRS samples included in the sample set for the trained panel were given a mean log rancidity score of .30 which was a "very rancid" concept score. Comments on samples from the trained panel were few except that an oxidized flavor was noted in the induced rancid sample from Farm A in addition to rancid flavor. This may be due to a 1 hour interval between homogenization and pasteurization when the sample was uncovered and light induced oxidation may have been initiated.

The large difference in ADV would appear to indicate that there would, of course, be a difference in flavor in the samples which were compared. However, samples with high ADV do not always receive a rancid flavor response as has already been demonstrated. Laboratory-prepared rancid samples were given higher log rancidity scores than were farm samples with similar ADV on many occasions (Figure 1).

The question becomes "Is there a different rancid flavor in LPRS than in farm samples?".

Different rancid flavors were reported by Willie and Duthie (19) when 3 milk flavor judges compared LPRS prepared by different methods but with similar ADV. Two distinct descriptions of the rancid off-flavor were noted: sickening and unclean. If there are different qualities of rancid, then standards currently being used to train sensory panels, including dairy products judging teams, quality control personnel, and other professionals in the dairy manufacturing industry, may not be providing the flavors associated with farm samples. The log rancidity scores for rancid flavors in the farm samples were not as high as those for LPRS, indicating that flavors found in farm samples were not as intense as those found in the LPRS. Another possibility may be that rancid flavors were masked by interactions with other flavors found in farm milk. The panelists evaluated rancid flavor in farm milk based on what was recognized from their training. It is important that the standards used for the training represent the flavors that are evaluated in the farm sample.

Many of the evaluations for rancid flavor were completed on LPRS, including the development of the BDI method of ADV, the determination of threshold levels and

descriptor terms associated with rancidity and related to ADV (17), and the determination of fatty acids responsible for rancid flavor (1, 8, 15). It is important to determine if the laboratory prepared rancid standards used for these analyses were representative of rancid flavor as found on the farm if these methods and terms are to be relevant to the determination of rancid flavor in farm samples.

V. CONCLUSIONS

The ADV method does not measure rancid flavor in farm samples or in LPRS. It may be a measure of the long chain FFA released through hydrolysis but does not permit the measurement of all FFA. Consumers could detect a difference between milk samples with low ADV and milk samples with very high ADV and a difference in intensity of rancid flavor was detected by the trained panel in those samples; however, there were many samples with high ADV which did not receive a rancid flavor criticism. The ADV, which is currently used as a quality control measure for rancid flavor, should not be relied upon because of its low correlation with rancid flavor scores. Evaluation of other methods is needed to determine a more reliable quality control method for the analyses of components responsible for rancid flavor. The development of a rancid flavor

standard which is representative of the rancid flavor on the farm is also needed to ensure that sensory panelists and others needed for dairy flavor research and quality control are searching for the correct off-flavor.

Terms associated with standard methods ADV as listed in Richardson (13) do not reliably describe the presence or intensity of rancid flavor in milk. Acid degree values associated with descriptor terms, as listed in *Standard Methods for the Examination of Dairy Products* (13) should be reevaluated for reference to farm samples until an alternative method of evaluating rancid flavor can be validated. The ADV procedure does provide a method of monitoring change in FFA concentration which is important in fluid milk quality and milkfat characteristics.

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APPENDIX C

Table 1. Means and probability of difference in paired *t*-test for ADV between raw and fresh pasteurized milk and for ADV or mean log rancidity scores between fresh and stored pasteurized milk.

Variable[1]	Raw	Fresh	Stored	p
ADV	.91	1.63		.0001
ADV		1.63	2.53	.0001
Mean log rancidity score		.13	.15	.0012

Flavor descriptor	Concept score[2]
Unpalatable	.44
Very rancid	.34
Moderately rancid	.24
Slightly rancid	.12
Not at all rancid	0

[1] N=59 samples.
[2] N=30.

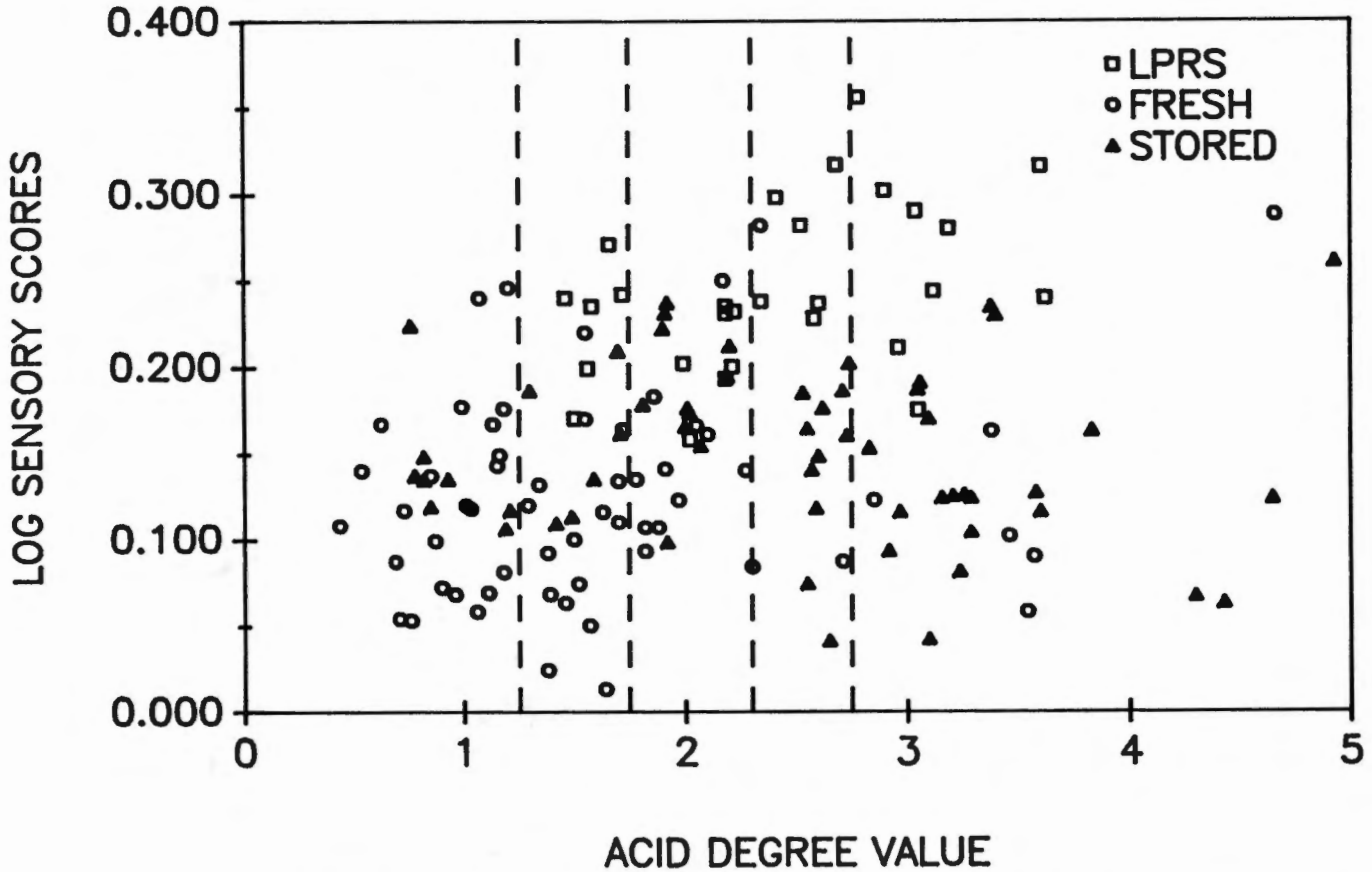

Figure 1. ADV and log rancidity scores for fresh (r=.22, p=.09), stored (r=-.12, p=.35), and laboratory-prepared rancid (r=.17, p=.002) samples (LPRS) in 5 ADV groups (designated by broken lines).

