CHAPTER I

INTRODUCTION

1.1 Problem Definition

Milk is an essential diet for human. For countless generation it has formed an important part of man's diet, not only for the infants but also for every age. Milk is often described as a complete food because it contains protein, carbohydrate in form of lactose (the milk sugar), fats, vitamins and minerals.

In Thailand, The dairy cows have been domesticated for a long time before World War II. At that time, most dairy breeds were *Bos indicus* that could produce a little raw milk and had short period of milking. After World War II, a new breed of dairy cow that entered Thailand was Holstein-Friesian, which could produce a lot of raw milk over a long period of milking, but it had weak health in hot climate. Later on, the farmers bred Holstein-Friesian to local breeds. The calves are healthier, have long period of milking and produce higher quantity of raw milk. However, quantity of raw milk produced in Thailand is insufficient to the consumer's demand. The government, there, allows import milk from foreign countries. Table 1.1 shows the consumer's demand of raw milk, drinking milk, and consumption rate of drinking milk in Thailand, 1999-2005.

Department of Livestock and related organizations were developed and continuously supported to raise dairy cows in order to increase milk production and decrease milk import. Until 2004, milk production has increased sufficiently to produce drinking milk with some excess for exporting to neighbor countries. Milk products in Thailand should meet international standard. The best method to increase milk production is to improve both quality and quantity of feedstuff, dispose of hygiene of dairy farm, look after of cow health as well as, other factors affecting to milk composition and yield. However, Thailand is located in the tropical area where the quality of feedstuff is low and variable, consequently the milk composition varied seasonally.

Fresh milk composition and properties are not always exactly the same. The main factors affecting the compositions and the properties of the milk are cow species, breed, individuality, health, milking efficiency, milking frequency, milking intervals, milking yield, feeds, climate, lactation stage, cow age, number of lactation, estrus, gestation, cow illness, and mastitis.

Table 1.1 Consumer's demand of drinking milk in Thailand, 1999-2005.

Year	Demand of raw milk (Tons)	Quantity of raw milk for manufactory (Tons)	Needy raw milk (Tons)	Consumption of drinking milk (Tons)	Consumption rate of drinking milk
1999	537,637	445,933	91,704	550,150	8.90
2000	596,895	499,310	97,585	572,460	9.17
2001	627,769	564,200	63,569	602,070	9.57
2002	679,740	633,885	45,855	651,910	10.19
2003	703,510	702,646	864	674,700	10.46
2004	796,120	797,730	-1,310	763,526	12.03
2005	840,730	868,800	-28,070	806,312	12.63
% increase	7.52	11.93	-44.47	6.78	6.19

Source: Office of Agricultural Economics, 2005

The milk composition from different cows varies enormously. In order to control quality of milk, many organizations have determined standard for fresh whole milk. The characteristics of good quality milk are flavor/odor and nutritive value (milk composition). Quality of good milk is a very bland with a slightly sweet taste, very little odor and a smooth, rich feel in the mouth. The origin of milk flavor/odor is volatile components in milk, as nutritive value depends on types and amounts of milk composition (protein and fat, etc.). Therefore, the method that determines volatile components and fatty acids will points to milk quality in Thailand.

In analysis of volatile components, because of their concentrations are low, so sample preparation and preconcentration techniques are necessary, for example, headspace sampling, dynamic headspace (purge and trap), Solid phase microextraction (SPME). The analysis of fatty acids in milk is complicated, because milk contains a great number of components. Liquid-Liquid Extraction (LLE) has been used in the extraction of milk fat from milk matrix. Gas chromatography is commonly selected used as the analytical technique for volatile components and fatty acids. Nevertheless, fatty acids have low sensitivity for GC analysis, leading to necessity the fatty acids derivatization to their ester derivatives, in order to increase the sensitivity of GC analysis.

1.2 Literature Review

The study of volatile components and fatty acids was studied by several researchers including the development and comparison of sample preparation techniques for analysis of volatile components in milk, flavor and off-flavor in milk, effect of diet on flavor and fatty acids in milk, and, etc.

