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Comparison of the physical, chemical and sensory properties of butters made from milks differing in their atherogenicity index

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Comparison of the physical, chemical and sensory properties of butters made from milks differing in their atherogenicity index

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INTRODUCTION

Dietary factors are believed to be linked with the incidence of coronary heart disease (CHD), which in most instances is due to obstruction of coronary vessels by atherosclerosis or thrombosis, singly or in combination. An index of atherogenicity (IA) was proposed by Ulbricht and Southgate (1991) to evaluate the athrogenic ability of a given diet. The fatty acid composition of the food must be determined to evaluate its potential effect on atherosclerosis, which could lead to CHD.

One important source of dietary fat in America is butter. The IA values of the milk used to make butter vary along with the season and the species of the cows. The purposes of my research work are:

1. Make butters from high-IA, low-IA, and bulk-tank milks; test the IA of these butter samples.
2. Evaluate the texture and flavor differences of high-IA, low-IA, and bulk-tank butters.
3. Obtain the fatty acid profiles and triacylglycerol (TAG) profiles of these butter samples, both complete and after fractionation by silver-ion thin-layer chromatography (TLC).

LITERATURE REVIEW

Index of atherogenicity

"Atherosclerosis is an insidious disease characterized by the development, over 30-40 years, of fibrofatty lesions in the intimal lining of large and medium-sized arteries, most typically in the coronary circulation" (Griffin, p163, 1998). One direct result of atherosclerosis is the development of coronary heart disease (CHD). Coronary arteries could suffer an impaired blood supply due to the occlusion of their lumen and a predisposition to thrombosis, which can lead to fatal myocardial infarction. According to Ulbricht and Southgate (1991), seven dietary factors contribute to atherosclerosis and CHD. Among these factors, two are promotive in the development of CHD – cholesterol-raising saturated fatty acids (SFA), and thrombogenic SFA. The other five factors protect against CHD. They are polyunsaturated fatty acids (PUFA) of the n-6 (linoleic) acid series, PUFA of the n-3 (linolenic) acid series, monounsaturated fatty acids (MUFA), dietary fiber, and antioxidants. CHD, as manifested in myocardial infarction, is the effects of two or more of those factors. In order to take into account the effect of the first five of the above factors, an index of atherogenicity (IA) has been proposed so as to allow comparison of different foods and diets for their potential in the development of atherosclerosis.

A great deal of epidemiological and experimental evidence indicates that a diet rich in SFA is associated with high levels of serum cholesterol, which in turn is correlated to high incidences of CHD (Becker et al., 1983; Mattson et al., 1985; Schaefer et al., 1981;

Shepherd et al., 1978). Elevated levels of serum cholesterol, especially of low density lipoprotein (LDL) cholesterol, appear to be important in atheroma, oxidized LDL is believed to be taken up by macrophages and deposited in the arterial plaques (Goldstein et al., 1979; Nagelkerke et al., 1983; Parthasarathy et al., 1986). Rajman et al. (1994) indicated LDL particle size and density have great effects on atherogenicity of circulating LDL particles. Smaller LDL particles are more easily oxidized, penetrate intima more easily due to a smaller particle diameter, and may have greater atherogenic potential.

SFA do not contribute equally to hypercholesterolemia. Some work indicated that diets high in stearic acid (C18:0) do not raise serum cholesterol (Horliek, 1959; Hegsted et al., 965; Keys et al., 1965; Bonanome et al., 1988). The data, obtained in several species (dog, rat hamster, and rabbit) all show that C18:0 or glycerides are less efficiently absorbed and when fed with cholesterol are less cholesterolemic and atherogenic than are fats containing lauric, myristic, or palmitic acids (Kristchevsky, 1994). Secondly, short chain SFA (C10:0 and shorter) do not raise blood cholesterol. Thus, the putative atherogenic SFA are C12:0 (lauric), C14:0 (myristic), and C16:0 (palmitic), which was recognized by Keys et al. in 1965. Nicolosi (1998) found C14:0 caused a much more significant rise in plasma total cholesterol compared with C8:0 and C18:1. Hegsted thought that C14:0 was the most atherogenic, with about four times the cholesterol-raising ability of C16:0. Thus, the ratio of PUFA to SFA (the P/S ratio) which is often used as an indicator or measure of whether a diet is atherogenic or CHD-

promoting, is obviously inappropriate, because not all SFA are in fact hypercholesterolemic and their atherogenic potential varies.

PUFA can be divided into two categories, the n-6 and n-3 series, depending on whether the double bond counting from the methyl end of the fatty acid is located at the C-6 or C-3 respectively. Humans are incapable of synthesizing n-6 and n-3 PUFA de novo, so these fatty acids must be taken from the diet. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are the principal essential unsaturated fatty acids in vegetable oil. They can be elongated and desaturated into longer-chain derivatives by most animals: arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3).

The n-6 fatty acids have long been known for their ability in lowering serum cholesterol (Ulbricht et al., 1989). However, diets high in these PUFA depress the LDL and high-density lipoproteins (HDL). But HDL are protective against CHD (Shepherd et al., 1978; Martson et al., 1985; Grundy, 1986). Crouse (1989) pointed out that a high level of LDL is not a risk factor for women, whereas a low level of HDL is; therefore, diets that reduce both types of lipoprotein will not benefit women.

α–Linolenic acid, C18:3, is the principal PUFA occurring in the green tissue of plants. In animals, α-linolenic acid is converted to a series of longer-chain PUFA, of which the most important are EPA (C20:5), and DHA (C22:6). The former is the precursor of the series three prostanoids. These fatty acids occur in animal tissues. In ruminants, the amounts of dietary n-3 PUFA are diminished by hydrogenation in the rumen, and the amount of n-3 PUFA will also depend on the relative proportions of

dietary forage and other feedstuffs in their diets. α–Linolenic acid is also synthesized in phytoplankton. So, EPA and DHA are found in fish. The only concentrated edible sources of these PUFA are oily fish and the oils derived from them. Lately there has been a great interest in these PUFA (Anonymous, 1988; Herold et al., 1986). Former work indicated that diets containing fish oil lowered LDL and total serum cholesterol but not HDL. It now appears that total cholesterol is reduced by fish consumption by depression of LDL, only if initial levels of cholesterol and LDL are high. At the same time, HDL is increased and triacylglycerols decline (Saynor, 1988). In fact, depression of LDL concentrations is due to a reduction in very-low-density lipoprotein (VLDL) and this in turn may be due to a reduction in the rate of synthesis of apolipoprotein B (apo B). Apo B (a principal component of LDL) may be the primary influence on atherogenesis. While high levels of apo(a), a genetic trait, increase the likelihood of thrombosis of the resulting atheromatous plaques (Anonymous, 1991). Thus, the n-3 PUFA are antiatherogenic but act differently from the n-6 acids.

Recently, it was found that diets high in MUFA are as effective as those rich in PUFA in lowering serum cholesterol, but in contrast to the effect of n-6 PUFA, MUFA do not lower HDL (Marston et al, 1985; Grundy, 1986; Mensink et al., 1989; Wardlaw et al., 1990). In the analysis and discussion of the Seven Countries Study, Keys *et al* stated that there was a negative correlation between MUFA consumption (and also the MUFA/SFA ratio) and death rate. The overall CHD death rates were the lowest in cohorts with olive oil consumption (Keys et al., 1986).

As was pointed out earlier, the PUFA/SFA ratio is not suitable for measuring the atherogenicity of a diet or foods. To arrive at a reasonable IA, Ulbricht and Southgate (1991) inverted the PUFA/SFA ratio, so that the IA would be highest for the most atherogenic dietary components. The lower chain length SFA and stearic acid should be omitted from "SFA", for reasons previously stated, and "PUFA" was broadened to include the MUFA. The ratio therefore becomes:

$$IA = \frac{aS'+bS''+cS'''}{dP + eM + fM'}$$

Where S′ = C12:0, S″ = C14:0 and S‴= C16:0; P = sum of n-6 and n-3 PUFA; M = C18:1; and M′ = sum of other MUFA. a-f are empirical constants; b has been set at 4, for reasons already given; and a, c, d, e, and f have been provisionally set at unity because we have no firm evidence to assign other values. The results are shown in Table 1.

In Table 1, the value of IA for the British diet is near unity, and one can readily see that increasing the consumption of dairy products would make the diet more atherogenic. Consuming more palm oil or lamb would have little effect, and eating more of other meats or hard margarine would reduce the IA. Eating more PUFA margarine or fish would reduce IA considerably, and increased sunflower and olive oil consumption would reduce the atherogenicity of our diet most of all.

Ulbricht and Southgate (1991) pointed out the most obvious possible errors in the proposed index are:

Table 1. Indices of Atherogenicity and Indices for Some Foods and Diets

	Index of atherogenicity (IA)
Coconut oil[a]	13.63
Milk, butter, cheese	2.03
Palm oil	0.88
Lamb:	
Roast breast, lean & fat	1.00
Chop, lean only	1.00
Beef:	
Topside roast, lean	0.72
Topside roast, lean[b]	0.70
Raw mince	0.72
Grilled sausages	0.74
Pork:	
Roast leg, lean	0.60
Grilled sausages	0.58
Fried steaky bacon, lean & fat	0.69
Hard margarine (veg oils only)	0.56
Stewed ox liver	0.41
Chicken, roast, meat & skin	0.50
PUFA margarine	0.35
Olive oil	0.14
Sunflower oil	0.07
Raw mackerel	0.28
Eskimo diet[c]	0.39
Danish diet[c]	1.29
British diet[d,e]	0.93

a. All data from ref Paul et al. (1980) unless otherwise stated.
b. Data from Prof. M. Crawford (1987).
c. From Bang et al. (1980)
d. From Bull et al. (1983)
e. Later date show a small reduction (Dr. O. H. Buss, unpublished)

1. If there is confirmation of the work showing that in human C18:0 acts like oleic acid in reducing LDL, then C18:0 should appear in the denominator.
2. The PUFA have been weighted equally, but this can hardly be correct. Although n-6 PUFA are more antiatherogenic that the n-3 PUFA, there is also a difference between the parent members of the two series, linoleic acid and linolenic acid, as

well as their longer chain homologues, such as AA, EPA, and DHA, which occur in meat or fish. The parent compounds are poorly converted in human whereas their homologues are much more efficiently utilized. It is probable, therefore, that the values in Table 1 exaggerate the beneficial effects of unsaturated vegetable oils and margarines compared with meat and fish.

3. No allowance has been made for the effects of *trans* fatty acids because of conflicting evidence.

Fatty acid composition of butterfat

The composition of milk fat is somewhat complex. Although dominated by triacylglycerols, which constitute some 98% of milk fat (with small amounts of diacylglycerols, monoacylglycerols, and free fatty acids), other lipid classes are also present in measurable amounts. It is estimated that about 500 separate fatty acids have been detected in milk lipids, and it is probable that additional fatty acids remain to be identified. Of these, about 20 are major components, and the remainder are minor and occur in small or trace quantities (Morrison, 1970; Jensen, 1973). Other lipid components in milk include phospholipids, cerebrosides, and sterols (cholesterol and cholesteryl esters), small amounts of fat-soluble vitamins (mainly A, D, and E), antioxidants (tocopherol), pigments (carotene), and flavor components (lactones, aldehydes, and ketones). The composition of the lipids of whole bovine milk is given in Table 2 (Morrison, 1970; Jensen, 1973).

Table 2. Composition of lipids in whole bovine milk

Lipid	Weight Percent
Hydrocarbons	Trace
Triacylglycerols	97-98
Diacylglycerols	0.28-0.59
Monoacylglycerols	0.016-0.038
Free fatty acids	0.10-0.44
Free sterols	0.22-0.41
Phospholipids	0.2-1.0

In milk, triacylglycerols are normally defined with respect to their carbon number (CN), i. e., the number of fatty acid carbon atoms present in the molecule. The three carbon atoms of the glycerol moiety are ignored. Because the fatty acid spectrum of milk fat is dominated by acids containing an even number of carbon atoms, so is the triacylglycerol spectrum. However, the proportion of triacylglycerols with odd carbon number is about three times greater than the proportion of odd-numbered fatty acids. Although an obvious correlation exists between fatty acid composition and triacylglycerol distribution, detailed information is lacking that would enable the triacylglycerol distribution to be predicted from the fatty acid composition. Much more needs to be understood of the strategy used in the bovine mammary gland in assembling a complex array of fatty acids into triacylglycerols. This is not an arcane study; it is necessary if processes such as fractionation are to yield products with consistent qualities throughout the year. In effect, the detailed structure of milk fat is not yet understood. Perhaps this is not surprising for if we consider only the 15 major fatty acids; there are 15^3 (3375) possible triacylglycerol structures using a purely random model.

