Preparing Samples of Rancid Milk for Milk Judging Contests

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ABSTRACT

Due to the growing concern of raw milk, there is a growing need for other methods of preparing rancid milk for milk judging. The origin of rancidity in milk is due to lipolysis caused the lipase enzyme. However, with pasteurization, the lipase enzyme is inactivated. The purpose of this experiment was to compare the effect of using lipase powder to other current methods of preparing rancid milk, such as utilizing raw milk. Result showed that all treatments caused desired rancidity. The second purpose was to compare the rancidity between Method A, two to three hour incubation at room temperature, and Method B, twenty-four hour refrigerated. Results showed that there is no statistically significant difference between the two methods.

Key Words: milk judging, rancid, lipase, lipolysis, Future Farmers of America

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Flavor of milk dictates much of consumer acceptability. Off-flavors in milk also indicate possible defects in milk and lead to economic losses. The National FFA Organization (also know as Future Farmers of America) has included milk judging to train students and promote uniform application of judging practices through organoleptic techniques. Judging milk includes recognition of rancid off-flavors. The current common practice of using raw milk to induce rancidity has caused concern due to potential microbial and health consequences. The objective of this study is to evaluate the use of lipase powder in inducing rancidity and its comparison with other methods for FFA milk judging purposes.

LITERATURE REVIEW

Rancidity

Rancidity is an infamous off-flavor of milk and undesirable to consumers (Clark, 2014). It is important to note that the term rancidity encompasses both oxidative rancidity and hydrolytic rancidity. While oxidative rancidity is due to fat's uptake of oxygen, hydrolytic rancidity results from fat's uptake of water. Oxidative rancidity occurs when oxygen reacts with a double bond of a fatty acid and becomes part of the molecule. Light, oxidative enzymes and heavy metals catalyze this reaction. The products peroxides, hydroperoxides and other compounds contribute to the "papery", cardboard, or tallow off-flavors in milk (USDA, 2014). Catalyzed my heat or lipase, hydrolytic rancidity in milk results from hydrolysis of the ester bonds between the glycerol and fatty acids

(Bruhn, 1995; Clark, 2014; Ishler and Roberts, 2014; Jensen, 1964; USDA, 2014). When catalyzed by lipase, hydrolysis of the fat is called lipolysis. This chemical defect has been characterized as a bitter, sharp, astringent, soapy off-flavor with unpleasant aroma (Deeth, 2006; Russell et. al, 2009; Tarassuk and Henderson, 1942), resembling stale fat (USDA, 2014) or baby burp (Ishler and Roberts, 2014). The aroma is similar to Romano, Cotija, or Blue cheese.

Hydrolytic Rancidity

Many factors contribute concurrently to the susceptibility of lipolysis. The conditional determinants in production include the season, feed, stage of lactation, mastitis, and the individual cow. Aging, rapid cooling, temperature fluctuations, homogenization, agitation, improper pasteurization, air leaks in pipeline systems also influence the milk's susceptibility to lipolysis during processing. Due to dry feed, cows' milk in winter season occasionally is highly susceptible to lipolysis (Chandan and Shahani, 1964; Fredeen et. al, 1950; Jensen, 1964; Tarassuk and Henderson, 1942). The move to more hay and concentrate mixture feeding has increased rancid flavor problems (Bruhn, 1995). Milk from cows in late stages of lactation is more susceptible to rancidity than cows in earlier stages of lactation (Bruhn, 1995; Fredeen et. al, 1950; Tarassuk and Henderson, 1942). Milk from individual cows in a herd, even individual quarters of a cow's udder, can vary in susceptibility to lipolysis (Bruhn, 1995; Fredeen et. al, 1950).