1.2.1 The Literature Review for the analysis of volatile components

Gordon, D.T. and Morgan, M.E. (1972) *(1)* studied volatile compounds in milk. Twenty-two compounds were detected in milk distillates. Eighteen compounds were identified by gas-liquid chromatography (GLC) retention times, selective pre-column qualitative reaction, and mass spectrometry. Quantitative analysis of six major compounds contributed to feed flavor were methyl sulfide, acetone, butanone, ethanol, 1-propanol, and 2-propanol in the concentration range of 25 to 45 ppb, 3.6 to 4.8 ppm 0.5 to 1.0 ppm, 10 to 20 ppm, 1.2 to 2.5 ppm and 0.3 to 0.4 ppm, respectively.

Bendall, G.J. (2001) *(2)* extracted volatile compounds from fresh milk produced by New Zealand cows, using the newly developed solvent-assisted flavor evaporation (SAFE) technique. The two samples that were used came from cows that had been fed on different diets. Using gas chromatography-olfactometry (GC-O), 71 aroma compounds were found from the milk extracts, 66 of which were identified. All of the aroma compounds were common to both extracts. Only one compound, γ-12:2

lactone, was significantly odor active for the extract of milk from cows fed by a supplement diet, but was not found for the differences in milk flavor which are primarily caused by concentration differences of a common set of flavor compounds, rather than by the occurrence of compounds uniquely associated with a particular feed.

Bendall, J.G. and Olney, S.D. (2001) *(3)* determined the concentration of hept-cis-4-enal in fresh milk. Hept-cis-4-enal concentrations were found at the low to medium picogram per gram range, and increased during refrigerated storage. There are many other aroma compounds present in fresh milk, which also contribute towards milk flavor. Therefore, the contribution that hept-cis-4-enal makes toward the flavor of fresh homogenized/pasteurized milk was considered extremely important. Milk samples with 61 and 212 pg/g of added hept-cis-4-enal were more intense for tallow flavor than the control sample of unadulterated milk. The milk with 425 pg/g of added hept-cis-4-enal was also more intense in tallow flavor, but less creamy, less grassy, and less sweet than the control sample.

Contarini, G., Povolo, M., Leardi, R., and Toppino, P.M. (1997) *(4)* studied the influence of heat treatments (pasteurization, direct ultrahigh-temperature method, and in-bottle sterilization) on volatile compounds of milk. Using dynamic headspace capillary gas chromatography coupled with multivariate statistical techniques was applied to distinguish milks. Volatile components were acetone, 2-butanone, 2-pentanone, 2-heptanone, 3-methylbutanal, pentanal, hexanal, heptanal, dimethy disulfide, toluene, and limonene. The origins of volatile compounds were: acetone and 2-heptanone had both 2 natural and 2-pentanone and 2-heptanone had both a natural and a technological; 3-methylbutanal could be found in raw milk as a consequence of microbial growth of *Streptococcus lactis* var. *maltigens,* but its presented in heated milk is due to nonenzymic browning reaction of leucine. The other aldehydes were formed in raw milk and heat treatment by autoxidation of unsaturated fatty acids. Dimethyl disulfide was found in raw milk as a consequence of feed while it's presence in heat-treated milk due to the oxidation of methanethio. In raw milk, toluene were a product of degradation of β-carotene, limonene was transferred from forages. Moreover, it was found the heat treatment did not significant the affect the milk volatile components.

Valero, E., Sanz, J., Castro, I.M. (1999) *(5)* studied the effect of heating modes on volatile components in raw milk. Raw milk was heated in different condition: at 120°C using closed vessel and at 70-80°C using a continuous flow device. In order to comparise volatile composition, milk was heated by microwave and by conventional heating method (a glycerol bath or a water bath). The volatile components were quantified by GC and identified by GC-MS. Similar volatile components were detected from both heating systems. In continuous flow treatment, the difference among treatments was slight and not significant because of the low thermal production of volatiles, which has been proved to give reproducible and uniform temperatures. However, the differences are quantitative and seem to be dependent on the heating intensity.