The great variety of fatty acids in butterfat cannot be treated in detail here. Reference will be made to only a few of the many available reports. Octadecadienoic acids are present in significant amounts; there are traces of hexadecadienoic acid, octadecatrienoic acids, and highly unsaturated C20 and C22 acids. Traces of dihydroxystearic acid and hydroxypalmitic acid have been detected (Henderson, 1944; Jack, 1945). A small proportion of the octadecenoic acid consists, not of oleic acid, but of a *trans*-11,12 isomer, vaccenic acid (Henderson, 1944; Jack, 1945). One report states that about 66% of one octadecadienoic acid content is normal linoleic acid, and the remainder consists of the *cis*-9, *trans*-12 or the *trans*-9, *cis*-12 isomers (Wolff et al., 1995). But other positional and geometric isomers undoubtedly are also present (Morrison, 1970). The positional and geometric isomers of fatty acids from the bovine milk produced in June and December are presented in Table 3.

There is a pronounced seasonal change in the fatty acid composition of butterfat. It is normally more unsaturated in the summer than in the winter. In colder climates, the difference seems to be slightly larger. The change is usually associated with the difference in the feed of the animals in different seasons, but not completely. Cows put on green pasturage produce softer butterfat even if their feed has previously consisted of hay or silage comparable in solid composition with the green feed.

Triacylglycerol composition of butterfat

Butterfat from bovine milk represents one of the most complex mixtures of natural triacylglycerols (TAGs). The component fatty acids range from C2 to C26, including

Table 3. Fatty acid composition of milk and butterfat[a]

Fatty acid[b]	June[c]	December[d]	Average[e]
4:0	4.22	3.51	3.57
6:0	2.53	2.24	2.22
8:0	2.34	1.07	1.17
9:0	0.05	0.05	0.03
10:0	2.24	2.57	2.54
10:1	0.32	-	-
11:0	0.34	0.29	0.33
12:0	2.40	2.77	2.81
13:0 (12:1)	0.29	0.29	0.33
14 (br)[f]	0.23	0.14	0.17
14:0	9.01	10.58	10.06
14:1 (15 br)	1.54	1.61	1.63
15:0	1.29	1.11	1.09
16 (br)	0.42	0.39	0.38
16:0	22.05	25.98	24.97
16:1 (17 br)	2.29	2.98	2.55
17:0	0.69	1.08	0.91
17:1 (18 br)	-	-	-
18:0 (br)	0.31	0.40	0.38
18:0	14.27	11.58	12.07
18:1	30.41	24.75	27.09
18:2	1.23	2.75	2.39
18:3 (20:0)	2.61	2.30	2.06

[a] Hettinga, 1996. All data are in weight percentage.
[b] Structural assignments are not necessarily authentic, but represent, in almost all instances, the most likely structure for the fraction.
[c] Data from the Department of Animal Industries, Storrs (Conn.) Agricultrual Experiment Station; 408 samples of milk plant production from June 1960 to June 1961.
[d] Data from Storrs Agriculture Experiment Station; 4-8 samples.
[e] For 108 samples.
[f] Branched chain.

even and odd carbon numbers, straight and branched chains, numbers of double bonds from zero to six and *cis* and *trans* isomers. The number of possible triacylglycerols calculated by Walstra & Jenness (1984) totaled more that 1300. Nutter & Privett (1967) described 168 molecular species of short-chain fatty acids in the whey of cows milk.

The triacylglycerol composition of milk fat has been analyzed in order to help determine the origin of the milk fat. The ratios of fatty acids can be of great help in detecting mixtures of milks of different animal species (Farag et al., 1984) and in detecting the possible adulteration of milk fat with other animal or vegetable fats (Soliman et al., 1986). Determination of classes of TAGs according to their total number of carbon atoms, or ACN, has also been used to detect the presence of non-milk fats (Timms, 1980; Pinto et al., 1987). TG compositions of total butterfat according to the total ACN distribution are given in Table 4. Molar percentages were calculated from chromatographic peak areas without applying any correction factors according to chain length or/and degree of unsaturation.

Table 4. TG composition of Total Butterfat TAGs Analyzed by Capillary GC[a]

Acyl carbon number	Calculated[b]	Carbon number	Calculated[b]
24	0. 08	40	11. 46
26	0. 33	41	0. 93
27	0. 02	42	6. 85
28	0. 81	43	0. 57
29	0. 07	44	4. 96
30	1. 42	45	0. 85
31	0. 14	46	5. 21
32	2. 57	47	1. 19
33	0. 52	48	5. 73
34	5. 34	49	1. 33
35	1. 29	50	7. 73
36	10. 04	51	1. 08
37	2. 03	52	8. 01
38	13. 45	53	0. 20
39	1. 38	54	4. 41

[a] Maniongui et al., 1991

[b] Calculated from the relative proportions and TG compositions of the 46 RPLC (Reverse-phase liquid chromatography) fractions of butterfat.

MATERIALS AND METHODS

GC conditions for fatty acid methyl esters and fatty acid butyl esters analysis

Fatty acid methyl esters (FAMEs) or fatty acid butyl esters (FABEs) were analyzed by a Hewlett Packard 5890A series II Gas Chromatograph) equipped with a flame ionization detector, a Hewlett Packard 3396A Integrator, and a 0.25μM I.D. fused-silica capillary column (SP^{TM}-2330, SUPELCO, Bellefonte, PA). Both 15 m and 30 m column was used. The carrier gas was helium at a flow-rate of 3 mL/min. Both the injector and detector were maintained at 250°C. The temperature programs for GC analysis were showed in Table 5.

Table 5. GC temperature program for FAMEs and FABEs analysis

	FAMEs	FABEs
Initial temperature (°C)	50	70
Initial time (minute)	0	4
Rate (°C/min)	20	20
Final temperature (°C)	200	230
Final time (minute)	5	5

For the FABEs analysis, three odd-chain fatty acids, pentanoic acid, undecanoic acid, and nonadecanoic acid (all three fatty acids came from Sigma Chemical Co., St. Louis, MO) were added in as internal standards. The weight percentage of C4 and C6 fatty acids were corrected according to a C5:0 standard; fatty acids with chain length between C8 and

C14 (including C8 and C14) were corrected according to C11:0 standard; fatty acids with chain length more than C14 were corrected according to C19:0 standard.

Preparation of butyl esters for milk and butter

Bovine milk (200 μl) or butter (about 10 mg) was suspended in 300 μl 1-butanol containing three internal standards, pentanoic acid, undecanoic acid, and nonadecanoic acid (all three fatty acids came from Sigma Chemical Co., St. Louis, MO) in a glass vial with Teflon-lined screw cap. Next, 500 μl of additional 1-butanol and 50 μl acetyl chloride were added, and the vial was filled by a small amount nitrogen gas to protect the fatty acid from being oxidized. The vial was votexed for 3 minutes and placed in a steam bath for 1.5 hr. At the end of the reaction, 3.3 mL 6% potassium carbonate water solution and 630 μL hexane were added, the whole mixture was vortexed for 1 minute and centrifuged for 15 minutes at 400× *g* in a Fisher Centrific™ Centrifuge. The lower aqueous layer was removed by a Pasteur pipette and discarded. The hexane phase was washed with 3.3 mL distilled water, and the whole mixture was centrifuged again. In order to remove most of the butanol in the sample, 100 μL sample of the top layer was withdrawn and applied to a 1000-mg silica cartridge (Chrom. Tech. Inc., Apple Aly, MN) placed in a SUPELCO, VISIPREP™ vacuum manifold (Bellefonte, PA) and pre-washed with 5 mL hexane. The cartridge was washed with 2.3 mL 5% diethyl ether in hexane solution and the eluate was discarded. Then the cartridge was washed again with 1.7 mL

5% diethyl ether in hexane solution; the eluate was collected in sample vial for GC analysis.

Preparation of methyl esters for butterfat

According to the method provided by Christie (1982), the FAMEs were prepared as follows: about 50 mg butter was dissolved in 1 ml tetrahydrofuran (Fisher Scientific, Fair Lawn, NJ) in a Teflon capped vial. Then 2 ml 5% methanolic hydrogen chloride was added. The vial was placed in a steam bath for about 2 hr. Next, 5 mL 5% sodium chloride was added and votexed for 1 min. The methyl esters were extracted by 5 mL hexane. The hexane layer was washed with 2% potassium bicarbonate and the solvent was removed under a stream of nitrogen. The methyl esters were weighed and redissolved in hexane at 100 mg/mL.

IA determination and cow selection

The milks were collected from the cows in two farms: The Answer Farm of Land O'Lakes at Webster City, IA, and Iowa State University Dairy Breeding Farm of Ankeny, IA. Milk from each cow was collected separately and frozen until butyl esters were prepared and analyzed by GC. To determine the range of IA actually achieved in the butter, three odd chain fatty acids, pentanoic acid (C5:0), undecanoic acid (C11:0), and nonadecanoic acid (C19:0), were added during the preparation of FABEs as internal

standards. Data were expressed as weight percent. The IA of each milk sample was calculated according to the following formula:

$$IA = \frac{\%C12:0 + 4 \times \%C14:0 + \%C16:0}{\%C18:1 + \%C18:2 + \%C18:3 + \%C10:1 + \%C12:1 + \%C14:1 + \%C16:1}$$

For each farm, four cows were selected for their highest IA, and four cows were selected for their lowest IA. Typically, about 10 kg milk was collected for each cow for butter making. The milk from the low-IA cows was pooled as was the milk from the high-IA cows. Bulk-tank samples from each farm were also pooled.

Butter making

Butters were made from low IA, high IA, and bulk tank milk, respectively. The butters were prepared once at a time on separate days. The milks from the two farms were well mixed before vat pasteurization at 63°C for 30 min. After separating the cream with a cream separator (Elecrem Company, Vanves, France), the fat content of the cream was determined by the standard Babcock test (Hunziker, 1927), and the cream was adjusted to 30% fat with skim milk. The cream was stored at 4°C in cold room overnight and was placed in 10°C water bath (HAAKE, Berlin, Germany) for at least half an hour before churning. Each butter sample was made by churning 1.82 kg of cream in a 4-quart electric churn (GEM DANDY, Alabama Manufacturing Co.) at room temperature while letting the cream temperature rise until the butter was formed. After churning, the fat content of each

butter sample was measured by the standard method for the examination of dairy products (Hunziker, 1927) and adjusted to 80% fat by mixing water back into the butter using a heavy duty mixer (KitchenAid, Troy, Ohio). To prevent the texture changes the butter sample was kept in cold water below the final churning temperature when performing the fat determination. Water was incorporated into the butter with a mixer. During this process the speed of the stirrer was kept as low as possible to prevent air from being incorporated into the butter. The butters were kept at 4°C in cold room in plastic containers.

Procedure of texture analyzing

The firmness of butters was measured on samples tempered to 4°C with a Texture Analyzer TA-XT2 (Stable Micro Systems, London, UK) by the application program "Spreadability/Softness of Margarine using a conical probe ('Traditional' penetrometer text)". A 40° conical probe was forced into the butter sample at speed of 0.5 mm/sec until a force of 300 g was reached. The penetration distance was recorded. The Texture Analyzer was programmed to maintain a force of 300 g for 30 sec, and the distance the probe traveled while maintaining the force was recorded as creep. Three tests were conducted for each sample. Two samples of commercial butter (CubFood butter and Land O'Lakes butter) and two samples of commercial margarine (Corn oil margarine from Crystal Farms and Soybean oil margarine from Land O'Lakes) with 80% fat content were also tested for comparison.

Sensory evaluation of butter flavor

A sensory panel of 11 people was selected for the sensory evaluation of the butter samples. The panel was trained in two sessions in which butter was examined. The panel agreed that they perceived two basic flavors: a typical creamy/milky flavor and an aftertaste. The evaluation form for the panelists was illustrated in Fig. 1. The panelists practiced estimating the strength of the flavors and marking them on a 14-cm line scale (Fig.1), labeled "undetectable" on the left, "strong" on the right.