The intensity of rancidity increases with storage of milk (Bruhn, 1995; Tarassuk and Henderson, 1942) possibly because of time for lipase activity. Cooling of fresh raw milk quickly causes lipase to be adsorbed into the fat globule. The rate of lipolysis in

milk cooled slowly is less than the rate for milk cooled immediately (Jensen, 1964; Bachman and Wilcox, 1990). With the development of the bulk tank system that can cool milk quickly, overly rapid cooling may lead to more interaction with lipase and the fat globule, globule surface shifting, or both. Often additions of warm milk to already cold milk induce temperature fluctuations (Bruhn, 1995; Tarassuk and Henderson, 1942). Homogenization increases the surface area of fat globule (Tarassuk and Henderson, 1942) thereby increasing the chance for lipase to interact with the fat. Incorporating air, extreme agitation of warm raw milk also increases surface area of milk and causes foaming (Tarassuk and Henderson, 1942; USDA, 2014). The fat globules become hydrophobic as the air bubbles burst (King, 1955). These factors in milk production and processing may induce the activation of lipase due to disruption, partial replacement or distortion of the original layer on the fat globules (Tarassuk and Henderson, 1942).

Lipolysis

The cause of rancidity in milk is due to the enzymatic hydrolysis of lipids or glycerides (Figure 1), commonly referred to as lipolysis (Deeth, 2006; Jensen, 1964). The comprehensive term lipolysis refers to milk in which any lipolysis has taken place without the milk becoming rancid; therefore, Jensen (1964) suggests to use the phrase "hydrolytic rancidity" - defined as released free, or nonesterified fatty acids, and the formation of partial glycerides, mono- and diglycerides that are recognized as rancidity by flavor and functionality change (Deeth, 2006; Jensen, 1964). Milk fats are composed of a glycerol and esterified with fatty acids by ester bonds. Lipase catalyzes the hydrolysis of the ester bonds, freeing the fatty acids. Milk fats are found in discrete

globule structures. The discrete globules are dispersed in the milk as an emulsion and stabilized by a layer of protein, called phospholipids, and glycerides (Jensen, 1964). In fresh milk, protein around the fat globule prevents lipolysis of the fat due to the enzymes' lack of contact with the fat (Bruhn, 1995; Jensen, 1964).

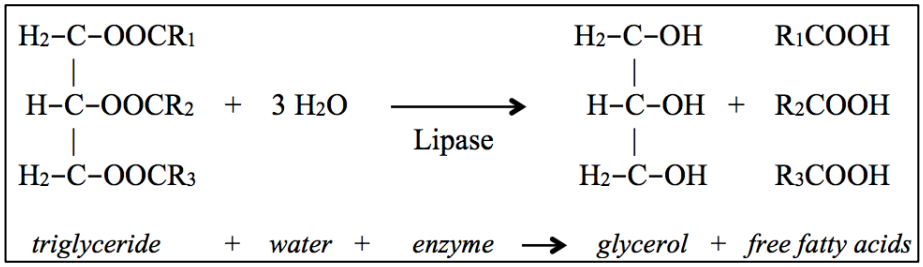

Figure 1. Hydrolysis of triglyceride

The hydrolytic rancid off-flavors and aromas in milk are caused by the aromatic and volatile free short-chain fatty acids, including butyric, caproic, caprylic, and capric acids (Bruhn, 1995; Clark, 2014; Ishler and Roberts, 2014). In milk, the strong flavors of the free fatty acids (FFAs) are undesirable by consumers. However, lipolyzed fat in certain cheeses, like Blue cheese or hard Italian cheese, the flavors are desirable (Deeth & Fitz-Gerald, 1995). Lipolysis in milk will also alter the functionality of its foaming ability. Because the partial glycerides produced by lipolysis are surface active, the foam-stabilizing proteins form bubbles at the air-water interface (Buchanan, 1965). Creaming ability can also be impaired during separation (Deeth & Fitz-Gerald, 1995). The activation of lipolysis may be from cooling or other processing treatments.

Spontaneous lipolysis. Spontaneous lipolysis is defined as rancidity developed without treatment besides cooling (Bruhn, 1995). Cooling is needed for activation (Tarassuk and Frankel, 1957). Spontaneous lipolysis is not a serious cause of off-flavor for commercial milk supplies (Bruhn, 1995). There is higher appearance of spontaneous

lipolysis in milk from cows on dry hay or in the latter part of the lactation cycle (Chanadan and Shahani, 1964).

