

BOUND ALDEHYDES IN BUTTEROIL

In connection with studies on off-flavor development in dry whole milk, substantial quantities of fatty aldehyde 2,4-dinitrophenylhydrazones were obtained by passing the extracted lipids (2) through an acid solution of 2,4-dinitrophenylhydrazine, according to the procedure of Schwartz *et al.* (8). Based on previously reported (6) threshold studies, and the flavors of the products investigated, it was concluded and later substantiated that the greater majority of these aldehydes were bound in butteroil.

At the time of this find, bound aldehydes in butteroil had not been reported, although van Duin (10) reported the liberation of aldehydes from the plasmalogen fraction of butter serum. However, during the later stages of this investigation, Schogt *et al.* (7) reported the isolation of a phosphorus-free lipid from butteroil which contained aldehydes bound to glycerol in an enol-ether linkage. This finding confirmed our belief as to the nature of the aldehyde precursor, based on the observation of Day and Lillard (1) that phosphorus was not detectable in butteroil. Schogt *et al.* (7) reported 40 mg. of bound aldehyde per kilogram of fat expressed as myristaldehyde, which compares favorably with our finding of 0.2 μ M bound aldehyde per gram of butteroil.

Our preliminary results on the bound, saturated aldehydes as their 2,4-dinitrophenylhydrazones suggested a complex mixture which was not resolved by the available column chromatographic techniques. Therefore, gas chromatographic techniques were introduced.

The butteroil used in these studies was obtained by melting sweet cream butter at 100° F. and centrifuging in an International centrifuge. The butter was prepared by churning cream obtained from fresh milk. No more than two days time elapsed between milking and conversion to butteroil. The oil was analyzed immediately after preparation. Analysis of oil stored at 0° F. for 2 mo. yielded the same results as the fresh oil.

The method for isolating the aldehydes liberated from the nonphospholipid material was as follows: 800 ml. of a 25% solution of butteroil in carbonyl-free hexane¹ was dried with sodium sulphate, filtered, and passed through

¹ Hexane (Phillips, High Purity Grade) scrubbed through a 5-ft. column of concentrated H₂SO₄, followed by redistillation from KOH pellets (3). Analysis of the purified hexane indicated that it contained 0.65 μ M of carbonyl per liter.

² The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

a chromatographic tube containing three layers of analytical grade Celite² (Johns-Manville Company) separated by glass wool. Layers One and Three contained 20 g. of Celite impregnated with 12 ml. of a saturated solution of sodium bisulphite, whereas Layer Two consisted of 20 g. of celite containing 20 ml. of a 50% solution of phosphoric acid. The column was packed so that a flow rate of less than 1 ml. per minute was attained. Following passage of the butteroil-hexane solution, the packings were washed free of fat with purified hexane. The lower bisulphite packing was removed from the column and the aldehydes freed by decomposing the bisulphite with 20% sodium carbonate. The aldehydes were extracted from the packing and solution with purified hexane. Control samples were attained by omitting the phosphoric acid in Packing No. 2. All investigations showed that undetectable quantities of aldehydes were extracted from the control samples by the lower bisulphite packing. The 2,4-dinitrophenylhydrazones were prepared on a Schwartz *et al.* (8) reaction column and the aldehyde 2,4-dinitrophenylhydrazones purified on adsorptive magnesia, according to the method of Schwartz *et al.* (9). The free aldehydes were obtained by regeneration with levulinic acid by the method of Keeney (4).

Gas chromatographic analyses were performed on a Barber-Colman Model 20 and also on a Research Specialties instrument. The Model 20 employed a 100-ft. capillary column coated with Apiezon L, a Tritium ionization chamber detector, and argon as the carrier gas. The Research Specialties was equipped with a 6-ft. stainless steel column containing a packing of 60-80 mesh, acid-washed Celite coated with Apiezon L. Strontium⁹⁰ was employed as the source for the ionization chamber detector and argon served as the carrier gas. The nature of the complex mixture necessitated varied column temperatures and pressures, in order to resolve and detect the individual aldehydes.

The straight-chain bound aldehydes of butteroil were identified by retention time, using authentic aldehydes as standards. The branched-chain saturated aldehydes were tentatively identified by plotting chain length vs. retention time on semilog paper.

