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I am submitting herewith a thesis written by Chow-Ming Lee entitled "Selection, Training and Utilization of a Sensory Panel for the Evaluation of Lipolyzed Flavor in Milk." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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SELECTION, TRAINING AND UTILIZATION OF A SENSORY PANEL FOR THE EVALUATION OF LIPOLYZED FLAVOR IN MILK

A Thesis
Presented for the
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Chow-Ming Lee
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DEDICATION

This thesis is dedicated to my grandma, Chew Kim Keok, and my parents, Lee Chin Kian and Yeoh See Suan, for their encouragement, love, financial support and belief in higher education.

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ABSTRACT

This research was an attempt to establish guidelines for selecting and training of sensory panelists for the evaluation of lipolyzed flavor in milk. Trained panelists were utilized in the evaluation of laboratory-prepared lipolyzed samples, retail samples, and laboratory-pasteurized samples. Gas chromatography and a titration procedure were used to evaluate the samples simultaneously with the sensory panelists.

Lipolyzed flavors in varying intensities of laboratory-prepared lipolyzed samples were not detected by the chemical methods but the trained panelists recognized the changes in the intensities. Both sensory and chemical evaluations of retail milk samples suggested that lipolyzed flavor is not a common problem in the Knoxville, TN area. Laboratory-pasteurized milk samples were slightly lipolyzed after one day of storage and moderately lipolyzed after fifteen days of storage. Good correlation between sensory scores and free fatty acids was limited to 1-day samples. Even though the titration method provided a more accurate measurement of lipolyzed flavor, it did not sufficiently account for the variation in lipolyzed flavor as evaluated by the panelists. Difficulties in sampling and the presence of other off-flavors in the 15-day laboratory-pasteurized milk may have misled the panelists, thus confounding the results. The use of samples that resemble authentically lipolyzed flavor is important.

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CHAPTER I

INTRODUCTION

The flavor of milk is one of the factors important in determining milk quality. Hydrolysis of milk fat results in a flavor defect known as hydrolytic rancidity. Shipe et al. (1978) recommended that this flavor be called lipolyzed. Under favorable conditions, lipase can act on the milk fat globule resulting in the release of free fatty acids (Bodyfelt et al., 1988), of which the even-numbered short chain acids contribute most to the lipolyzed flavor characteristic (Scanlan et al., 1965). Some of the possible factors contributing to lipolysis in raw milk are excessive agitation, homogenization, alternate warming to about 32°C and cooling after that, excessive air incorporation, and raw milk left sitting for an extended time (Bodyfelt et al., 1988).

For more than 35 years, acid degree value (ADV) has been the standard method for determining the degree of lipolysis (Thomas et al., 1955). However, recent research by Duncan and Christen (1991) indicates that ADV fails to recover short-chain length fatty acids, though providing good recovery of medium- and long-chain length fatty acids. They were unable to provide evidence of correlation between ADV and subsequent lipolysis flavor scores (Duncan et al., 1991).

In our laboratory a rapid method is being sought to recover free fatty acids from milk that correlates to lipolyzed flavor of milk. Previous methods such as the silica gel method (Harper et al., 1956), colorimetric ultramicro method (Novak, 1965), and automatic titration by photocolorimetry (Noble, 1966) were adapted to milk and tested but were unsuccessful in achieving high recovery of the short chain free fatty acids or were too cumbersome for routine analysis (Christen 1990a,b). Similar results were obtained by Christen and Shen (1991) with the copper soap method.

The objective of this research project was to select, train, and utilize sensory panelists for evaluation of lipolysis in milk. Results were compared to chemical extraction/titration of fatty acids and free fatty acid profiles determined by gas chromatography.

CHAPTER II

REVIEW OF LITERATURE

LIPOLYSIS IN MILK

Milk fat is composed primarily of triglycerides which mostly are contained inside a protective fat globule membrane (Kintner and Day, 1965). Under certain conditions, the membrane is broken and subsequent reactions lead to the release of free fatty acids (FFA) by the native lipolytic enzyme known as lipase (Bodyfelt et al., 1988). This is known as lipolysis, more commonly as "hydrolytic rancidity" or simply "rancid".

The existence of one or more lipases in bovine milk has been reviewed (Herrington, 1954; Jensen and Pitas, 1976). Castberg et al. (1975) suggest that there may be only one lipase in milk, that is, lipoprotein lipase. The enzyme is basically inactive unless external factors act upon the milk system and damage the fat globule membrane (Herrington, 1954). Lipase activity is naturally impaired by its association with the casein micelles and by unidentified inhibitors (Downey, 1980). The lipase-membrane system may be stabilized by storing at 0-4°C for 24 h prior to homogenization or pasteurization (Tarassuk and Frankel, 1955). Increasing amounts of lipase may be present in milk from cows in late

lactation (Hileman and Courtney, 1935). The amount of FFA does not influence lipase activity (Driessen and Stadhouders, 1974). Once activated, lipase acts upon position one and three of the triglyceride, forming FFA, diglycerides and monoglycerides (Kuzdzal-Savoie, 1980).

Free fatty acids impact the bland flavor of fresh milk leading to off-flavors commonly perceived as "goaty", "rancid", "bitter" or "soapy" (Shipe et al., 1978), and frequently to consumer rejection. Lipolysis also results in processing problems in the manufacturing of dairy products as well as flavor impairment of these products (Deeth and Fitz-Gerald, 1976). It is important to detect and correct for lipolysis at its earliest stage of development.

The flavor of lipolyzed milk is mainly due to short-chain FFA such as butyric, caproic, caprylic, capric and lauric acids (Al-Shabibi et al., 1964; Bills et al., 1969; Scanlan et al., 1965). The sensory threshold for butyric is the lowest; increasing thresholds have been found up to lauric acid (McDaniel et al., 1969). The long-chain FFA do not contribute significantly to the off-flavor (Scanlan et al., 1965) while preferential hydrolysis of short-chain FFA (Jensen, 1964; Kuzdzal-Savoie, 1980) may enhance the impact of short-chain FFA on the flavor.

Surveys of raw and pasteurized-homogenized milk in the United States found milk quality with varying degrees of lipolysis. Surveys using acid degree value (ADV) as the

primary tool tend to indicate a serious problem with lipolysis (Bandler and Wolff, 1979; Barnard and Moir, 1987; Senyk et al., 1982, 1985). Milk quality evaluated using ADV and a trained panel also found a wide occurrence of lipolysis (Barnard and Moir, 1987). Most surveys which employ sensory evaluation by "experts" or trained panels found no significant presence of retail milk with lipolyzed flavor defect (Buenaventura et al., 1991; Bruhn et al., 1988; Potter and Hankinson, 1960). On the contrary, Barnard (1979) conducted a survey using three expert panelists and found lipolysis a common problem in the milk supply. The type of survey tool used may be responsible for the different results and their reliability should be carefully examined.

DEVELOPMENT OF LIPOLYSIS

Lipolysis may occur spontaneously or be induced by external factors. Factors impacting lipolysis may be physiological, mechanical, thermal, biochemical, or microbiological. Physiological changes account primarily for spontaneous lipolysis while mechanical, thermal, and other factors result in induced lipolysis. Lipolysis may be controlled by the milk producer and processor with proper care, but the quality of freshly pasteurized-homogenized milk does not predict the chances for lipolysis (Barnard, 1972). Instead, post-processing factors at and beyond the retail

level contribute to the problem of lipolysis (Bruhn et al., 1988). Thus, consumer education may play an important role beyond the best effort of milk producers and processors to control lipolysis.

Spontaneous lipolysis occurs after milking upon cooling the milk and is not caused by external factors (Deeth and Fitz-Gerald, 1983). The susceptibility of such milk is often traced to physiological aspects of the cows. Some research indicated that stage of lactation is crucial since late lactation may yield milk with less protective fat-globule membrane (Colmey et al., 1957; Connolly et al., 1979; Jellema, 1975; Ortiz et al., 1970). In contrast, Chen and Bates (1962) and Guthrie and Herrington (1960) found no relationship between the stage of lactation and spontaneous lipolysis. Afternoon milk is also more prone to spontaneous lipolysis than morning milk (Ahrné and Björck, 1985; Herringson and Krukovsky, 1939). Spontaneous lipolysis is found in milk from cows with improper nutrition (Jellema, 1975) and with mastitis (Guthrie and Herrington, 1960; Tallamy and Randolph, 1969). Bovine serum, though not commonly present, acts as an activator of lipase (Clegg, 1980; Jellema, 1975; Jensen, 1964). The differences in feed and some unknown factors between summer and winter milk may also promote spontaneous lipolysis in the summer (Buenaventura et al., 1991; Chazal and Chilliard, 1986; Chen and Bates, 1962). Others reported that such effect is not as dominant as the stage of lactation

(Speer et al., 1958). Furthermore, spontaneous lipolysis is related to mastitis (Chazal and Chilliard, 1986; Ortiz et al., 1970). Some cows may exhibit the tendency to produce spontaneously lipolyzing milk without known cause (Bodyfelt et al., 1988). The problem persists even when the animal is fed increasing vitamin E or different kinds of feed (Hansen and Wesen, 1987). In such cases, spontaneous lipolysis may be decreased by mixing the lipolyzed milk with normal milk within one hour before or immediately after cooling (Tarassuk and Henderson, 1942). Henningson and Adams (1967) observed a relationship between spontaneous lipolysis and milk fat melting point. Spontaneous lipolysis is not affected by the condition of the body of the cow (Ortiz et al., 1970).

During the time between milking and consumption, lipolysis may be induced by one or more of the following factors: mechanical action, thermal activation, and/or other changes. Induced lipolysis occurs rapidly for a short period but terminates soon after initiation (Downey, 1980). Mechanical factors such as improper installation of pipes, mixing freshly arrived raw milk with stored raw milk, excessive agitation, delay of processing, homogenization and foaming lead to increasing chances of induced lipolysis Bandler et al., 1989; Barnard, 1972; Chen and Bates, 1962; Gholson et al., 1966; Herrington and Krukovsky, 1939; King, 1955; Speer et al., 1958). Homogenization pressure does not

significantly affect induced lipolysis (Shipe and Senyk, 1981).

Alternating the temperature of milk from 4°C to 30°C then back to 4°C significantly induces lipolysis (Bandler et al., 1989; Jensen, 1964; Wang and Randolph, 1978). The temperature of milk during storage or during piping also affects the induction of lipolysis (Henningson and Adams, 1967; Wang and Randolph, 1978). Shipe and Senyk (1981) stated that the minimum temperature for pasteurization does not sufficiently control lipolysis, despite varying the time between 16-24 s.

Other changes such as increase in temperature and pH to the optimum for lipase, lead to increased lipase activity and cause increased lipolysis (Driessen and Stadhouders, 1974; Wang and Randolph, 1978). Induced lipolysis is not related to the fat content of milk (Thomas et al., 1954). Microbiological changes, especially the increasing presence of lipolytic bacteria around and beyond 10^5-10^6 CFU/mL milk often lead to lipolysis (Downey, 1980; Jellema, 1975). Heat-resistant lipase and post-pasteurization contamination by lipase-producing bacteria are primarily responsible for lipolysis during retail sales of milk (Bandler et al, 1989).

SENSORY EVALUATION OF LIPOLYZED MILK

Free fatty acids and mono- and diglycerides are responsible for lipolyzed flavor (Downey, 1980; Jensen, 1964).

Short-chain FFA between butyric acid and lauric acid are believed responsible for lipolyzed flavor, though an agreement on the domination of one or more of them is not present (Al-Shabibi et al., 1964; Kuzdzal-Savoie, 1980; Scanlan et al., 1965; Woo and Lindsay, 1983). There also may be some interaction and/or unknown compounds responsible for lipolyzed flavor (Kolar and Mickle, 1963; Scanlan et al., 1965; Shipe et al., 1978). Shipe et al. (1978) failed to produce a simulation of naturally lipolyzed milk by adding FFA in similar proportions.

With proper supervision and training, sensory perception may be the most sensitive tool for the evaluation of lipolyzed milk flavor. Traditional evaluations has been done by a few "expert" judges of unknown training and ability. The accuracy obtained by using too few judges and too few samples, such as those by Thomas et al. (1955), may be too tenuous for conclusions to be drawn. During the past two decades, the use of a trained panel, whether large or small, has become common (Barnard, 1972; Barnard and Moir, 1987; Bills et al, 1969; Buenaventura et al., 1991; Connolly et al., 1979; Duncan, 1989). However, the procedures for selection, training and verification of such panels are often omitted in the literature. In the evaluation of frankfurters, trained panelists were observed to perform better than semi-trained panelists (Chambers et al., 1981).

Developing a panel requires the selection of panelists. Thresholds for FFA vary from one panelist to another. The flavor threshold for individual FFA varies and thresholds increase from butyric to octanoic acid (Duncan et al., 1991; McDaniel et al., 1969). Lipolysis may have occurred and FFA accumulated before it can be tasted by panelists since short-chain FFA form less flavorful salts and the long-chain FFA are dissolved in the lipid phase (Kintner and Day, 1965). Lipolysis may be possible without the perception of lipolyzed flavor (Jensen, 1964). The complexity of lipolyzed flavor suggests the need for careful selection and training of panelists for the evaluation of lipolyzed milk flavor. Selection methods such as the use of basic tastes by Connolly et al. (1979) may not account for the complex flavor notes of lipolyzed milk.