Induced lipolysis. Induced lipolysis is defined as rancidity developed due to agitation, homogenization at temperatures below 130°F, or temperature manipulation between 40-80°F (Hetrick and Tracy, 1948; Tarassuk and Frankel, 1957). These activation treatments release lipase from the casein micelle, encourage adsorption of lipase on the fat globules, and shift the fat globule membrane (Jenness and Patton, 1959).

Lipase

Lipolysis is caused by lipase that is secreted by spoilage bacteria (Alvarez, 2008). Lipase reacts with emulsified glyceride molecules separated by an interface from water (Desnuelle, 1961). Milk enzymes include membrane lipase, plasma lipase and milk lipoprotein lipase (Chandan and Shahani, 1964; Jensen, 1964; Tarassuk and Frankel, 1955). The optimum pH range for these lipases is about 8 to 9 (Jensen, 1964; Jensen and Pitas, 1976). These lipases are present in all raw milk (Tarassuk and Henderson, 1942).

Membrane lipase. When milk is cooled, membrane lipase, also called naturally active lipase, is adsorbed onto the fat globule membrane (Chandan and Shahani, 1964; Jensen, 1964). They irreversibly incite spontaneous lipolysis. Low amounts of membrane lipase are found in normal milk (Tarassuk and Frankel, 1955).

Plasma lipase. Plasma lipase, also called lipase of normal milk, stays associated with casein in the plasma (Chandan and Shahani, 1964). After an activation treatment, the plasma lipase is adsorbed onto the fat globule (Tarassuk and Frankel, 1955). They incite induced lipolysis.

Lipoprotein lipase. Lipoprotein lipase only hydrolyzes fat bound to protein when cooled (Jensen, 1964). Compared to plasma lipase, there is a relatively high concentration of lipoprotein lipase in milk. There is greater lipase activity in milk from short milking intervals and cows in late lactation. When milk is cooled, lipoprotein lipase activity increased in the milk fat globule membrane. Storing milk at 31°C causes loss in lipoprotein lipase activity (Dickow et. al, 2011). Serum or apoproteins and heparin stimulate activity. Albumin is required as a fatty acid acceptor. Protamine and sodium chloride inhibit activity (Jensen and Pitas, 1976).

Rancidity Prevention

The first place to start prevention of rancidity is on the dairy farm with the raw milk by reducing the amount of lipase activity through proper production and storage practices. Preventing and reducing foaming or flooding in pipeline and receiver jar during milking (Ishler, 2014), using stainless steel equipment, and eliminating contaminating metals (Bruhn, 1995) in addition to emptying and washing raw milk storage tanks regularly reduces rancidity. Cows that produce rancid milk from cooling should be detected, specially cows in late lactation. It may be necessary to sample milk from all four quarter of cows in late lactation to check for rancid taste 48 hours later (Ishler, 2014). Late-lactation milk should be eliminated from the bulk tank (Ishler, 2014; Jensen, 1964; Tarassuk and Henderson, 1942). The somatic cell counts need to be maintained below 250,000 per mL to ensure healthy cows and quality milk (Ishler, 2014). To decrease the incidence of hydrolytic rancidity, plate coolers and refrigerated receive jars can be used to cool bulk raw milk (Bachman and Wilcox, 1990). Intermingling of milk or

with normal milk before cooling decreases the chances for an individual cow's milk to be in a favorable environment for developing rancidity (Jensen, 1964; Tarassuk and Henderson, 1942). Tarassuk and Henderson (1942) found that mixing about four volume units of susceptible milk per one volume unit of normal milk within one hour will prevent development of rancidity.

