Quantitative Studies on Methyl Ketone Formation in Butteroil: Effect of Temperature

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Abstract

A quantitative study of the effect of various heat treatments on methyl ketone formation in butteroil was conducted. Methyl ketone formation was found to follow first-order reaction kinetics. The energy of activation was calculated to be approximately 26.55 kcal/mole. The ketones were formed in a fairly constant molar ratio approximating to the following C_{15},2:C_{13},1:C_{11},1:C_9,1:C_7,2:C_5,1. The temperature-time conditions had little or no effect on the ratio.

Introduction

IT HAS BEEN WELL ESTABLISHED by a number of different techniques that a series of odd-numbered (from C_5 through and including C_{15}) methyl ketones can be produced in butterfat, butteroil or some fat-containing dairy products when the proper heat treatment and exposure is applied (1,4, 6–11,14,15). Temperatures in the vicinity of 100C produce the ketones relatively rapidly. Lower temperatures require longer exposure periods, (31) whereas saponification causes rapid appearance of the ketones (11). Even mild thermal exposure (i.e., 40C) under reduced pressure, conditions which are widely utilized for the isolation of flavor volatiles, may produce the ketones as artifacts (7).

The precursors of the methyl ketones in butterfat have been identified as a series of β-keto esters (14) and more specifically as β-keto acid-containing glycerides (10). This being the case, hydrolysis of the β-keto acid from the glyceride is a prerequisite for methyl ketone formation; thus, sufficient water must be present in the fat for the phenomenon to occur (6,14).

To the authors' knowledge no report has appeared describing a parallel situation in other fats and oils and butterfat may be unique in this regard.

Although the formation of methyl ketones in butterfat has been known for some time, no fundamental work has been documented to date on the kinetics of thermal breakdown of the precursors. Adequate methods for quantitative carbonyl analysis developed in this Laboratory (12) lend themselves readily to a study of this type and form the basis of this manuscript.

Experimental

Preparation of Butteroil

Butteroil was prepared by churning fresh cream (from mixed herd milk, Beltsville, Md.), melting the resulting butter at 40C and centrifuging the oil layer until clear. Twelve milliliter portions of the oil were transferred to 15 ml ampoules with a hypodermic syringe, nitrogen bubbled through the oil for 10 min and the ampoules sealed immediately and stored at −18C until needed. Adequate precaution was exercised to insure that none of the oil was exposed to the heat of the flame during the sealing operation.

Heat Treatment of Butteroil

The ampoules were incubated at 115C, 100C, 77C and 50C in a constant temperature bath or room for various periods of time. At the end of the desired exposure period the ampoule was removed and either analyzed immediately or stored at −18C until needed.

Analysis of Methyl Ketones

For the analysis of methyl ketones produced in a heated sample, the ampoule was crushed under carbonyl-free hexane (13), and the solution passed over a column of Celite impregnated with a phosphoric acid solution of 2,4-dinitrophenylhydrazine as described by Schwartz and Parks (13). The amount of oil having passed over the column was determined by weighing the residue from an aliquot of the effluent. The remainder of the procedure for the isolation and fractionation of the 2,4-dinitrophenylhydrazone derivatives was conducted as described by Schwartz et al. (12). Briefly, the derivatives in the fat-hexane solution were adsorbed onto a column of Seasorb 43: Celite 545 (1:1) and the fat washed through with hexane. The hydrazones, adsorbed as a purple band, were desorbed with 25% nitromethane in $CHCl_3$ and the solvents removed under a stream of N_2. The residue was taken up in hexane and passed over a column of 6% hydrated alumina. The classical monocarbonyl fraction (containing the methyl ketones) was selectively eluted with a benzene:hexane (1:1) solution. The residue obtained after evaporation of the solvents was taken up in $CHCl_3$ and separated into classes on Seasorb 43: Celite 545 (1:1) using a gradient of MeOH in $CHCl_3$. The methyl ketone band (gray on the column) was collected and the amount present determined spectrophotometrically in $CHCl_3$ at 362 mμ using a molar absorptivity of 22,500.

Resolution of the methyl ketone fraction into its individual components was carried out by liquid-liquid column partition chromatography in the acetonitrile-hexane system as described by Corbin et al. (2). The C_{15} and C_{13} ketone bands which do not completely separate in this system were collected and

TABLE I
Effect of Temperature and Time on Methyl Ketone Formation in Butteroil

Temperature							
50C		77C		100C		115C	
Time, hr	Methyl ketones, μmoles/10 g	Time, hr	Methyl ketones, μmoles/10 g	Time, hr	Methyl ketones, μmoles/10 g	Time, hr	Methyl ketones, μmoles/10 g
336	2.49	17	2.01	1	2.52	.25	2.23
1008	6.09	41	3.53	3	4.16	1.0	4.60
2352	8.13	161	8.71	5	5.38	3.0	9.20
		218	9.00	8	7.24	7.0	11.00
		264	9.80	16	10.40	24.0	11.90
		504	11.10	24	11.20		
				92	11.80		

