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CHEMICAL EVALUATION OF LIPID CLASSES IN THE FRESH AND STORED COW BUTTER

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The lipid fractions from fresh and stored butter were found to be (92.0 % and 91.6 %) neutral lipids and polar lipids (7.92 % and 8.4 %) respectively. The main fatty acids in the neutral and polar lipid fractions were palmitic and oleic acids. Amounts of lower fatty acids i.e. C_4-C_{10} were found only in fresh butter. The enzymatic hydrolysis of the triacyl glycerol fractions showed that they contained large amounts of oleic acid at the Sn-2-position. Palmitic, stearic and other saturated fatty acids were almost exclusively distributed in the Sn-1 and Sn-3 positions. Oleic acid represented 75.5 % and 81.8 % of the fatty acids at the Sn-2-position in fresh as well as in stored butter respectively.

INTRODUCTION

Traditionally about 75 % the rural population of Pakistan, is engaged in agricultural persuits and till very recently it has been using butter fat as the cooking medium. In recent years, however, this situation has changed and people have slowly switched over to hydrogenated fats as they can sell fresh milk at attractive rates. In spite of this there still is a large cross-section of the rural population that uses butter fat for their edible needs. In remote northern areas of the country, where the animal population is not very large, there is a tradition to store butter as such and to use it after many years. A common belief exists in these areas that the older the butter the better are its properties. Also it is believed that a family that can store the butter for a longer period is socially higher.

The present study was undertaken with the view to analysing stored as well as fresh butter for their physico-chemical properties and to compare their chemical composition. This paper also reports upon the structure of fresh and stored butter triacyl glycerols. The distribution of fatty acids in the 2-MG, and by difference in the 1(3) positions of TG was determined by hydrolysis with lipase.

MATERIALS AND METHODS

Stored and fresh* butter used for analysis in the present study were obtained from Gilgit. Standard lipids for thin layer chromatography were prepared by the method of Thomas and Ralston [1], and the other reagents were prepared according to the AOAC methods [2]. Di*iso*propyl ether was used as the reaction medium as little acyl migration occurs in the partial glycerides in the solvent. Pancreatic lipase was used for the lipolysis of fresh and stored butter triacyl glycerols.

Physico-chemical values of the oils. Physico-chemical values such as free fatty acids, iodine value, saponification value, peroxide value, **Stored butter is ten years old. Fresh butter is one day old.* Reichert Meissl value, Polenski value and refractive index of fresh as well as stored butter were determined by standard methods [3] and the results are given in Table 1.

Chromatographic analysis. Crude lipids of stored and fresh butter were separated into neutral and polar fractions by silicic acid adsorption column chromatography using hexane/diethyl ether (7:3, v/v) as the eluting solvents [4]. Neutral and polar lipids were weighed and their percentages were determined. Separated polar lipids were further fractionated by re-chromatography in a column of silicic acid using acetone for glycolipids and methanol for phospholipids as eluting solvents [5].

The non-polar classes of lipids were isolated by thin layer chromatography. Aliquots of the oil (75-100 mg) were streaked on glass plates (20 x 20 cm) coated with 0.5 mm silica gel. Chromatograms were developed in hexane/diethyl-ether/acetic acid (80:20:2, v/v/v) mixture and the resulting bands were made visible under UV lamps by spraying with 2', 7'-dichloro fluorescein in methanol. Lipid classes were identified by the comparison of the R_f values with those of the standards under identical conditions. The bands, made visible under UV lamp by spraying with 2', 7', dichloro fluorescein, were marked and then scraped from plates. Lipids of various classes such as hydrocarbon-wax-ester, triacylglycerol, free fatty acid,

Table 1. Physico-Chemical values of fresh and stored butter

No.	Name	Free fatty acid (%)	Iodine value	Saponification value	Peroxide value	Reichert meissel value	Polanski value	Melting point (°C)	Refractive index
1	2	3	4	5	6	7	8	9	10
1.	Fresh butter	2.96	33.70	229.17	0	26.30	0.90	40.0	1.4645
2.	Stored butter	11.56	29.30	207.79	253.22	0.90	0.70	46.0	1.4690

1, 3 (1, 2) diacyl glycerol, monoacyl glycerol (1 & 2) were scraped into 50 ml. conical flasks. containing 1 ml of internal standard (1 % w/v margaric acid dispersed in diethyl ether) and 25 ml of diethyl ether. The contents of the flasks and additional 25 ml washings were filtered through a fritted disc funnel and the filtrate evaporated to dryness in a rotary evaporator under nitrogen. The dried residues were dissolved in 1.5 ml of chloroform: methanol and methylation was carried out by the mehtod of Kumar and Tsunoda [6]. Samples (10-50 mg) of the glycolipids, phospholipids and of total lipids were also dissolved in the above solvent and methylated.