To prevent hydrolytic lipolysis in processing and throughout commercial milk's shelf life, inactivation of lipase is required. Rancid milk is prevented because of intact membranes around the fat globules (Ishler and Roberts, 2014), which prevents lipase to come in contact with the fat globules. Because lipase is a protein, the enzyme can be inactivated by heat (Clark, 2014). The standard pasteurization conditions are sufficient to prevent lipolysis (Hetrick and Tracy, 1948). Therefore properly pasteurized and packaged milk will not develop rancidity (Bruhn, 1995; Chandan and Shahani, 1964). Raw milk ideally should be pasteurized immediately after milking; however, this is not the case in current practice in the United States and industrialized countries (Tarassuk and Henderson, 1942). In developing a process flow, cooled milk warmed for processing, such as separation, should not be cooled again without pasteurization. For example, homogenized milk should be pasteurized before, or immediately after homogenization and before cooling. Because, the lipase is activated by the low heat, it needs to be inactivated before the cooling environment allows the enzyme to produce more rancidity. It is not advisable to mix pasteurized milk with raw milk (Bruhn, 1995). This would re-introduce new lipase from raw milk into the lipase-free pasteurized milk. If milk is found rancid during storage, the milk may have been improperly pasteurized or been

contaminated after pasteurization. A phosphatase test is legally used to indicate if milk was properly pasteurized (Fasken and McClure, 1940).

Rancidity Tests

Two currents test to evaluate milk fat quality includes determining Peroxide Value and Free Fatty Acid Content. Peroxide Value indicates deterioration of fat caused by lipid oxidation. The method measures formation of peroxides, a product of oxidation, in the sample. A high peroxide value indicates high oxidation. The free fatty acid content (often called acid degree value, ADV) indicates the deterioration of fats caused by hydrolytic rancidity. The method measures the amount of free fatty acids in the sample. A fluid fat sample is titrated with a standard NaOH solution. A high free fatty acid content indicates high hydrolysis and, therefore, high rancidity

Preparing Rancid Milk for FFA

Currently there are several methods of preparing rancid milk for judging. The most popular method to produce rancidity is through adding raw milk to pasteurized milk. The bacteria enzymes that are present in non-pasteurized milk are available to act upon the fat globules. USDA advises to add ten percent raw milk to homogenized milk, heating the mixture to body temperature, and then refrigerating it overnight. Another method is to added two drops of butyric acid per pint of milk early on in the preparation to increase the rancid flavor. Rancidity is often described having a butyric acid taste; therefore, research has found that butyric acid contribute to the rancid flavor. Other methods include adding Romano, Parmesan, Blue Cheese or Cotija cheese extract to

normal milk, since these cheeses already possess the rancid flavor. The Iowa FFA Association suggests another method of adding ¼ cup of grated Romano cheese to one cup of milk and refrigerate overnight. After filtering out the cheese, the rancid milk should be combined with the original milk. Iowa FFA Association, however, does not include the ratio of original milk to rancid milk from the cheese. Vinegar also has been used to prepare rancid milk (Pinchin, 2013). The acid environment promotes acid hydrolysis of lipids. However, details of the sample preparation were not mentioned.

METHODS AND PROCEDURES

Experimental Design

Unhomogenized milk or cream top was used to access the fat more easily in the milk. Each time the tests were done, a control was included to monitor the original milk's rancidity level via acid degree value method. Then the traditional practice of including raw milk to produce rancidity was also included to compare the effects of lipase (Danisco's Lipase Powder 300). To observe the effects of lipase on smaller, more evenly disbursed fat globules, homogenized (Ralphs, Vitamin D Milk) and ultra-pasteurized homogenized milk (Kroger Co., Whole Milk) was induced with lipase. Using cheese to induce the rancid flavor, the water soluble-nitrogen extraction of Romano (Digiorno, Sell by June 06 2014), Cotija (Cacique, Part skim milk cheese, Batch 20, November 11 14) or Blue Cheese (Saputo, Stella® Blue Cheese Deli Cup) was added to create a 1.0% cheese milk sample. The study the effects of temperature versus time, samples were incubated at refrigerated temperatures (4°C) overnight or at room temperatures (22°C) for 2 h. Each sample was only tested in triplicate due time constraints.