Training of panelists is the next step in preparing for the evaluation of lipolyzed milk flavor. Authentic lipolyzed milk samples such as those prepared by mixing raw and homogenized-pasteurized milk (Bodyfelt et al., 1988; Shipe et al., 1978) are often used to train and familiarize panelists with the characteristics of a naturally lipolyzed flavor. It has been postulated, however, that the authentic lipolyzed flavor may be significantly different from the naturally lipolyzed flavor (Duncan et al., 1990). Willey and Duthie (1969) classified lipolyzed flavor into two categories: 1) "sickening" type from milk prepared by mixing raw and

homogenized milk, churning, excessive agitation by blending and temperature fluctuation; and 2) "unclean" type resulting from foaming or spontaneous lipolysis of milk. The mechanism of lipolysis due to homogenization and temperature fluctuation are different (Tarassuk and Frankel, 1955). Thus, a naturally lipolyzed milk due to foaming may be different from that resulting from excessive agitation. Additionally, the flavor of authentic milk samples is related to the milk source (Fitz-Gerald, 1974).

Evaluation of lipolyzed milk requires the use of a scorecard with an appropriate scale and wording as described by O'Mahony (1979). It is also important that ideal conditions for a good sensory panel be observed. Kramer (1969) reported that the correlation between sensory scores and a mechanical or chemical method is a function of the range of the sensory criterion evaluated. A narrow range not covering the normal samples would yield a poor correlation while an unrealistically wide range beyond the normal would yield good correlation but one that is unrealistic.

CHEMICAL EVALUATION

Sensory response may be highly sensitive but is subject to variations among panelists and by interference from the environment. Chemical methods, on the other hand, are less subjective. The ADV procedure (Case et al., 1985; Thomas et

al., 1955) is the dairy industry's standard procedure for evaluating lipolysis in milk. It is a measurement of the amount of alkali required to titrate 100 g of fat after extraction from milk by a detergent. Lipolyzed flavor is said to be perceived by most evaluators at around ADV of 1.2 (Case et al., 1985). Poor correlation between lipolyzed flavor scores and ADV have been reported (Bell and Parsons, 1977; Duncan et al., 1991). ADV measures long-chain FFA but fails to recover short-chain FFA, thus not measuring the FFA most important to lipolyzed flavor (Duncan and Christen, 1991; Duncan et al., 1990). Also, milk of the same ADV may have different flavor intensity (Duncan et al., 1991; Shipe et al., 1978). The ADV procedure was originally developed by evaluation of laboratory-prepared lipolyzed samples of unspecified source by two sensory "experts". Based on subsequent evaluations, it has been recommended that ADV be related to fat hydrolysis but not specifically to perceived flavor (Bradley et al., 1992).

Several methods (Deeth et al., 1975; Dole, 1956; Harper et al., 1956; Kason et al., 1972; Noble, 1966, Novak, 1965; Salih et al., 1977; Shipe et al., 1980) have been evaluated in our laboratory for measuring lipolysis (Christen, 1990a,b; Christen and Shen, 1991). Methods for evaluation were selected based on simplicity, safety and convenience for use in a milk processing plant. The methods of Dole (1956) and Noble (1966) were selected for further evaluation and

modification by Christen and Shen (1991). This titration method is simple and fast and provides reasonable recovery of short-chain FFA. Good recovery of short-chain FFA is necessary because of their low threshold and association with lipolyzed flavor characteristics (Duncan and Christen 1991; Scanlan et al., 1965).

Quantitation of total FFA and individual FFA is commonly done by gas-liquid chromatography (GLC). FFA are present in normal milk at a concentration of approximately 0.25 meq/100g fat (Kuzdzal-Savoie, 1980). Increasing FFA is proportional to an increase in lipolyzed flavor (Bell and Parsons, 1977; Kolar and Mickle, 1963) and a decrease in consumer acceptance (McDaniel et al., 1969). GLC is required to identify individual FFA that may be responsible for the lipolyzed flavor. Deeth et al. (1983) described a GLC method that is reported to be able to provide good recovery of the FFA in milk. This method has been applied to previous research in our laboratory (Duncan, 1990; Breeding, 1989).

SUMMARY AND OBJECTIVE

Sensory evaluation may be the most sensitive tool for evaluation of lipolyzed flavor in milk. With proper selection and training, a group of panelists should produce reliable and repeatable results. However, panelists are subject to many variations and may be subject to constant turnover. A

chemical method is always available and provides repeatable results without fatigue. The use of ADV as a predictor of lipolysis without accompanying independent sensory evaluation may have exaggerated the problem of lipolyzed flavor in milk markets by not measuring accurately the short-chain FFA. The method proposed by Christen and Shen (1991) was evaluated and compared with sensory results from a trained panel and to gas chromatographic estimates of free fatty acids. Laboratory prepared lipolyzed milk, retail milk and farm bulk tank milk were evaluated.

REFERENCES

Ahrné, L. and Björck, L. 1985. Lipolysis and the distribution of lipase activity in bovine milk in relation to stage of lactation and time of milking. J. Dairy Res. 52: 55-64.

Al-Shabibi, M.M.A., Langner, E.H., Tobias, J., and Tuckey, S.L. 1964. Effect of added fatty acids on the flavor of milk. J. Dairy Sci. 47: 295-296.

Bandler, D.K. and Wolff, E.T. 1979. Characterization and extent of hydrolytic rancidity in fresh and aged pasteurized milk. J. Dairy Sci. 62(Supplement 1): 34.

Bandler, D.K., Barnard, S.E., Hinz, C.W., and Wolff, E.T. 1989. Guidelines for preventing rancid flavors in milk. Northeast Dairy Practices Council. Publication NDPC 23. Cornell University, Ithaca, NY.

Barnard, S.E. 1972. Importance of shelf life for consumers of milk. J. Dairy Sci. 55: 134-136.

Barnard, S.E. 1979. Quality and flavor of store purchased milk samples. J. Dairy Sci. 62(Supplement 1): 34.

Barnard, S.E. and Moir, L.M. 1987. Bacterial quality and flavor of store purchased milk samples in Pennsylvania. J. Dairy Sci. 70(Supplement 1): 74.

Bell, L.I and Parsons, J.G. 1977. Factors affecting lipase flavor in butter. J. Dairy Sci. 60: 117-122.

Bills, D.D., Scanlan, R.A., Lindsay, R.C., and Sather, L.A. 1969. Free fatty acids and the flavor of dairy products. J. Dairy Sci. 52: 1340-1345.

Bodyfelt, F.W., Tobias, J., and Trout, G.M. 1988. *The Sensory Evaluation of Dairy Products*. Van Nostrand Reinhold, New York.

Bradley, R.L., Jr., Arnold, Jr, E., Barbano, D.M, Semerad, R.J., Smith, D.J., and Vines, B.K. 1992. Chemical and Physical Methods. Ch. 18, In *Standard Methods for the Examination of Dairy Products*, R.T. Marshall (Ed.). American Public Health Association, Washington, D.C. In press.

Breeding, C.J. 1989. Comparison of free fatty acid concentrations from the action of *Pseudomonas fluorescens* and milk lipases on butteroil. M.S. thesis. The University of Tennessee, Knoxville.

Bruhn, J.C., Franke, A.A., Reif, G.D., and Frazeur, D.R. 1988. Milk quality surveys in California: 1974 and 84. Dairy, Food and Environmental Sanitation. 8: 404-408.

Buenaventura, M.L., Smith, D.E., Villela, A.M., Tatini, S.R., and Reineccius, G.A. 1991. Keeping quality of fluid milk from various regions of the United States. Dairy Food and Environmental Sanitation. 11: 82-86.

Case, R.A., Bradley Jr., R.L., and Williams, R.R. 1985. Chemical and Physical Methods. Ch. 18, In *Standard Methods for the Examination of Dairy Products*, G.H. Richardson (Ed.), 327-404. American Public Health Association, Washington, DC.

Castberg, H.B., Egelrud, T., Solberg, P., and Olivecrona, T. 1975. Lipases in bovine milk and the relationship between the lipoprotein lipase and tributyrate hydrolysing activities in cream and skim-milk. J. Dairy Res. 42: 255-266.

Chambers, E., IV, Bowers, J.A., and Dayton, A.D. 1981. Statistical designs and panel training/experience for sensory analysis. J. Food Sci. 46: 1902-1906.

Chazal, M.P. and Chilliard, Y. 1986. Effect of stage of lactation, stage of pregnancy, milk yield and herd management on seasonal variation in spontaneous lipolysis in bovine milk. J. Dairy Res. 53: 529-538.

Chen, J.H.S. and Bates, C.R. 1962. Observations on the pipeline milker operation and its effect on rancidity. J. Milk & Food Technol. 25: 176-182.

Christen, G. 1990a. Evaluation and development of chemical methods for measurement of lipolytic flavor of milk. National Dairy Board. Semi-Annual Progress Report, July 9th. Arlington, VA.

Christen, G. 1990b. Evaluation and development of chemical methods for measurement of lipolytic flavor of milk. National Dairy Board. Semi-Annual Progress Report, November 27th. Arlington, VA.

Christen, G.L. and Shen, N. 1991. Comparison of methods to extract free fatty acids from milk. J. Dairy Sci. 74(Supplement 1): 130.

Clegg, R.A. 1980. Activation of milk lipase by serum proteins: possible role in the occurrence of lipolysis in raw bovine milk. J. Dairy Res. 47: 61-70.

Colmey, J.C., Demott, B.J., and Ward, G.M. 1957. The influence of the stage of lactation on rancidity in raw milk. J. Dairy Sci. 40: 608-609.

Connolly, J.F., Murphy, J.J., O'Connor, C.B., and Headon, D.R. 1979. Flavor impairment of milk and milk products due to lipolysis. VIII. Relationship between free fatty acid levels of milk and butter and lipolysed flavour. Int. Dairy Fed. Bull. 118: 67-76.

Deeth, H.C. and Fitz-Gerald, C.H. 1976. Lipolysis in dairy products: a review. Aust. J. of Dairy Technol. 31: 53-63.

Deeth, H.C. and Fitz-Gerald C.H. 1983. *Lipolytic Enzymes and Hydrolytic Rancidity in Milk and Milk Products*. Ch. 6, In *Developments in Dairy Chemistry-2*, P.F. Fox (Ed.), 195-239. Applied Science Publishers, New York.

Deeth, H.C., Fitz-Gerald, C.H., and Snow, A.J. 1983. A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. N. Z. J. Dairy Sci. Technol. 18: 13-20.

Deeth, H.C., Fitz-Gerald, C.H., and Wood, A.F. 1975. A convenient method for determining the extent of lipolysis in milk. Aust. J. Dairy Technol. 30: 109-111.

Dole, V.P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35: 150-154.

Downey, W.K. 1980. Review of the progress of dairy science: flavour impairment from pre- and post- manufacture lipolysis in milk and dairy products. J. Dairy Res. 47: 237-252.

Driessen, F.M. and Stadhouders, J. 1974. A study of spontaneous rancidity. Neth. Milk Dairy J. 28: 130-145.

Duncan, S.E. 1989. Relationship of free fatty acids and acid degree values to lipolytic flavor of milk. Ph.D thesis. The University of Tennessee, Knoxville.

Duncan, S.E. and Christen, G.L. 1991. Sensory detection and recovery by acid degree value of fatty acids added to milk. J. Dairy Sci. 74: 2855-2859.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1990. Acid degree value - does it really predict rancid flavor in milk? Dairy, Food and Environmental Sanitation. 10: 715-718.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1991. Rancid flavor of milk: relationship of acid degree value, free fatty acids, and sensory perception. J. Food Sci. 56: 394-397.

Fitz-Gerald, C.H. 1974. Milk lipase activation by agitation-influence of temperature. Aust. J. Dairy Technol. 29:28-32.

Gholson, J.H., Gelpi, A.J., and Frye Jr., J.B. 1966. Effect of a high-level and a low-level milk pipeline on milk fat acid degree values. J. Milk & Food Technol. 29: 248-250.

Guthrie, E.S. and Herrington, B.L. 1960. Further studies of lipase activity in the milk of individual cows. J. Dairy Sci. 43: 843.

Hansen, A.P. and Wesen, D.P. 1987. Variations in acid degree values of individual and groups of cows from a herd producing rancid milk. J. Dairy Sci. 70(Supplement 1): 82.

Harper, W.J., Schwartz, D.P., and Hagarawy, E. 1956. A rapid silica gel method for measuring total free fatty acids in milk. J. Dairy Sci. 39: 46-50.

Henningson, R.W. and Adams, J.B. 1967. Influence of the melting point of milk fat and ambient temperature on the incidence of spontaneous rancidity of cow's milk. J. Dairy Sci. 50: 961-962.

Herrington, B.L. 1954. Lipase: a review. J. Dairy Sci. 37: 775-789.

Herrington, B.L. and Krukovsky, V.N. 1939. Studies of lipase action. I. Lipase action in normal milk. J. Dairy Sci. 22: 127-135.

Hileman, J.L. and Courtney, E. 1935. Seasonal variations in the lipase content of milk. J. Dairy Sci. 18: 247-257.

Jellema, A. 1975. Note on susceptibility of bovine milk to lipolysis. Neth. Milk Dairy J. 29: 145-152.