Valero, E., Villamiel, M., Miralles, B., Sanz, J., and Castro, I.M. (2001) *(6)* studied the changes in flavor and volatile components during storage of whole and skimmed UHT milk by purge and trap coupled to GC-MS instrument. The samples were stored at 25 ± 2°C for 4 months. Non-casein nitrogen (NCN) and sensorial analyses were carried out on packs opened every month. Volatile compositions were analyzed every 15 days. NCN was found to increase during stages, especially in skimmed milk samples. Sensory characteristics were slightly better in the whole samples. Quantification of about 40 volatile components in whole milk samples showed no change upto 90 days; the main change was the increase of methyl ketones. New volatile components appeared in skimmed samples after 65 days storage; they could be related to both proteolysis and Maillard reaction.

Kim, Y.D. and Morr, C.V. (1996) *(7)* used dynamic headspace technique for analysis of light activated flavor (LAF) in milk. The samples stored in pyrex test tubes and exposed to 200 foot-candles of fluorescent light for 6 to 48 hours at 0-5°C, with and without headspace during storage. Major volatile compounds were recovered from more than twelve hours light exposed milk. The compounds are products of photosensitization and decomposition of riboflavin and oxidation of lipids and sulfur compounds and included hexanal, pentanal, dimethyl disulfide, 2-butanone, and 2-propanol. A trained sensory panel was unable to detected LAF after 6 or 12 hours

light–exposure. However dynamic headspace was able to detect increasing trends of pentanal and hexanal.

Whited, L.J., Hammond, B.H., Chapman, K.W., and Boor, K.J. (2002) *(8)* determined the effects of light oxidized flavor in milk. The samples of whole, reduced fat, and non fat milk were exposed to fluorescent light (either 1000 or 2000 lx) at time intervals of 2, 4, 8, or 16 hours. Measurable vitamin A losses occurred at 2, 4 and 16 hours at 2000 lux. Moderate light-oxidized flavors were detected after 4 hours of light exposure in the whole and reduced fat milk and after 8 hours in nonfat milk. The presence of milk fat appears to protect against vitamin A degradation but adversely affects the flavor quality of milk after exposure to light. Nutritional value and flavor quality was reduced by light exposure.

Marsili, R.T. (1999) *(9)* studied off-flavors in milk by solid phase microextraction, mass spectrometry, and multivariate analysis (SPME-MS-MVA). A 1 m × 0.25 mm deactivated fused-silica column used instead of an analytical column to transfer volatiles extracted from milk samples with a carboxen-PDMS fiber to the mass spectrometer. Principal component analysis based on SPME-MS-MVA provided rapid different ratio of control reduced-fat milk (2% butter fat content) samples from which were abused by light, heat, copper, and microbial contamination. The three psychotropic bacteria studied included: *Pseudomonas fluorescens, Pseudomonas aureofaciens,* and *Pseudomonas putrefaciens.* The major metabolites found in the microbially abused milk were dimethyl sulfide, dimethy disulfide, 2-pentanone, 2-heptanone, 2-nonanone, 2- undecanone, 3-methybutanal, 2-pentanol, 2-nonanol, furfuryl alcohol, acetic acid, butyric acid, hexanoic acid and octanoic acid. Some volatiles commonly found in control milk were actone, 2-butanone, limonene, toluene, xylene isomers, styrene, cholroform, trimethylbenzene.

Urbach, G. (1987) *(10)* described the dynamic headspace method for investigation of volatile compounds in milk. The volatiles were removed from milk by a stream of helium and trapped on 40 mg of NIOSH charcoal. They were thermally desorbed and re-trapped on Tenax-GC coated with 1% OV–275 as GC packing at room temperature. The volume of 10 mL milk was recommended as it gave higher recovery and better chromatographic separation than the use of 100 mL volume. The

addition of potassium carbonate increased the yield of volatiles from 100 mL milk but not from 10 ml milk. These conditions trapped over 90% of the quantity of the lowest boiling compounds swept from the milk such as acetaldehyde and ethanol, and retained 100% of the total quantity of acetone, propanol and higher boiling compounds, from the gas stream.

Imhof, R. and Bosset, T.O. (1994) *(11)* applied a standard addition method to quantified volatile organic compound in pasteurized milk and fermented dairy products. Thirty-three volatile organic compounds was analyzed and defected by a combination of dynamic headspace analysis and GC-MS. Fifteen compounds could be quantified as significantly present in pasteurized milk, include, 2-propanol, 1-propanol, 2-butanone, 2,3–pentanedione, pentanal, penten-2-one-4-ol, toluene, hexanal, ethylbenzene, heptanal, benzaldehyde, octanal, nonanal, decanal, and undecanal.