Sample Preparation

The non-homogenized milk (Trader Joe's Cream Top) was placed at room temperature until warmed to room temperature and gently shaken well to disperse the top cream layer before various treatments (Table 1). The samples were stored in 50 mL-BD Falcon™ tubes. The raw milk sample was prepared following USDA's traditional ratio of one part raw milk to every nine parts pasteurized milk. The raw milk was collected at Cal Poly Dairy's holding tank and stored in the refrigerator. The raw-pasteurized mixture was also mixed in a 50 mL-BD Falcon™ tubes, heated to 37°C in a water bath, and then refrigerated or left at room temperature.

To prepare the one percent lipase milk, 10 g of six percent lipase in water solution was added 50 g of the milk. Similarly, the lipase shake sample only differed with the regular lipase sample in the added milk. The milk of the lipase shake sample was vigorously shaken by hand for 30 s before adding the lipase. The preparation for the homogenized and ultra-pasteurized milk was the same ratios as the one percent lipase milk. However, instead of non-homogenized milk, commercial homogenized HTST pasteurized milk and ultra-pasteurized homogenized milk were added. To prepare the milk with cheese, a water-soluble nitrogen (WSN) extraction was performed. 30 g of cheese were stomached with 15 g of deionized water in a Whirl-pak® bag for 3 m. After placing in a hot water bath at 40°C for 1 h, the liquid portion of the sample was pipetted out to make the milk-cheese samples or into a Falcon tube and store in refrigerator for later use.

Table 1. Summary of Sample Formulations

Milk Sample	Sample Preparation
Control	Unadulterated non-homogenized milk
Raw milk	10% raw milk in non-homogenized milk, warmed to 37°C
Lipase	1.0% lipase in non-homogenized milk
Lipase Shake	Vigorously hand-shaken milk, 1.0% lipase
Homogenized	1.0% lipase in homogenized milk
Ultra-pasteurized	1.0% lipase in ultra-pasteurized homogenized milk
Romano cheese	1.0% WSN in non-homogenized milk
Cotija cheese	1.0% WSN in non-homogenized milk
Blue cheese	1.0% WSN in non-homogenized milk

Preparing Reagents for Acid Degree Value Method

The acid degree value (ADV) – defined as the milliliters of 1N base required to neutralize the acids in 100 g fat – was measure in the samples using the procedure described in the Standard Methods for the Examination of Dairy Products (Wehr and Frank, 2004). None of the steps were blanketed with dry nitrogen or flushed continuously with dry nitrogen as suggested by the procedure.

Bureau of Dairy Industry (BDI) reagent. 10 mL were needed per sample. The BDI was prepared in a 100 mL volumetric flask. Three grams of Triton X-100 and seven grams of sodium hexametaphosphate were weighed into the volumetric flask and topped with HPLC grade water the 100 mL mark. The contents of the flask were shaken to dissolve and stored at room temperature in the volumetric flask and capped tightly.

Phenolphthalein (1%). Five drops were needed per sample. One gram of phenolphthalein was weighed into a 100 mL volumetric flask. Methanol was added to the

phenolphthalein to the 100 mL mark. The solution was then transferred to 50 mL BD Falcon™ tubes and stored at room temperature.

Alcoholic potassium hydroxide (KOH) solution. Around 0.1 to 0.7 mL of 0.02N KOH was needed per sample. A 1N alcoholic KOH solution was first made in a 100 mL volumetric flask. KOH (5.611 g) was accurately weighed into the volumetric flask. Methanol was added to fill to the 100 mL mark. Then 4 mL of the 1N KOH solution was diluted with more methanol to 0.02N in a 200 mL volumetric flask. The 0.02N KOH was stored in a glass jar at room temperature.

Fat solvent. Five milliliters were needed per sample. In two 50 mL BD Falcon™ tubes, 20 mL of petroleum ether and 5 mL n-propanol were pipetted and mixed. Seven milliliters of aqueous methanol were needed per sample. In a glass jar, 35 mL methanol and 35 mL of HPLC grade water was pipetted and mixed.