Jensen, R.G. 1964. Lipolysis. J. Dairy Sci. 47: 210-215.

Jensen, R.G. and Pitas, R.E. 1976. Milk lipoprotein lipases: a review. J. Dairy Sci. 59: 1203-1214.

Kason, C.M., Pavamani, I.V.P., and Nakai. S. 1972. Simple test for milk lipolysis and changes in rancidity in refrigerated pasteurized milk. J. Dairy Sci. 55: 1420-1423.

King, N. 1955. Microscopic appearance of fat on the milk surface as affected by mechanical disturbance of the surface. J. Dairy Res. 55: 328-335.

Kintner, J.A. and Day, E.A. 1965. Major free fatty acids in milk. J. Dairy Sci. 48: 1575-1581.

Kolar, C.W. and Mickle, J.B. 1963. Relationships between milk fat acidity, short-chain fatty acids, and rancid flavors in milk. J. Dairy Sci. 46: 569-571.

Kramer, A. 1969. The relevance of correlating objective and subjective data. Food Technol. 23: 926-928.

Kuzdzal-Savoie, S. 1980. Flavor impairment of milk and milk products due to lipolysis. VII. Determination of free fatty acids in milk and milk products. Int. Dairy Fed. Bull. 118: 53-66.

McDaniel, M.K., Sather, L.A., and Lindsay, R.C. 1969. Influence of free fatty acids on sweet cream butter flavor. J. Food Sci. 34: 251-254.

Noble, R.P. 1966. Automatic titration of plasma fatty acids by photocolorimetry. J. Lipid Res. 7: 745-749.

Novak, M. 1965. Colorimetric ultramicro method for the determination of free fatty acids. J. Lipid Res. 6: 431-433.

O'Mahony, M. 1979. Psychophysical aspects of sensory analysis of dairy products: a critique. J. Dairy Sci. 62: 1954-1962.

Ortiz, M.J., Kesler, E.M., Watrous, Jr., C.H., and Cloninger, W.H. 1970. Effect of the cow's body condition and stage of lactation on development of milk rancidity. J. Milk Food Technol. 33: 339-342.

Potter, F.E. and Hankinson, D.J. 1960. The flavor of milk from individual cows. J. Dairy Sci. 43: 1887.

Salih, A.M.A., Anderson, M., and Tuckley, B. 1977. The determination of short- and long-chain free fatty acids in milk. J. Dairy Res. 44: 601-605.

Scanlan, R.A., Sather, L.A., and Day, E.A. 1965. Contribution of free fatty acids to the flavor of rancid milk. J. Dairy Sci. 48: 1582-1584.

Senyk, G.F., Murphy, S.C., Barbano, D.M., and Shipe, W.R. 1985. Sources of high acid degree values in raw milk supplies. J. Dairy Sci. 68(Supplement 1): 73.

Senyk, G.F., Zall, R.R., and Wolff. E. 1982. Assessment of raw milk quality in New York State. Dairy and Food Sanitation. 2: 318-320.

Shipe, W.F. and Senyk, G.F. 1981. Effects of processing conditions on lipolysis in milk. J. Dairy Sci. 64: 2146-2149.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Morgan, M.E., Nelson, J.H., and Scanlan, R.A. 1978. Off flavors of milk: nomenclature, standards, and bibliography. J. Dairy Sci. 61: 855-869.

Shipe, W.F., Senyk, G.F., and Fountain, K.B. 1980. Modified copper soap solvent extraction method for measuring free fatty acids in milk. J. Dairy Sci. 63: 193-198.

Speer, J.F., Watrous, G.H., and Kesler, E.M. 1958. The relationship of certain factors effecting hydrolytic rancidity in milk. J. Milk Food Technol. 21: 33-37.

Tallamy, P.T. and Randolph, H.E. 1969. Influence of mastitis on properties of milk. IV. hydrolytic rancidity. J. Dairy Sci. 52: 1569-1572.

Tarassuk, N.P. and Frankel, E.N. 1955. The mechanism of activation of lipolysis and the stability of lipase systems of normal milk. J. Dairy Sci. 38: 438-439.

Tarassuk, N.P. and Henderson, J.L. 1942. Prevention of development of hydrolytic rancidity in milk. J. Dairy Sci. 25: 801-806.

Thomas, W.R., Harper, W.J., and Gould, I.A. 1954. Free fatty acid content of fresh milk as related to portions of milk drawn. J. Dairy Sci. 37: 717-723.

Thomas, E.L., Nielson, D.J., and Olson, Jr., J.C. 1955. Hydrolytic rancidity in milk - a simplified method for estimating the extent of its development. Am. Milk Rev. 17: 50-52, 85.

Wang, L. and Randolph, H.E. 1978. Activation of lipolysis I. distribution of lipase activity in temperature activated milk. J. Dairy Sci. 61: 874-880.

Willey, H.A. and Duthie, A.H. 1969. Evidence for existence of more than one type of rancid flavor. J. Dairy Sci. 52(Supplement 1): 277.

Woo, A.H. and Lindsay, R.C. 1983. Statistical correlation of quantitative flavor intensity assessments and individual free fatty acid measurements for routine detection and prediction of hydrolytic rancidity off-flavors in butter. J. Food Sci. 48: 1761-1766, 1771.

CHAPTER III

SELECTION AND TRAINING OF SENSORY PANELIST FOR THE EVALUATION OF LIPOLYZED MILK

ABSTRACT

Sensory evaluation of lipolyzed milk flavor is a complicated and difficult task. Reports of previous research involving sensory evaluation of lipolyzed milk flavor do not include details regarding selection and training of panelists. The choice of panelists and depth of training will influence outcome.

Laboratory-prepared lipolyzed samples served as an available source of lipolyzed milk. Duo-trio tests were used for panelist selection. Thirteen out of 28 panelists were selected based on their ability to identify the sample similar to the reference sample in at least four out of six duo-trio tests.

Panelists were trained to recognize common off-flavors in milk and lipolyzed milk of varying intensity and in the use of a line-scale scorecard. After six sessions of each, panelists were evaluated. Panelists improved in their ability to recognize common off-flavors in milk and milk samples of varying intensity of lipolysis. Subsequent performance evaluation using control laboratory-prepared lipolyzed samples indicated that, despite previous performance, some panelists

failed to perform consistently in identifying the lipolyzed sample.

Chemical evaluations of laboratory-prepared lipolyzed samples of varying intensity indicated inconsistency in their free fatty acids composition. Sensory panelists responded to the increasing intensity of laboratory-prepared lipolyzed samples while chemical methods were insensitive to such small changes in free fatty acid concentrations and perhaps interactions among free fatty acids.

INTRODUCTION

Lipolyzed milk flavor is characterized as "rancid", "goaty", "soapy", "butyric" and "bitter" (Shipe et al., 1978). Free fatty acids (FFA) of all lengths are released by the action of lipoprotein lipase on milk fat globules roughly to the proportion present in intact triglycerides (Bodyfelt et al., 1988). However, the shorter chain free fatty acids (C_4, C_6, C_8, C_{10} and C_{12}) are primarily responsible for the lipolyzed flavor (Scanlan et al., 1965). Past research on lipolysis in milk has concentrated on chemical evaluations and little has been reported, if any, of the details of the selection and training of sensory panelists.

Sensory response to milk flavor may be the most sophisticated instrument that has yet been reported in detail. Careful selection and training of sensory panelists in the

evaluation of lipolyzed milk flavor may be the key to successful research with this characteristic. Lipolysis is a common milk defect (Barnard and Moir, 1987; Senyk et al., 1985), and sensory panelists who can correctly identify the defect and its intensity are needed.

Most sensory evaluation of milk flavor involves only a few panelists or the use of an "expert". Recently, more information has been published regarding panel selection or training (Buenaventura et al., 1991; Connolly et al., Duncan et al., 1991; Scanlan et al., 1965; Shipe et al., 1978). Still, selection and training procedures are often briefly reported and without detail. Difficulties and opportunities encountered in the selection and training for sensory panelists in the evaluation of lipolyzed milk flavor will be reported.

MATERIALS AND METHODS

Initial Triangle Testing

Triangle tests were first used to select panelists. Testing consisted of a total of six triangles, each containing three samples of milk. Samples used were either lipolyzed or non-lipolyzed. The lipolyzed sample was prepared by blending a mixture of 100 mL commercially pasteurized-homogenized milk and 20 mL raw milk for 2 min at high speed using a Waring Commercial Blendor (Dynamics Corporation of America, New

Hartford, CT). The mixture was incubated at 4°C for 24 h and batch pasteurized at 66°C for 3.5 min. Following pasteurization, the mixture was cooled and added to 480 mL of commercially pasteurized-homogenized milk. The lipolyzed sample was refrigerated immediately and was consumed within 24 h after pasteurization. A non-lipolyzed sample was prepared. This was done by pasteurizing the same mixture of pasteurized-homogenized milk and raw milk immediately after blending. Samples (20 mL) were dispensed into clear plastic cups (Solo Cup Co., Urbana, IL) labelled with 3-digit random numbers, capped and stored at 14°C until ready to be evaluated. Storage was never more than 3 h.

Triangle samples were served in random order under white fluorescent lighting in individual sensory booths. Water and crackers (unsalted) were provided as rinses. A scorecard (Fig. III.1) was also provided. Prospective panelists were asked to identify the odd sample in each of the three triangles and describe any difference perceived. Three triangles were served per day with a total of six triangles conducted. Panelists were selected on the basis of their ability to identify the odd sample in at least four out of six triangles.

The initial triangle test provided information and experience that was applied to the subsequent procedures for sensory evaluation.

TRIANGLE DIFFERENCE TEST SCORECARD II

Name: _____ Panelist Number _____

Product: MILK

There are three samples in each of the three triangles for you to evaluate. Two of these samples are duplicates. Taste the samples in the order indicated and identify the odd sample. Rinse your mouth with water and a cracker between triangles.

	Code	Check odd sample	Describe the difference observed
Triangle 4	_____	_____	
	_____	_____	_____
	_____	_____	
Triangle 5	_____	_____	
	_____	_____	_____
	_____	_____	
Triangle 6	_____	_____	
	_____	_____	_____
	_____	_____	

Fig. III.1--Triangle scorecard used in the initial selection of sensory panelists.

Laboratory-prepared Lipolyzed Sample (LPLS)

Laboratory-prepared lipolyzed sample (LPLS) was prepared as a source of lipolyzed milk sample. A mixture of 100 mL pasteurized-homogenized milk and 20 mL raw milk was blended for 2 min using a Waring Commercial Blendor at high speed. The mixture was incubated at 4°C for 72 h and batch pasteurized at 66°C for 3.5 min. Following pasteurization, the mixture was cooled and added to 480 mL of pasteurized-homogenized milk. This served as stock LPLS (100%). Stock LPLS was refrigerated immediately and was always consumed within 24 h after pasteurization. Dilution of stock LPLS with pasteurized-homogenized milk was performed when needed to achieve mixtures of 25, 50, 75% LPLS.

Sensory Evaluation

Sensory evaluation criteria were established after initial triangle tests and prior to beginning the rest of the research. Samples were equilibrated in the dark at 17°C in 60-mL clear glass bottle with teflon-lined screw-caps (Baxter Healthcare Corporation, McGaw Park, IL). Samples were served in randomized order under white fluorescent lighting in individual sensory booths. Water and crackers (unsalted) were provided. Rinsing with water was required between samples with a waiting period of 30 s. However, use of crackers was optional. Panelists were asked to expectorate the samples.

Panelist Selection

Difficulties encountered with the triangle test prompted changes in some conditions and the use of a duo-trio test to select panelists. Thirty-milliliter samples were equilibrated at 17°C and 60-mL clear glass bottles were used. Two unknown samples labelled with 3-digit random numbers, and a reference sample labelled as "R" were presented. Panelists were asked to smell and taste the sample, and rinse their mouths with crackers (if desired) and water between samples. Panelists indicated the sample similar to the reference sample and described any difference perceived on the scorecard (Fig. III.2). Only three duo-trio sets were served per day to reduce panelist fatigue but a total of six were conducted. A non-lipolyzed sample was prepared similarly as the LPLS except that pasteurization was performed immediately after mixing raw milk and pasteurized-homogenized milk. Lipolyzed sample (100% LPLS) and the non-lipolyzed sample had equal chances of being the reference sample and the unknown samples. Panelists were selected if they could correctly identify at least four of six duo-trio sets.

Panelist Training

Panelist training was conducted in three steps: 1) recognition of common off-flavors in milk, 2) recognition of varying intensity of lipolyzed milk flavor, and 3) line-scale scorecard training. Four common off-flavors of milk are

DUO-TRIO DIFFERENCE SCORECARD

NAME: _____ Panelist: no._____

On your tray you have three sets of samples. In each set there is a reference sample labeled "R", and two other samples labeled with different codes. One is identical to R and the other is different than "R". Smell each sample and taste it. Which of the coded samples differs from "R"? Indicate below. You may retaste the samples as you need. Please use the water and crackers between sets to cleanse your palate. Expectorate samples and rinse water into the styrofoam cup.