Table 2. Mean log rancidity scores for fresh and stored milk samples in 5 ADV groups and for laboratory-prepared rancid samples and concept scores associated with rancid flavor intensity.

ADV group	ADV range	Mean log rancidity scores			
		Fresh	N	Stored	N
1	< 1.26	.12[a]	23	.14[a]	8
2	1.26-1.75	.10[a]	16	.15[a]	6
3	1.76-2.30	.14[a]	12	.18[a]	11
4	2.31-2.75	.18[a]	2	.14[a]	11
5	> 2.76	.14[a]	6	.14[a]	23
LPRS[1]	2.61[2]	.24	30		

[1] LPRS=laboratory-prepared rancid samples.
[2] Mean of 30 samples; range of ADV for LPRS is 1.46-3.62 with one sample ADV at 7.87.
[a] Scores associated with ADV groups with the same letter in the same column are not significantly different ($p>.05$).

Table 3. Examples of individual farm samples with similar ADV but different log rancidity scores[1] in each ADV group.

		Slightly rancid		Moderately-very rancid	
ADV group	ADV range	Mean log score	ADV	Mean log score	ADV
1	< 1.26	.06	1.06	.24	1.07
2	1.26-1.75	.07	1.52	.22	1.55
3	1.76-2.30	.10	1.92	.25	2.17
4	2.31-2.75	.07	2.55	.28	2.34
5	> 2.75	.08	3.24	.24	3.38

[1] Concept scores provided in Table 1.

Table 4. Standard ADV descriptors[1].

ADV	Descriptors
< .4	Normal
.7 - 1.1	Borderline(indefinite)
1.2	Slightly lipolyzed
> 1.5	Unsatisfactory (extremely lipolyzed)

[1] (13).

Table 5. Paired *t*-tests for individual panelist performance and correlation for each panelist between ADV and log rancidity score for laboratory-prepared rancid samples.

Panelist	*t*	p	r	p
1	-.58	.57	.25	.07
2	.79	.44	.16	.31
3	-.27	.79	.14	.34
4	-.16	.87	.42	.003
5	-1.56	.13	.03	.81

Table 6. Training evaluation of panel performance.

Panelist	Mean log rancidity score[1]	
	Initial evaluation[2]	Second evaluation[3]
1	.10[a]	.19[a]
2	.29[c]	.25[a]
3	.07[a]	.25[a]
4	.18[b]	.20[a]
5	.21[bc]	.23[a]
6	.24[bc]	.26[a]

[1] Concept scores provided in Table 1.
[2] Mean of log rancidity scores for each panelist over 5 dilutions of laboratory-prepared rancid samples (LPRS). Dilutions were 0, 25, 65, 85, and 100%.
[3] Mean of log rancidity scores for each panelist over 4 dilutions of LPRS. Dilutions were 0, 25, 75, and 100%.
[a,b,c,] Scores with the same letter within the same column are not significantly difference ($p>.05$).

Table 7. Evaluation of difference in farm milk with low ADV (Farm A), farm milk with high ADV (Farm B), and farm milk with induced high ADV (Farm A, induced) by consumer panel (n=72) using paired-comparison test.

Comparison	Diff.[1]	No diff.[2]	No response	p
Farm A (low ADV) vs Farm B (high ADV)	46	25	1	<.05
Farm B (high ADV) vs Farm A (induced)	33	39		>.05
Farm A (low ADV) vs A (induced)	46	26		<.05

[1] Diff. Number of positive responses to "Is there a difference?"
[2] No diff. Number of responses indicating there was no difference.

Table 8. ADV and log rancidity score of farm milk with low ADV (Farm A), farm milk with high ADV (Farm B), and farm milk with induced high ADV (Farm A, induced).

Measurement	Farm A	Farm B	Farm A (induced)	LPRS
ADV	1.57	5.37	7.67	
Log rancidity score	.08	.22	.18	.30

APPENDIX D

ANOVA FOR LOG RANCIDITY SCORE

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	71	0.20873511	0.00293993
ERROR	46	0.15960146	0.00346960
CORRECTED TOTAL	117	0.36833658	

MODEL F = 0.85 PR > F = 0.7385

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.566697	42.9236	0.05890329	0.13722814

SOURCE	DF	TYPE III SS	F VALUE	PR > F
ADVGRPX	4	0.01537484	1.11	0.3643
STORAG	1	0.00407915	1.18	0.2839
FARM	17	0.04554618	0.77	0.7134
ADVGRP*STOR	4	0.00521725	0.38	0.8246
ADVGRP*FARM	21	0.04109854	0.56	0.9220
STORAG*FARM	13	0.01981667	0.44	0.9460
ADVGRP*STOR* FARM	7	0.02212216	0.91	0.5067

ANOVA FOR LOG RANCIDITY SCORE (2)

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	26	0.10748234	0.00413394
ERROR	91	0.26085424	0.00286653
CORRECTED TOTAL	117	0.36833658	

MODEL F = 1.44 PR > F = 0.1048

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.291805	39.0153	0.05353999	0.13722814

SOURCE	DF	TYPE III SS	F VALUE	PR > F
ADVGRPX	4	0.01357683	1.18	0.3232
STORAG	1	0.00538195	1.88	0.1740
FARM	17	0.05256696	1.08	0.3863
ADVGRP*STOR	4	0.01269320	1.11	0.3581

ANOVA FOR LOG RANCIDITY SCORE (3)

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	22	0.09478913	0.00430860
ERROR	95	0.27354744	0.00287945
CORRECTED TOTAL	117	0.36833658	

MODEL F = 1.50 PR > F = 0.0939

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.257344	39.1031	0.05366048	0.13722814

SOURCE	DF	TYPE III SS	F VALUE	PR > F
ADVGRPX	4	0.02027992	1.76	0.1432
STORAG	1	0.01207527	4.19	0.0433
FARM	17	0.05494401	1.12	0.3449

ANOVA FOR LOG RANCIDITY SCORE (4)

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	5	0.3984512	0.00796902
ERROR	112	0.32849145	0.00293296
CORRECTED TOTAL	117	0.36833658	

MODEL F = 2.72 PR > F = 0.0235

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.108176	39.4648	0.05415680	0.13722814

SOURCE	DF	TYPE III SS	F VALUE	PR > F
ADVGRPX	4	0.02227571	1.90	0.1156
STORAG	1	0.01109043	3.78	0.0543

ANOVA FOR PANEL TRAINING EVALUATION

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	195	4.59082893	0.02354271
ERROR	128	0.80736303	0.00630752
CORRECTED TOTAL	323	5.39819197	

MODEL F = 3.73 PR > F = 0.0001

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.850438	32.5338	0.07941992	0.24411503

SOURCE	DF	TYPE III SS	F VALUE	PR > F
PANEL	5	0.20213704	6.41	0.0001
REP	28	0.32028809	1.81	0.0140
DUP	1	0.00481817	0.76	0.3838
DUP*REP	28	0.23159098	1.31	0.1576
DUP*PANEL	5	0.01904351	0.60	0.6971
PANEL*REP	128	2.03617981	2.52	0.0001

ANOVA FOR INITIAL EVALUATION OF PANEL TRAINING (1)

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	46	1.09624443	0.02383140
ERROR	25	0.17990542	0.00719622
CORRECTED TOTAL	71	1.27614986	