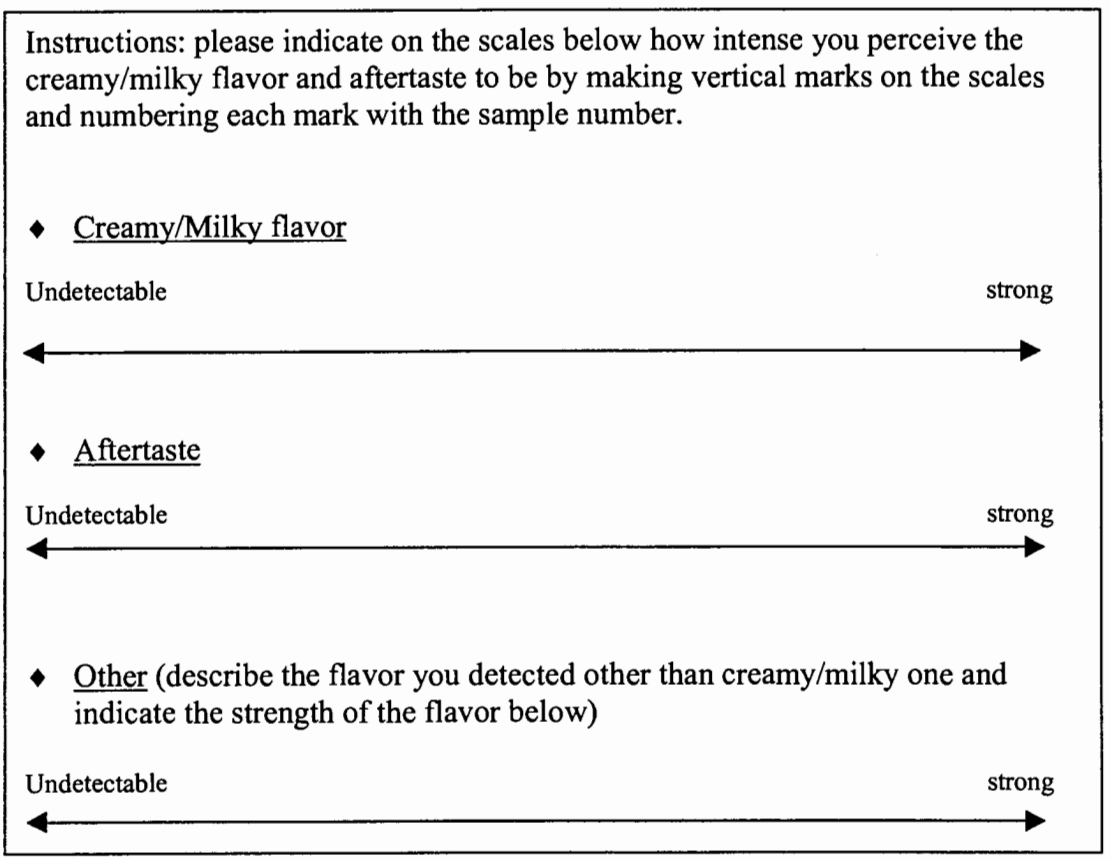


Fig. 1. The evaluation form for the sensory evaluation of butter samples

After training, the two low-IA, four high-IA, and four bulk-tank butters were evaluated by the panelists. The butter was cut into approximate 1-inch square blocks and placed in room temperature for the tasting. Since we have ten butter samples, five samples were presented to the panelists each time. The order of sample presentation was selected according to a random table. The data were grouped as high-IA butter, low-IA butter, or bulk-tank butter. Both the creamy/milk flavor and aftertaste of each sample were recorded. The strength of the flavor was expressed as a percentage of the total line scale starting from the right, which was labeled no flavor.

Statistical analyses of the data from sensory evaluation and texture analyses

The data from sensory evaluation and texture analyses were evaluated by several statistical methods, including multiple comparison and paired comparison. The general linear model was used to determine the variance among the samples. The means for each type of butter or margarine were compared by a least square difference calculation. All statistical calculations were carried out with SAS application program (SAS Institute, 1992).

Fractionation of FAMEs by silver-ion TLC

The silica gel plates, 20cm × 20cm with 500 micron silica gel soft layer, were purchased from Alltech Company (Deerfield, IL). The silica gel plates were impregnated by silver ion (Wolff et al., 1995): the silica plate was soaked in 5% silver nitrate in

acetonitrile for about 20 min, partially dried in air, and activated at 120°C for 20 min. After cooling, the plates were stored in dry and dark place. For the fractionation, about 10 mg FAMEs in hexane were applied to a plate by a streaker (Applied Science Laboratories Inc., State College, PA), then developed by hexane/diethyl ether (90:10, vol/vol) in a glass tank. After development, the plate were dried in air for a few minutes and sprayed with 0.1% (wt/vol) 2', 7'-dichlorofluorescein (Sigma Chemical Co., St. Louis, MO) in methanol. The plates were examined under ultraviolet (UV) light and each visualized silica band was scraped off onto a piece of aluminum foil and transferred to a test tube. The silica gel containing methyl esters were washed with methanol (3 mL), hexane (4 mL), and 5% sodium chloride (3 mL) in sequence. Thorough mixing followed each addition. The whole mixture was centrifuged briefly, and the hexane phase was withdrawn and concentrated under a nitrogen stream for GC analysis.

Butterfat triacylglycerol (TAG) extraction and purification

One gram butter was suspended in 10 mL methanol and 20 mL chloroform in a separatory funnel. After standing for about 1 hr, the chloroform phase was collected and filtered, and the solvent was removed under a nitrogen stream. The butterfat was weighed and redissolved in hexane at 100 mg/mL. About 20 mg butterfat was applied on a 500 μm silica gel plate, developed by hexane/diethyl ether (90:10), sprayed with 0.1% (wt/vol) 2', 7'-dichlorofluorescein in methanol. Under UV light, the TAG band was scraped off and

recovered by the same method as used for recovering FAMEs. The purified butter TAG was redissolved in hexane at 100 mg/mL.

Fractionation of butter TAG by silver-ion TLC

The procedure of fractionation of butter TAG by Ag-TLC is essentially the same as that of fractionation of FAMEs, except that the development solvent was chloroform/toluene (50:50, vol/vol). Each silica band visible under UV light on the plate was scraped off, and the lipids were recovered by the same method used for recovering FAMEs. The solvent was removed by a stream of nitrogen and the TAG was ready for FABEs preparation.

FABEs preparation of butter TAG

Fractionated butter TAG was dissolved in 100 μL 1-butanol containing three standard fatty acids: pentanoic acid, undecanoic acid, and nonadecanoic acid. Next, 20 μL acetyl chloride was added as catalyst. After heating in a steam bath for 2 hr, 3 mL 6% potassium carbonate solution was added to terminate the reaction, and 500 μL hexane was added to extract the butyl esters. The whole mixture was votexed for 1 min and centrifuged briefly. The hexane phase containing FABEs of butter TAG was ready for injection into GC.

GLC analysis of butter TAG

The TLC-purifed butter TAG was analyzed by the same gas chromatography system as used for FAMEs and FABEs analyses except a SUPELCO SPB^{TM}-1 column (Bellefonte, PA) was used, which was 15 m × 0.25 I.D. The temperature of injector and detector was maintained at 300°C. The GC temperature program was as follows:

Initial temperature: 100°C
Initial time: 0 min
Rate: 10°C/min
Final temperature: 350°C
Final time: 5 min

GC/MS analysis of milk sample 116

During IA determination of the milk samples, we found for some low IA milk samples there was a separated peak that eluted just before *cis*-18:1. We suspected it would be one of the isomers of *trans*-18:1. In order to identify the position of double bond in this fatty acid, picolinyl ester derivative of this fatty acid was made and analyzed by GC/MS. The procedure is as follows: the butterfat was extracted from the milk sample by chloroform:methanol (2:1 vol/vol) and transesterified to methyl esters by the method stated above. Next, 10 mg methyl esters were applied onto 500 μm silver-ion plate and developed by hexane:diethyl ether (90:10 vol/vol). Under the UV light, The second band corresponding to the *trans* fatty acids methyl esters was scraped off, and the lipids were recovered. Finally, the methyl esters were dissolved in hexane at 10 mg/mL. To obtain the free fatty acids from the methyl esters, the hexane solution of methyl esters was

saponified by 1 M potassium hydroxide in ethanol. The free fatty acids were extracted with 500 μL hexane. The picolinyl ester derivatives from the free fatty acids were made according the procedure from Christie (1992). In brief: 50 μL hexane solution of free fatty acids were blown to dry in a 1.5 mL eppendorf tube under a stream of nitrogen. Then thionyl chloride (100 μL) (ACROS Organics, New Jersey) was added. The mixture was left to stand at room temperature for 1 min, then the solvent was evaporated by a stream of nitrogen. Next 100 μL 1% 3-pyridyl carbinol (Aldrich Chemical Company, Inc., Milwaukee, WI) in acetonitrile was added, the mixture was shaken briefly and held at room temperature for 1 min again. The solvent was evaporated under a stream of nitrogen gas again and the residue in the tube was dissolved in 20 μL N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) (Sigma Chemical Co., St. Louis, MO). The mixture was heated to 60°C for about 10 min and the derivative was ready for injection. The capillary column was the same model used for TAG analysis. The MS test was performed on a Hewlett Packard 5970 Series Mass Selective Detector. The temperature program is as follows:

Injector temperature: 300°C

Initial temperature: 100°C

Initial time: 0 min

Rate: 10°C/min

Final temperature: 300°C

Final time: 5 min

RESULTS AND DISCUSSION

The cows selected for butter making

The milk from individual cows was analyzed by gas chromatography (GC) of the fatty acid butyl esters and their index of atherogenicity (IA) were determined. Four cows were selected for their highest IA from the each of the two herds, and four cows were selected for their lowest IA. The selected cows were showed in Table 6. Both the identification numbers and IA values were given out.

From each cow about 10 kg of milk was collected. At the same time, bulk-tank milk representing milk from the whole herd was collected at each farm.

Table 6. Cows selected to provide milk for butter making

Land O'Lakes herd				Ankeney herd			
Low-IA cows		High-IA cows		Low-IA cows		High-IA cows	
ID	IA	ID	IA	ID	IA	ID	IA
114	1.155	1314	3.410	4617	1.109	4454	3.604
93	1.216	1250	3.359	4827	1.133	5044	4.049
1285	1.384	1670	3.247	4529	1.251	4156	3.953
1340	1.288	5	3.215	5050	1.303	4995	3.285

The butter making

The butters were made twice, because the butter from the first experiment varied in texture due to varied storage time. So the experiment was repeated. The flavor evaluation was done on the butter from the first experiment and the texture on the butter from the second experiment. In the first time, we obtained four high-IA, four bulk-tank, and two low-IA butter samples from about 80 kg milk in each IA rank. The second

time, we adjusted the amount of low-IA milk obtained two butter samples in each IA rank. For low-IA milk, one 1.82 kg sample of cream was obtained because the fat content was relatively low for the low-IA milk. After churning, the butter was normally between 520 to 580 g, and the fat content was between 82 to 85%.

Fatty acid composition of the butter samples

The composition of the major fatty acids from high-IA, low-IA, and bulk-tank butters determined by GC of the butyl esters are summarized in Table 7 and Table 8. Table 7 shows the fatty acid profiles of the butter samples made from the first batch of milk. Since four high-IA, four bulk-tank, and two low-IA butters were obtained, the fatty acid composition for the high-IA and bulk-tank butter sample are from different batches of cream. In Table 8, all the butter samples for each treatment, i.e. bulk, low-IA, and high-IA, were made from the same batches of cream.

By the Student's t-test, the coefficient of variance for the two butter samples in each IA rank are 0.97, 0.999, and 0.998 respectively. So the two butter samples made from the same batch of cream are almost identical in fatty acid composition.

IA value of our butter samples

According to the fatty acid compositions from Table 7 and Table 8, the IA values of high-IA, low-IA, and bulk-tank butters are calculated and summarized in Table 9.