Acid Degree Value Method

Using a 17.6 mL pipet, 35.2 mL of the milk samples were placed in 18 g milk Babcock bottles. 10 mL of BDI reagent were added with a pipet and mixed with the milk by shaking. The bottles were placed in a bath of gently boiling water. A rack was placed above the water to prevent bottles from overturning. After the first five minutes in the water bath, the Babcock bottles were taken out, shaken to mix contents, and then replaced in the water bath for another five minutes. The process was repeated, and the bottles were centrifuged for one minute. Methanol was added to bring the fat column at the seven mark in the graduated portion of the bottle neck using a pipet. The bottles were centrifuged for another minute. The bottles were then transferred to a water bath of 57°C

± 3°C. The water level was above the fat column. One milliliter of fat at 57°C was transferred to a 50-mL Erlenmeyer flask, using a 1-mL pipet. The fat was weighed and recorded. The fat then was dissolved in five milliliters of fat solvent and five drops of 1% phenolphthalein. The fat was titrated with the alcoholic KOH using a 10-mL microburet with 1% phenolphthalein as indicator until a faint but definite color change. A blank titration was made on only the fat solvent and five drops of phenolphthalein indicator. The ADV was calculated (Figure 2) and the results interpreted as described (Table 2) in the Standard Methods For the Examination of Dairy Products (Wehr and Frank).

$$ADV = \frac{(mL\ KOH\ for\ sample - mL\ KOH\ for\ blank)\ x\ N\ x\ 100}{Weight\ of\ fat}$$

Figure 2. Acid degree value formula

Table 2. Acid degree value interpretation

ADV	Interpretation
< 0.7	Normal
0.7 to 1.1	Slightly hydrolyzed
1.2 to 1.4	Definitely hydrolyzed
> 1.4	Extremely hydrolyzed

RESULTS AND DISCUSSION

All of the adulterated milk samples resulted in rancidity (Table 3). As expected the control was normal. A two-tailed Student's t-Test (McDonald, 2009) was performed to compare the difference between the control and the treatments (Table 4). Only Lipase, Ultra-pasteurized, and Romano treatments from Method 1 and Ultra-pasteurized treatment from Method 2 showed statistically significant difference ($P < 0.05$) from the

control. There was mostly no significant different ($P < 0.05$) between the samples stored at room temperature for 2 - 3 h and the samples refrigerated for 24 h (Table 5). In the Method 1 samples, the raw and lipase treatments resulted in the highest in extreme hydrolysis, while the Romano treatment ranged the lowest of the samples in the slight to definitely hydrolyzed. In Method 2, the raw and ultra-pasteurized treatments achieved the highest in extreme hydrolysis; the Blue Cheese treatment relatively had the lowest hydrolysis. The use effect of using Cotija cheese was extremely hydrolyzed in both methods. There is not a significant difference ($P < 0.05$) in using homogenized and non-homogenized milk. Both can be used for the purpose of making rancid milk for judging. Varying room temperatures between trials may have contributed to varying results. More research should be pursued to compare the ADV of these samples to sensory evaluation.

Table 3. Sample means of acid degree values for milk treated with various lipolytic agents and incubated at refrigeration or room temperatures

Milk Sample	**Method 1** *2-3 h, room temperature*	**Method 2** *24 h, refrigerated*
Control	0.43 ± 0.10	0.48 ± 0.25
Raw	3.11* ± 2.76	2.76* ± 1.85
Lipase	3.51* ±1.63	2.26* ± 1.36
Lipase Shake	2.19* ± 1.00	1.65* ± 0.34
Homogenized	2.26* ± 1.47	4.88* ± 3.82
Ultra-pasteurized	2.75* ± 0.68	2.70* ± 0.76
Romano	1.13*** ± 0.34	2.07* ± 0.98
Cotija	1.74* ± 0.99	3.53* ± 3.31
Blue Cheese	1.63* ± 0.34	1.23** ± 0.82