MODEL F =	3.31	PR > F =	0.0010

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.859025	47.0788	0.08483052	0.18018848

SOURCE	DF	TYPE III SS	F VALUE	PR > F
REP	1	0.00923342	1.28	0.2661
PANEL	5	0.42739806	11.88	0.0001
TRTMNT	5	0.25916633	7.20	0.0003
REP*PANEL	5	0.05814594	1.62	0.1924
REP*TRTMNT	5	0.00663021	0.18	0.9659
PANEL*TRTMN	25	0.35567047	1.87	0.0628

ANOVA FOR INITIAL EVALUATION OF PANEL TRAINING (2)

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	10	0.68656439	0.06865644
ERROR	61	0.58958547	0.00966534
CORRECTED TOTAL	71	1.27614986	

MODEL F = 7.10 PR > F = 0.0001

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.537997	54.5609	0.09831244	0.18018848

SOURCE	DF	TYPE III SS	F VALUE	PR > F
PANEL	5	0.42739806	8.84	0.0001
TRTMNT	5	0.25916633	5.36	0.0004

ANOVA FOR SECOND EVALUATION OF PANEL TRAINING (1)

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	47	1.14140572	0.02428523
ERROR	37	0.40244659	0.01087693
CORRECTED TOTAL	84	1.54385231	

MODEL F = 2.23 PR > F = 0.0065

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.739323	44.7525	0.10429254	0.23304292

SOURCE	DF	TYPE III SS	F VALUE	PR > F
REP	2	0.04492487	2.07	0.1412
PANEL	5	0.06358048	1.17	0.3427
TRTMNT	4	0.55651742	12.79	0.0001
REP*PANEL	9	0.05800009	0.87	0.5611
REP*TRTMNT	7	0.08273510	1.09	0.3915
PANEL*TRTMN	20	0.21479009	0.99	0.4971

ANOVA FOR SECOND EVALUTION OF PANEL TRAINING (2)

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOG RANCIDITY SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	9	0.73208828	0.08134314
ERROR	75	0.81176403	0.01082352
CORRECTED TOTAL	84	1.54385231	

MODEL F = 7.52 PR > F = 0.0001

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.474196	44.6425	0.10403615	0.23304294

SOURCE	DF	TYPE III SS	F VALUE	PR > F
PANEL	5	0.05851375	1.08	0.3777
TRTMNT	4	0.67417060	15.57	0.0001

APPENDIX E

PREPARATION OF OFF-FLAVORED SAMPLES

Oxidized - Homogenized milk was placed in a clear glass bottle under direct fluorescent light for 1-2 hours.

Feed - Homogenized milk was placed in a clear glass bottle in a covered 5 gallon plastic bucket containing silage for 24-36 hours.

Cooked - Homogenized milk was heated to 75°C for 30 minutes and cooled.

Rancid - 30 ml raw milk were mixed with 750 ml homgenized milk and incubated at 4°C for 24-36 hours. Milk was pasteurized at 66.5°C for 3.5 minutes.

(3).

PANEL TRAINING: INFORMATION ABOUT OFF-FLAVORS

CHARACTERISTICS OF MILK OFF-FLAVORS

Typical Milk Flavor (No Criticism) - Very little distinct odor; Excellent milk is pleasantly sweet and leaves only a clean, pleasing sensation after tasting. The flavor is quite subtle in character.

Cooked - Note odor. Sweetish nature. Pleasant. Slightly nutty-like. Lightly heated.

Cause: Over-pasteurization, higher heat treatment or/and for longer time period.

Feed -Odor. Aromatic, usually pleasant. Hay, alfalfa, silage (frequently slightly unclean). Usually clears up very readily after discharge from mouth.

Cause: Cow consumes particular feed prior to milking, inhales odors from barn or feed lot; the feed, etc. transmits to milk.

Oxidized Sunlight - Odor, burnt-protein, burnt-feathers, or "medicinal"-like flavor. Sunlight or fluorescent light-activated. Slight burnt taste.

Cause: UV-rays from sunlight or fluorescent lighting catalyze oxidative reaction in unprotected milk. (Clear glass or plastic containers).

Rancid - Odor. Butyric acid flavor, soapy, blue cheese-like aroma, slight bitter. Foul, pronounced aftertaste; does not clear up readily.

Cause: Hydrolysis of milk fat by lipase enzyme. Often due to rough handling, improper cooling and excessive pumping of milk. Mixing of homogenized and raw milk is possible cause.

(3).

PANEL TRAINING: SCORECARD

Identify the flavor characteristic in each milk sample as oxidized, rancid, cooked, or feed.

Sample No.	Characteristic
1	_____
2	_____
3	_____
4	_____
5	_____
6	_____
7	_____
8	_____

SAMPLE EVALUATION: SCORECARD

In front of you is a series of bottles filled with milk. Your task is to tell how rancid they seem by assigning numbers proportional to rancidity. If the second stimulus is nineteen times as rancid as the first, assign it a number nineteen times as large. If it seems one-eleventh as rancid, assign it a number one-eleventh as large, and so forth. Use numbers, fractions, and decimals, but make each assignment proportional to the intensity of rancid flavor as you perceive it. Zero represents the absence of rancid flavor. Use positive numbers to represent any rancid flavor. Please wait 30 seconds between samples. Make comments regarding any additional off-flavors present.

Sample Code	Intensity Score
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Given the scaling method you have just used, indicate the intensity score you would give for a milk sample that is

unpalatable	_____
very rancid	_____
moderately rancid	_____
slightly rancid	_____
not at all rancid	_____

CONSUMER PANEL: SCORECARD

You have three pairs of samples on the tray. Taste each pair in the order indicated below. Indicate whether the samples in each pair are different or not. If you find a difference, please describe it. You may swallow the sample or expectorate it into the styrofoam cup. Rinse your mouth with water between pairs.

Set 1 Samples _____ _____

Are they different? Yes _____

No _____

Describe the difference. _____

_____

_____

Set 2 Samples _____ _____

Are they different? Yes _____

No _____

Describe the difference. _____

_____

_____

Set 3 Samples _____ _____

Are they different? Yes _____

No _____

Describe the difference. _____

_____

_____

CONSUMER PANEL: DEMOGRAPHIC INFORMATION

Thank you for serving on the milk panel. Your input has been a very valuable part of our project. To assist in analysis of the data we need some additional information from you. Please answer the following questions.

1) Female _____ Male _____

2) Age: _____ less than 18
_____ 18-24
_____ 25-34
_____ 35-44
_____ 45-54
_____ 55 or over

3) What is your ethnic background?
_____ White/Caucasian
_____ American Indian or Alaskan Native
_____ Black
_____ Hispanic
_____ Asian or Pacific Islander

4) Mark the term that best describes the location of your home.
_____ urban
_____ rural nonfarm
_____ farm

5) Please rate how well you like milk.

Like extremely _____
Like very much _____
Like moderately _____
Like slightly _____
Dislike slightly _____
Dislike moderately _____
Dislike very much _____
Dislike extremely _____

6) How often do you drink milk?

_____ Several times a day
_____ Several times a week
_____ Several times a month
_____ Several times a year

7) What kind of milk do you most frequently drink?