Table 7. The fatty acid profiles of the butter samples made from the first experiment

	Weight percentage of the major acid (%)					
	High-IA butter 1	High-IA butter 2	Low-IA butter 1	Low-IA butter 2	Bulk-tank butter 1	Bulk-tank butter 2
C4:0	3.72	3.76	3.98	3.91	3.94	4.03
C6:0	2.16	2.20	1.79	1.77	2.12	2.14
C8:0	1.25	1.27	0.89	0.90	1.19	1.22
C10:0	2.75	2.80	1.70	1.67	2.53	2.50
C12:0	3.02	3.07	1.78	1.77	2.73	2.70
C14:0	9.62	9.81	6.76	6.37	9.08	8.86
C14:1	1.32	1.35	0.81	0.79	1.13	1.09
C16:0	32.73	32.52	28.18	27.78	30.22	30.74
C16:1	3.17	3.01	2.98	4.16	2.86	2.90
C18:0	9.66	9.68	13.02	12.97	12.04	11.95
C18:1	24.91	24.91	32.32	31.90	26.45	26.39
C18:2	2.60	2.59	2.90	2.85	2.71	2.73
C18:3	0.36	0.40	0.38	0.38	0.41	0.31

Table 8. The fatty acid profiles of the butter samples made from the second experiment

	Weight percentage of the major fatty acid (%)					
	High-IA butter 1	High-IA butter 2	Low-IA butter 1	Low-IA butter 2	Bulk-tank butter 1	Bulk-tank butter 2
C4:0	3.32	3.52	3.53	3.56	3.56	3.38
C6:0	2.04	2.07	1.84	1.88	1.93	1.77
C8:0	1.22	1.29	1.02	1.08	1.02	1.00
C10:0	2.70	2.93	2.21	2.25	2.21	2.08
C12:0	2.88	2.99	2.32	2.33	2.47	2.33
C14:0	9.18	9.21	7.00	6.97	8.56	8.58
C14:1	1.17	1.19	1.06	1.07	1.62	1.48
C16:0	34.25	34.05	30.11	29.83	26.16	26.75
C16:1	4.23	3.66	3.40	3.37	4.56	5.82
C18:0	9.35	9.22	12.79	12.82	10.76	10.84
C18:1	24.15	24.40	29.04	29.21	24.56	24.52
C18:2	2.65	2.77	2.71	2.74	2.96	2.98
C18:3	0.49	0.44	0.45	0.44	1.43	1.92

Table 9. The IA of the high-IA, low-IA, and bulk-tank butters made from the first experiment and second experiment

	Weight percentage (%)					
	High-IA butter		Low-IA butter		Bulk-tank butter	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
First batch	2.29	2.32	1.45	1.32	2.06	2.06
Second batch	2.26	2.28	1.65	1.63	1.79	1.73

Sensory evaluation of butters

The sensory data came from the butters we made the first batch of milk. Because we have 10 butter samples, five samples were presented to the panelists each time. The order of sample presentation was selected according to a random table. The strength of the creamy/milky flavor and aftertaste of the ten butter samples were listed in Table 10 and Table 11, respectively. All the values in Table 10 and Table 11 were expressed as percentages of the total line scale starting from the right, which was labeled no flavor. S1, S2, S3, and S4 are the sample names.

Then sensory data were pooled into three main groups – high-IA butter, low-IA butter and bulk-tank butter by SAS programming, the least square means (LSMEAN) were calculated for IA group, and the difference among the LSMEAN were compared in Table 12 and Table 13 in the creamy/milky flavor and aftertaste.

In Table 12 and 13, all the P values (level of significance) are greater than 0.05, so there was no significant difference for either the creamy/milky or aftertaste among the butter types. The IA differences of the milks and of the butters had no detectable impact on the butter flavor as judged by the sensory panel.

Table 10. The strength of the creamy/milky flavor of bulk-tank, high-IA, and low-IA butters

	High-IA butter				Low-IA butter		Bulk-tank butter			
Panelist	S1	S2	S3	S4	S1	S2	S1	S2	S3	S4
1	49	41	51	59	47	41	47	55	47	47
2	4	31	37	41	49	24	27	25	23	9
3	4	23	4	13	16	7	23	41	16	13
4	24	17	11	26	16	26	28	18	40	21
5	55	51	28	45	23	49	41	50	22	21
6	37	42	35	30	61	59	56	52	50	31
7	24	22	41	24	28	28	22	24	22	50
8	16	28	37	46	44	32	64	45	50	24
9	76	54	81	86	69	80	74	66	61	53
10	17	11	29	73	63	63	24	57	46	71
11	30	53	51	46	51	59	49	35	43	39

Table 11. The strength of the aftertaste of bulk-tank, high-IA, and low-IA butters

	High-IA butter				Low-IA butter		Bulk-tank butter			
Panelist	S1	S2	S3	S4	S1	S2	S1	S2	S3	S4
1	30	16	30	30	20	16	20	30	20	30
2	31	21	55	41	25	35	20	26	23	21
3	3	18	21	4	5	9	5	3	5	4
4	16	8	6	9	4	11	16	14	25	19
5	10	26	10	11	22	15	10	21	29	12
6	20	39	20	18	58	53	55	36	51	29
7	14	6	14	18	6	6	6	18	6	18
8	28	9	28	57	34	18	41	30	6	51
9	34	14	34	30	30	37	21	14	17	11
10	17	12	17	51	43	64	37	59	50	46
11	10	14	10	12	4	4	4	9	4	16

Table 12. Comparison of the strength of the creamy/milky flavor among bulk-tank, high-IA, and low-IA butters.

	[a]LSMEAN of strength %	[b]T for H_0 : LSMEAN (i) = LSMEAN (j) / Pr > |T|		
		High IA butter	Low IA butter	Bulk butter
High IA butter	36.43	-	-1.240/0.218	-0.677/0.500
Low IA butter	42.50	1.240/0.218	-	0.687/0.493
Bulk butter	39.14	0.677/0.500	-0.687/0.493	-

[a] Least square mean from 11 panelists. Strength was expressed as percentages of the total line scale starting from the right

[d] T test for null hypothesis H_0. The difference was considered significant if $Pr < 0.05$.

Table 13. Comparison of the strength of the aftertaste flavor among bulk, high-IA, and low-IA butters.

	[a]LSMEAN of strength %	T for H_0 : LSMEAN (i) = LSMEAN (j) / *Pr > |T|		
		High-IA Butter	Low-IA Butter	Bulk Butter
High IA butter	20.955	-	-0.663/0.509	-0.462/0.645
Low IA butter	23.591	0.663/0.509	-	0.286/0.777
Bulk butter	22.455	0.462/0.645	-0.286/0.777	-

[a] Least square mean from 11 panelists. Strength was expressed as percentages of the total line scale starting from the right

[d] T test for null hypothesis H_0. The difference was considered significant if $Pr < 0.05$.

Texture Analyses

The texture data came from the butters we made the second batch of milk. The typical graph of penetration test was shown in Fig. 2.

Each butter sample was tested six times, both the penetration distance and creep compliance distance were recorded. The data were shown in Table 14 and Table 15 respectively. Also, the testing data from the commercial butters and margarine were put in Table 16. Three tests were performed for each sample.

The data from the butters in the same IA rank were pooled together by SAS programming and their least square means were compared in Table 17 and Table 18 for penetration test and creep.

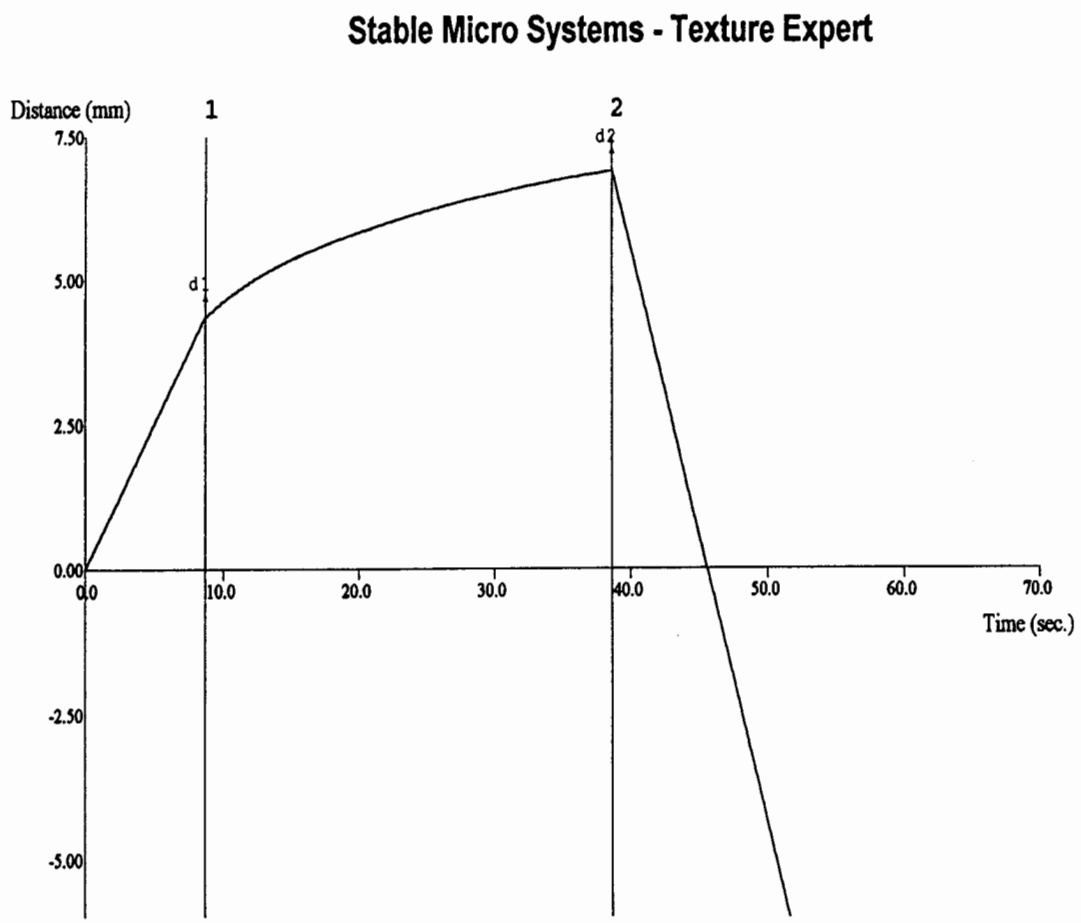


Fig. 2. The typical graph of penetration test by the Texture Analyzer TA-XT2. At zero time, the cone touched the surface the butter sample. Then the cone was penetrated into the butter sample at constant speed of 0.5 mm/s until it reached 300 g force. At this point, the penetration distance was recorded as distance 1 (d1). Then the cone was maintained at 300 g constant force for 30 sec and reached distance 2 (d2). The gradient between d1 and d2 was recorded as creep compliance distance.

Table 14. The penetration distance of the butter samples

	Penetration distance (mm)					
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Bulk-tank butter 1	4.88	5.05	5.45	5.31	5.27	5.24
Bulk-tank butter 2	5.11	5.36	5.19	5.59	5.44	5.60
High-IA butter 1	4.91	5.12	5.14	5.42	5.66	5.39
High-IA butter 2	4.94	4.71	4.72	5.34	5.14	5.31
Low-IA butter 1	5.04	5.06	4.95	5.44	5.60	5.79
Low-IA butter 2	5.47	5.34	5.43	5.46	5.51	5.53

Table 15. The creep compliance of the butter samples

	Creep (mm/s)					
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Bulk-tank butter 1	0.087	0.088	0.083	0.095	0.093	0.094
Bulk-tank butter 2	0.094	0.088	0.095	0.094	0.092	0.100
High-IA butter 1	0.073	0.081	0.084	0.091	0.088	0.092
High-IA butter 2	0.088	0.087	0.089	0.091	0.091	0.092
Low-IA butter 1	0.086	0.087	0.098	0.085	0.096	0.100
Low-IA butter 2	0.089	0.094	0.093	0.090	0.092	0.091

Table 16. Penetration and creep compliance of some commerical butter or margarine samples

	Penetration distance (mm)			Creep (mm/s)		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Cub Food butter	4.75	4.97	4.96	0.081	0.081	0.082
Land O'Lakes butter	5.27	5.13	4.92	0.085	0.081	0.077
Corn margarine	10.40	10.30	10.30	0.083	0.116	0.074
Soybean margarine	8.55	8.48	8.52	0.069	0.073	0.086

Table 17. Texture difference of high-IA, low-IA and bulk-tank butters in terms of penetration distance

	[a]LSMEAN of penetration Distance (mm)	[b]T for H_0 : LSMEAN (i) = LSMEAN (j) / *Pr > |T|		
		Bulk butter	High IA butter	Low IA butter
Bulk butter	5.289	-	1.406/0.170	-0.9568/0.346
High IA butter	5.149	-1.406/0.170	-	-2.3623/0.025#
Low IA butter	5.384	0.957/0.346	2.363/0.025#	-

[a] Least square means of penetration distance from all tests for same kind of butter.
[b] T test for null hypothesis H_0. The difference was considered significant if $Pr < 0.05$. # Significant

Table 18. Texture difference of high-AI, low-AI and bulk-tank butters in terms of creep compliance distance under constant load

	[a]LSMEAN of creep (mm/s)	[b]T for H_0 : LSMEAN (i) = LSMEAN (j) / *Pr > |T|		
		Bulk butter	High AI butter	Low AI butter
Bulk butter	0.0919	-	2.356/0.025#	0.084/0.934
High IA butter	0.0873	-2.356/0.025#	-	-2.271/0.030#
Low IA butter	0.0918	-0.084/0.934	2.271/0.030#	-

[a] Least square means of creep compliance distance from all tests for each kind of butter.
[b] T test for null hypothesis H_0. The difference was considered significant if $Pr < 0.05$.