A chromatographic analysis of the saturated aldehydes on the packed column revealed the presence of straight-chain aldehydes varying from nine to 18 carbons in chain length. In addition to the homologous series of straight-chain aldehydes, branched-chain aldehydes containing 11 to 18 carbons, with the exception of a C₁₂ aldehyde, constitute a major portion of the bound carbonyls. A semilog plot of

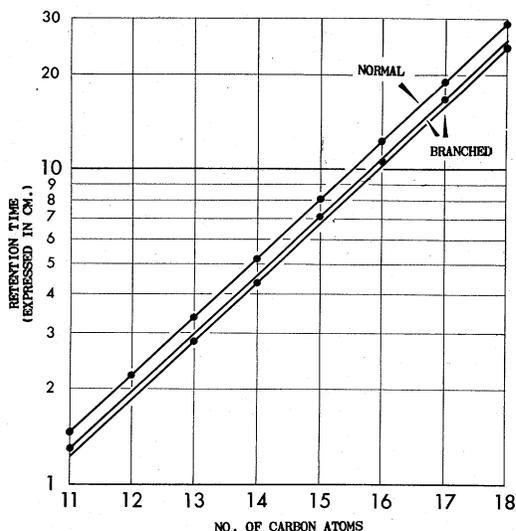


FIG. 1. Semilog plot of retention time vs. carbon chain of bound saturated aldehydes from butteroil.

carbon chain vs. retention time (Figure 1) revealed that two types of branching were present in the complex mixture. This was further substantiated on the capillary column which separated the branched aldehydes containing 13, 15, and 16 carbons into two distinct peaks. Although not reporting the aldehydes bound in butteroil, Schogt *et al.* (7) proposed that the presence of aldehydes of less than 12 carbons in chain length is a result of autoxidation of the butteroil. The method reported herein compensates for any autoxidation and the results indicate that small amounts of C_6 to C_{11} , and possibly lower molecular weight normal and branched aldehydes, are bound in butteroil. A complete summary of the bound aldehydes detected in butteroil is presented in Table 1.

Direct reaction of butteroil with 2,4-dinitrophenylhydrazine also revealed the presence of small amounts of unsaturated aldehydes. The presence of three enals (approximate chain lengths of 12, 18, and 20 carbons) and three dienals (C_{16-20}) was established by paper chromatography, according to the method of Klein and de Jong (5), by ultraviolet studies in 95% ethanol, and by magnesia class separation (9). Smaller amounts of other enals ranging from 13-16 carbons in chain length also were observed.

As a comparative study, the aldehyde 2,4-dinitrophenylhydrazones from the plasmalogens were obtained according to the procedure of van Duin (10), separated into classes on magnesia, and the saturated aldehydes regenerated and gas-chromatographed. Table 1, summariz-

ing the saturated aldehydes from the plasmalogen fraction of butter serum, shows that the saturated aldehydes bound in this lipid fraction are similar to those in butteroil. However, the gas chromatograms in Figure 2 illustrate that the relative amounts of the higher molecular weight aldehydes (C_{13-18}) differ considerably within these fractions. Whereas the greater majority of bound, saturated aldehydes in butteroil range in chain length from 14 to 16 carbons, the C_{13-18} aldehydes dominate the bound, saturated aldehydes of the plasmalogens. Furthermore, the plasmalogens also contain a variety of unsaturated aldehydes, although still in relatively minor amounts. Evidence based on paper chromatography, behavior on magnesia, and ultraviolet studies indicate that enals of 16 to 18 carbons and eight to 12 carbons in chain length are dominant, with barely detectable amounts of C_{13-15} unsaturated aldehydes. Although all necessary precautions were observed to prevent autoxidation, the presence of the lower molecular weight unsaturated aldehydes as a result of chemical deterioration can not be disregarded.

TABLE 1

Summary of data on the bound, saturated aldehydes in butteroil and the plasmalogen fractions of milk

No. of carbon atoms	Butteroil		Plasmalogens	
	n-Aliphatic	Branched	n-Aliphatic	Branched
18	+	-	+	-
17	+	+	+	-
16	+	+	+	+
15	+	+	+	-
14	+	-	+	+
13	+	+	+	-
12	+	-	-	-
11	+	+	-	-
10	+	-	-	-
9	+	-	-	-

The significance of bound aldehydes in the off-flavor development of various dairy products is under investigation at the present time.

OWEN W. PARKS

MARK KEENEY

DANIEL P. SCHWARTZ

Dairy Products Laboratory
Eastern Utilization Research
and Development Division
Agricultural Research Service
U. S. Department of
Agriculture
Washington 25, D. C.