_____ Vitamin D pasteurized
_____ 2%
_____ Skim or lowfat milk

MICROBIOLOGICAL AND QUALITY DATA FOR RAW, FRESH AND STORED MILK SAMPLES

Sample	LBC[1]	SPC[2]	PBC[3]	CC[4]	SCC[5]	ADV[6]	Phos[7]
101R[8]	LE[11]	8,100	74	56	-[12]	.42	-
F[9]	LE	16,200	< 1	< 1	-	.62	-
S[10]	LE	18,000	159	< 1	-	.82	-
129R	LE	22,400	29	17	-	.47	-
F	LE	5,000	< 1	< 1	-	.69	-
S	LE	>300,000	>30,000	< 1	-	.76	-
138R	LE	4,800	68	3	-	.52	-
F	LE	1,510	< 1	< 1	-	.73	-
S	LE	2,520	< 1	< 1	-	.78	-
147R	LE	7,100	76	11	-	1.33	-
F	LE	270	< 1	< 1	-	1.55	-
S	LE	>300,000	>30,000	< 1	-	1.70	-
156R	LE	31,000	760	300	-	.35	-
F	LE	210	< 1	< 1	-	.85	-
S	LE	217,000	>30,000	< 1	-	.85	-
291R	LE	10,000	780	33	-	.59	-
F	LE	170	< 1	< 1	-	.96	-
S	LE	750	12	< 1	-	.93	-
301R	LE	510,000	270	480	-	.53	-
F	LE	140	< 1	< 1	-	2.85	Neg.
S	LE	4,600	133	< 1	-	2.92	-
311R	LE	69,000	500	24	-	.56	-
F	LE	2,800	< 1	< 1	-	1.34	Neg.
S	LE	>300,000	>30,000	< 1	-	1.92	-
329R	LE	1,200	9	5	-	.42	-
F	LE	250	1	< 1	-	1.78	Neg.
S	LE	100	1	< 1	-	2.73	-
338R	LE	7,700	179	13	-	.96	-
F	LE	230	1	< 1	-	1.88	Neg.
S	LE	220	1	< 1	-	2.00	-

Sample	LBC[1]	SPC[2]	PBC[3]	CC[4]	SCC[5]	ADV[6]	Phos[7]
347R	LE	2,000	16	2	-	.37	-
F	LE	190	< 1	< 1	-	.71	Neg.
S	LE	50	1	< 1	-	.82	-
356R	LE	12,800	1,610	16	-	1.38	-
F	LE	480	< 1	< 1	-	1.70	Neg.
S	LE	330	10	< 1	-	1.90	-
410R	LE	1,900	11	1	406,805	1.29	-
F	LE	560	< 1	< 1	-	1.82	Neg.
S	LE	210	10	< 1	-	3.21	-
429R	LE	12,300	3,000	347	446,780	1.44	-
F	LE	500	2	< 1	-	1.91	Neg.
S	LE	520	< 1	< 1	-	2.83	-
438R	LE	4,800	40	127	178,495	.50	-
F	LE	27	< 1	< 1	-	1.13	Neg.
S	LE	100	30	< 1	-	2.01	-
510R	LE	61,000	124	84	1,892,250	.63	-
F	LE	700	20	< 1	-	1.18	Neg.
S	LE	770	11	< 1	-	1.91	-
529R	LE	150,000	220	1	403,600	3.05	-
F	LE	111	10	< 1	-	4.66	Neg.
S	LE	260	50	< 1	-	4.93	-
538R	LE	1,700	390	23	451,907	.38	-
F	LE	45	< 1	< 1	-	1.38	Neg.
S	LE	100	11	< 1	-	2.19	-
610R	LE	35,000	550	340	2,060,175	.43	-
F	LE	730	< 1	< 1	-	1.18	Neg.
S	LE	410	2	< 1	-	1.71	-
629R	LE	4,000	20	7	474,385	1.16	-
F	LE	780	< 1	< 1	-	1.86	Neg.
S	LE	250	4	< 1	-	2.74	-
638R	LE	>300,000	4,100	630	341,150	.53	-
F	LE	202	< 1	< 1	-	1.07	Neg.
S	LE	270	36	< 1	-	2.07	-

Sample	LBC[1]	SPC[2]	PBC[3]	CC[4]	SCC[5]	ADV[6]	Phos[7]
710R	180	14,500	500	43	840,850	1.67	-
F	170	440	< 1	< 1	-	2.17	Neg.
S	180	9,300	1	< 1	-	3.38	-
729R	<10	12,300	370	131	474,500	.61	-
F	230	100	< 1	< 1	-	1.15	Neg.
S	90	140	9	< 1	-	2.57	-
738R	260	37,350	21,600	15	298,990	.37	-
F	40	130	< 1	< 1	-	1.82	Neg.
S	70	23	4	< 1	-	3.10	-
214R	410	1,800	83	11	254,000	.78	-
F	250	33	< 1	< 1	-	1.29	Neg.
S	8	36	LE	< 1	-	3.06	-
215R	690	1,400	610	43	330,000	.47	-
F	250	59	< 1	< 1	-	1.55	Neg.
S	1	139	LE	< 1	-	2.71	-
216R	530	13,800	440	95	1,816,500	.74	-
F	170	480	< 1	< 1	-	1.52	Neg.
S	LE	>300,000	LE	< 1	-	3.24	-
221R	220	11,900	128	10	565,000	.27	-
F	LE	9	< 1	< 1	-	.43	Neg.
S	LE	>300,000	>30,000	< 1	-	1.21	-
222R	LE	4,400	890	33	522,000	1.28	-
F	LE	650	< 1	< 1	-	2.34	Neg.
S	7	570	34	< 1	-	3.40	-
223R	LE	104,000	1,110	103	2,526,000	.28	-
F	LE	770	2	< 1	-	1.20	Neg.
S	LE	>300,000	>30,000	< 1	-	2.02	-
228R	400	1,200	570	142	1,397,500	.37	-
F	6	218	2	< 1	-	.52	Neg.
S	9	3,000	630	< 1	-	1.19	-
229R	40	< 100	104	10	308,500	.41	-
F	< 1	5	< 1	< 1	-	1.63	Neg.
S	< 1	130	46	< 1	-	2.97	-

Sample	LBC[1]	SPC[2]	PBC[3]	CC[4]	SCC[5]	ADV[6]	Phos[7]
230R	26	1,500	48	10	432,000	.57	-
F	< 1	1	< 1	< 1	-	1.01	Neg.
S	3	40	24	< 1	-	1.81	-
417R	10	2,170	6,100	64	1,256,000	.56	-
F	2	18	< 1	< 1	-	3.57	Neg.
S	1	32	17	< 1	-	3.58	-
418R	< 1	4,000	1,620	1	700,000	.94	-
F	5	4	1	< 1	-	3.54	Neg.
S	< 1	5	5	< 1	-	3.60	-
419R	110	10,700	280	170	710,500	1.58	-
F	10	60	1	< 1	-	3.38	Neg.
S	3	110	28	< 1	-	3.83	-
425R	29	450	89	17	368,400	1.31	-
F	< 1	15	< 1	< 1	-	.90	Neg.
S	LE	7	< 1	< 1	-	2.60	-
426R	15	990	77	8	700,400	.93	-
F	< 1	19	< 1	< 1	-	1.06	Neg.
S	LE	>300,000	>30,000	< 1	-	2.55	-
427R	300	10,300	3,500	220	620,400	2.71	-
F	3	81	< 1	1	-	1.97	Neg.
S	LE	>300,000	>30,000	< 1	-	3.05	-
428R	180	5,700	2,510	5	291,000	1.15	-
F	3	24	< 1	< 1	-	1.39	Neg.
S	LE	60	10	< 1	-	2.55	-
429R	400	8,400	1,080	76	818,900	1.72	-
F	2	21	< 1	< 1	-	1.11	Neg.
S	LE	>300,000	>30,000	< 1	-	3.16	-
502R	160	3,400	1,800	< 1	390,366	.91	-
F	< 1	138	< 1	< 1	-	.99	Neg.
S	60	18,700	16,200	LE	-	1.49	-
503R	250	8,700	1,380	111	749,875	1.21	-
F	7	99	1	< 1	-	1.50	Neg.
S	170	6,100	6,600	LE	-	2.20	-