Marsili, R.T. and Miller, N. *(12)* determined the cause of off-flavor development in milk by dynamic headspace GC/MS and multivariate data analysis. Free fatty acids were analyzed in control samples and abused samples. The abused conditions were exposition to light, copper, and sanitizer, at various levels. The abused samples provided the basis for KNN and SIMCA classification modeling. Results showed that multivariate analysis could accurately predict the type of sample abused responsible for off-flavors.

Friedrich, J.E. and Acree, T.E. (1998) *(13)* analyzed the aroma of dairy products by gas chromatography-olfactometry (GC/O). Seven common odorants were found in four different types of raw milk: dimethyl sulfone, ethyl butanoate, ethyl hexanoate, heptanal, indole, nonanal, and 1-octen-3-ol. The effect of heating cows milk changed the aroma profile of the milk, resulting in the formation of four common odor-potent compounds: hexanal, 2-nonanane, benzothiazole, and δ-decalactone. Fermented dairy products were found four common odorants: 1-octen-3-one, methional, 3-methylbutanal, and butyric acid.

Capone, S., Epifani, M., Quaranta, F., Siciliano, P., Taurino, A., Vasanelli, L. (2001) *(14)* reported the use of semiconductor thin films based electronic nose to recognize the rancidity of two different kinds of milk (UHT and pasteurized) during their ageing days. The employed sensor array consists of five different SnO_2 thin films prepared by means of the sol-gel technology. Comparative analysis making use to traditional analytical techniques like GC/MS should be performed at this stage in order to recognize and quantify the volatile compounds. These measurements performed day by day will allow identifying what was changing in the headspace and what is related to the increasing of rancidity. The possibility to use instrumentation for the control of food properties having low cost, good reliability and reduced size can find many important applications in many food industries.

Contarini, G. and Povolo, M. (2002) *(15)* validated the results obtained previously by purge and trap *(13)* and investigate the ability of solid phase microextraction (SPME). The result was validated to be very satisfactory, that is applicable by different operators at different times, and the statistical model was also reliable for milk sample, produced from different factories. The yield of extraction by the two techniques was dependent upon the molecular weight of compounds. The purge and trap was shown to be able to better extract the compounds having a smaller weight (acetone and 2-butanone). Conversely, the SPME technique, tested with a three-phase fiber (divinylbenzene/carboxen/polydimethylsiloxane), provided better recovery of those compounds having a higher carbon number (2-heptanone, 2-nonanone, and 2-undecanone). Both techniques seemed to have comparable recovery of 2-pentanone. The same behavior was also observed for mean value. Similar precision of both methods were compare by coefficients of variation.

1.2.2 The Literature Review for Fatty Acid Analysis

Kennelly, J.J. (1996) *(16)* studied the influence of feeding oilseed on the fatty acid composition of milk fat. The long-chain fatty acids of dietary origin could be in corporate directly into milk fat. The opportunity was existed to alter the ratio of short and long-chain fatty acids as well as the degree of saturation of milk fat. The fatty acid composition of milk with amenable to alteration by the feeding of oilseed was illustrated by cows fed protected lipid supplements containing linseed oil & safflower

oil. It was found that produced milk containing in excess of 30% and 20% respectively of C18:2 and C18:3, in contrast, milk from control cows contained only 3% and 1% respectively. The influence of feeding graded levels of canola seed (unprocessed whole canola seed; WCS, ground canola seed; GCS and a protected lipid supplement; Protec®), indicated that feeding of Protec® did significantly increase C18:2 and C18:3 concentrations compared to WCS or GCS diets. Indeed, the C16/C18 ratio in milk from cows feed 60 g/kg GCS was actually lower than that observed for 60 g/kg Protec®.