Examination of Table 17 and Table 18, in terms of both penetration and creep showed that, low-IA butter and high-IA butters were significantly different, and High-IA butter was harder than low-IA butter. In terms of penetration, bulk-tank butter was not statistically different from high-IA butter or low-IA butter, and the mean penetration of bulk-tank butter was between that of high-IA and low-IA butters. In terms of creep, the bulk-tank butter and high-IA butters were also statistically different, implying the texture of bulk-tank butter was softer than the high-IA butter. Comparisons with commercial butter and margarine suggest that all our butters were about like Land O'Lakes butter, softer than Cub Foods butter, and harder than margarines.

The fatty acid composition of each of the four FAMEs bands as fractionated by silver-ion TLC

To distinguish *cis* and *trans* isomers, butter methyl esters made from the second batch of butter samples were fractionated on silver-ion plates. Five bands appeared on the plate after spraying with 2′,7′-dichlorofluorescein. The relative position of these five bands on the plate was illustrated in Fig. 3.

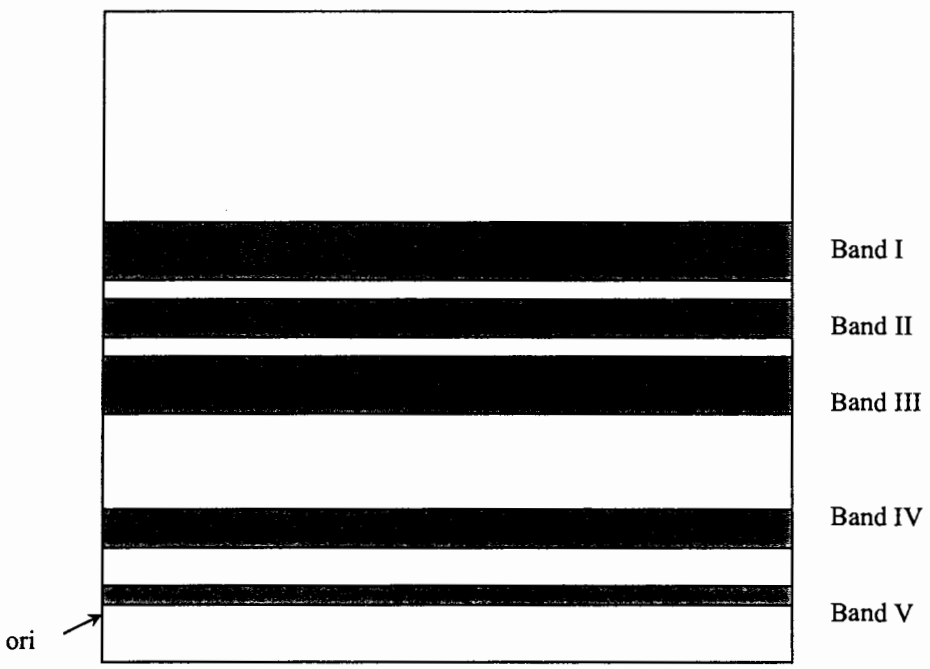


Fig. 3. The illustration of silver-ion fractionation of butterfat methyl esters. Band I represents saturated fatty acids; band II represents *trans* monoene; band III represents *cis* monoene; band IV and band V represent polyunsaturates.

The chromatograms of all five bands are shown in Fig. 4. The short chain fatty acids (C4, C6) were totally lost because of their great volatility. C8:0 was added in the samples for band II and III samples before GC analysis as an internal standard. The amount of each peak was corrected according to C8:0.

The first band is the long-chain saturated fatty acids fraction. C16:0 was the most abundant ester, the next was C18:0. Most of these fatty acids are even-numbered withstraight chain, from C10:0 to C20:0. Between these peaks, there are some odd-numbered, branched fatty acids. They are most abundant for C15:0 and C17:0.

Band II represents the *trans* fatty acids. In our graph, two groups of peaks were observed: C16:1 and C18:1. In bovine milk, the predominant *trans* fatty acid is *trans*-18:$1^{\Delta 11}$ (Wolff, 1995; Wolff, 1994; Parodi, 1976), and *trans*-18:$1^{\Delta 16}$ peak was eluted behind *trans*-18:$1^{\Delta 11}$ and totally separated from other positional isomers of *trans*-18:1. Also, in our test, *trans*-18:$1^{\Delta 9}$ was eluted just before *trans*-18:$1^{\Delta 11}$ but barely separated. So, the big peak in the chromatograph representing *trans*-18:1 isomers in band II was identified as *trans*-18:$1^{\Delta 11}$, and the small peak representing *trans*-18:1 isomers was identified as *trans*-18:$1^{\Delta 16}$. The weight percentage of these two group of *trans* fatty acids to *cis* 18:1 in our butter samples were compared in Table 19.

Obviously the *trans*-18:$1^{\Delta 11}$ was the most abundant *trans* fatty acid in butter. The proportion of *trans*-18:$1^{\Delta 9}$ to *cis*-18:1 was about the same for high-IA butter and low-IA butter. The proportion of *trans*-18:$1^{\Delta 11}$ to *cis*-18:1 was relatively low for bulk-tank butter. The *trans*-16:1 acids are relatively higher for high-IA butter compared to *cis*-18:1. There are several isomer of *trans*-16:1 that have not been identified individually.

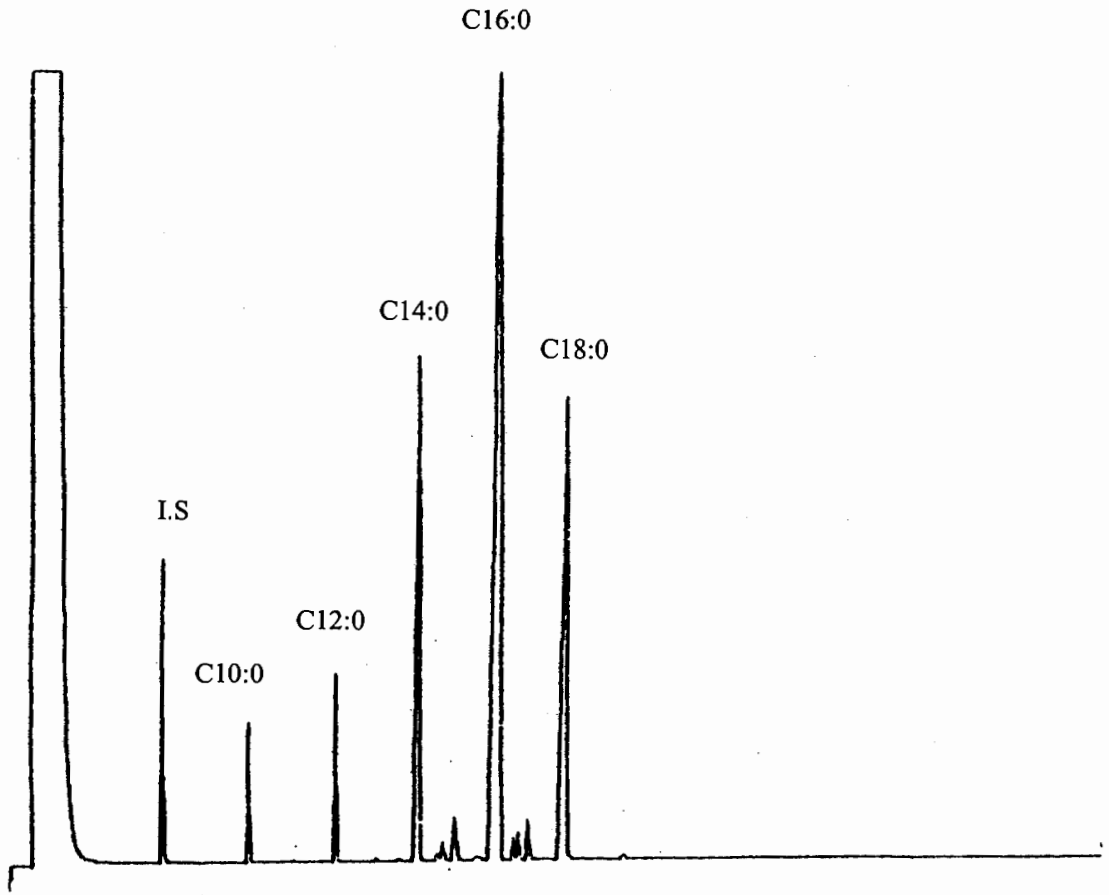
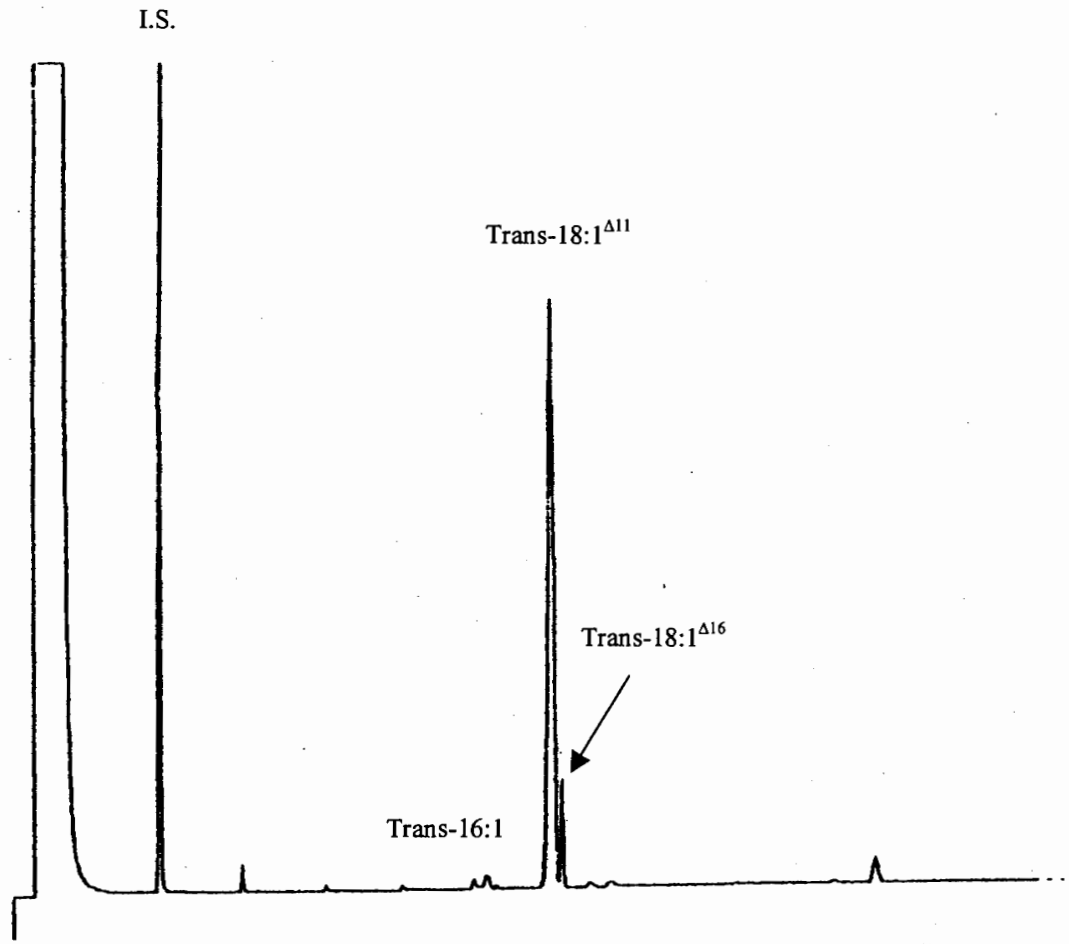
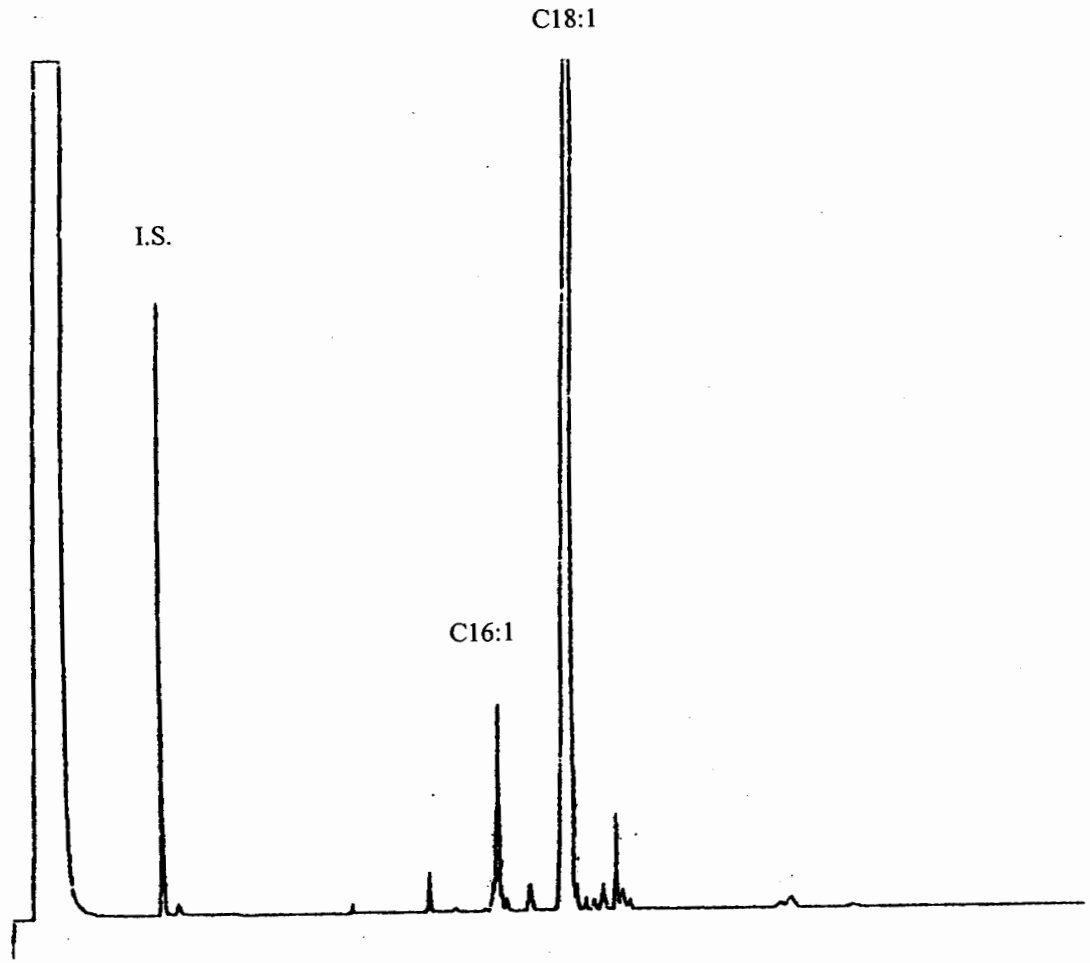