Sample	LBC[1]	SPC[2]	PBC[3]	CC[4]	SCC[5]	ADV[6]	Phos[7]
504R	90	1,480	52	11	296,275	.57	-
F	< 1	41	< 1	< 1	-	2.30	Neg.
S	310	26,300	23,500	LE	-	3.29	-
505R	40	4,400	3,370	6	127,050	.53	-
F	< 1	12	< 1	1	-	1.72	Neg.
S	200	59,000	51,000	LE	-	3.29	-
509R	9	580	49	20	221,025	.57	-
F	< 1	28	< 1	< 1	-	.76	Neg.
S	1	86	10	< 1	-	1.30	-
510R	50	690	46	2	255,025	.54	-
F	< 1	14	< 1	< 1	-	.87	Neg.
S	3	86	80	< 1	-	1.42	-
511R	150	5,800	225	190	637,025	2.08	-
F	< 1	42	< 1	< 1	-	2.10	Neg.
S	110	940	290	< 1	-	2.62	-
512R	40	900	370	8	274,525	.99	-
F	7	79	< 1	< 1	-	1.46	Neg.
S	50	3,600	2,040	< 1	-	1.92	-
513R	LE	LE	LE	LE	244,525	.66	-
F	6	94	< 1	< 1	-	1.16	Neg.
S	1	240	290	< 1	-	1.59	-
516R	-	4,200	1,310	280	617,725	1.68	-
F	-	75	1	< 1	-	2.27	Neg.
S	620	40,000	31,000	< 1	-	4.43	-
517R	-	1,970	158	11	368,625	.53	-
F	-	22	< 1	< 1	-	2.71	Neg.
S	170	7,400	5,600	< 1	-	4.30	-
518R	-	42,000	860	31	916,100	.94	-
F	-	18	< 1	< 1	-	3.46	Neg.
S	70	2,250	690	< 1	-	5.21	-
519R	-	6,300	1,180	109	221,975	.54	-
F	-	130	< 1	< 1	-	1.70	Neg.
S	560	64,000	64,000	< 1	-	4.65	-

Sample	LBC[1]	SPC[2]	PBC[3]	CC[4]	SCC[5]	ADV[6]	Phos[7]
523R	30	560	122	7	553,350	.61	-
F	< 1	28	< 1	< 1	-	1.57	Neg.
S	1	5	< 1	< 1	-	2.53	-
524R	80	17,000	1,840	27	1,187,025	1.22	-
F	< 1	6	< 1	< 1	-	1.38	Neg.
S	< 1	19	20	< 1	-	2.59	-
525R	100	1,760	380	2	399,800	.67	-
F	< 1	17	< 1	< 1	-	1.03	Neg.
S	< 1	22	20	< 1	-	2.65	-
526R	210	9,600	450	560	818,100	2.01	-
F	7	221	< 1	< 1	-	2.05	Neg.
S	13	180	80	< 1	-	3.26	-
527R	90	4,400	1,570	< 1	286,500	1.16	-
F	4	27	< 1	< 1	-	1.64	Neg.
S	8	190	620	< 1	-	3.10	-

[1] LBC = lipophilic bacteria count (CFU/ml)
[2] SPC = standard plate count (CFU/ml)
[3] PBC = psychrotrophic bacteria count (CFU/ml)
[4] CC = coliform count (CFU/ml)
[5] SCC = somatic cell count (cells/ml)
[6] ADV = acid degree value (meq/100 g fat)
[7] Phos= phosphatase test
[8] R = raw sample
[9] F = fresh sample
[10] S = stored sample
[11] LE = laboratory error
[12] - = test not completed

CHAPTER V

CORRELATION OF FATTY ACID CONCENTRATION TO ACID DEGREE VALUE AND SENSORY DETECTION OF RANCID FLAVOR IN MILK

I. ABSTRACT

Gas chromatographic analysis was completed on 44 milk samples collected from area (Maryville, TN) farms and on 12 laboratory-prepared rancid samples to determine concentration of 10 major fatty acids (C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{15}, C_{16}, $C_{18:0}$, $C_{18:1}$). Acid degree value and magnitude estimation were completed as chemical and sensory measures of rancid flavor. Concentrations of individual fatty acids and sum of concentrations for shorter chain fatty acids (C_4-C_{10}), longer chain fatty acids (C_{12}-$C_{18:1}$), and total fatty acids (C_4-$C_{18:1}$) were correlated to acid degree value and to rancidity scores. Concentration of even-chain fatty acids increased as acid degree value increased for farm samples ($r>.82$, $p=.0001$) but significant differences among mean concentrations in acid degree value groups (<1.26, 1.25-1.75, 1.76-2.30, 2.31-2.75, >2.75 meq/100 g fat) were found only for C_4, C_{14}, $C_{16:0}$, $C_{18:0}$,

and $C_{18:1}$. Sums of concentrations for fatty acids (C_4-C_{10}, C_{12}-$C_{18:1}$, C_4-$C_{18:1}$) had positive high correlations to acid degree value (r=.93, p<.0001) for farm milk samples. For laboratory-prepared rancid samples, a positive moderate correlation was obtained between acid degree value and the sum of concentrations for the shorter chain fatty acids (r=.53, p=.07) but the correlation between longer chain fatty acids and acid degree value was low (r=.23, p=.47). Significant differences in concentration of C_{16} and $C_{18:1}$ for laboratory-prepared rancid samples with acid degree value of 2.31-2.75 as compared to samples with acid degree values greater than 2.75 were found. The concentrations for C_4 and C_8 were more closely related to sensory detection of rancid flavor than were concentrations of other fatty acids but correlations between concentration and rancidity scores were low (<.41) for all individual fatty acids and groups of fatty acids.

II. INTRODUCTION

Free fatty acids (FFA) are the components responsible for the development of lipolytic flavor in milk. These FFA are liberated from glycerides by lipase, which may be inherent in milk or produced by psychrotrophic microorganisms. An increased concentration of FFA

contributes a rancid, bitter, unpleasant taste to milk which is objectionable to many consumers.

Certain FFA have been implicated in the development of rancid flavor. Scanlan et al. (11) reported that there was no single FFA responsible for rancid flavor in milk but FFA of shorter chain length, primarily C_4-C_{12}, were all important in characterizing the flavor. Those FFA with chain length of 14 or more carbons were not associated with rancidity. Al-Shabibi et al. (1) found that C_6, C_8, C_{10}, and C_{12} contributed to rancid flavor but C_{10} and C_{12} contributed the most characteristic rancid flavors.

The measurement of rancid flavor may be completed by chemical or sensory testing. The most common chemical method for quality control testing is the Bureau of Dairy Industries (BDI) method of acid degree value (ADV) as listed in *Standard Methods for the Examination of Dairy Products* (10). This method liberates the milkfat by heat and detergent, FFA in a weighed sample of fat are titrated, and ADV results reported as meq FFA/100 g fat. Milk with an ADV of 1.5 meq/100 g fat or greater is reported as "extemely lipolyzed" (10). The relationship between rancid flavor and ADV is conservative due to the limitations of the methodology, however. As reported in Chapter III, the ADV procedure does not extract and measure all FFA equally. The short-chain FFA (C_4-C_8) are partly distributed in the

skim milk phase and are not completely detected by ADV. ADV does not account for selective solubility, molecular weight, and flavor potency of individual FFA (15). Thus, some short-chain fatty acids are not measured by ADV but may contribute to rancid flavor. ADV does not measure and identify specific fatty acids so it is not possible to determine which fatty acids are contributing to the titration of a milk sample with high ADV.