* Extremely hydrolyzed
** Definitely hydrolyzed
*** Slight hydrolyzed

Table 4. Comparison between control and treatments by *P* value

Milk Sample	**Trial 1** *2-3 h, room temperature*	**Trial 2** *24 h, refrigerated*
Raw	0.0785	0.0586
Lipase	0.0171*	0.0603
Lipase Shake	0.0515	0.0747
Homogenized	0.0867	0.0977
Ultra-pasteurized	0.0134*	0.0170*
Romano	0.0408*	0.0796
Cotija	0.0821	0.1177
Blue Cheese	0.0152*	0.1800

* Means differ significantly ($P < 0.05$)

Table 5. Comparison between methods

Milk Sample	***P* value**
Control	0.6480
Raw	0.7100
Lipase	0.4142
Lipase Shake	0.2691
Homogenized	0.3289
Ultra-pasteurized	0.9501
Romano	0.1274
Cotija	0.5437
Blue Cheese	0.2806

CONCLUSION

Besides using raw milk to induce rancidity for milk judging training, other methods are available. Using one percent lipase in the milk sample has a similar effect as using raw milk. Both homogenized and non-homogenized milk can be used for preparation of rancid milk for dairy products judging. Romano, Cotija, and Blue cheeses also have a similar effect as raw milk in preparing rancid milk. Any room temperature milk treatment 2 – 3 h before judging is not significantly different from preparing the same treatment to milk 24 h ahead of judging.

REFERENCES

Alvarez, V. B. 2008. Sensory evaluation of fluid milk and cream products. Pages 103-119 in The Sensory Evaluation of Dairy Products, 2nd ed. S. Clar, M. Costello, and F. Bodyfelt, ed. Springer Science Business Media, Inc., New York.

Bachman, K. C. and C. J. Wilcox. 1990. Effect of time of onset of rapid cooling on bovine milk fat hydrolysis. J. Dairy Sci. 73:617-620.

Bandler, D. K. and Inc. Int. Assoc. Milk Food Environmental Sanitarians. 1979. Rancid milk - Is it here to stay? J. Food Prot. 42.10:840.

Bruhn, J. C. 1995. Milk flavors. The Dairy Research & Information Center, UC Davis. Accessed Feb. 23, 2014. http://drinc.ucdavis.edu/dairyp/dairyp3.htm

Buchanan, R. A. 1965. Lipolysis and the frothing of milk-Steam frothing test. Aust. J. Dairy Technol. 20:62-66.

Cartier, P. and Y. Chilliard. 1990. Spontaneous lipolysis in bovine milk: Combined effects of nine characteristics in native milk. J. Dairy Sci. 73:1178-1186

Chandan, R. C. and Shahani, K. M. 1964. Milk lipase: a review. J. Dairy Sci. 47:471-480.

Clark, S., compiler. 2014. ISU dairy foods manual: National FFA organization dairy foods career development event manual. Department of Food Science and Human Nutrition, Iowa State University.

Deeth, H. C. 2006. Lipoprotein lipase and lipolysis in milk. Int. Dairy J. 16.6:555-562.

Deeth, H. C. and Fitz-Gerald, C.H. 1995. Lipolytic enzymes and hydrolytic rancidity in milk and dairy products. Pages 247-308 in Advanced Dairy Chemistry, Vol. 2: Lipids, 2nd ed. P. F. Fox, ed. Chapman & Hall Publishers, London, UK.

Desnuelle, P. 1961. Pancreatic lipase. Advances in Enzymology. 23:129

Dickow, J. A., L. B. Larsen, M. Hammershøj, and L. Wiking. 2011. Cooling causes changes in the distribution of lipoprotein lipase and milk fat globule membrane proteins between the skim milk and cream phase. J. Dairy Sci. 94:646-658.

Duncan, S. E. and G. L. Christen. 1991. Sensory detection and recovery by acid degree value of fatty acids added to milk. J. Dairy Sci. 74.9:2855-2859.