The methyl esters of the fatty acids in the non – polar and polar lipid fractions were separated and quantified through GC analysis. The relative weight percent of each fatty acid, as determined from peak area, was used to calculate the proportions of non polar lipid fraction. The results of GC anaysis are given in Tables 2 and 3.

Table 2. Percentage of the various fractions and the mole percentage of fatty acids in each of these fractions in fresh butter

Lipid classes		Percentage in lipids	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:0}$	$C_{18:0}$
1.	Hydrocarbons and waxes	5.17	2.5	2.1	1.40	3.2	5.08	8.92	31.68	8.81	33.80	3.77
2.	Triacylglycerol	45.3	2.1	1.7	1.60	3.77	4.98	8.50	34.65	8.78	30.75	3.21
3.	Free fatty acid	6.44	2.3	2.0	1.00	2.86	4.86	9.99	33.02	8.30	32.37	3.3
4.	1, 3-Diacyl glycerol	12.95	2.5	2.18	1.49	3.26	4.43	9.19	32.99	8.43	31.16	3.5
5.	1, 2-Dyacyl glycerol	12.86	2.7	1.8	1.83	3.60	5.85	8.82	32.00	8.87	30.63	3.9
6.	Monoacyl glycerol	2.58	2.2	1.75	1.80	3.07	4.92	9.25	33.20	8.50	31.50	3.8
7.	Monoacyl *1* glycerol	6.56	1.87	1.5	1.50	3.70	5.00	9.00	30.61	8.50	34.72	3.6
8.	Glycolipids *2*	3.70	2.3	1.8	1.50	3.86	5.52	9.13	30.51	8.40	33.08	3.9
9.	Phospholipids	4.22	2.5	1.7	1.59	3.81	4.86	8.99	31.05	8.53	33.26	3.7
0.	Total lipids	–	2.1	1.6	1.54	3.91	5.88	9.70	30.90	8.30	32.1	3.97

Table 3. Percentage of the various fractions and the mole percentage of fatty acids in each of these fractions in stored butter

Lipid classes		Percentage in lipids	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
1.	Hydrocarbons and wax esters	3.14	6.82	10.05	34.43	9.21	35.20	4.29
2.	Triacyl-glycerol	32.53	7.10	10.00	32.88	9.19	36.24	4.63
3.	Free fatty acid	12.0	6.78	10.49	33.98	8.85	34.52	4.38
4.	1, 3-Diacyl-glycerol	12.25	6.69	10.21	33.79	9.11	35.17	4.29
5.	1, 2-Diacyl-glycerol	13.31	7.02	10.13	33.26	9.18	36.12	4.36
6.	Monoacyl-glycerol *1*	10.17	7.09	10.23	33.40	9.20	36.00	4.10
7.	Monoacyl-glycerol *2*	8.2	6.48	10.91	34.01	9.24	35.90	4.20
8.	Glycolipids	4.0	6.82	10.53	34.26	8.99	34.99	4.30
9.	Phospholipids	4.0	6.55	10.60	33.50	9.25	35.90	4.19
10.	Total lipids	–	6.58	10.50	33.10	10.70	36.27	4.30

Enzymatic hydrolysis. Enzyme powder (100-200 mg) was placed in a 25 ml screw capped flask and to this was added water (25 μl), care being taken that the enzyme powder is not wetted. The substrate solution (2.5 ml) was then pipetted into the reaction flask and mixed well before incubating in a shaking water bath at 40° for one hr [7]. The reaction mixture was then cooled and diluted with an additional 2.5 ml of the solvent and centrifuged for 2 min. 2.5 ml of the supernatant was transferred into 10 ml ethanol in a 25 ml titration flask and titrated against 0.1 N NaOH using thymolphthalein as indicator [8]. Blanks obtained above, but with the omission of the triacylglycerol, were substracted and the percentage hydrolysis of the substrate was calculated assuming that 33.1 ml of 0.1 N NaOH will be required for neutralizing fatty acids released on the complete hydrolysis of 1.0 g of butter (fresh and stored). The supernatent (5 μl) as obtained above was applied to freshly activated TLC plates coated with 0.5 mm thick layer of silica gel. The lipid classes – triacyl glycerol, free fatty acid and 2-monoacyl glycerol – were separated and the relative percentages were determined as given already.

Methyl esters of the fatty acids of the lipid class were separately prepared and the results of GLC analysis are given in Table 4. GLC analysis was carried out on a Pye Unicam 204 Series unit using a glass column (1.5 x 4 mm) packed with 20 % PEGS on diatomite (80-100 mesh). Column temperature was maintained at 200° and nitrogen was used as the carrier gas at the flow rate of 40 ml/min. Detection was made by flame ionisation detector and the detector was maintained at 250°. The percentages of individual fatty acids of 1, 2 and 3 positions, of glycerides of fresh and stored butter were calculated [8] and the results are given in Tables 4 and 5.