	SAMPLES	CHECK ODD SAMPLE	DESCRIBE DIFFERENCE OBSERVED
SET I	_____	_____	
	_____	_____	_____
SET II	_____	_____	
	_____	_____	_____
SET III	_____	_____	
	_____	_____	_____

Fig. III.2--Duo-trio scorecard used in the selection of sensory panelists.

cooked, feed, light-oxidized and lipolyzed. "Cooked" milk flavor was prepared by heating 600 mL of pasteurized-homogenized milk at 80°C for 1 min. "Feed" milk was prepared by placing 600 mL of pasteurized homogenized milk in a glass bottle, uncapped and without contact in a polyethylene bag of silage. The milk sample and silage were in a closed environment at 4°C for six hours. Pasteurized-homogenized milk (600 mL) was exposed 150 mm from a white fluorescent light for six hours at 4°C to achieve "light-induced oxidation". Lipolyzed milk samples were prepared as described previously and the 100% LPLS was used.

In six group sessions, panelists were introduced to the four common milk off-flavors. Open discussion was conducted and the panelists evaluated labeled samples.

These training sessions were followed by another six sessions of training on lipolyzed milk of varying intensity. Panelists were introduced to samples of 0, 25, 50, 75 and 100% LPLS in capped one-quart (≈ 1 L) glass bottles and appropriately marked. Open discussion was again encouraged.

After 12 sessions of training on common milk flavor defects and on lipolyzed milk of varying intensity, panelists were introduced to the line-scale scorecard (Fig. III.3). Training on the use of scorecard began with the association of the five levels of lipolyzed flavor (0, 25, 50, 75, 100% LPLS) with the zero, quarter, half, three-quarter and full points, respectively, along the line-scale. Panelists were also

SENSORY EVALUATION OF RANCID MILK

Panelist I.D. ☐

You will first receive two reference samples: not rancid and rancid. These samples represent the ends of the scale. Please smell and taste the samples.

Next, you will be given five (5) samples of milk. Please shake the bottle, uncap the bottle and smell the sample. Taste the sample and record the degree of rancidity, if any, with reference to the anchor samples by drawing a vertical line on the scale corresponding to the sample code. Rinse your mouth with some water, chew some crackers if necessary, and wait 30 seconds before evaluating the next sample.

Sample:_____

Not Rancid _____ Rancid

Sample:_____

Not Rancid _____ Rancid

Sample:_____

Not Rancid _____ Rancid

Sample:_____

Not Rancid _____ Rancid

Sample:_____

Not Rancid _____ Rancid

Fig. III.3--Line scale scorecard used in the evaluation of lipolyzed milk samples. (The original line scale length was 15 cm).

advised concerning procedures for sensory evaluation, especially in rinsing, use of optional crackers, use of scorecard and tasting procedure. Due to the increasing complexity of samples involved, sample size was raised to 40 mL. For each sample, panelists were asked to uncap the bottle, smell and taste the sample, and follow with the rinsing procedure. Scorecard training lasted for another six sessions using 0, 25, 50, 75, and 100% LPLS.

Performance Evaluation

After the training sessions, panelist performance was evaluated. Tests were conducted to investigate the ability of the panelists to recognize the common milk off-flavors, and varying intensities of lipolyzed flavor. Additionally, during experimentation using the panelists, their continual performance with control LPLS in the presence of other milk samples was determined. Initially, panelists were asked to record the off-flavor of four milk samples in clear glass sample bottles identified with 3-digit random codes. At the same session, five samples of 0, 25, 50, 75 and/or 100% LPLS in glass sample bottles labelled with 3-digit random codes were presented. Panelists were asked to mark the scorecard knowing that the choices were at zero, quarter, half, three-quarter and full points on the line-scale. Data collected were used to evaluate the performance of panelists prior to their utilization in evaluation of pasteurized-homogenized

samples from the retail market and raw milk samples from the farm which were pasteurized and homogenized in our laboratory (Chapters IV AND V).

Control samples (50% LPLS) were presented among actual test samples in subsequent research. Also, samples of 0, 25, 50, 75 and 100% LPLS were randomly presented among test samples in subsequent research to achieve four replications.

Titration Method

A titration method for determining the extent of lipolysis has been developed by Christen and Shen (1991) as a modification of the methods of Dole (1956) and Noble (1966). FFA are extracted using a mixture of isopropyl alcohol, hexane and 0.1*N* sulfuric acid in a ratio of 40:10:1 (v/v/v). The extraction mixture (15 mL) was added to 5 mL of milk sample in a test tube. After mixing for 15 s using a Vortex, hexane (16.5 mL) and water (6 mL) were added. The test tubes were placed horizontally in a basket and were shaken for 15 min using a Garver shaker (Garver Mfg. Co., Union City, IN) at full speed. The upper hexane layer (20 mL) was titrated against 0.001*N* KOH in 95% ethanol to an endpoint of pH 11.30 using an Ag/AgCl glass body combination electrode (Fisher Scientific, Atlanta). Lauric acid was used to standardize the procedure. One-half milliliter of 0.005*N* solution lauric acid in hexane was added to 4.5 mL water in the extraction test tube. Extraction was performed as for the samples. A blank

consisted of 0.5 mL hexane and 4.5 mL water and was extracted as for the samples. Microequivalent (μeq) of FFA per milliliter of milk was determined using the following formula:

$$\frac{\mu\text{eq FFA}}{\text{mL milk}} = \frac{(A \times B) \times 1000}{\text{sample size (mL)}}$$

Where:

A = volume of KOH titrated - the volume of KOH titrated for the blank; *B* = normality of KOH as determined by standardization using lauric acid.

Gas-Liquid Chromatographic (GLC) Method

Free fatty acids were extracted according to the procedure described by Deeth et al. (1983). Modifications of the method included the conditioning of alumina at 173°C for 24 h prior to extraction. Also, pentanoic acid ($C_{5:0}$) was used as the only internal standard and was added into the formic acid-diisopropyl ether (6%, v/v).

Free fatty acids in diethyl ether were separated and quantified using an HP 5890 Series II gas chromatograph (Hewlett-Packard Co., Kennett Square, PA) equipped with 25 m × 0.32 mm × 0.5 μm HP FFAP column (Hewlett-Packard Co., Kennett Square, PA), an HP 7673 (Hewlett-Packard Co., Kennett Square, PA) automatic injector, HP 3365 ChemStation (Hewlett-Packard Co., Kennett Square, PA) software, and a flame ionization detector. Sample (3 μL of extract) was injected initially in the splitless mode with a purge time of 0.02 min.

After that time, the injection port reverted to split mode. Initial temperature was set at 110°C and temperature programming was 8°C/min for 10 min to 190°C, followed by 4°C/min for 6.25 min to 215°C, then the column was held at 215°C for 22 min. Nitrogen was used as the carrier gas at a flow rate of 25 mL/min. Injector and detector temperatures were 250°C and 260°C, respectively. Retention times and quantities of known FFA were determined and used to develop concentration equations to quantify free fatty acids from samples (Duncan and Christen, 1991). Individual and total FFA are expressed as μequivalent per milliliter of milk.

Statistical Analyses

Results from post-training evaluation of lipolyzed samples of varying degree of lipolysis were transformed into ranks. Panelists scores were first ranked as 1, 2, 3, 4 and 5 for those marked on the zero, quarter, half, three-quarter and full points of the line-scale. Mean ranks were then calculated.

Subsequent performance of panelists was evaluated using General Linear Models (GLM) (SAS Institute Inc., 1985). Least-squares means (LSMEAN) of sensory scores, titration value, individual FFA and total FFA were determined and reported. Pearson correlation coefficients were determined among sensory scores, titration values and total FFA. Significance was pre-established at α=0.05.

RESULTS AND DISCUSSION

Initial Triangle Testing

Initial testing using the triangle test resulted in selection of only 4 panelists out of 21 screened. Several conditions of sensory evaluation led to the unsuccessful trials. Feedback from the panelists showed that the 20-mL sample size was inadequate. Additionally, the plastic caps did not adequately retain the volatile components of the milk flavor. Comments by many panelists indicated the evaluation temperature of 14°C was too cold for evaluating fluid milk. Incubation of raw milk with pasteurized-homogenized milk for 24 h was insufficient to achieve adequate differences between lipolyzed and non-lipolyzed samples. The change from triangle test to duo-trio test was suggested to reduce the amount of re-tasting required, thus reducing panelist fatigue. This feedback was implemented during the duo-trio tests and subsequent sensory testing.

Panelist Selection and Training

Of the 28 panelists screened, 13 (3 males, 10 females) were selected based on ability to correctly identify at least four of six duo-trio sets. Comments on the scorecard also indicated that some of the selected panelists necessarily did not describe the lipolyzed sample/reference pair as "rancid" or "lipolyzed". Terms such as "sweet", "sour", "soapy",

"Parmesan cheese", "oxidized", "strange aftertaste" and "bitter" were used instead to describe the samples.

The ability of panelists to correctly identify common milk off-flavors improved with training (Table III.1). "Cooked" flavor was identified early in training and remained fairly consistent. Lipolyzed flavor was more difficult for the panelists. Percentage correct for lipolyzed flavor started low but improved during training. Similar results were obtained for feed and light-induced oxidized samples.

After each off-flavor recognition session, panelists also evaluated unknown samples containing either 0, 25, 50, 75 or 100% LPLS. Sensory scores were on the zero, quarter, half, three-quarter and full points of the line-scale, and were ranked as 1, 2, 3, 4, and 5 respectively. Mean ranks of each session are reported (Table III.2). During any session a correct response would be rank of 1, 2, 3, 4, 5 for 0, 25, 50, 75 and 100% LPLS, respectively. Improvement can be seen as panelists became familiar with the LPLS. In the last three sessions, consistency was established with almost perfect average performance. Panelists were capable of performing satisfactorily and actual milk samples were introduced.

Performance of panelists was continually evaluated in subsequent parts of the research. The first continuing evaluation was determination of the ability of panelists to recognize the control (50% LPLS samples) placed among unknown samples from the market and from the farm (Table III.3). A

Table III.1--Ability of panelists to correctly identify common milk off-flavors during eight sessions[1]

	Off-Flavor				
Session	Cooked	Lipolyzed	Feed	Light-induced oxidized	Average
	----------Percentage Correct----------				
1	92	54	38	46	58
2	92	50	50	58	63
3	100	69	77	69	79
4	78	56	56	56	62
5	64	73	55	55	62
6	89	78	78	67	78
7	90	60	90	70	78
8	89	100	89	89	92
Average	87	68	67	64	-

[1]n=17.

Table III.2--Mean ranks of 0, 25, 50, 75 and 100% laboratory prepared lipolyzed samples (LPLS)[1]

Session	LPLS (%)				
	0	25	50	75	100
	Mean Ranks[2]				
1	2.2	3.7	2.6	3.6	3.0
2	2.6	2.6	2.7	3.1	4.0
3	2.6	2.6	3.2	3.2	3.5
4	1.4	2.9	2.6	4.3	3.9
5	2.2	2.2	3.5	3.5	3.6
6	1.4	2.6	2.3	4.1	4.6
7	1.9	2.7	2.8	3.5	4.1
8	1.3	2.3	3.1	3.6	4.7

[1]n=17.
[2]An appropriate rank for 0, 25, 50, 75, and 100% LPLS would be 1, 2, 3, 4, 5 respectively.

Table III.3--Ability of panelists to recognize control sample (50% laboratory-prepared lipolyzed samples (LPLS)) as lipolyzed when samples were placed among authentic milk samples from the market or from the farm[1]

Panelist	Percentage recognizing 50% LPLS as lipolyzed when placed among:	
	Market Samples	Farm Samples
1	92	93
2	100	—[2]
3	67	53
4	100	93
5	33	41
6	89	—[2]
7	58	88
8	83	82
9	100	—[2]
10	100	75
11	33	41
12	100	73
13	75	41

[1]n=12 for market, n=18 for farm.
[2]Did not participate in this phase of research.

correct response was noted if the panelist marked a sensory score of more than zero on the line-scale. Panelists were evaluated in two separate situations; one where other samples were from the retail market and another where samples were from lab-scale homogenized-pasteurized farm milk (other results from these studies are reported in Chapters IV and V). Panelists performed consistently throughout the two phases. Panelists No. 5 and 11 performed poorly in both cases while panelist No. 8 had a tendency to assign higher lipolysis scores than the other panelists. Panelists 5, 8 and 11 were deleted from the LSMEAN sensory scores for all subsequent data. Since training of panelists does not guarantee their consistent performance, it may be more appropriate to conduct trained panels with larger numbers rather than using a few "trained" or "expert" panelists unless skill is adequately documented.

Panelists were served samples of 0, 25, 50, 75 and 100% LPLS among samples prepared from farm milk during that study (Chapter V). Results from these samples are shown in Table III.4. LSMEAN sensory scores indicate panelists were unable to differentiate among 25%, 50% and 75% or among 50%, 75% and 100% LPLS. Panelists were able to distinguish samples of equal to or more than 25% LPLS from the 0% sample. Sensory scores assigned correlated well with quantity of LPLS in the sample ($r=0.75$; $p<0.005$) (Table III.5).