Fig. 4. The chromatograph of five bands of fatty acid methyl esters fractionated by silver-ion plate. I.S. means internal stand.



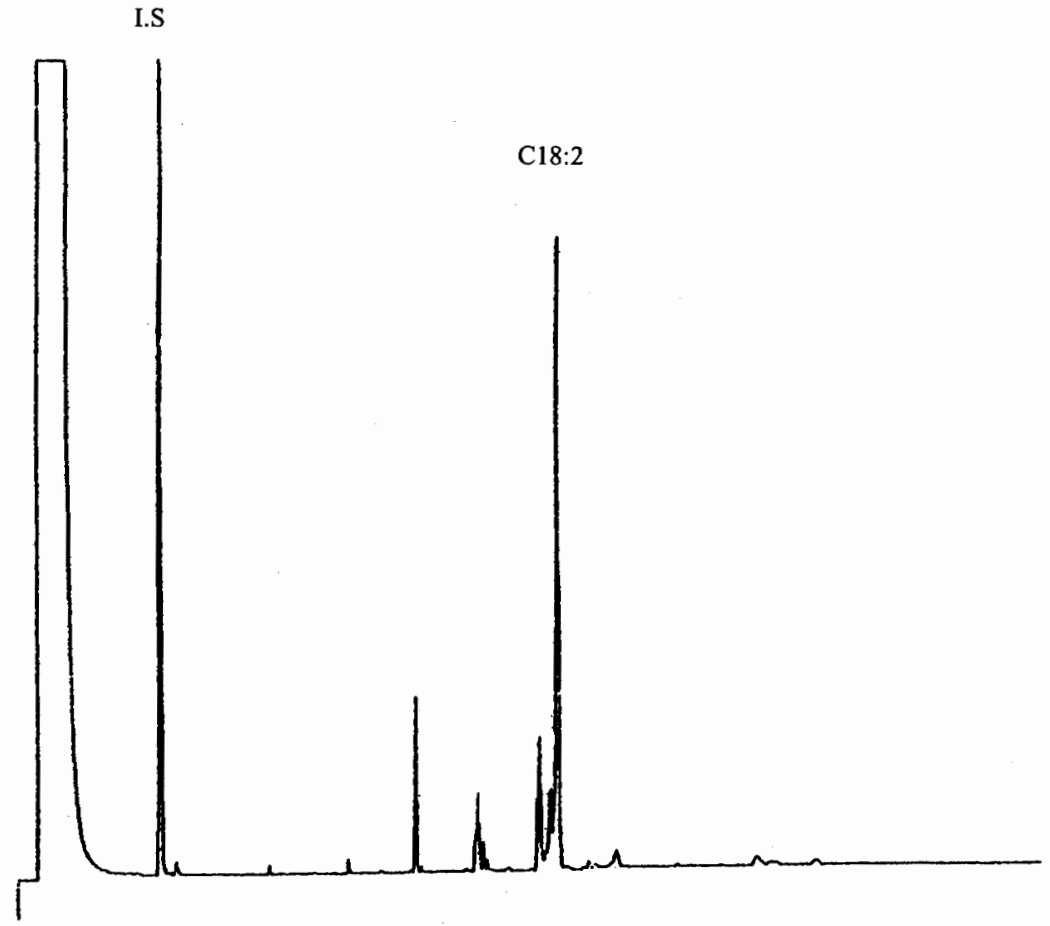
Band II

Fig. 4. (continued)



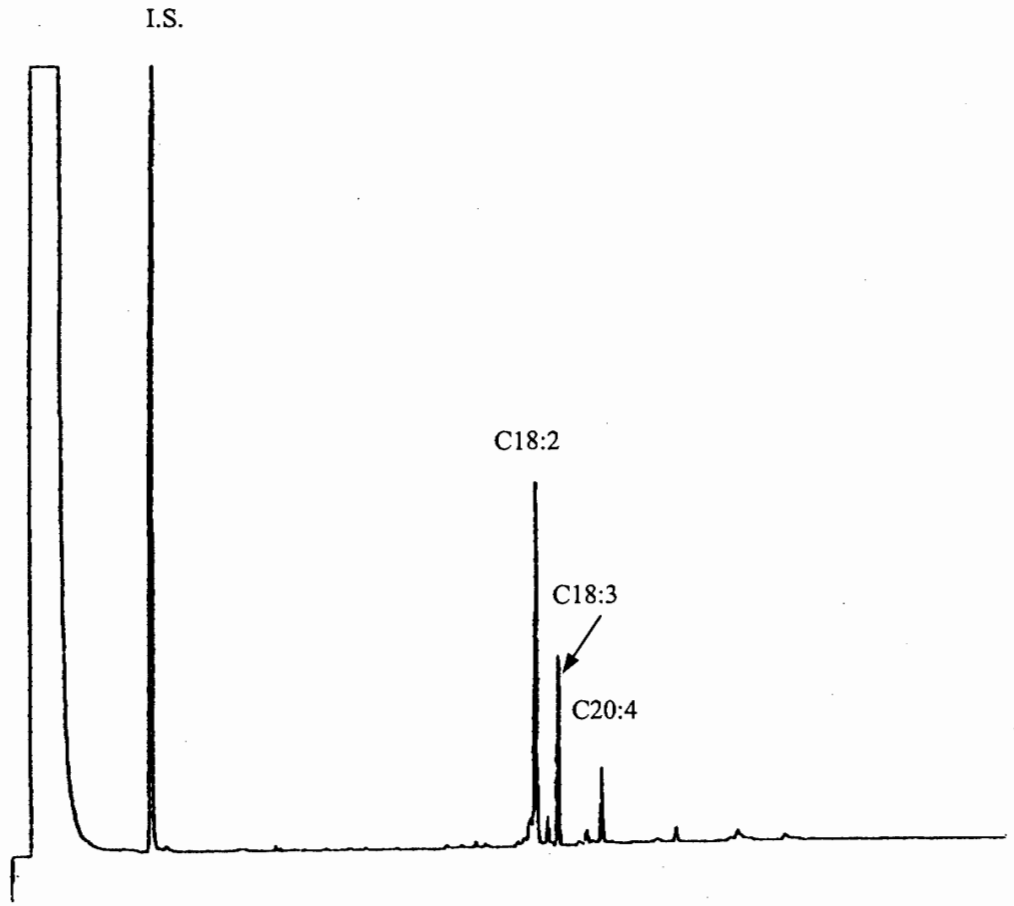
Band III

Fig. 4. (continued)



Band IV

Fig. 4 (continued)



Band V

Fig. 4 (continued)

Table 19. The relative abundance of *trans* fatty acids in high-IA, low-IA, and bulk-tank butterfat

	Weight percentage to *cis*-18:1%		
	High-IA butter	Low-IA butter	Bulk-tank butter
Trans-16:1	2.12	0.74	0.49
Trans-18:1$^{\Delta 11}$	36.77	34.21	18.47
Trans-18:1$^{\Delta 16}$	1.90	2.36	1.48
Total	40.79	37.84	20.4

The isomer eluted just behind trans-18:1$^{\Delta 11}$ is trans-18:1$^{\Delta 16}$ (Wolff et al., 1995) with double bond at C16 counting from the carboxyl end.

Band III represents *cis*-monoenoic acids. The major fatty ester in this fraction was (*cis*-9) 18:1. Some odd chain fatty acids such as 15:1, 17:1 were also detected, but in trace amount. At least two isomers were separated from major *cis*-16:1 peak, which are probably Δ7 and Δ11 eluted before and after the major peak (Hay, 1970). There is a small peak on the shoulder on the descending edge of the major cis-18:1 peak, which is probably the Δ11 isomer.

The dienoic acids and polyunsaturated fatty esters are included in band IV and band V. The cis-18:2n-6 dienoic acid fractions has several isomers eluted on its shoulder of the leading edge in band. Also, the retention time is different for dienoic acids for band IV and band V, suggesting there are different isomers of dienoic acids for band IV and band V. All the cis-18:3n-3 acid are present in band V with no obvious isomers. The biggest peak eluted after cis-18:3n-3 is 20:4 very likely, considering its retention time. The other peaks are unidentified.

GC analysis of triacylglycerol of butterfat

The TAG from butterfat was purified by thin-layer chromatography. The TAG from three butter samples (high-IA butter, low-IA butter, and bulk-tank butter) was analyzed by GC and their TAG profiles were summarized in Table 21.

All the TAG peaks were eluted according to the acyl carbon number (ACN), from 24 to 54. The cholesterol peak was eluted between C24 and C26. The most abundant TAG for all three butter samples is C38. Due to the high resolution of capillary GC, the TAG with same ACN number appear as several overlapped peaks in the chromatograph. Those peaks with in the same ACN number reflect the different fatty acids or different arrangement of the three fatty acids in one glycerol backbone. The odd-numbered TAG was eluted between even-numbered TAG peaks, and their percentages are usually below 1%.

For all three butter samples, C38 are the most abundant triaclyglyceride, followed by C36 and C40. There are only a small amount of TAG with ACN number below C34. C23 is only in trace amount. C54 is also very scarce in butterfat, and its content is relatively higher in bulk-tank butterfat than in the other two butter samples. Low IA butter was lowest in cholesterol and in peaks C41-C52. It was highest in the middle even chain length C34-C40. The reverse was true for high-IA. Bulk-tank was intermediate in all instances. This suggest that C18:1 tends to concentrate in the middle chains lengths and C16:0 in the high chain lengths.

