Normalcy of Some Non-volatile Carbonyl Compounds and Carbonyl Precursors in the Fat of Synthetically-fed Cows

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Ketoglycerides,[1] bound aldehydes,[2,3] and β-ketoglycerides [4,5] are minor constituents of butterfat. Ketoglycerides occur in butterfat to the extent of about 5—15 μmoles/g [1] and constitute the major carbonyl-containing moiety in butterfat. The bound aldehydes (phosphorus-free analogues of the plasmalogens) have been reported to occur in butterfat to the extent of 0.1 to 0.2 μmole/g [2,3] and can give rise to a series of long-chain saturated and unsaturated, and branched and unbranched aldehydes under the proper conditions.[2,3] β-Ketoglycerides occur in butterfat to the extent of about 0.4 to 1.7 μmole/g.[6] The β-keto acids are hydrolyzed from the glycerides under various heating and storage conditions [6] and the free β-keto acid is then decarboxylated to give methyl ketones. In butterfat the methyl ketones formed from this process are the C_5, C_7, C_9, C_{11}, C_{13}, and C_{15}.

Since the bound aldehydes and β-ketoglycerides can potentially effect the flavor of some dairy products, it was considered important to ascertain whether the milk fat of cows wholly deprived of grass-feeding contained these constituents and also to what extent. Moreover, the role that the natural feeding plays in the elaboration of these constituents was unknown and could be settled through this investigation.

Experimental. Preparation of butteroil. Five liters of fresh milk from the cow Metta,[7] an animal long adapted to synthetic feeding ** was separated and the cream churned in a centrifuge by spinning it in polyethylene bags placed in plastic cups for 40 min at about 4°C and a speed of 6000 rpm. At the end of this time, the buttermilk was drained, the butter placed in stainless steel centrifuge tubes, and centrifuged at 6000 rpm at 40°C for 30 min to yield the clear oil. The same procedure was followed using the milk from a herd of Ayrshires on a normal feeding regime.

Determination of bound aldehydes. The concentration of bound aldehydes was determined on 60 ml of a 10 % solution of the butteroil in carbonyl-free hexane [8] according to the method of Parks *et al.*[2]

Determination of total ketoglycerides. Total ketoglyceride content of the butteroils was determined as follows: 500 mg of butteroil in 20 ml of pure hexane was passed over a column of 2,4-dinitrophenylhydrazine in aqueous phosphoric acid impregnated on Celite as described by Schwartz and Parks.[8] The optical density of the effluent was ascertained and the concentration of carbonyl 2,4-dinitrophenylhydrazones was calculated using $E = 22\,500$. The effluent was then recycled through the column and reread in order to ascertain whether complete reaction of the ketoglycerides had occurred. It was found that a single pass under the conditions employed (5 g column, flow rate = 20—25 ml/h) gave quantitative derivative formation on the above concentration of butteroil. The total amount of carbonyl found minus the bound aldehyde determined on another sample gives the ketoglyceride content of the oil.

Determination of β-ketoglycerides. The β-ketoglyceride content of the butteroils was determined by sealing the butteroil in ampoules in the presence of N_2 and sufficient water to saturate the fat at 100°C. The ampoules were then heated at 100°C for 16 h and the heated oil analyzed for monocarbonyls as described

Table 1. Concentration of bound aldehydes, total ketoglycerides, and β-ketoglycerides in the fat phase of milk from a synthetically-fed cow and that from normally-fed cows.

	Synthetically-fed cow (Metta) μmole/g	Normally-fed-cows μmole/g
Bound aldehydes	0.12	0.11
Total ketoglycerides	5.84	8.47
β-Ketoglycerides	0.60	0.56

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** This milk is called zero milk (0-milk) in this laboratory.

by Schwartz *et al.*[9] Since over 95 % of the carbonyls formed under these conditions are methyl ketones, the increase in the monocarbonyl content over that of an unheated sample is taken as methyl ketones formed.[10]

Results and discussion. Table 1 summarizes the results obtained. All samples were run in duplicate and agreed very closely.

Inspection of the data indicates that all values obtained are in the normal range reported for these constituents of cow's milk fat. Although only the fat from one synthetically-fed cow was analyzed, the results indicate that these constituents are not derived from the feed. One may also expect dairy products made from the milk of synthetically-fed cows to undergo the same elaboration of flavor constituents arising from the classes studied as would dairy products made from normal milk.

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1. Keeney, M., Katz, I. and Schwartz, D. P. *Biochem. Biophys. Acta* **62** (1962) 615.
2. Parks, O. W., Keeney, M. and Schwartz, D. P. *J. Dairy Sci.* **44** (1961) 1940.
3. Schogt, J. C. M., Haverkamp Begemann, P. and Koster, J. *J. Lipid Res.* **1** (1960) 446.
4. Van der Ven, B., Haverkamp Begemann, P. and Schogt, J. C. M. *J. Lipid Res.* **4** (1963) 91.
5. Parks, O. W., Keeney, M., Katz, I. and Schwartz, D. P. *J. Lipid Res.* **5** (1964) 232.
6. Schwartz, D. P., Parks, O. W. and Yoncoskie, R. A. *J. Am. Oil Chemists' Soc.* **43** (1966) 128.
7. Virtanen, A. I. *Science* **153** (1966) 1603.
8. Schwartz, D. P. and Parks, O. W. *Anal. Chem.* **33** (1961) 1396.
9. Schwartz, D. P., Haller, H. S. and Keeney, M. *Anal. Chem.* **35** (1963) 2191.
10. Schwartz, D. P., Spiegler, P. S. and Parks, O. W. *J. Dairy Sci.* **48** (1965) 1387.

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