Table III.4--Least-squares means (LSMEAN) of sensory scores, titration values, and total free fatty acids (Total FFA) values for laboratory-prepared lipolyzed samples (LPLS) of varying intensity[1]

LPLS	Sensory scores[2]	Titration values	Total FFA
		(μeq FFA/mL)	(μeq/mL)
0	0.8[a]	0.86[a]	0.87[a]
25	4.8[b]	1.36[a]	1.25[a]
50	5.9[bc]	1.29[a]	1.33[a]
75	7.3[bcd]	1.05[a]	1.55[a]
100	8.3[cd]	1.21[a]	2.47[a]

[1]n=4.
[2]On a line scale of 0="not rancid" to 15="rancid".
[abcd]LSMEAN within a column with different superscripts differ ($P<0.05$).

Table III.5--Pearson correlation coefficients (r) and p-values (p) among sensory scores, titration values, total free fatty acid (Total FFA) and amount of laboratory-prepared lipolyzed sample (LPLS)[1]

	Titration values		Total free fatty acids		LPLS level	
	r	p	r	p	r	p
Sensory scores	-0.05	0.83	0.25	0.32	0.78	0.00[2]
Titration values	-	-	0.17	0.51	0.07	0.78
Total FFA	-	-	-	-	0.49	0.04

[1]n=20.
[2]<0.005.

Titration Values

LSMEAN titration values were obtained for each mixture of LPLS (Table III.4). Titration values did not differ significantly among the various mixtures of LPLS and untreated milk. Additionally, the Pearson correlation coefficient between sensory scores and titration values for LPLS mixtures was not significant ($r=-0.05$; $p=0.8274$) (Table III.5). Inconsistency in stock LPLS (100%) may account for subsequent inconsistency among 25, 50 and 75% dilutions. Despite such inconsistency, the LSMEAN titration values for LPLS mixture was usually more than 1.00 while that of 0% LPLS (untreated pasteurized-homogenized milk), was less than 1.00 (Table III.4). Previous discussion of the sensory results indicated that the samples were increasingly lipolyzed with increasing LPLS concentration. Correlation between titration value and the concentrations of LPLS was poor ((Table III.5, $r=0.07$; $p=0.7803$), suggesting that the titration method was poor for LPLS. Willey and Duthie (1969) suggested that more than one type of lipolyzed flavor may exist. They suggested that lipolyzed flavor may be described as "sickening" or "unclean". The former is formed by mixing raw milk and pasteurized-homogenized milks, or by excessive agitation such as that by a Waring Commercial Blendor. The latter is formed by excessive foaming. Apparently, the titration method was unable to accurately measure the free fatty acids associated with the former type of lipolysis. When lipolysis resulting

from foaming was evaluated (Chapter V), different results were reported.

Gas-Liquid Chromatography

Total FFA of LPLS of varying intensity were not significantly different ($p<0.05$) (Table III.4). The relationship between total FFA and quantity of LPLS was significant but small ($r=0.49$; $p=0.0388$) (Table III.5). Mixtures of LPLS had increasing total FFA with increasing LPLS percentage but these were not significantly different (Table III.4). Correlation between total FFA and sensory scores was not significant ($r=0.25$; $p=0.3203$) (Table III.5), yet sensory evaluations indicated differences in lipolyzed flavor. These data support the data obtained by titration. Apparently, lipolysis induced by excessive agitation in a blender was difficult to measure chemically. Correlation between the total FFA and titration value was not significant ($r=0.17$; $p=0.5122$) (Table III.5). Few differences in individual free fatty acids concentration exist among LPLS of varying intensities (Table III.6). Although all fatty acids increased as amount of LPLS increased, significant differences were found between 0% LPLS and 100% LPLS only for the free fatty acids C_6, C_8, C_{12} and C_{15}. Again, this method of preparing lipolyzed milk samples was not readily measured chemically.

Table III.6--Least-squares means (LSMEAN) of individual free fatty acids at different concentrations of laboratory-prepared lipolyzed samples (LPLS)[1]

FFA	LPLS (%)				
	0	25	50	75	100
	----------μeq FFA/mL----------				
C_4	0.07	0.12	0.11	0.13	0.13
C_6	0.03[a]	0.06[ab]	0.06[ab]	0.07[b]	0.07[b]
C_8	0.02[a]	0.04[ab]	0.04[ab]	0.04[ab]	0.06[b]
C_{10}	0.03	0.04	0.04	0.05	0.08
C_{12}	0.02[a]	0.04[ab]	0.04[ab]	0.05[ab]	0.08[b]
C_{14}	0.07	0.11	0.12	0.12	0.20
C_{15}	0.00[a]	0.01[ab]	0.01[ab]	0.01[ab]	0.02[b]
C_{16}	0.23	0.33	0.38	0.38	0.76
C_{18}	0.16	0.18	0.21	0.27	0.51
$C_{18:1}$	0.20	0.31	0.32	0.35	0.49
$C_{18:2}$	0.02	0.01	0.01	0.08	0.06

[1]n=4.
[ab]LSMEAN within a row with different superscripts differ significantly ($p<0.05$).

CONCLUSIONS

The selection and training of sensory panelists serves as the base for successful research in the evaluation of lipolyzed milk. The process is long, involving training on the recognition of common milk off-flavors, lipolyzed milk of varying intensity, and usage of a line-scale scorecard. Though selected panelists may perform well during training sessions, these results are not a guarantee of consistent performance after training. Panelists' consistency may be a problem and should be investigated further.

Evaluation of LPLS indicates that although panelists were able to recognized samples of varying intensity, differences in intensities of lipolysis were not detected by the chemical methods. Sensory panelists may respond to interactions among free fatty acids or to slight changes in concentrations that can not be detected by chemical methods.

REFERENCES

Barnard, S.E. and Moir, L.M. 1987. Bacterial quality and flavor of store purchased milk samples in Pennsylvania. J. Dairy Sci. 70(Supplement 1): 74.

Bodyfelt, F.W., Tobias, J., and Trout, G.M. 1988. *The Sensory Evaluation of Dairy Products*. Van Nostrand Reinhold, New York.

Buenaventura, M.L., Smith, D.E., Villela, A.M., Tatini, S.R., and Reineccius, G.A. 1991. Keeping quality of fluid milk from various regions of the United States. Dairy Food and Environmental Sanitation. 11: 82-86.

Christen, G.L. and Shen, N. 1991. Comparison of methods to extract free fatty acids from milk. J. Dairy Sci. 74(Supplement 1): 130.

Connolly, J.F., Murphy, J.J., O'Connor, C.B., and Headon, D.R. 1979. Flavor impairment of milk and milk products due to lipolysis. VIII. Relationship between free fatty acid levels of milk and butter and lipolysed flavour. Int. Dairy Fed. Bull. 118: 67-76.

Deeth, H.C. and Fitz-Gerald, C.H. 1976. Lipolysis in dairy products: a review. Aust. J. of Dairy Technol. 31(2): 53-63.

Deeth, H.C., Fitz-Gerald, C.H., and Snow, A.J. 1983. A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. N. Z. J. Dairy Sci. Technol. 18: 13-20.

Dole, V.P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35: 150-154.

Duncan, S.E. and Christen, G.L. 1991. Sensory detection and recovery by acid degree value of fatty acids added to milk. J. Dairy Sci. 74: 2855-2859.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1991. Rancid flavor of milk: relationship of acid degree value, free fatty acids, and sensory perception. J. Food Sci. 56: 394-397.

Larmond, E. 1982. *Laboratory Methods for Sensory Evaluation of Food*. Publ. 1637, Canada Dept. Agric. Ottawa, Ontario.

Noble, R.P. 1966. Automatic titration of plasma fatty acids by photocolorimetry. J. Lipid Res. 7: 745-749.

SAS Institute Inc. 1985. *SAS/STAT Guide for Personal Computers, Version 6 Edition*. SAS Institute Inc., Cary, NC.

Scanlan, R.A., Sather, L.A., and Day, E.A. 1965. Contribution of free fatty acids to the flavor of rancid milk. J. Dairy Sci. 48: 1582-1584.

Senyk, G.F., Murphy, S.C., Barbano, D.M., and Shipe, W.R. 1985. Sources of high acid degree values in raw milk supplies. J. Dairy Sci. 68(Supplement 1): 73.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Morgan, M.E., Nelson, J.H., and Scanlan, R.A. 1978. Off flavors of milk: nomenclature, standards, and bibliography. J. Dairy Sci. 61: 855-869.

Willey, H.A. and Duthie, A.H. 1969. Evidence for existence of more than one type of rancid flavor. J. Dairy Sci. 52(Supplement 1): 277.

CHAPTER IV

EVALUATION OF RETAIL MILK SAMPLES FOR LIPOLYSIS

ABSTRACT

Evaluations of 47 samples of retail pasteurized-homogenized milk in the Knoxville, TN area showed that lipolysis in not a common problem. Sensory and chemical analyses suggest that the retail milk samples were similar to that for good quality milk. Mean sensory score for 47 samples was 1.4 (on a scale of 1=not rancid to 15=rancid). Total free fatty acids determined by titration and by gas-liquid chromatography were 0.60 and 0.71 μeq/mL, respectively

INTRODUCTION

The quality of fluid milk is often compromised by the presence of undesirable off-flavor(s). The flavor of milk is mild and is easily altered by microbial or enzymatic actions within the system. Lipolysis results from action of lipase on milk fat globules, releasing free fatty acids (FFA) (Bodyfelt et al., 1988). Scanlan et al. (1965) reported that even-numbered FFA between butyric acid and lauric acid (C_4, C_6, C_8, C_{10} and C_{12}) contribute most to the lipolyzed flavor.

The quality of retail milk in some regions of the United States has been studied. Lipolyzed flavor in milk was reported as common (Barnard & Moir, 1987) and more prevalent in winter (Buenaventura et al., 1991). A survey by Senyk et al. (1985) found a number of raw milk suppliers producing milk with extensive lipolysis. Lipolyzed milk flavor is objectionable to many consumers and lipolytic action results in processing difficulties and quality problems in the manufacture of milk products (Kirst, 1986).

The standard method for determining the extent of lipolysis is acid degree value (ADV) (Richardson, 1985). The relationship between ADV and lipolyzed milk flavor score has long been questioned (Duncan et al., 1990, 1991; Earley and Hansen, 1982; Rerkrai et al., 1987). Many methods have been proposed in place of the acid degree value (Deeth et al., 1975; Harper et al., 1956; Kason et al., 1972; Nakai et al., 1970; Shipe et al., 1980). Each has limitations which have prevented substitution for ADV. Additionally, each was found to correlate to some extent with ADV, whose reliability is now under question.

The objective of this study was to utilize a trained sensory panel to evaluate retail pasteurized-homogenized whole milk samples and to determine the relationship between lipolyzed flavor score and a titration method proposed by Christen & Shen (1991).

MATERIALS AND METHODS

Collection of Samples

Forty-seven (47) samples of pasteurized-homogenized whole milk were randomly purchased from retail stores in the Knoxville, TN area. These were in paperboard or pigmented plastic containers (1 pt, 1 qt, 1/2 gal, or 1 gal). Code dates showed samples of varying age. All samples were held at 4°C until evaluated.

Sensory Evaluation

A panel of 13 students and staff, 10 females and 3 males, participated in this study. They were selected and trained as discussed previously (Chapter III). Panelists were trained to utilize a line-scale scorecard (Fig. III.3) to indicate the level of lipolyzed milk flavor. Efficacy of training was reported (Chapter III). Panelists were presented with five test samples and two anchor samples. Samples were equilibrated to 17°C and served as described previously (Chapter III). The two anchor samples, namely 0% laboratory-prepared lipolyzed samples (LPLS) and 100% LPLS (Chapter III), were first smelled and tasted by the panelist to re-familiarize them with the ends of the line scale ("not rancid" and "rancid", respectively). These references thus served as the basis for evaluating other samples. Fifty milliliters of each reference sample was provided.

The five samples consisted of four retail samples and one control sample (50% LPLS). The inclusion of a 50% LPLS served partially as a basis for further evaluation of sensory panelists as described previously (Chapter III). Forty milliliters of each of the five milk samples were presented in random order in 60-mL clear glass bottles with teflon-lined screw-caps (Baxter Healthcare Corporation, McGaw Park, IL). The bottles were labelled with 3-digit numbers selected randomly. Panelists evaluated the flavor of milk directly from the glass bottles by first smelling the sample and then tasting it. The intensity of lipolyzed milk flavor, if any, was registered on the line scale with reference to the 0% and 100% anchors.

Titration Method

The titration method was completed as previously described (Chapter III) but the blank and standard were not included. Quantities of 0.001*N* KOH were recorded and used to estimate microequivalents of free fatty acids per milliliter of milk. The average blank value and KOH normality were calculated using data collected for Chapters III and V.

Gas-liquid chromatographic (GLC) Method

Extraction and quantitation of free fatty acids were performed as previously described (Chapter III).

Microbiological Analyses

Standard plate, coliform, lipolytic bacteria, proteolytic bacteria and psychrotrophic bacteria counts were performed by standard methods (Richardson, 1985) on all retail milk samples at least 24 h before sensory evaluation.

Statistical Analyses

Statistical analyses by least-squares means (LSMEAN), Pearson correlation coefficient and general linear models (GLM) procedures were performed using PC-SAS (SAS Institute Inc., 1985). LSMEAN of sensory scores, titration values, free fatty acids and total free fatty acids were determined. Pearson correlation coefficients among titration values, sensory scores and total FFA values were calculated. Correlation coefficients were also determined between individual FFA (C_4, C_6, C_8, C_{10}, C_{11}, C_{12}, C_{14}, C_{15}, C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$) concentrations and the above three variables.