Table 21. The TAG profiles from high-IA, low-IA, and bulk-tank butter

	Area %		
ACN number[a]	Low-IA butter	High-IA butter	Bulk-tank butter
C24	0.30	0.17	0.12
Cholesterol	0.28	0.54	0.46
C26	0.59	0.50	0.42
C27	0.06	0.05	0.04
C28	1.19	1.10	0.93
C29	0.07	0.09	0.06
C30	1.78	1.87	1.56
C31	0.14	0.25	0.18
C32	2.89	2.96	2.71
C33	0.28	0.43	0.56
C34	6.09	5.60	5.58
C35	0.82	1.09	1.36
C36	11.87	10.43	11.38
C37	1.24	1.36	1.11
C38	16.19	12.90	14.42
C39	0.96	1.02	1.01
C40	12.43	10.24	10.99
C41	0.51	0.72	0.36
C42	6.73	6.86	6.53
C43	0.29	0.68	0.47
C44	5.47	6.36	5.69
C45	0.63	0.69	0.68
C46	6.52	7.91	6.59
C48	7.49	9.45	7.94
C50	8.12	8.96	8.87
C51	0.07	0.53	0.58
C52	6.16	6.67	7.60
C54	0.82	0.57	1.79

[a] ACN = acyl carbon number

Silver-ion TLC fractionation of triacylglycerol from butterfat

The triacylglcerol from butterfat was fractionated into eight visible bands under UV light. The fatty acid composition of the TAG from each band was summarized in Table 22. All the values are the weight percentage of the individual fatty acid. Only the main fatty acids are included in the table, the other fatty acids in small and trace amounts have not been counted.

There was almost no unsaturated fatty acid in band 1, which was expected for silver-ion TLC. The shortest saturated fatty acid, C4:0, appeared first in band 2, which means C4:0 was absent in fully saturated TAG molecules. The C18:1 began to appear in band 2, then increased with band number to become the most abundant fatty acid from band 5 to band 9. On the other hand, the amount of C16:0 tends to decrease from band 1 to band 9. The amount of C18:0 decreases from band 1 to 5 and then remained relatively low. Since the C18:1 and C16:0 are the most abundant fatty acids except for band 1 which has no C18:1, the proportion of these two fatty acids is the main factor to determine the degree of saturation of each band of fatty acids. The C18:2 is found in bands 6 to 9 and C18:3 is present only in band 9.

The short-chain fatty acids tend to be in the more saturated triacylglycerols and those shorter than C14:0 do not appear in band 9. The bulk butter values tend to fall between these of high- and low-IA. Band 3 is about 30% unsaturated and is probably mostly disaturated-monounsaturated. From band 6 to 9 one has mostly monosaturated-diunsaturated species.

Table 22. The fatty acid composition of nine TAG bands as fractionated by silver-ion TLC

	Band 1			Band 2			Band 3		
	High	Low	Bulk	High	Low	Bulk	High	Low	Bulk
C4:0	-	-	-	-	6.57	7.64	-	4.02	4.64
C6:0	-	-	2.02	4.04	4.68	3.39	-	-	-
C8:0	-	-	3.18	-	2.07	1.51	3.30	-	-
C10:0	3.13	6.06	6.72	2.75	3.13	2.90	2.62	2.40	2.47
C12:0	4.62	4.63	5.86	3.54	3.43	2.96	2.76	2.42	2.66
C14:0	7.60	12.22	15.63	12.25	13.31	9.24	9.19	8.02	8.56
C14:1	-	-	-	-	-	0.91	-	-	-
C16:0	67.12	53.38	45.61	53.59	42.99	49.83	35.68	28.10	34.27
C16:1	-	-	-	-	-	-	-	-	-
C18:0	17.53	23.71	19.70	14.99	16.24	14.93	15.73	20.39	17.37
C18:1	-	-	-	8.84	5.47	6.68	29.14	34.65	30.02
C18:2	-	-	-	-	-	-	-	-	-
C18:3		-	-	-	-	-	-	-	-

	Band 4			Band 5			Band 6		
	High	Low	Bulk	High	Low	Bulk	High	Low	Bulk
C4:0	-	-	-	4.74	5.80	5.53	1.47	-	-
C6:0	1.24	1.26	1.77	2.31	6.88	2.72	1.03	-	-
C8:0	0.90	0.78	1.25	-	-	-	0.69	-	-
C10:0	2.51	2.14	2.72	1.78	1.40	1.81	1.50	1.08	-
C12:0	2.77	2.16	3.05	1.78	1.43	1.55	2.56	1.07	-
C14:0	8.46	6.92	7.91	6.46	4.89	4.89	4.76	3.78	5.01
C14:1	1.11	0.62	0.91	2.37	1.45	1.49	1.61	0.99	2.69
C16:0	34.88	34.25	33.06	25.56	22.61	26.84	22.40	21.61	18.72
C16:1	3.29	2.75	2.76	6.77	3.37	4.22	5.81	3.79	4.18
C18:0	10.75	12.60	11.86	6.93	8.47	8.25	7.07	8.81	8.57
C18:1	32.86	35.93	33.91	41.30	43.71	42.69	43.58	52.25	51.56
C18:2	-	-	-	-	-	-	6.67	6.62	9.27
C18:3	-	-	-	-	-	-	-	-	-

Table 22. (continued)

	Band 7			Band 8			Band 9		
	High	Low	Bulk	High	Low	Bulk	High	Low	Bulk
C4:0	3.88	5.08	-	2.20	-	-	-	-	-
C6:0	-	-	-	-	-	-	-	-	-
C8:0	-	-	-	-	-	-	-	-	-
C10:0	1.31	-	1.54	1.34	-	-	-	-	-
C12:0	1.26	-	1.42	1.30	-	-	-	-	-
C14:0	4.08	3.31	4.54	4.94	4.91	5.49	-	2.96	4.83
C14:1	2.72	2.11	0.98	2.18	-	-	-	-	0.40
C16:0	17.94	18.89	21.84	24.28	23.54	19.79	44.48	22.70	17.82
C16:1	6.88	-	3.37	8.27	3.80	-	-	-	3.36
C18:0	9.23	7.15	8.97	12.27	9.56	10.73	-	9.44	9.34
C18:1	42.03	53.42	50.32	31.17	44.88	48.49	55.52	41.92	40.14
C18:2	10.67	10.04	7.03	10.52	13.31	15.50	-	15.21	13.17
C18:3	-	-	-	-	-	-	-	7.76	7.85

GC/MS analysis of milk sample 116

The butyl esters were prepared from milk sample 116 by and the chromatograph was shown in Fig. 5. This sample contained relatively large amounts of an unidentified peak that eluted between C18:0 and C18:1. Silver-ion TLC indicated it was a *trans* monoene isomer. In order to identify the position of the double bond in the *trans* isomers, picolinyl esters were prepared for mass spectroscopy. Because the molecular weight of picolinyl ester derivatives made from isomers of C18:1 is 373, peaks containing this mass in the mass spectrum were identified and the result is shown in Fig. 6. The peak we needed to identify was eluted at about 10.40 min. The mass spectrum of the peak was showed in Fig. 7.

The characterized ions formed from picolinyl ester derivatives were illustrated in Fig. 8. All these characterized ions were found in our mass graph, indicating the

picolinyl ester derivatives were made successfully. Counting down from the ion at *m/z* 373, each ion decreased by 14 in *m/z* until to the ion at *m/z* 288. The difference in MW of ion at *m/z* 288 and ion at *m/z* 276 is only 12. The next major peaks is at 262, and 288–262 = 26 represents – CH = CH –. Thus, the double bond locates between carbon 11 and carbon 12 counting from the carboxyl end of the fatty acid. So, the mass spectrum represents the picolinyl derivative of vaccenic acid, *trans*-octodecenoic acid.

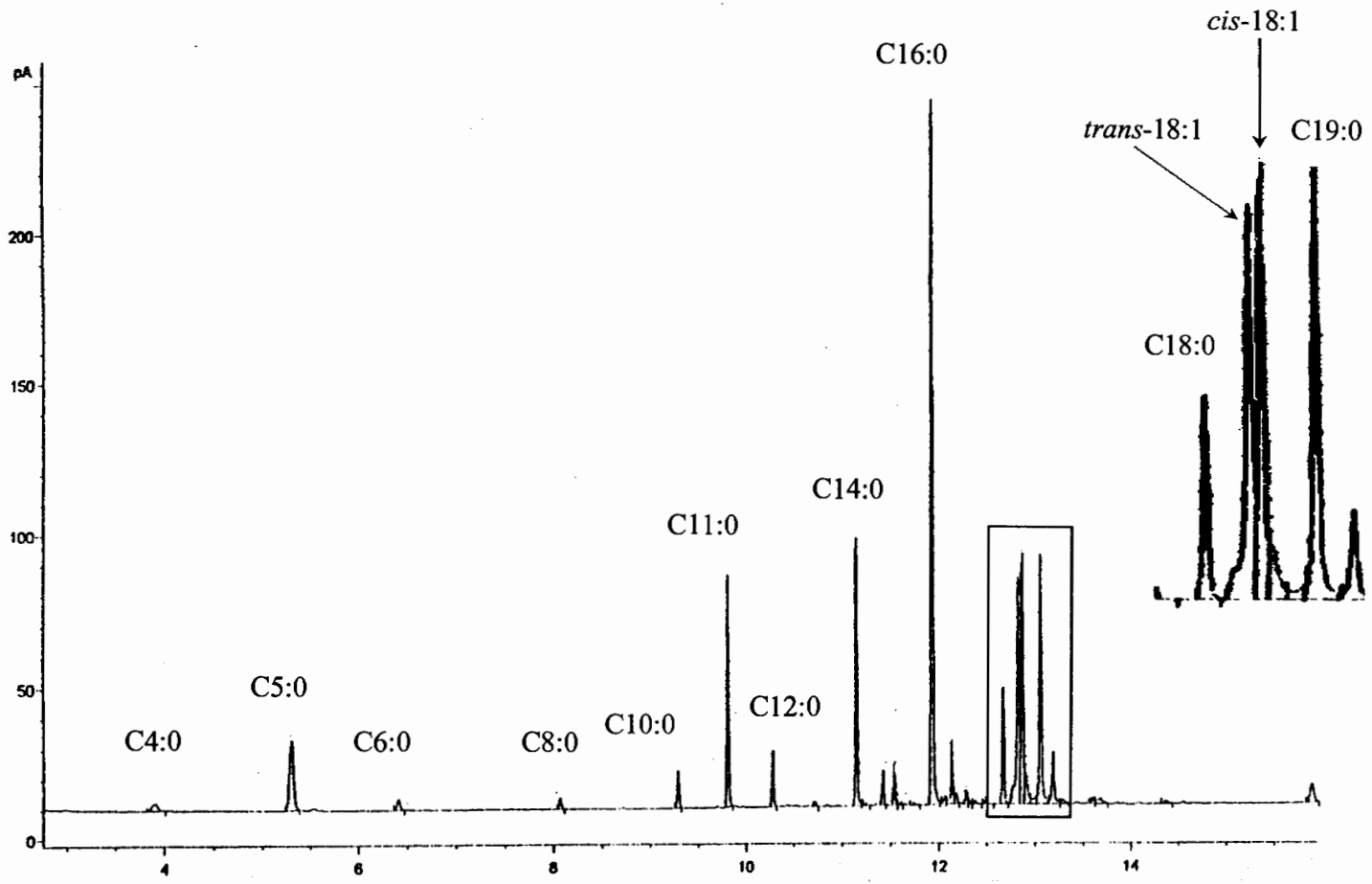


Fig. 5. The chromatograph of milk sample 116 by GC analysis of FABEs. C5:0, C11:0, and C19:0 are the internal standards. The *trans*-18:1 was eluted just before *cis*-18:1.