RESULTS AND DISCUSSION

Chemical analyses of fresh and stored butter were carried out for the various constants as described under the Materials and Methods. As it is established that the acid value increases with the age of fat; therefore, the amount of free fatty acid was higher in stored butter than in fresh butter. Iodine and saponification values were low in stored butter whereas the peroxide value was very high, which showed a change in the relative proportion of the component fatty acids. The Reichert-Meissel and Polenski value were also low in stored butter showing a decrease in low molecular and volatile fatty acids as compared to fresh butter. Melting point was high in stored butter as compared to fresh butter.

Lipid Composition. The lipid fractions of the fresh and stored butter were found to be composed of (92.0 % and 91.6 %) neutral lipids and (7.92 % and 8.4 %) of polar lipids. The relative proportions of the classes of neutral

Table 4. Analysis of monoacyl glycerol and fatty acids from lipase hydrolysis of stored butter

Stored butter	Fatty acid composition					
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
1. Triacyl glycerol	7.10	10.0	32.88	9.19	36.24	4.63
2. Monoacyl glycerol (2-position)	0.48	3.41	10.31	1.72	81.80	2.29
3. % in 2-position (mg)	0.07	11.9	10.89	6.60	75.91	19.3
4. Fatty acids	6.78	10.49	33.98	9.85	34.52	4.38
5. Fatty acids (1, 3) Calc	9.20	13.80	45.16	13.8	12.47	5.32
6. % in 1, 3 position (mg)	97.93	88.3	89.12	92.02	24.1	80.6

Table 5. Analysis of monoacylglycerol and fatty acids from lipase hydrolysis of fresh butter

Fresh butter	Fatty acid composition									
	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
1. Triacyl glycerol	2.10	1.70	1.60	3.73	4.98	8.50	34.65	8.78	30.75	3.21
2. Monoacyl glycerol (2-position)	1.35	0.80	1.16	6.6	2.31	4.75	9.11	2.77	69.60	1.57
3. % in 2-position (mg)	24.64	17.76	24.34	20.52	15.51	19.45	8.96	10.63	75.50	18.20
4. Fatty acids	2.30	2.0	1.0	2.86	4.86	9.99	33.02	8.30	32.38	3.3
5. Fatty acids (1, 3) calc	5.2	2.47	1.13	3.41	6.16	12.07	45.09	11.12	11.90	4.05
6. % in 1, 3 position (mg)	75.36	82.33	75.66	79.48	84.49	80.55	91.04	89.35	24.50	81.8

lipids shown in Tables 2 and 3 were quite similar except that high amounts of triacyl glycerol were found in fresh butter but in stored butter the amounts of free fatty acids were high.

Fatty acid composition. The fatty acid composition of all lipid classes of fresh and stored butter and their total lipids are given in Tables 2 and 3. The short-chain fatty acids (C_4-C_{10}) were found only in the lipids of fresh butter whereas in stored butter they disappeared and, therefore, the percentages of other fatty acids were increased as compared to fresh butter. The results of enzymatic hydrolysis of stored and fresh butter are also given in Tables 4 and 5. The Sn-2-positions were occupied mostly by oleic acid and the percentages determined at this position were (75.91 % and 75.5 %) respectively in the stored and fresh butter samples. The Sn-1 and Sn-3 positions were occupied by palmitic and stearic acids and their percentages were (89.12 % and 91.04 %) for palmktic acid and (92.02 % and 89.35 %) for stearic acid respectively in the stored and the fresh butter samples. The results of the present study show that extensive hydrolysis has occurred in the stored fat and it is also devoid of the lower (C_4-C_{10}) fatty acids. Consequently, it is concluded that there are not any obvious advantages in storing butter for long periods.

REFERENCES

1. A.E. Thomas, J.E. Scharon and H. Ralston, JAOCS., 42, 789 (9163).
2. *"Official Methods of Analysis of the Association of Official Analytical Chemists"* (A.O.C.S., Washington, DC., 1975), 12th ed.
3. F.R. Earle, E.H. Melvin, L.H, Mason, *et al.*, JAOCS, 36, 304 (1959).
4. R.E. Wuthier, J. Lipid Res., 7, 558 (1966).
5. G. Rouser, G. Kritchensky, G. Simon and G. J. Nelson, Lipids, 2, 37 (1967).
6. P.R. Kumar and S. Tsunoda, JAOCS, 55, 320 (1978).
7. F.H. Mattson and R.A. Volpenhein, J. Biol. Chem., 236, 1891 (1961).
8. F.H. Mattson and R.A. Volpenhein, J. Lipid Res., 4, 392 (1963).