RESULTS AND DISCUSSION

An average of 7.18 mL of 0.001*N* KOH was required to titrate the retail milk samples. This translates into approximately 0.60 μeq FFA/mL of milk (Table IV.1). Total FFA determined by GLC was 0.71 μequivalent/mL. The LSMEAN sensory score of the retail samples was 1.4 on a scale of 1-15, indicating they were not lipolyzed. The LSMEAN sensory

Table IV.1--A comparison between retail milk samples[1] and 0% laboratory-prepared lipolyzed samples (LPLS)

Sample type	Least-squares means		
	Sensory scores[2]	Titration value	Total FFA
		(μeq FFA/mL)	(μeq FFA/mL)
Retail milk	1.4	0.60[3]	0.71
0% LPLS[4]	0.8	0.86	0.87

[1]n=47.
[2]On a line-scale of 15 cm with 0 cm as "not rancid" and 15 cm as "rancid".
[3]Estimated titration value.
[4]Data from Chapter II.

score, estimated LSMEAN titration value and LSMEAN total FFA of the retail milk samples was similar to that of 0% LPLS (Table IV.1). The LSMEAN of individual FFA concentrations were converted to mole percentages and compared to quantities of total fatty acids in Canadian milk fat as reported in the literature (Breckenridge and Kuksis, 1968, 1969). Results were similar except that fatty acids with 18 carbons were slightly higher in the retail milk samples (Table IV.2). This comparison indicates that FFA in the retail milk samples were present approximately in the same proportion as they exist in the milk fat.

The titration values and sensory scores of retail milk samples were not correlated ($r=0.17$; $p=0.2504$) (Table IV.3). The titration method also was not correlated ($r=-0.24$; $p=0.1125$) with the total FFA (Table IV.3). Significant correlation ($r=0.57$; $p<0.005$) was found between sensory scores and total FFA (Table IV.3). The lack of correlation between the titration method and sensory results may be explained by the lack of range (0-4.74) in lipolyzed flavor scores for the retail milk samples (Kramer, 1969). Although the titration method and the GLC method were not significantly correlated (Table IV.3), both gave similar mean values for total FFA concentrations (Table IV.1).

Microbiological analyses of the retail samples revealed that most were within acceptable microbiological standard

Table IV.2--Means of free fatty acids (FFA) concentrations (mol %) in retail milk samples[1] compared to the literature[1]

FFA	Retail milk (mol %)	Literature[2] (mol %)
C_4	8.3	9.3
C_6	4.2	4.4
C_8	2.2	1.9
C_{10}	3.6	3.3
C_{12}	3.1	3.5
C_{14}	8.4	9.9
C_{15}	0.7	0.5
C_{16}	25.8	23.7
$C_{18:0}$	19.4	11.8
$C_{18:1}$	19.5	23.9
$C_{18:2}$	4.1	1.6
C_4-C_{12}[3]	21.5	22.4

[1]n=47.
[2]From Breckenridge and Kuksis (1968;1969).
[3]Sum of concentrations of free fatty acids between butyric acid and lauric acid (C_4, C_6, C_8, C_{10}, C_{12}).

Table IV.3--Pearson correlation coefficients (r) and p-values (p) among sensory scores, titration values and total free fatty acid of retail milk samples[1]

	Titration value		Total free fatty acids	
	r	p	r	p
Sensory score	-0.17	0.25	0.57	0.00[2]
Titration value	-	-	-0.24	0.11

[1]n=47.
[2]<0.005.

(data in Appendix). Two samples were slightly high in coliform count while one was much higher than 1 $\log_{10}$CFU/mL.

CONCLUSIONS

Sensory and chemical evaluations of retail milk samples suggest that lipolyzed flavor is not a common problem in the Knoxville, TN area. On the average, sensory scores, estimated titration values and free fatty acids resembled those of 0% LPLS (Chapter III). The titration method was not significantly correlated with sensory scores or total FFA. The lack of range in lipolysis in the retail milk samples may account for this. Total FFA were well correlated with sensory scores on these milk samples.

REFERENCES

Barnard, S.E. and Moir, L.M. 1987. Bacterial quality and flavor of store purchased milk samples in Pennsylvania. J. Dairy Sci. 70(Supplement 1): 74.

Bodyfelt, F.W., Tobias, J., and Trout, G.M. 1988. *The Sensory Evaluation of Dairy Products*. Van Nostrand Reinhold, New York.

Breckenridge, W.C. and Kuksis, A. 1968. Specific distribution of short-chain fatty acids in molecular distillates of bovine milk fat. J. Lipid Res. 9: 388-393.

Breckenridge, W.C. and Kuksis, A. 1969. Structure of bovine milk fat triglycerides: II. long chain lengths. Lipids 4: 197-204.

Buenaventura, M.L., Smith, D.E., Villela, A.M., Tatini, S.R., and Reineccius, G.A. 1991. Keeping quality of fluid milk from various regions of the United States. Dairy Food and Environmental Sanitation 11: 82-86.

Christen, G.L. and Shen, N. 1991. Comparison of methods to extract free fatty acids from milk. J. Dairy Sci. 74(Supplement 1): 130.

Deeth, H.C., Fitz-Gerald, C.H., and Wood, A.F. 1975. A convenient method for determining the extent of lipolysis in milk. Aust. J. Dairy Technol. 30: 109-111.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1990. Acid Degree Value - Does it really predict rancid flavor in milk? Dairy, Food and Environmental Sanitation 10: 715-718.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1991. Rancid flavor of milk: relationship of acid degree value, free fatty acids, and sensory perception. J. Food Sci. 56: 394-397.

Earley, R.R. and Hansen, A.P. 1982. Effect of process and temperature during storage of ultra-high temperature steam-injected milk. J. Dairy Sci. 65: 11-16.

Harper, W.J., Schwartz, D.P., and Hagarawy, E. 1956. A rapid silica gel method for measuring total free fatty acids in milk. J. Dairy Sci. 39: 46-50.

Kason, C.M., Pavamani, I.V.P. and Nakai, S. 1972. Simple test for milk lipolysis and changes in rancidity in refrigerated pasteurized milk. J. Dairy Sci. 55: 1420-1423.

Kirst, E. 1986. Lipolytic changes in the milk fat of raw milk and their effects on the quality of milk products. Food Microstructure 5: 265-271.

Nakai, S., Perrin, J.J. and Wright, V. 1970. Simple test for lipolytic rancidity in milk. J. Dairy Sci. 53: 537-540.

Rerkrai, S., Jeon, I.J., and Bassette, T. 1987. Effect of various direct ultra-high temperature heat treatments on flavor of commercially prepared milks. J. Dairy Sci. 70: 2046-2054.

Richardson, G.H. 1985. *Standard Methods for the Examination of Dairy Products*. 15th ed. American Public Health Association, Washington, DC.

SAS Institute Inc. 1985. *SAS/STAT Guide for Personal Computers, Version 6 Edition*. SAS Institute Inc., Cary, NC.

Scanlan, R.A., Sather, L.A., and Day, E.A. 1965. Contribution of free fatty acids to the flavor of rancid milk. J. Dairy Sci. 58: 1582-1584.

Senyk, G.F., Murphy, S.C., Barbano, C.M., and Shipe, W.R. 1985. Sources of high acid degree values in raw milk supplies. J. Dairy Sci. 68(Supplement 1): 73.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Morgan, M.E., Nelson, J.H., and Reveres, R.A. 1978. Off flavors of milk: nomenclature, standards, and bibliography. J. Dairy Sci. 61: 855-869.

Shipe, W.F., Senyk, G.F., and Fountain, K.B. 1980. Modified copper soap solvent extraction method for measuring free fatty acids in milk. J. Dairy Sci. 63: 193-198.

APPENDIX

Table IV.4--Microbiological data for retail milk samples

Sample Number	APC[1]	CC[2]	LBC[3]	SM[4]	PBC[5]
	----------log$_{10}$CFU/mL----------				
253	2.43	<0 Est.[6]	0.70	-[7]	-
520	2.40	0	0.30	-	-
532	4.54	<0 Est.	0.30	-	-
946	1.83	0	<0 Est.	-	-
059	1.51	<0 Est.	1.18	-	-
341	1.00	<0 Est.	1.15	-	-
603	4.07	1.41	3.78	-	-
916	1.48	<0 Est.	1 -	-	
015	3.58	<0 Est.	- -	<0 Est.	
506	1.62	<0 Est.	- -	<0 Est.	
830	1.64	<0 Est.	- -	<0 Est.	
969	>2.40 Est.	<0 Est.	- -	>4.40 Est.	
608	2.43	<0 Est.	>4.40 Est.	-	<0 Est.
282	1.59	<0 Est.	>4.40 Est.	-	<0 Est.
124	4.36	<0 Est.	>4.40 Est.	-	<0 Est.
115	>4.40 Est.	<0 Est.	>4.40 Est.	-	<0 Est.
077	>4.40 Est.	1.38	LE[8]	>4.40 Est.	>4.40 Est.
185	2.18	<0 Est.	LE	<1 Est.	<0 Est.
575	>4.40 Est.	<0 Est.	LE	<1 Est.	<0 Est.
102	1.88	<0 Est.	<3 Est.	<0 Est.	<0 Est.
664	1.81	<0 Est.	<3 Est.	1.49	<0 Est.
722	>4.40 Est.	3.46	>4.40 Est.	>4.40 Est.	>4.40 Est.
771	5.06	<0 Est.	<3 Est.	4.16	<0 Est.
828	1.68	<0 Est.	<0 Est.	1.76	<0 Est.
908	4.06	<0 Est.	<0 Est.	4.07	>4.40 Est.
946	2.03	<0 Est.	<0 Est.	1.98	2.51
981	1.86	<0 Est.	<0 Est.	2.18	<0 Est.
046	4.17	<0 Est.	<3 Est.	4.10	4.09
543	1.60	<0 Est.	<3 Est.	0.78	<0 Est.

Table IV.4-- (cont.)

Sample	APC	CC	LBC	SM	PBC
	----------$\log_{10}$CFU/mL----------				
598	>4.40 Est.	<0 Est.	6.10	>4.40 Est.	>4.40 Est.
762	>4.40 Est.	LE	>4.40 Est.	<0 Est.	>4.40 Est.
094	3.30	<0 Est.	<3 Est.	>4.40 Est.	<0 Est.
675	>4.40 Est.	<0 Est.	>4.40 Est.	>4.40 Est.	>4.40 Est.
727	2.56	<0 Est.	<3 Est.	0.60	<0 Est.
813	3.81 Est.	<0 Est.	<3 Est.	4.17	4.31
095	4.06	<0 Est.	<3 Est.	4.04	3.59
366	3.09	<0 Est.	<3 Est.	4.09	<0 Est.
432	2	<0 Est.	<3 Est.	2.28	<0 Est.
747	2.03	<0 Est.	<3 Est.	2.61	<0 Est.
130	2.41	<0 Est.	<3 Est.	2.48	2.43
210	>4.40 Est.	<0 Est.	>4.40 Est.	>4.40 Est.	>4.40 Est.
348	>4.40 Est.	<0 Est.	>4.40 Est.	>4.40 Est.	>4.40 Est.
533	3.35	<0 Est.	<3 Est.	3.40	3.23
137	3.64	<0 Est.	<3 Est.	4.49	3.62
179	3.98	<0 Est.	<3 Est.	3.92	3.73
223	3.80	<0 Est.	<3 Est.	3.88	3.65
725	2.79	<0 Est.	<3 Est.	2.76	2.52

[1] APC = aerobic plate count.
[2] CC = coliform count.
[3] LBC = lipoplytic bacteria count.
[4] SM = proteolytic bacteria count.
[5] PBC = psychrotrophic bacteria count.
[6] Est.= Estimate.
[7] - = test not completed.
[8] LE = laboratory error.

CHAPTER V

EVALUATION OF LABORATORY-PASTEURIZED FARM MILK FOR LIPOLYSIS

ABSTRACT

Raw milk samples (48) collected from six East Tennessee farms were homogenized and pasteurized in a laboratory scale system. Sensory evaluation, a titration method, and gas-liquid chromatography were utilized in the evaluation of samples for lipolysis. Samples were evaluated after one and fifteen days of storage at 4°C. Lipolyzed flavor was present at slight (2.1) and moderate (6.6) levels (on a scale of 1=not rancid to 15=rancid) for 1-day and 15-day samples, respectively.

Relationships among sensory scores, titration values and free fatty concentrations were also determined. At one day after processing, the titration method was significantly correlated with sensory scores, short-chain free fatty acid concentrations and total free fatty acid concentrations. Total free fatty acids were not correlated with sensory scores after one day. After fifteen days, more samples were lipolyzed and the flavor intensity of the samples may have been confusing to the panelists. Neither chemical methods correlated with the sensory score when data collected after

fifteen days were analyzed. The two chemical methods were correlated with one another. When all data was combined there was a significant relationship among sensory scores, titration values, and free fatty acid concentrations.