Duncan, S. E., M.P Penfield, and G.L Christen. 1991. Rancid flavor of milk: Relationship of acid degree value, free fatty acids, and sensory perception. J. Food Sci. 56.2:394-397.

Dunkley, W. L. and L. M. Smith. 1951. Hydrolytic rancidity in milk. IV. Relation between tributyrinase and lipolysis. J. Dairy Sci. 34.9:940-947.

Fasken, J. E. and A. D. McClure. 1940. Phosphatase test in pasteurization of milk. Can. J. Comp. Med. 4:128-133

Fredeen, H., and J. E. Bowstead, W.L. Dunkley and L.M. Smith. 1951. Hydrolytic rancidity in milk. II. Some management and environmental factors influencing lipolysis. J. Dairy Sci. 34.6:521-528.

Hetrick, J. H. and P. H. Tracy. 1948. Effect of high-temperature short-time heat treatments on some properties of milk. II. Inactivation of the lipase enzyme. J. Dairy Sci. 31.10:881-887

Ishler, V. and B. Roberts. 2014. Troubleshooting milk flavor problems. Dairy Herd Management, Penn State Extension. Accessed Feb. 23, 2014. http://extension.psu.edu/animals/dairy/health/nutrition/nutrition-and-feeding/troubleshooting-guides/troubleshooting-milk-flavor-problems

Jenness, R. and S. Patton. 1959. Principles of Dairy Chemistry. John Wiley & Sons, Inc. New York, NY. pp 193.

Jensen, R. G. 1964. Lipolysis. J. Dairy Sci. 47.2:210-215.

Jensen, R. G. and R. E. Pitas. 1976. Milk lipoprotein lipase: A review. J. Dairy Sci. 59.7:1203-1214.

King, N. 1955. Microscopic appearance of fat on the milk surface as affected by mechanical disturbance of the surface. J. Dairy Research. 22:328.

McDonald, J. H. 2009. Student's t-test. Pages 118-122 in Handbook of Biological Statistics. 2nd ed. Sparky House Publishing. Baltimore, MD.

Pinchin, K. 2013. Does this taste funny to you? Teens face off in competitive dairy tasting. Accessed Mar. 15, 2014. http://modernfarmer.com/2013/10/teenage-dairy-tasters-ffa/

Product description - Lipase Powder 600. 2012. *www.thecheesemaker.com*. Accessed Jan. 15, 2014. http://www.thecheesemaker.com/pdf/specs_mild_lipase.pdf

Reeder, J., and Inc Int Assoc Milk Food Environmental Sanitarians. 1985. Rancid flavors of milk: Causes and prevention. J. Food Prot. 48.10:912.

Russell, K. M., S. Barkman, J. Chambers, M. Bozell, P. Robinson, P. Baker, E. Daming. 2009. 4-H 945 4-H/FFA Dairy foods contest coaches' guide. 4-H Department,

Purdue University. Accessed Feb. 24, 2013. http://www.four-h.purdue.edu/downloads/judging/945.pdf

Tarassuk, N. P. and E. N. Frankel. 1955. On the mechanism of activation of lipolysis and the stability of lipase systems of normal milk. J. Dairy Sci. 38:438.

Tarassuk, N. P. and E. N. Frankel. 1957. The specificity of milk lipase. IV. Partition of the lipase system in milk. J. Dairy Sci. 40:418.

Wehr, H. M. and J. F. Frank. 2004. Acid degree value method (Class O). Pages 510-512 in Standard Methods for the Examination of Dairy Products. Am. Publ. Health Assoc., Washington DC.

Tarassuk, N. P. and J. L. Henderson. 1942. Prevention of development of hydrolytic rancidity in milk. J. Dairy Sci. 25.9:801-806.

United States Department of Agriculture, Agricultural Marketing Service, Bulletin AMS 2259. 2014. Judging and scoring milk and cheese. Dairy Standardization Branch. Accessed Feb. 24, 2014. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004794