Chilliard, Y., Ferlay, A., and Doreau, M. (2001) *(17)* summarized the known effects of forages, animal fats or marine oils on bovine milk fat secretion and composition. Milk fat from pasture fed cows seemed to have higher in linolenic acid than milk fat from cows receiving preserved grass or maize. But the magnitude of this difference was limited. Indirect comparisons showed that milk fat from maize silage diets is richer in short-chain fatty acid and linoleic acid when compared to grass silage diets. Compared to fresh grass, grass silage favors myristic and palmitic at the expense of mono-and polyunsaturated fatty acid, including conjugated linoleic acid (CLA). The potential of marine oil supplementation to increased the mean CLA content in cow milk fat. A specific role for dietary C20:5 (n=3) in the sharp decreased milk fat secretion after fish oil supplementation.

Collomb, M., Butikofer, U., sieber, R., Jeangros, B., and Bosset, J.O. (2002) *(18)* studied the relationships between fodder plants and the fatty acid composition of milk fat in lowland (600 – 650 m), mountain (900 – 1210 m) and highland (1275 – 2120 m) areas of Switzerland. The intake of different plant families and species could be related to the fatty acid composition and the concentration of fatty acid in milk fat. If the relatively low levels of unsaturated fatty acids in the milk fat from the lowlands are due to the relatively high production of endogenous fatty acids in the mammary gland. Nevertheless, the increase in the content of trans-vaccenic acid and CLA as a function of a high rate of biohydrogenation was occurred in the rumen of the cows grazing in the Highlands. This effect could be related to a higher content of polyunsaturated fatty acids in the fat of the plant species in the highlands. Furthermore, Collomb, M. et al (2002) *(19)* determined the composition of fatty acids

in milk from lowland, mountain and highland. The largest relative increases as a function of the altitude of these three sites were those of the concentration of conjugated linoleic acids (0.87 , 1.61, and 2.35 g / 100 g), especially of the cis (c) 9 – trans (t) 11 isomer (0.81, 1.50 and 2.18 g/100 g), and the fatty acid C18:1 t10 + t11 (2.11, 3.66 and 5.10 g / 100 g).

Secchiari, P., Antongio vanni, M., Mele, M., Serra, A., Buccioni, A., Ferruzzi, G., Paoletti, F., and Petacchi, F. (2003) *(20)* studied the effect of the four different fat sources on milk yield and composition and on the quality of milk fat in term of safety for the consumer health, with particular attention to trans fatty acids and to conjugated linoleic acid isomers (CLA). The four fats were: toasted full fat soy bean diet; WS, toasted full fat linseed diet; WL, calcium soap of palm oil diet; PS, and calcium soap of olive oil diet; OS. Diet OS was induced the highest milk yield, while PS diet gave the highest milk content of both saturated and medium chain fatty acids. Saturated to unsaturated ratio of milk fat was decreased when cows were fed WS diet, while the ratio was increased with cows fed on PSdiet.

Wiking, L., Bjorck, L., and Nielsen, J.H. (2003) *(21)* studied the influence of feed composition on stability of fat globules during pumping of raw milk. The different fatty acid compositions; one high in saturated lipids, another high in unsaturated lipids and the last one simulating high *de novo* synthesis, was resulted in milk with fat contents of 5.0%, 3.7% and 4.0%, respectively. The milk fat globules (MFGs) were significantly larger in the milk with the highest fat content. The fat globules were more unstable at a pumping temperature of 31 °C compared with lower temperatures. Likewise, an increase was found in free fatty acids for milk containg the largest MFGs, indicating that milk with a high fat content was more stable when exposed to mechanical stress.

Collomb, M., Sollberga, H., Butikofer, U., Sieber, R., Stoll, W., and Schaeren, W. (2004) *(22)* studied the impact of a basal diet of hay and fodder beet supplemented with rapeseed, linseed and sunflower seed on the fatty acid composition of milk fat. A control diet was comprised of hay ad libitum and 15 kg fodder beet, supplemented with either 1 kg ground rapeseed (RAP1), 1 or 1.4 kg ground linseed (LIN1 or LIN 1.4), or 1 or 1.4 kg ground sunflower seed (SUN1 or SUN 1.4). The content of fatty

acids (FAs) was determined using high resolution gas chromatography. The concentration of saturated fatty acids (SFAs) in milk fat from cows fed the control diet was very high and decreased as a function of increased dairy intake of oleic, linoleic and α-linolenic acids of the oilseeds. The highest concentrations of unsaturated fatty acids (UFAs) and conjugated loinoleic acids (CLAs) were found in milk from cows fed the SUN 1.4 diet. A supplement of linseed was induced the highest content of α-linolenic acid in milk fat. Sunflower seed was the best of the three oilseeds for counter balancing the high content of SFAs and the relatively low content of UFAs in milk fat.