Gas chromatographic procedures are available which may be used to quantitate fatty acids and identify the relationship of fatty acids in milk samples with different ADV. Deeth et al. (3) developed a gas chromatographic method which minimized hydrolysis of lipids and loss of short-chain fatty acids, problems which had been evident in other procedures (2,4,6,7,14). Reported recoveries using this method (3) were greater than 92% for all major fatty acids. Ikins et al. (5) measured fatty acid concentration by gas chromatography (3) to determine the correlation between ADV and concentration of different fatty acid groups in cheddar cheese. Correlation between ADV and fatty acid concentration were .739 for total FFA, .561 for fatty acids from C_4-C_{10}, .470 for C_4, and .750 for fatty acids with longer carbon chains (C_{12}-C_{18}).

The objectives of this research were to determine which fatty acids were important in sensory detection of

rancid flavor and which contributed to ADV for laboratory-prepared rancid samples (LPRS) and farm milk samples.

III. MATERIALS AND METHODS

Collection of Samples

Sample collection was completed as previously described in Chapter IV. Twenty-two samples were collected over a 6 week period.

Sample Preparation

Sample preparation was completed as previously described in Chapter IV.

Chemical and Microbiological Analyses

Chemical and microbiological analyses were completed as previously described in Chapter IV.

Sensory Evaluation

Panel training, performance evaluation, and sample evaluation were completed as previously described in Chapter IV.

Gas Chromatographic Method

Fatty acid extraction and isolation was completed as previously described for milk samples in Chapter III.

Calculation of Fatty Acid Concentration

Tentative identification of fatty acids present in milk samples was based on retention times of fatty acid standards injected into the gas chromatograph under the same conditions (Table 1). Concentration of each major fatty acid was calculated based on relative molar response (RMR) of the representative fatty acid standard as described in Chapter III.

Statistical Analyses

Analysis of the relationship between ADV and concentration of 10 major fatty acids (C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{15}, C_{16}, C_{18}, $C_{18:1}$) and between $\log_{10}$ rancidity scores and concentration of fatty acids was attempted using factor analysis and stepwise regression (SAS Institute, Cary, NC). Correlations between ADV and concentration of each major fatty acid and sum of concentrations for short chain fatty acids (C_4-C_{10}), long chain fatty acids (C_{12}-$C_{18:1}$), and total fatty acids (C_4-$C_{18:1}$) were obtained. Correlations were also completed between $\log_{10}$ rancidity scores and concentration of fatty acid in the same manner.

General Linear Model (GLM) was used to determine significant differences in individual fatty acid concentrations for samples in 5 ADV groups (<1.26, 1.26-1.75, 1.76-2.30, 2.31-2.75, >2.75) and for farm and LPRS samples evaluated by sensory measurement as "slightly", "moderately", "very rancid", or "unpalatable". Least-squares means (LS Means) were calculated and differences ($p<.05$) noted.

IV. RESULTS AND DISCUSSION

Objectives of this phase of research were to determine which fatty acids had the greatest influence on ADV and sensory detection of rancid flavor in LPRS and in milk samples collected from farm bulk tanks. Factor analysis, or principal components regression, was initially attempted to describe interrelationships among concentrations of fatty acids for LPRS and farm samples. Redefining values for concentration of fatty acids by linear transformation to a new set of variables, called principal components, could reduce multicollinearity and help describe interrelationships among fatty acids (SAS Instituted, Cary, NC). However, factor analysis did not yield any information which could be applied (Table 2). Factor 1 for both LPRS and farm samples had high principal components

for all major fatty acids expect C_{15} in farm samples or $C_{16:1}$ in LPRS. Pentadecanoic acid (C_{15}) was not included in factor analysis of LPRS and $C_{16:1}$ was not included in the factor analysis of farm samples because they were not found in all samples by gas chromatography. Factor 2 for LPRS showed a tendency for shorter chain fatty acids (C_4-C_{10}) to move in an opposite direction than C_{14}, $C_{16:1}$, and C_{18}, and C_{12}, C_{16}, and $C_{18:1}$ were neutral (near 0). Principal components for Factor 2 for farm samples were all neutral (close to 0) except that for C_{15}, providing no further understanding of the interrelationships among fatty acids. Due to the relative lack of information available from this method of statistical analysis, it was determined that stepwise regression of fatty acid concentration against ADV and against log rancidity scores might be a better alternative for analysis of the data.

Farm samples were randomly divided into 2 subsets, one for determination of a regression equation and the other for verification of the equation. Stepwise regression was attempted using the 2 data subsets and the entire data set but different regression equations were obtained each time until all 10 fatty acids were included in the equation. Variability among fatty acids in individual milk samples and relatively few sample numbers limited the application of each regression equation to those samples from which it

was derived. The relationship between fatty acid concentration and ADV or fatty acid concentration and sensory perception of rancid flavor could not be generalized to other samples based on these equations.

Simple correlations were calculated for concentration of each major fatty acid and ADV (Table 3) and between mean fatty acid concentration of samples in 5 ADV groups for LPRS and farm samples. The relationship between ADV and concentration was high ($r>.83$, $p<.0001$) for all individual and groups of fatty acids in farm samples except for C_{15} ($r=.11$, $p=.46$). These positive high correlations indicated that ADV did increase as concentration of each fatty acid increased. The correlations between fatty acid concentration and ADV were more variable for LPRS, however. The group of shorter chain fatty acids (C_4-C_{10}) had a positive moderate correlation ($r=.53$, $p=.07$) but the correlation between ADV and longer chain fatty acids (C_{12}-$C_{18:1}$) was lower ($r=.23$, $p=.47$). The correlation between sum of total FFA (C_4-$C_{18:1}$) and ADV was .27 ($p=.40$). The concentrations of individual fatty acids, C_{12}, C_{16}, and $C_{18:1}$ had weak positive correlations ($r=.31$, .29, .37, respectively) but these were not significant. Ikins et al. (5) reported high positive correlations of .74 and .75 between concentration and ADV for total FFA (C_4-C_{18}) and longer chain fatty acids (C_{12}-C_{18}) in cheese. Correlations

for C_4 and C_4-C_{10} were similar to those found for LPRS in this study. The concentration of shorter chain fatty acids found in LPRS had stronger significant correlations to ADV than concentration of longer chain fatty acids.

Table 4 shows the LS Means for concentrations of individual fatty acids for farm samples and LPRS in each ADV group. The concentration of individual even-numbered monosaturated fatty acids increased as ADV increased in farm samples. The concentration of minor fatty acids (C_{11}, C_{13}, C_{15}, $C_{16:1}$) did not increase as ADV increased. Significant differences ($p<.05$) were found in mean concentrations of both C_4 and $C_{18:0}$ between the low ADV group (<1.26 meq/100 g fat) and high ADV group (>2.75 meq/100 g fat). Significant differences in mean concentrations of C_{14} between the high ADV group and all other ADV groups (<1.26, 1.26-1.75, 1.76-2.30, 2.31-2.75 meq/100 g fat) were found. Differences ($p<.05$) were found in mean concentrations of both $C_{16:0}$ and $C_{18:1}$ among the low ADV group (<1.26 meq/100 g fat), group 3 (1.76-2.30 meq/100 g fat), group 4 (2.31-2.75 meq/100 g fat), and group 5 (>2.75 meq/100 g fat). Laboratory-prepared rancid samples were divided into ADV groups 4 or 5. Significant differences in mean fatty acid concentration were found for only $C_{16:0}$ and $C_{18:1}$ in LPRS.