INTRODUCTION

Milk fat globules are protected by a membrane which may be disrupted by physiological or mechanical means, thus leading to the hydrolysis of triglycerides by lipoprotein lipase (Bodyfelt et al., 1988). Lipolyzed triglycerides result in the release of free fatty acids (FFA) and mono- and diglycerides (Kuzdzal-Savoie, 1980). Short-chain FFA between butyric and lauric acids are primarily responsible for the lipolyzed flavor but no clear conclusion has been drawn on the dominant acid(s) (Al-Shabibi et al., 1964; Bills et al., 1969; Scanlan et al., 1965). It has also been shown that the short-chain FFA may be preferentially lipolyzed (Jensen, 1964; Kuzdzal-Savoie, 1980).

Lipolysis is commonly measured by the acid degree value (ADV) as described in the *Standard Methods for the Examination of Dairy Products* (Richardson, 1985). Recent research has shown that ADV may not accurately measure the short-chain FFA and has a poor relationship to lipolyzed flavor (Bell and Parsons, 1977; Duncan et al., 1990, 1991). Christen (1990a, b) examined various methods for measuring lipolysis (Deeth et

al., 1975; Dole, 1956; Harper et al., 1956; Kason et al., 1972; Noble, 1966; Novak, 1965; Salih et al., 1977; Shipe et al., 1980). The methods of Dole (1956) and Noble (1966) were incorporated into a titration method that is safe and simple and provides good recovery of short-chain FFA (Christen and Shen, 1991).

While chemical methods may provide accurate measurement, they are limited to their specificity for the compounds and are not capable of measuring other compounds or interactions among compounds (Burgard and Kuznicki, 1990). Sensory evaluation provides the necessary human tool for evaluating a complex food system like milk (Bodyfelt et al., 1988). Evaluation of milk may be performed by various types of panelists such as "experts", trained panelists, or consumer panelists (Stone and Sidel, 1985). The latter lack any form of training while "experts" or trained panelists received some form of training (Stone and Sidel, 1985). The procedure for panelist training is, however, seldom reported in detail (i.e., the extent of training or methodology used), while the "experts" are often quoted in the literature without documentation of experience. Panelists must be carefully selected, trained, evaluated after training and continuously during use (Rainey, 1986). It may be possible for a trained panelist to perform poorly in the evaluation of milk if other flavors for which the panel has not been trained are present (Rainey, 1986).

Individual FFA may be quantified by gas-liquid chromatography (GLC) (Deeth et al., 1983). However, equipment is relatively expensive, and the FFA extraction procedure is tedious and time-consuming. Thus, it is unsuitable for routine evaluation of lipolyzed milk flavor. In the research laboratory, however, GLC is a useful tool for evaluation of both the sensory and the titration results.

The objectives of this study were to utilize a trained sensory panel in the evaluation of farm-collected laboratory-pasteurized milk and to determine the efficacy of the titration procedure of Christen and Shen (1991). Individual FFA concentrations were measured to determine what, if any, relationship they may have to sensory scores and titration results.

MATERIALS AND METHODS

Collection and Processing of Samples

Forty-eight (48) raw milk samples (≈ 3750 mL) were collected at intervals from bulk tanks of six East Tennessee farms and stored at 4°C until processed. Within 24 h, samples were warmed (10°C), then homogenized (2200 psi) and pasteurized for 15 s at 72-74°C in a laboratory-scale system (Wadsworth and Bassette, 1985). Samples were collected in glass milk bottles (≈ 1 L) for evaluation within 24 h and in five amber glass bottles (250 mL) for storage (Baxter

Healthcare Corporation, McGaw Park, IL). Phosphatase tests and coliform counts (Richardson, 1985) were performed on pasteurized samples within 24 h and prior to sensory evaluation. Phosphatase positive samples or those containing coliforms were excluded from further analyses leaving 43 samples. Samples collected in amber glass bottles were stored at 4°C for 15 da.

Sensory Evaluation

A panel of 10 students and staff, 8 females and 2 males, participated in this study. They were selected and trained as previously described (Chapter III). Sensory evaluation of the laboratory-pasteurized farm milk samples was completed as in Chapter IV after one day and after 15 days of storage.

Titration Method

The titration method was completed as previously described (Chapter III).

Gas-Liquid Chromatographic (GLC) Method

Extraction and quantitation of FFA were performed as previously described (Chapter III).

Other Tests on Raw Milk

Somatic cell count and protein and fat contents were determined electronically on raw milk samples by the Dairy

Herd Improvement Association Testing Laboratory, Knoxville, TN. Standard plate, coliform, lipolytic bacteria, proteolytic bacteria and psychrotrophic bacteria counts were performed according to standard methods (Richardson, 1985).

Statistical Analyses

Statistical analyses by least-squares means (LSMEAN), Pearson correlation coefficient and general linear models (GLM) procedure were performed using PC-SAS (SAS Institute Inc., 1985). Mean titration value, electronic somatic cell count ($\log_{10}$), protein content, fat content, standard plate count, coliform count, lipolytic bacteria count, proteolytic bacteria count, and psychrotrophic bacteria count (all bacteria counts were converted to $\log_{10}$ CFU/mL) were calculated for raw milk samples. For pasteurized milk samples, LSMEAN for sensory scores, titration values, individual FFA and total FFA were computed. Pearson correlation coefficients among titration values, sensory scores and total FFA values were calculated, as well as between concentrations of 12 FFA (C_4, C_6, C_8, C_{10}, C_{11}, C_{12}, C_{14}, C_{15}, C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$).

RESULTS AND DISCUSSION

Quality of Raw, 1-Day and 15-Day Milk

Means of the raw milk quality tests are reported in Table V.1 (individual farm test results are in the Appendix). The

Table V.1--Quality of raw milk collected from bulk tanks of six East Tennessee farms[1]

Quality criteria	Means±S.D.
Titration values (μeq FFA/mL)	0.75±0.85
Electronic somatic cell count ($\log_{10}$ CFU/mL)	5.70±0.20
Protein content (%)	3.21±0.17
Fat content (%)	3.50±0.26
Standard plate count ($\log_{10}$ CFU/mL)	4.03±0.71
Coliform count ($\log_{10}$ CFU/mL)	1.86±1.16
Lipolytic bacteria count ($\log_{10}$ CFU/mL)	3.22±1.06
Psychrotrophic bacteria count ($\log_{10}$ CFU/mL)	2.56±0.78
Proteolytic bacteria count ($\log_{10}$ CFU/mL)	3.43±0.63

[1]n=48.

samples were of normally reported quality (Jenness, 1988). The raw milk samples averaged 0.75 μequivalents FFA/mL (Table V.1) which is comparable to that of a non-lipolyzed milk or retail milk samples (Table V.2). Microbiological results were within expected limits.

LSMEAN sensory scores from 1-day samples was 2.1 (Table V.2), indicating the milk was slightly lipolyzed. This score was greater than that of the retail milk samples or 0% laboratory prepared lipolyzed samples (LPLS). Similarly, the titration value and total FFA were higher than those for the retail milk samples. The LSMEAN sensory scores for 1-day samples would place it in a range between 0 and 25% LPLS (Table 4, Chapter III). However, the FFA as determined by titration or GLC were much higher than 0% LPLS and similar to 25-100% LPLS. Our method of sample preparation was less effective in eliminating subsequent lipolysis than was evident in commercial samples.

The 15-day samples were highly lipolyzed, with LSMEAN sensory scores, titration value and total FFA of 6.6, 3.06 and 4.27, respectively (Table V.2). Compared to retail milk samples (Chapter IV), laboratory-pasteurized farm milk samples after 15 days at 4°C had a greater lipolyzed flavor. Sensory evaluation indicates a lipolysis intensity near that of 50% and 75% LPLS (Chapter III), while the titration values and total FFA were much higher than the 0-100% LPLS. The panelists were able to identify lipolyzed flavor in LPLS with

Table V.2--A comparison between farm milk samples (1-day and 15-day)[1], retail samples, 0 and 100% laboratory-prepared lipolyzed samples (LPLS)

Sample type	Least-squares means		
	Sensory scores[2]	Titration value	Total FFA
		(μeq FFA/mL)	(μeq FFA/mL)
Farm sample (1-day)	2.1	1.22	2.41
Farm sample (15-day)	6.6	3.06	4.27
Retail samples[3]	1.4	0.60[4]	0.71
0% LPLS[5]	0.8	0.86	0.87
100% LPLS[6]	8.3	1.20	2.47

[1]n=43.
[2]On a line-scale of 15 cm with 0 cm as 'not rancid' and 15 cm as 'rancid'.
[3]Data from Chapter IV.
[4]Estimated titration value.
[5,6]Data from Chapter III.

a lower titration value and total FFA. It was observed that when the farm samples were homogenized and pasteurized, excessive foaming occurred during sample collection. Willey and Duthie (1969) proposed the possible existence of two types of lipolyzed flavor. The first is the "sickening" type prepared by mixing raw and homogenized milk, churning, agitating, and altering the temperature from 4°C to 30°C and back to 4°C. The second, the "unclean" type is caused by foaming or spontaneous lipolysis. Duncan et al. (1991) suggested differences among LPLS and farm samples processed in a manner similar to this study. Results from this study thus support their findings.

Relationship Between Sensory and Chemical Methods

For the 1-day samples, the sensory scores are significantly related to the titration value ($r=0.51$; $p<0.005$) (Table V.3). Duncan et al. (1991) found that the correlation between ADV and the sensory scores of fresh samples prepared in a similar manner and evaluated within 48 h was low ($r=0.22$; $p=0.09$). The titration method appears to provide a more competent measurement of the intensity of lipolyzed flavor in milk. Titration values were significantly ($p<0.005$) correlated with specific FFA, i.e. caproic (C_6), caprylic (C_8), capric (C_{10}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}) and oleic (C_{18}) acids (Table V.4). The relationship between the

Table V.3--Pearson correlation coefficients (r) and p-values (p) among sensory scores, titration values and total free fatty acid (Total FFA) of farm samples evaluated on the first day[1]

	Titration value		Total free fatty acids	
	r	p	r	p
Sensory score	0.51	0.00[2]	0.08	0.62
Titration value	-	-	0.37	0.02

[1]n=43.
[2]<0.005.

Table V.4--Least-squares means (LSMEAN) of free fatty acids (FFA), and their Pearson correlation coefficients (r) and p-values (p) with titration values and sensory scores of 1-day farm milk samples[1]

		Pearson correlation			
		Titration value		Sensory score	
FFA	LSMEAN	r	p	r	p
C_4	0.13	0.26	0.11	0.14	0.41
C_6	0.06	0.38	0.02	0.19	0.24
C_8	0.06	0.36	0.03	0.19	0.26
C_{10}	0.07	0.33	0.04	0.12	0.48
C_{12}	0.08	0.32	0.05	0.12	0.47
C_{14}	0.20	0.37	0.02	0.07	0.68
C_{15}	0.02	0.25	0.12	0.11	0.49
C_{16}	0.66	0.39	0.01	0.07	0.66
$C_{18:0}$	0.46	0.28	0.09	0.03	0.84
$C_{18:1}$	0.55	0.48	0.00[2]	0.11	0.51
$C_{18:2}$	0.11	0.02	0.89	0.00[2]	0.99
C_4-C_{12}[3]	0.40	0.35	0.03	0.16	0.34

[1]n=43.
[2]<0.005.
[3]Sum of concentrations of free fatty acids between butyric acid and lauric acid (C_4, C_6, C_8, C_{10}, C_{12}).

titration value and the short-chain FFA is moderate and significant (Table V.4).

Relationships among total FFA, sensory scores, short-chain FFA and long-chain FFA were not significant (Table V.3-4). Similar observations were found by Duncan et al. (1991) with farm milk samples evaluated within 48 h after homogenization and pasteurization in a laboratory system. Their method of assigning lipolyzed flavor scores differed from this research. Bell and Parsons (1977) also found no relationship between FFA and lipolyzed flavor score of butter. LSMEAN concentrations of the short-chain FFA (Table V.4) were similar to that for the 100% LPLS (Chapter III, Table 6), yet the LSMEAN for the sensory scores differ vastly (Table V.2). This supports the earlier conclusion that lipolyzed flavor in authentic milk samples may be different from that in LPLS.

After 15 days of storage, the relationship between sensory scores and titration values was not significant ($r=0.27$; $p=0.10$) (Table V.5). On the other hand, titration values, total FFA and short-chain FFA showed significant and stronger correlation ($r=0.52$; $p<0.005$, and $r=0.49$; $p<0.005$, respectively) (Table V.5-6) compared to those for the 1-day samples (Table V.3-4). The titration method appears to be measuring the appropriate factors for lipolyzed flavor, i.e. changes in FFA concentrations. After 15 days of storage, samples evaluated showed signs of flavor development other than lipolyzed flavor (based on comments by panelists). Some

Table V.5--Pearson correlation coefficients (r) and p-values (p) among sensory scores, titration values and total free fatty acid (Total FFA) of farm samples evaluated after 15 days[1]

	Titration value		Total free fatty acids	
	r	p	r	p
Sensory score	0.27	0.10	0.21	0.21
Titration value	-	-	0.52	0.00[2]

[1]n=43.
[2]<0.005.