Elgersma, A., Ellen, G., Van der Horst, H., Boer, H., Dekker, P.R., and Tamminga, S. (2004) *(23)* evaluated the effects on milk composition of transition from a fresh grass diet on pasture to a winter diet of mixed grass/maize silage. After transition, the average milk fat content was increased from 43.7 g/kg on day 0 to 54.9 g/kg on day 14 and the milk fatty acid composition altered within two days. The largest changes occurred in the proportions of C14:0 (From 89 g/kg milk fat on day 0 to 117 g/kg on day 14), C16:0 (226-348 g/kg), C18:0 (110-88 g/kg), and C18:1 cis-9 (238-170 g/kg). The average content of conjugated linoleic acid (CLA) was decreased from 24.3 g/kg milk fat on day 0 to 4.4 g/kg on day 14. The milk fatty acid profile of grazing cows was more favorable from a consumer health perspective than that of silage-fed cows.

Schmidt, A., Gabisch, C.S., and Bilitewski, U. (1996) *(24)* used microbial biosensor for free fatty acids. A microbial biosensor base on thick film technology was developed. The microorganisms, *Arthrobacter nicotianae*, were immobilized in Ca-alginate directly on the electrode surface. The respiratory activity of microorganisms was monitored by oxygen consumption at –600 mV vs. Ag/AgCl reference electrode. The sensor was used in a batch system and was applied to the determination of free fatty acids in milk. The sensor showed linearity over the concentration range 9.5-165.5 μM (r^2 = 0.9992). The response time of the sensor was approximately 3 min. No additional dialysis membrane was necessary, which led to a high sensitivity of the sensor and fast response times.

Gonzalez-Cordova, A.F., and Vallejo-Cordoba, B. (2001) *(25)* established a methodology for the quantification of short-chain free fatty acids (FFA) (C_4-C_{12}) in milk by solid-phase microextraction and gas chromatography. FFA extraction consisted of placing 40 ml of milk containing 28% NaCl at pH 1.5 in sealed vial and equilibrating for 30 min at 70°C. A polyacrylate fiber was exposed to the sample headspace for 60 min and desorbed for 5 min into the gas chromatograph. Calibration curves for FFA were followed linear relationships with highly significant ($P < 0.001$) correlation of less than 7.7% for FFA concentrations indicated that the technique was reproducible. The limits of quantification for butyric acid, caproic acid, caprylic acid, and capric acid were in the low parts per million level, which were below the concentration range found in fresh pasteurized milk (0.48-2.52 ppm) or rancid milk (4.73-32.31 ppm).

1.3 Purpose of the Study

From literature review, several volatile components are contributed to milk flavor. In this work, 7 volatile components were studied, that include acetone, butanone, butyric acid, caproic acid, caprylic acid, capric acid and lauric acid.

Recently, Thai farmers preferred to raise diary cows because of higher profit. However, some tends to aim toward high profit and willing to sacrifice yield over milk quality. Dairy farms are usually cotains a lot of cows, individual cow and many factors are key contributing to milk quality. In this study, factor affecting milk quality such as flavor/odor (volatile components) and fatty acid (especially, essential fatty acid) were studied.

In this study, solid phase microextraction (SPME) was parameter such as SPME fiber, sample volume, extraction temperature, and extraction time were studied. Quantitative analyses of some volatile components in cow milk were studied by SPME, gas chromatography (GC) equipped with a flame ionization detector (FID). Qualitative and quantitative of fatty acid in cow milk was also analyzed by gas chromatography coupled to mass spectrometer (GC/MS). As stated, many causes are contributed to different milk quality and milk composition. In this work some

influential factors such as number of lactation, stage of lactation, feed and farm environment were studied. Moreover, this work will trend to development of milk production in Thailand.