In both LPRS and farm samples, the correlation between $\log_{10}$ rancidity score and sum of concentrations for C_4-C_{10} was higher than for C_{12}-$C_{18:1}$ or total fatty acids but all correlations obtained were low (Table 5). Butyric acid (C_4) and C_8 had the highest positive correlation with log rancidity score. The mean concentration (LS Means) of total fatty acids for samples in each concept score range ("slightly", "moderately", "very rancid", "unpalatable") are provided in Table 6. The limited range in flavor intensity provided no samples with "very rancid" or "unpalatable" farm samples and no "slightly rancid" LPRS. There were no differences ($p>.05$) in concentration of total fatty acids between "slightly" and "moderately" rancid farm samples or among "moderately", "very", or "unpalatable" LPRS.

The shorter chain fatty acids seemed to have a stronger relationship to rancid flavor than did the longer chain fatty acids for both LPRS and farm samples but the correlations were not significant. The importance of shorter chain fatty acids to rancid flavor perception has been demonstrated in LPRS (1, 9, 11). The distribution of fatty acids and pH of the system in which they are found affects perception and recovery of fatty acids (2). A low pH, between 1.3-3.0, is needed to recover short-chain (C_4-C_8) fatty acids (7). As pH decreases, more fatty acid

enters the fat phase of milk (13). The low pH also increases detectability of the rancid flavor (12) by changing the soluble salt or dissociated fatty acid to the protonated form (2). The protonated form of the fatty acid has more odor. No acid is added in the ADV method so a decrease in pH probably does not occur, although pH measurements were not included in this study. Recovery of short-chain fatty acid is limited to those associated with the fat but some will stay in the charged (dissociated) form or as salts in the skim phase at the normal pH of milk.

The pH of the sample was altered in the gas chromatographic procedure (3) accounting for increased recovery of short-chain fatty acids by this method. The sample was acidified with HCl but no measurement of pH change has been reported. Incomplete recovery of fatty acids may still occur, however, possibly due to differences in acidification of the sample, by incomplete retention of fatty acids on the alumina column, loss of fatty acids from the alumina during the hexane-ether wash for removal of triglycerides (due to overloading), incomplete release of fatty acids from alumina with formic acid into the isopropyl ether, or volatilization of ethyl ether-FFA throughout the extraction.

The relationship between concentration of fatty acids in LPRS to ADV was different than that found for farm samples. This supports the hypothesis that there are differences found between the laboratory-prepared rancid standards and farm milk samples. The correlations between individual fatty acids and ADV for farm samples was at least .83 ($p<.0001$) except for C_{15} ($r=.11$, $p<.0001$) whereas the correlations between individual fatty acid concentration and ADV for LPRS were much lower and many were not significant. The individual shorter chain fatty acids (C_4, C_6, C_8, C_{10}) were moderately correlated with ADV for LPRS.

One possible reason for the observed differences in LPRS and farm samples with respect to correlations between ADV and FFA concentration may have been the difference in ADV range for each set of samples. The concentration of C_{14} and $C_{18:0}$ did not significantly increase within the narrow ADV range used for LPRS (2.68-3.04 meq/100 g fat, ADV groups 4 and 5 only) demonstrating a limited relationship and contributing to the low correlation between ADV and longer chain fatty acids. There was a significant increase in concentration of C_{14} between ADV groups 4 and 5 in farm samples. The range in ADV for farm samples was broader, encompassing all ADV groups (.76-5.21 meq/100 g fat), indicating an increase in longer chain

fatty acids. Significant increases in concentration of C_4, C_{14}, $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ were observed among ADV groups but no significant increases in C_6, C_8, C_{10}, or C_{12} were found (Table 4). The fatty acids which significantly increased in concentration as ADV increases for farm samples were those with highest recoveries (except C_4) when added to milk (Chapter III).

The differences observed in individual fatty acid concentrations with increasing ADV may have been caused by different lipase sources. The hydrolysis of fatty acids from glycerides in LPRS was primarily caused by milk lipase from the raw milk added to the homogenized milk. Milk lipase is a nonspecific lipase, releasing various fatty acids in nearly the same proportion that the acids are present in the intact fat (8). The high correlations obtained between individual fatty acid concentrations and ADV for farm samples may be related to the activity of lipases from psychrotrophic bacteria. Psychrotrophic bacteria (i.e. *Pseudomonas spp.*) have opportunity to proliferate during bulk storage on the farm. Many of these organisms produce heat resistant lipases with varying specificities.

The high correlation between concentration of individual fatty acids and ADV observed for farm samples may be attributed to the wide range of ADV and to the

activity of heat-resistant lipase from psychrotrophic bacteria. Greater concentrations of longer chain fatty acid were released, increasing the ADV. ADV and intensity of rancid flavor increased with storage (Chapter IV), indicating that FFA concentration increased over time, probably due to lipase from psychrotrophic microorganisms.

The relationship between concentration of fatty acids and flavor is difficult to explain. Shorter chain fatty acid concentration increased with ADV but rancid flavor intensity did not increase with ADV based on the low correlation reported in Chapter IV ($r=.13$, $p=.16$). It is possible that interactions among fatty acids diminished the flavor strength of the short chain fatty acids: as concentration of long chain fatty acids increased (and ADV increased), a greater concentration of short chain fatty acids may have been needed to impart a rancid flavor. The relationship between fatty acid concentration and rancidity scores observed for LPRS and farm samples was similar both in direction of change and magnitude of change. A ratio of the concentrations of short- to long-chain fatty acids may be of more value than concentrations of any single fatty acid in determining the relationship between fatty acid concentration and rancid flavor intensity.

The results of this study support the conclusions of others (1, 7, 11) that no single fatty acid is responsible

for rancid flavor but the shorter chain fatty acids have a stronger relationship than the longer chain fatty acids to rancid flavor intensity. The limited range of rancidity scores obtained for farm samples (Chapter IV) may have contributed to the low correlation between rancidity score and concentration of fatty acids. The observations indicated that, although the ADV range for farm samples was broad, the range for rancid flavor intensity, the dependent variable, was not. Most LPRS were characterized as "moderately" and "extremely rancid" even though the ADV were in the same range as the farm samples. Further research is needed to determine whether LPRS are representative of rancid flavor observed in pasteurized milk.

V. CONCLUSIONS

There was no single fatty acid which could be considered responsible for changes in ADV or rancid flavor intensity and the nature of the relationship between fatty acid concentration and ADV and between concentration and flavor is still in question. Differences in fatty acid concentrations with increasing ADV in LPRS and farm samples may possibly be attributed to source of lipase. Limited ADV range for LPRS may have also confounded the

relationship. The ratio between fatty acids important in rancid flavor (C_4-C_{12}) and longer chain fatty acids needs further investigation to determine if it may have a stronger relationship to rancid flavor intensity than concentration of individual or groups of fatty acid. Investigation of rancid flavor standards is needed to determine if those currently used to train for the detection of rancid flavor match the rancid flavor which is found in the consumer milk supply.

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APPENDIX F

Table 1. Relative molar response and retention times for fatty acid standards.

Fatty acid	Retention time (min.)	Relative molar response
C_4	2.16	.7451
C_5	2.93	1.000
C_6	3.75	1.257
C_8	5.52	1.722
C_{10}	7.44	2.160
C_{11}	8.50	2.289
C_{12}	9.35	2.477
C_{13}	10.50	2.701
C_{14}	11.30	2.887
C_{15}	12.18	3.086
C_{16}	13.30	3.285
$C_{16:1}$	14.55	4.244
C_{18}	16.45	3.352
$C_{18:1}$	17.71	3.618
$C_{18:2}$	18.10	3.539

Table 2. Factor analysis for fatty acids in laboratory-prepared rancid samples and farm milk samples.