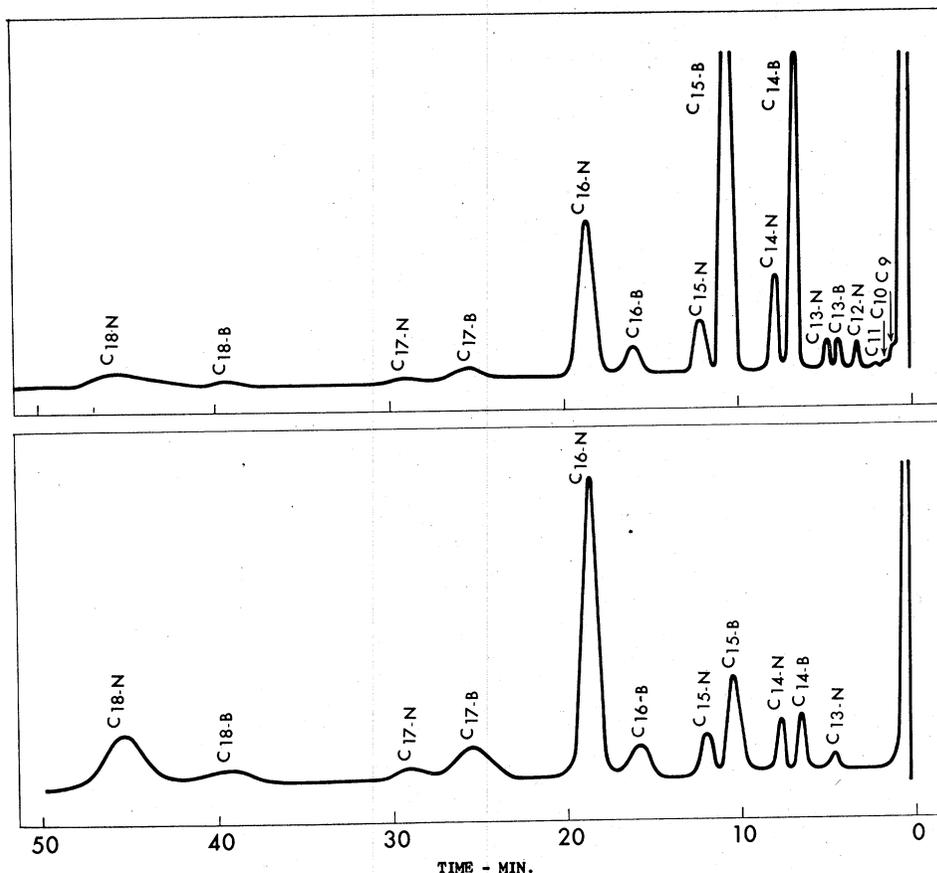


FIG. 2. Separation of bound saturated aldehydes from butteroil (top) and plasmalogens (bottom) on a 6-ft. stainless steel column containing 10% Apiezon L. on 60-80 mesh Celite, temperature 175°, argon pressure—30 p.s.i., voltage—1,000. C_x = No. of carbon atoms; N = Normal; B = Branch.

REFERENCES

- (1) DAY, E. A., AND LILLARD, D. A. Autoxidation of Milk Lipids. I. Identification of Volatile Monocarbonyl Compounds from Autoxidized Milk Fat. *J. Dairy Sci.*, 43: 585. 1960.
- (2) EMERY, S., AND SCHWARTZ, D. P. Method for the Quantitative Extraction of Fat from Dry Whole Milk. *J. Dairy Sci.*, 44: 721. 1961.
- (3) KEENEY, MARK. Unpublished data.
- (4) KEENEY, MARK. Regeneration of Carbonyls from 2,4-Dinitrophenylhydrazones with Levulinic Acid. *Anal. Chem.*, 29: 1489. 1957.
- (5) KLEIN, F., AND DE JONG, K. Paper Chromatography of 2,4-Dinitrophenylhydrazones of Aliphatic Carbonyl Compounds. *Rec. trav. chim.*, 75: 1285. 1956.
- (6) LEA, C. H., AND SWOBODA, P. A. T. The Flavour of Aliphatic Aldehydes. *Chemistry and Industry*, 1289-1290. 1958.
- (7) SCHOGT, J. C. M., BEGEMANN, P. HAVERKAMP, AND KOSTER, J. Nonphosphatide Aldehydogenic Lipids in Milk Fat, Beef Tallow, and Ox Heart. *J. Lipid Research*, 1: 446. 1960.
- (8) SCHWARTZ, D. P., HALLER, H. S., AND KEENEY, M. Method for the Direct Quantitative Isolation of the 2,4-Dinitrophenylhydrazone Derivatives of Carbonyls from Fats and Oils. Presented at the 136th Meeting, Am. Chem. Soc., Atlantic City, N. J. September, 1959. Abstracts of Papers, p. 15A.
- (9) SCHWARTZ, D. P., PARKS, O. W., AND KEENEY, M. Chromatographic Separation of 2,4-Dinitrophenylhydrazone Derivatives of Aliphatic Carbonyl Compounds into Classes on Magnesia. Presented 138th Meeting, Am. Chem. Soc., New York, New York, September, 1960. Abstracts of Papers, p. 15B.
- (10) VAN DUIN, H. Investigation into the Carbonyl Compounds in Butter. III. Phosphatide Bound Aldehydes. *Netherlands Milk and Dairy J.*, 12: 90. 1958.