Table V.6--Least-squares means (LSMEAN) of free fatty acids (FFA), and their Pearson correlation coefficients (r) and p-values (p) with titration values and sensory scores of 15-day farm samples[1]

		Pearson correlation			
		Titration value		Sensory score	
FFA	LSMEAN	r	p	r	p
C_4	0.29	0.43	0.01	0.51	0.00[2]
C_6	0.15	0.49	0.00[2]	0.51	0.00[2]
C_8	0.14	0.49	0.00[2]	0.45	0.00[2]
C_{10}	0.16	0.45	0.01	0.40	0.01
C_{12}	0.16	0.46	0.00[2]	0.39	0.02
C_{14}	0.38	0.45	0.00[2]	0.31	0.06
C_{15}	0.06	0.40	0.01	0.03	0.86
C_{16}	1.11	0.44	0.01	0.13	0.45
$C_{18:0}$	0.62	0.35	0.03	-0.08	0.63
$C_{18:1}$	1.07	0.52	0.00[2]	0.15	0.36
$C_{18:2}$	0.13	0.36	0.03	-0.08	0.63
C_4-C_{12}[3]	0.90	0.49	0.00[2]	0.50	0.00[2]

[1]n=43.
[2]<0.005.
[3]Sum of concentrations of free fatty acids between butyric acid and lauric acid (C_4, C_6, C_8, C_{10}, C_{12}).

panelists may have distinguished the presence of strong lipolyzed flavor despite these other flavors while some failed to do so. Confusion was reported by panelists regarding the presence of two or more off-flavors in milk. Thus, lipolyzed sensory scores assigned to 15-day samples may not be accurate.

GLC analyses indicated a moderate relationship ($r=0.50$; $p<0.005$) between the short-chain FFA and sensory scores (Table V.6) although no significant correlation was present between total FFA and sensory scores (Table V.5). Samples with higher lipolyzed flavor scores also had higher concentrations of FFA. Duncan et al. (1991) found no correlation between lipolyzed flavor scores and individual FFA or group of FFA concentrations. They had no samples that were very lipolyzed or unpalatable, while the present study had a greater range of samples.

In order to obtain a wider range of lipolyzed samples, data from 1-day and 15-day samples were combined. Significant relationships between titration values and sensory scores ($r=0.51$; $p<0.005$), and between titration values and total FFA were observed ($r=0.55$; $p<0.005$) (Table V.7). Additionally, the titration values correlated well with all FFA except for $C_{18:0}$ and $C_{18:2}$ (Table V.8), indicating that given a wider range of data, the titration method was related to increases in most FFA in authentic lipolyzed milk samples. Total FFA determined by GLC and sensory scores were significantly correlated ($r=0.35$; $p<0.005$) (Table V.7). Short-chain FFA were also well

Table V.7--Pearson correlation coefficients (r) and p-values (p) among sensory scores, titration values and total free fatty acid (Total FFA) of all (1-day and 15-day) farm samples[1]

	Titration value		Total free fatty acids	
	r	p	r	p
Sensory score	0.51	0.00[2]	0.35	0.00[2]
Titration value	-	-	0.55	0.00[2]

[1]n=86.
[2]<0.005.

Table V.8--Least-squares means (LSMEAN) of free fatty acids (FFA), and their Pearson correlation coefficients (r) and p-values (p) with titration values and sensory scores of all (1-day and 15-day) farm samples[1]

		Pearson correlation			
		Titration value		Sensory score	
FFA	LSMEAN	r	p	r	p
C_4	0.21	0.54	0.00[2]	0.59	0.00[2]
C_6	0.11	0.61	0.00[2]	0.61	0.00[2]
C_8	0.10	0.59	0.00[2]	0.56	0.00[2]
C_{10}	0.12	0.56	0.00[2]	0.53	0.00[2]
C_{12}	0.12	0.53	0.00[2]	0.47	0.00[2]
C_{14}	0.29	0.55	0.00[2]	0.43	0.00[2]
C_{15}	0.04	0.48	0.00[2]	0.15	0.20
C_{16}	0.88	0.50	0.00[2]	0.30	0.01
$C_{18:0}$	0.54	0.33	0.00[2]	0.08	0.50
$C_{18:1}$	0.81	0.59	0.00[2]	0.33	0.00[2]
$C_{18:2}$	0.12	0.22	0.06	-0.00	0.99
C_4-C_{12}[3]	0.66	0.60	0.00[2]	0.59	0.00[2]

[1]n=86.
[2]<0.005.
[3]Sum of concentrations of free fatty acids between butyric acid and lauric acid (C_4, C_6, C_8, C_{10}, C_{12}).

correlated ($r=0.59$; $p<0.005$) with sensory scores (Table V.8). This agrees with the findings of Al-Shabibi et al. (1964), Bills et al. (1969), and Scanlan et al. (1965). Kramer (1969) emphasized the importance of obtaining good correlations among results using samples of normal range. The range used in the combined study of 1-day and 15-day samples is within the possible normal range of lipolysis and is appropriate for conclusions. The amount of variation in the dependent variable explained by the independent variable is r^2 (Kramer, 1969). Thus, the titration method significantly accounts for 26% of the changes in lipolyzed flavor. In contrast, ADV provided only 12% of the variations in lipolyzed flavor (Duncan et al., 1991).

CONCLUSIONS

Raw milk collected from six East Tennessee farms was of good quality. These samples, after processing in a laboratory scale system, were slightly lipolyzed after one day and moderately lipolyzed after 15 days of storage at 4°C. The titration method was correlated with other measures of lipolysis. Total FFA or groups of FFA were not related to the intensities of lipolyzed flavor after one day of storage but were after 15 days. The titration method correlated well with total FFA and especially with short-chain FFA. Sensory scores on the 15-day samples were confounded by the presence of other

strong off-flavors for which the panel had not been trained. Over a wide range of samples, sensory scores, titration values, total FFA, and short-chain FFA were significantly related.

In conclusion, the titration method was found to be more efficient than ADV in that it recovers the short-chain FFA more effectively and provided a more accurate picture of the lipolyzed flavor. Difficulties such as the dissimilarity of LPLS to authentically lipolyzed milk samples, and the presence of other off-flavors in high intensity may have misled the panelists and confounded the results. It is more important to evaluate the method in terms of authentic samples such as that of 1-day farm collected samples where the samples resemble those in the milk processing plants with slight lipolysis. Although further study is required, the titration method provides closer agreement with lipolysis flavor scores on authentic milk samples than does ADV.

REFERENCES

Al-Shabibi, M.M.A., Langner, E.H., Tobias, J., and Tuckey, S.L. 1964. Effect of added fatty acids on the flavor of milk. J. Dairy Sci. 47: 295-296.

Bell, L.I and Parson, J.G. 1977. Factors affecting lipase flavor in butter. J. Dairy Sci. 60: 117-122.

Bills, D.D., Scanlan, R.A., Lindsay, R.C., and Sather, L.A. 1969. Free fatty acids and the flavor of dairy products. J. Dairy Sci. 52: 1340-1345.

Bodyfelt, F.W., Tobias, J., and Trout, G.M. 1988. *The Sensory Evaluation of Dairy Products*. Van Nostrand Reinhold, New York.

Burgard, D.R. and Kuznicki, J.T. 1990. *Chemometrics: Chemical and Sensory Data*. CRC Press, Inc., Boca Raton, FL.

Christen, G. 1990a. Evaluation and development of chemical methods for measurement of lipolytic flavor of milk. National Dairy Board. Semi-Annual Progress Report, July 9th. Arlington, VA.

Christen, G. 1990b. Evaluation and development of chemical methods for measurement of lipolytic flavor of milk. National Dairy Board. Semi-Annual Progress Report, November 27th. Arlington, VA.

Christen, G.L. and Shen, N. 1991. Comparison of methods to extract free fatty acids from milk. J. Dairy Sci. 74(Supplement 1): 130.

Deeth, H.C., Fitz-Gerald, C.H., and Wood, A.F. 1975. A convenient method for determining the extent of lipolysis in milk. Aust. J. Dairy Technol. 30: 109-111.

Deeth, H.C., Fitz-Gerald, C.H., and Snow, A.J. 1983. A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. N. Z. J. Dairy Sci. Technol. 18: 13-20.

Dole, V.P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35: 150-154.

Duncan, S.E. and Christen, G.L. 1991. Sensory detection and recovery by acid degree value of fatty acids added to milk. J. Dairy Sci. 74: 2855-2859.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1990. Acid Degree Value - Does it really predict rancid flavor in milk? Dairy, Food and Environmental Sanitation. 10: 715-718.

Duncan, S.E., Christen, G.L., and Penfield, M.P. 1991. Rancid flavor of milk: relationship of acid degree value, free fatty acids, and sensory perception. J. Food Sci. 56: 394-397.

Harper, W.J., Schwartz, D.P., and Hagarawy, E. 1956. A rapid silica gel method for measuring total free fatty acids in milk. J. Dairy Sci. 39: 46-50.

Jenness, R. 1988. Composition of Milk. Ch. 1, In *Fundamentals of Dairy Chemistry*, N.P. Wong, R. Jenness, M. Keeney, and E.H. Marth (Eds.), pp. 1-38. Van Nostrand Reinhold Company Inc., New York.

Jensen, R.G. 1964. Lipolysis. J. Dairy Sci. 47: 210-215.

Kason, C.M., Pavamani, I.V.P., and Nakai. S. 1972. Simple test for milk lipolysis and changes in rancidity in refrigerated pasteurized milk. J. Dairy Sci. 55: 1420-1423.

Kramer, A. 1969. The relevance of correlating objective and subjective data. Food Technol. 23: 926-928.

Kuzdzal-Savoie, S. 1980. Flavor impairment of milk and milk products due to lipolysis. VII. Determination of free fatty acids in milk and milk products. Int. Dairy Fed. Bull. 118: 53-66.

Noble, R.P. 1966. Automatic titration of plasma fatty acids by photocolorimetry. J. Lipid Res. 7: 745-749.

Novak, M. 1965. Colorimetric ultramicro method for the determination of free fatty acids. J. Lipid Res. 6: 431-433.

Rainey, B.A. 1986. Importance of reference standards in training panelists. J. Sensory Studies 1: 149-154.

Richardson, G.H. 1985. *Standard Methods for the Examination of Dairy Products*. 15th ed. American Public Health Association, Washington, DC.

Salih, A.M.A., Anderson, M., and Tuckley, B. 1977. The determination of short- and long-chain free fatty acids in milk. J. Dairy Res. 44(3): 601-605.

SAS Institute Inc. 1985. *SAS/STAT Guide for Personal Computers, Version 6 Edition*. SAS Institute Inc., Cary, NC.

Scanlan, R.A., Sather, L.A., and Day, E.A. 1965. Contribution of free fatty acids to the flavor of rancid milk. J. Dairy Sci. 48: 1582-1584.

Shipe, W.F., Senyk, G.F., and Fountain, K.B. 1980. Modified copper soap solvent extraction method for measuring free fatty acids in milk. J. Dairy Sci. 63: 193-198.

Stone, H. and Sidel, J.L. 1985. *Sensory Evaluation Practices*. Academic Press, Orlando, FL.

Wadsworth, K.D. and Bassette, R. 1985. Laboratory-scale system to process ultra-high temperature milk. J. Food Protect. 48: 530-531.

Willey, H.A. and Duthie, A.H. 1969. Evidence for existence of more than one type of rancid flavor. J. Dairy Sci. 52(Supplement 1): 277.

APPENDIX

Table V.9--Chemical and microbiological analyses of raw milk collected from individual farms[1]

Analyses	Farm Number					
	1	2	3	4	5	6
Fat[2]	3.31	3.52	3.46	3.54	3.76	3.39
Protein[2]	3.23	3.16	3.38	3.35	3.11	3.01
ESCC[3]	5.91	5.83	5.68	5.49	5.80	5.51
SPC[4]	4.17	3.66	4.21	4.52	4.00	3.64
LBC[5]	3.39	2.71	3.09	3.39	3.38	3.33
PBC[6]	3.70	3.32	3.20	3.87	3.52	2.98
CC[7]	1.50	1.32	1.68	3.01	2.34	1.30
PSBC[8]	2.63	2.55	2.87	3.43	2.40	1.74
Titration value[9]	0.78	1.54	0.45	0.22	1.20	0.32

[1]n=48.
[2]Percent (%).
[3]ESCC = electronic somatic cell count ($\log_{10}$ CFU/mL).
[4]SPC = standard plate count ($\log_{10}$ CFU/mL).
[5]LBC = lipolytic bacteria count ($\log_{10}$ CFU/mL).
[6]PBC = proteolytic bacteria count ($\log_{10}$ CFU/mL).
[7]CC = coliform count ($\log_{10}$ CFU/mL).
[8]PSBC = psychrotrophic bacteria count ($\log_{10}$ CFU/mL).
[9]Microequivalent (μeq) of FFA per milliliter of milk.

VITA

Chow-Ming Lee was born in Taiping, Perak, Malaysia on November 24, 1969. He received his elementary education in the Sekolah Kebangsaan Chung Hwa Wei Sin (Malaysia) and graduated from Maris Stella High School (Singapore) in December, 1985. Prior to coming to America, he attended the Temasek Junior College (Singapore) briefly and continued his post-secondary education at the University of Tennessee, Knoxville in 1987. Three years later, he received the degree of Bachelor of Science in Food Technology and Science and was partially Americanized. He continued his post-baccalaureate studies at the University of Tennessee in August, 1990 and graduated in August, 1992 with a Master of Science degree in Food Technology and Science.