	LPRS[1]		Farm	
Fatty acid	Factor 1	Factor 2	Factor 1	Factor 2
C_4	.753	-.254	.942	-.009
C_6	.800	-.342	.954	.004
C_8	.874	-.446	.940	-.036
C_{10}	.950	-.246	.947	.018
C_{12}	.976	.116	.960	.028
C_{14}	.832	.445	.879	-.072
C_{15}			.135	.990
C_{16}	.947	.035	.969	-.035
$C_{16:1}$	.266	.840		
$C_{18:0}$	.855	.445	.913	-.018
$C_{18:1}$	.959	-.034	.961	-.027

[1] LPRS=Laboratory-prepared rancid sample.

Table 3. Correlation[1] between ADV and concentration of major fatty acids for laboratory-prepared rancid samples and farm milk samples.

	LPRS[2]		Farm[3]	
Fatty Acid	r	Prob.[4]	r	Prob.
C_4	.45	.15	.93	<.0001
C_6	.59	.04	.88	<.0001
C_8	.55	.06	.90	<.0001
C_{10}	.46	.14	.87	<.0001
C_{12}	.31	.33	.94	<.0001
C_{14}	.08	.82	.84	<.0001
C_{15}	-.38	.22	.11	<.0001
C_{16}	.29	.35	.94	<.0001
$C_{18:0}$	-.01	.98	.83	<.0001
$C_{18:1}$	.37	.23	.89	<.0001
C_4-C_{10}[5]	.53	.07	.93	<.0001
C_{12}-$C_{18:1}$[6]	.23	.47	.93	<.0001
C_4-$C_{18:1}$[7]	.27	.40	.93	<.0001

[1] Pearson correlation coefficient.
[2] LPRS=Laboratory-prepared rancid samples, n=12.
[3] N=44.
[4] Prob>|r| under H_o: Rho=0.
[5,6,7] Sum of concentrations of fatty acids used for correlation.

Table 4. Least-squares means concentrations for fatty acids for farm samples and laboratory-prepared rancid samples in each ADV group.

	ADV group[1]					LPRS[2]	
Fatty Acid	1	2	3	4	5	4	5
				(μg/10 ml milk)			
C_4	.54[a]	.66[ab]	.94[ab]	1.01[bc]	1.37[c]	1.14	1.27
C_6	.23	.28	.41	.49	.65	.58	.67
C_8	.16	.17	.27	.31	.45	.39	.48
C_{10}	.21	.26	.38	.49	.77	.57	.74
C_{11}	.03	.09	.06	.04	.09	.12	.19
C_{12}	.21	.34	.42	.53	.86	.68	.85
C_{13}	.09	.11	.08	.06	.21	.17	.16
C_{14}	.58[a]	1.00[a]	1.09[a]	1.09[a]	2.06[b]	1.42	1.78
C_{15}	.34	.60	.41	.34	.59	.55	.58
$C_{16:0}$	1.42[a]	1.91[ab]	2.48[b]	3.81[c]	6.60[d]	3.32[a]	4.50[b]
$C_{16:1}$	.36	.35	.28	.14	.19	.53	.56
$C_{18:0}$	.68[a]	.94[ab]	1.03[ab]	1.84[bc]	2.46[c]	1.61	1.83
$C_{18:1}$	1.25[a]	1.72[ab]	2.30[b]	3.29[c]	4.39[d]	3.68[a]	4.74[b]
Group Mean	.25[a]	.68[b]	.78[b]	1.06[c]	1.62[d]	1.13[a]	1.42[b]

[1] ADV group 1=<1.26 meq/100 g fat; ADV group 2=1.26-1.75 meq/100 g fat; ADV group 3=1.76-2.30 meq/100 g fat; ADV group 4=2.31-2.75 meq/100 g fat; ADV group 5=>2.75 meq/100 g fat.

[2] LPRS=Laboratory-prepared rancid samples.

[a-d] Means within the same row under the same heading (farm, LPRS) with different letters are significantly different ($p<.05$).

Table 5. Correlation[1] between log rancidity scores and concentration of major fatty acids for laboratory-prepared rancid samples and farm milk samples.

	LPRS[2]		Farm[3]	
Fatty Acid	r	Prob.[4]	r	Prob.
C_4	.40	.19	.18	.25
C_6	.24	.45	.10	.52
C_8	.33	.30	.20	.19
C_{10}	.23	.47	.10	.50
C_{12}	.10	.77	.13	.41
C_{14}	.08	.80	-.03	.84
C_{15}	.18	.57	-.21	.18
C_{16}	.03	.93	.07	.65
$C_{18:0}$	.10	.76	-.06	.72
$C_{18:1}$	.19	.56	.10	.51
C_4-C_{10}[5]	.33	.29	.15	.33
C_{12}-$C_{18:1}$[6]	.11	.73	.03	.85
C_4-$C_{18:1}$[7]	.13	.69	.04	.80

[1] Pearson correlation coefficient.
[2] LPRS=Laboratory-prepared rancid samples, n=12.
[3] n=44.
[4] Prob>|r| under H_o: Rho=0.
[5,6,7] Sum of concentrations of fatty acids used for correlation.

Table 6. Mean concentrations of total fatty acids from farm and laboratory-prepared rancid samples with different intensities of rancidity.

Intensity	Farm	LPRS
	(μg/10 ml milk)	
Slightly rancid	0.99	----
Moderately rancid	1.05	1.42
Very rancid	----	1.31
Extremely rancid	----	1.57

APPENDIX G

ANOVA FOR FATTY ACID CONCENTRATION IN 5 ADV GROUPS

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: CONCENTRATION

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	64	928.48969350	14.50765146
ERROR	482	84.06748518	0.17441387
CORRECTED TOTAL	546	1012.55717867	

MODEL F = 83.18 PR > F = 0.0

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.916975	40.8531	0.41762887	1.02227078

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FAID	12	428.63425551	204.80	0.0
ADVGROUP	4	104.73179506	150.12	0.0
FAID*ADVGRP	48	202.33713380	24.17	0.0

ANOVA FOR FATTY ACID CONCENTRATION IN 5 RANCIDITY GROUPS

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: CONCENTRATION

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	25	609.37884130	24.37515365
ERROR	521	403.17833738	0.77385477
CORRECTED TOTAL	546	1012.55717867	

MODEL F = 31.50 PR > F = 0.0

R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.601822	86.0526	0.8969016	1.02227078

SOURCE	DF	TYPE III SS	F VALUE	PR > F
FAID	12	601.46548799	64.77	0.0
RANCIDITYGP	1	0.51041859	0.66	0.4171
FAID*RANGRP	12	2.57371238	0.28	0.9926

VITA

Susan E. Duncan was born in Mansfield, Ohio on June 23, 1959. She graduated from Crestview High School, Ashland, Ohio, in June 1977. She entered The Ohio State University in September 1977 and received a Bachelor of Science in Food Technology in June 1981. She worked for 2 years as a quality control technician at T. Marzetti Co., Columbus, Ohio.

Susan began working as a research and teaching assistant at Indiana University of Pennsylvania in January 1985 and pursued a Master's of Science degree in Food and Nutrition which was awarded in August 1987. She passed the registration examination for dietetics in April 1987. She accepted a research assistantship at The University of Tennessee, Knoxville, in September 1986 and received her PhD in Food Technology and Science in December 1989.

She is a member of Institute of Food Technologists, American Dairy Science Association, American Dietetic Association, and Gamma Sigma Delta. Susan will continue working at The University of Tennessee, Knoxville, in a postdoctoral position after graduation.