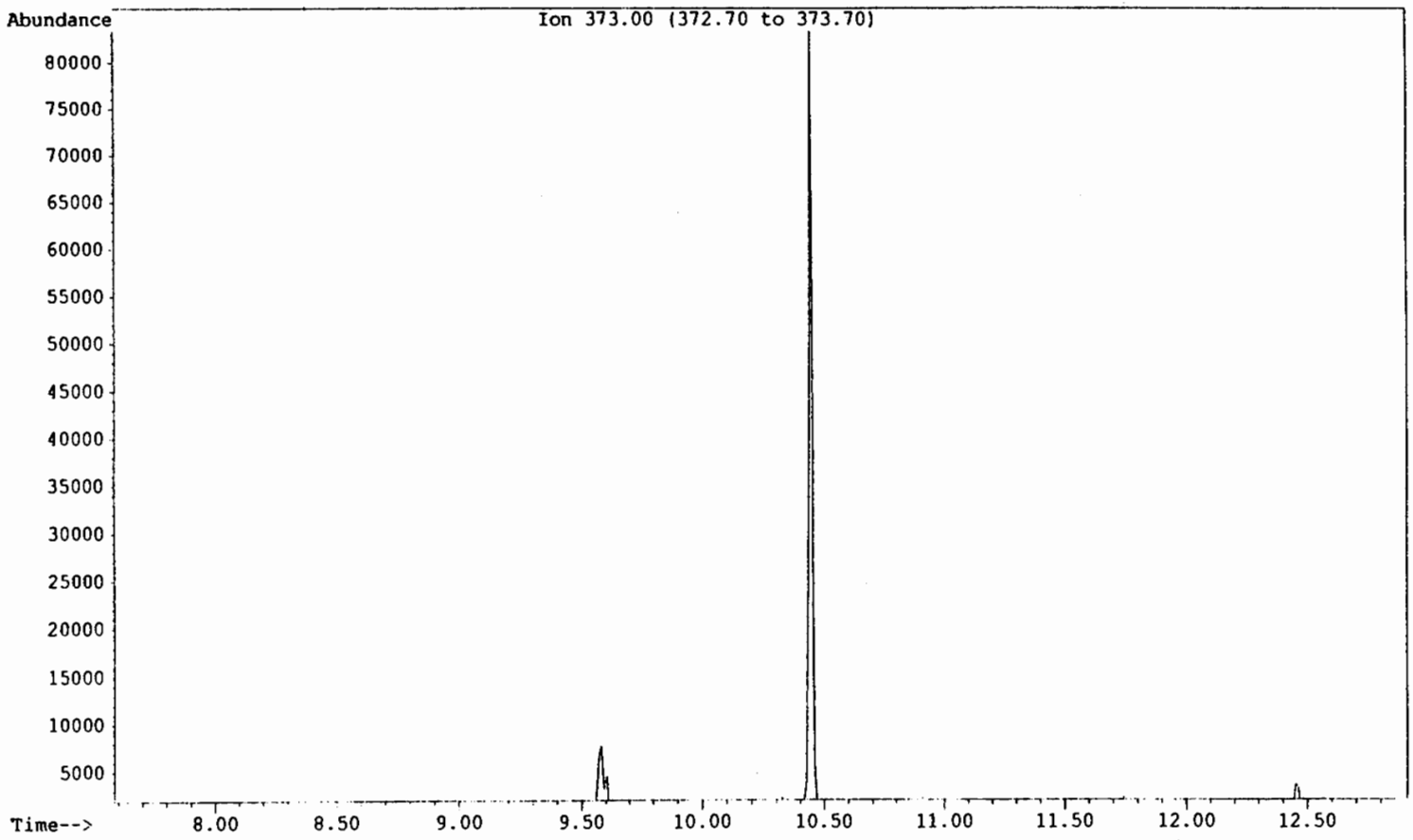


Fig. 6. The extracted chromatograph from the GC/MS containing the derivatives with MW at 373.

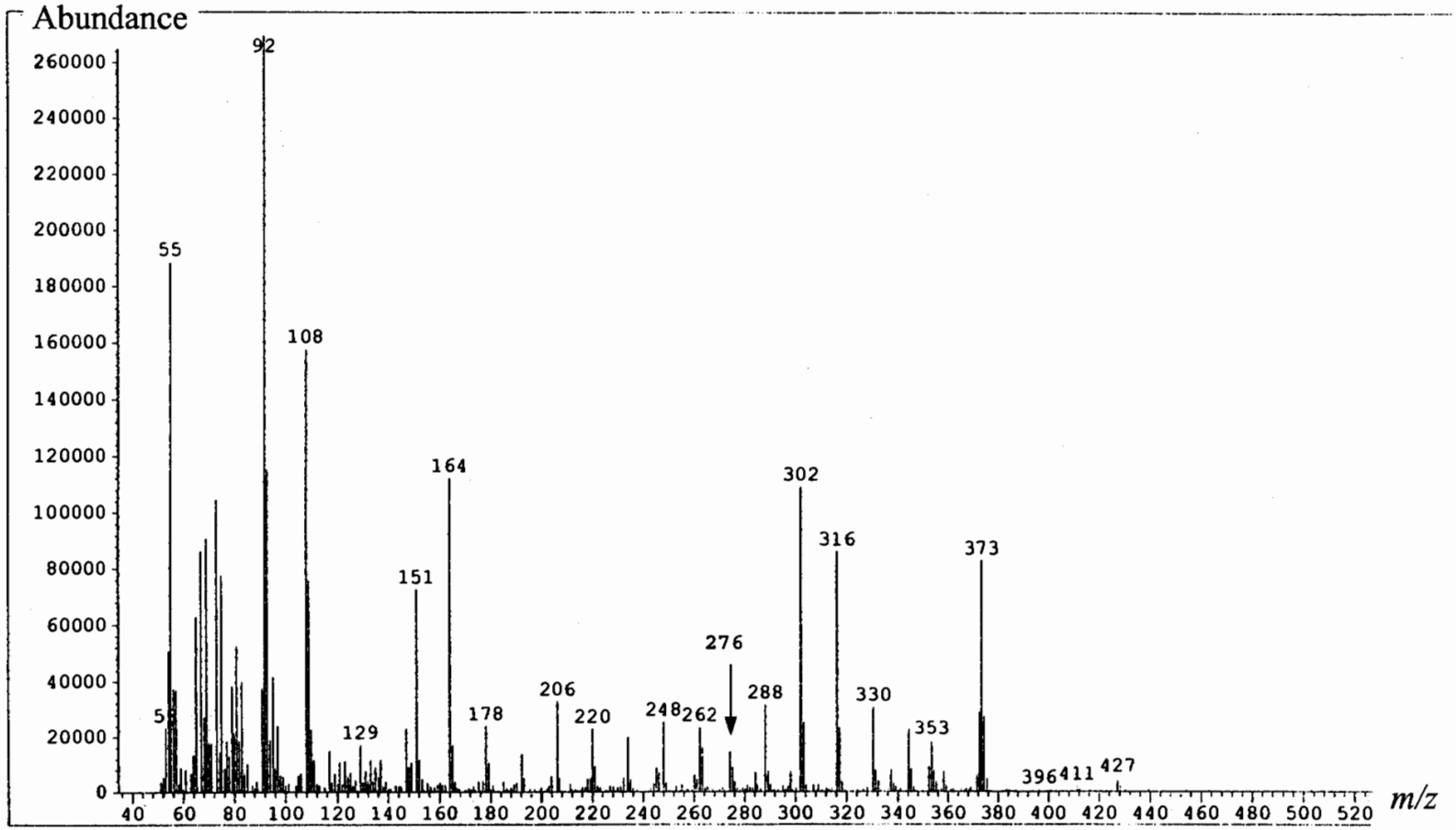


Fig. 7. The mass spectrum of the derivatives with MW at 373. The ions with m/z at 93, 108, 151, and 164 are the characterized ions formed by picolinyl ester derivatives. The ion with m/z at 373 is represent the whole molecule of *trans* 18:1. Since the difference between ions with m/z at 276 and 288 is only 12, the double bond in this fatty acid should be located between C11 and C12.

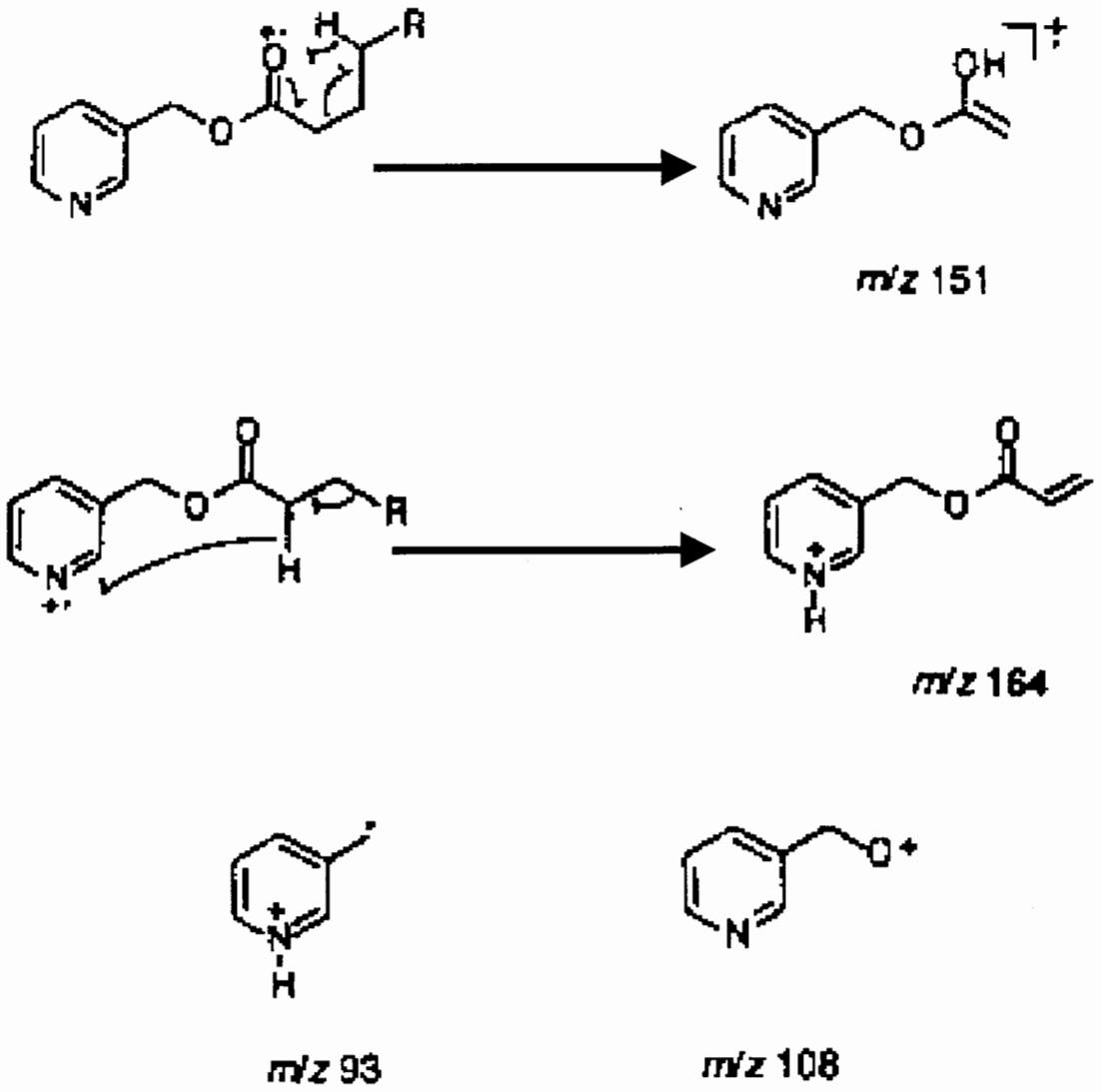


Fig. 8. Structures of the characterized ions formed by picolinyl ester derivatives. (Christie, 1992)

CONCLUSIONS

Milk from individual cows was analyzed by gas chromatography (GC) of the butyl esters and their indices of atherogenicity (IA) were determined. Butter samples were made from high-IA, low-IA, and bulk-tank milk. The IA values, fatty acid profile, and triacylglycerol composition were determined by GC.

The typical creamy/milky flavor and aftertaste of the butter samples were evaluated by a sensory panel of 11 people. The IA differences in the milks and the butters had no detectable impact on the butter flavor as judged by the sensory panel.

The hardness/softness was tested for butter samples in three IA classes. In term of penetration and creep, low-IA butter and high-IA butters were significantly different in texture.

The *cis* and *trans* isomers of fatty acids from butter samples were analyzed by making methyl esters from butterfat and separating them by silver-ion thin-layer chromatography (TLC). The *trans*-18:1$^{\Delta 11}$ was the most abundant *trans* fatty acid in butter. The proportion of *trans*-18:1$^{\Delta 11}$ to *cis* 18:1 was about the same for high-IA butter and low-IA butter. The *trans*-16:1 acids are relatively higher for high-IA butter compared to *cis* 18:1.

The triacylglcerols from butterfat were fractionated into eight bands by silver-ion TLC. The fatty acid composition of the TAG from each band was analyzed by GC. The shortest saturated fatty acid C4:0 appeared first in band 2, which means C4:0 was absent in fully saturated TAG molecules. The C18:1 began to appear in band 2, then

increased to become the most abundant fatty acid from band 5 to band 9. The bulk butter values tend to fall between these of high- and low-IA. Band 3 was about 30% unsaturated and was probably most disaturated-monounsaturated.

During IA determination of the milks, we found some low IA milk samples that had a separated peak that eluted just before *cis*-18:1 in the gas chromatograph. This peak was found in the *trans* monoene band by silver-ion chromatography. In order to identify the position of the double bond in this fatty acid, a picolinyl ester derivative of this fatty acid mixture was made and analyzed by GC/MS. The double band was located between carbon 11 and carbon 12 counting from the carboxyl end of the fatty acid. Thus, the mass spectrum represents vaccenic acid, *trans*-11-octodecenoic acid.

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