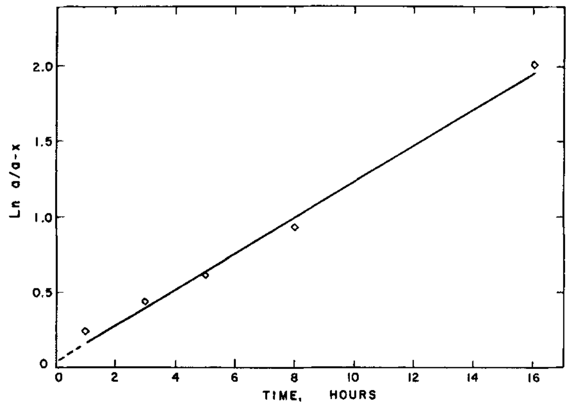

FIG. 1. First order reaction plot of methyl ketone formation in butteroil at 100C.

resolved by reverse-phase liquid-liquid partition chromatography on silaned Celite using dodecane as stationary phase (6 ml on 15 g silaned HyFlo SuperCel prepared according to Howard and Martin (5)) and 85% acetonitrile-15% as mobile phase. The concentration of the individual ketones isolated in both chromatographic procedures was determined spectrophotometrically in chloroform using a molar absorptivity of 22,500 at 362 mμ.

Results and Discussion

An analysis of the experimental results assembled in Table I showed the formation of methyl ketones to be kinetically of a first order type and could be expressed mathematically by $\frac{-dc}{dt} = kc$ where c is the concentration of the reacting precursor. The proportionality constant k is the specific reaction rate or rate constant.

A more convenient form for expressing the above differential rate equation is $\frac{dx}{dt} = k(a\text{–}x)$ where a is the initial concentration and x is the amount of product formed at time t. At time t, a–x is the concentration of the reactant remaining. Upon integration and rearranging of terms. a more useful equation is obtained as follows:

$$k = \frac{1}{t_2 - t_1} \ln \frac{(a - x_1)}{(a - x_2)}$$

The above equation, suitable for calculating k between any time intervals can be simplified by making t_1 equal to zero time. After this modification the equation becomes reduced to $k = \frac{1}{t} \ln \frac{a}{(a - x)}$. Using this equation and plotting each value of ln a/(a–x) against each value of t, the slope of a straight line corresponding to the reaction rates at 50C, 77C, 100C and 115C was obtained. An example of how well the experimental data fit the above equation is shown in Figure 1 for the reaction rate at 100C. Reaction rates at the four studied temperatures are given in Table II.

The energy of activation for the formation of methyl ketones was obtained from the reaction rate constants using the Arrhenius equation. The equation relating the energy of activation to reaction rate is as follows: $k = Ae^{-E/RT}$. Taking the logarithms of the equation there is obtained the following form: $\log k = \log A\left(\frac{-E}{2.303RT}\right)$. A plot of log k against 1/T, shown in Figure 2 illustrates the validity of the Arrhenius equation in the temperature range studied. The slope being equal to –E/2.303RT was used to calculate the energy of activation for the formation of methyl ketones. A value of 26.55 kcal/mole was obtained.

TABLE II

Specific Reaction Rates of Methyl Ketone Formation

Temperature, C	Reaction rate k (sec^{-1})
50	1.16×10^{-7}
77	1.68×10^{-6}
100	3.39×10^{-5}
115	9.80×10^{-5}

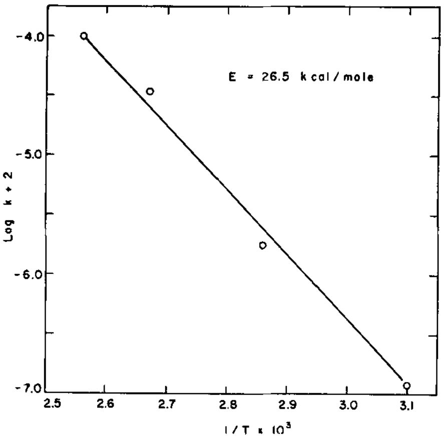

FIG. 2. Energy of activation plot for methyl ketone formation in butteroil.

Analyses of the methyl ketone hydrazones isolated from the heated fat samples showed that the ketones were formed under any time-temperature conditions in a fairly constant molar ratio. This ratio approximated very closely to the following: C_{15},2:C_{13},1:C_{11}, 1:C_9,1:C_7,2:C_5,1, for this particular fat. Analyses of other butteroil samples indicated that this ratio prevailed in almost all instances, although slight deviations were noted. In all cases, however, the C_{15} and C_7 ketones were produced in greatest amounts, an observation recorded several times in the literature (6,14).

The maximum potential of this particular sample of butteroil to produce methyl ketones was of the order of 1.2 μmoles per gram or butteroil. Analyses of some other samples of butteroil in this Laboratory indicated maximum potentials of from 0.4 to 0.6 μmoles per gram. Langler and Day (6) give a value of approximately 1.7 μmoles per gram on the sample of butteroil which they analysed. Considerable variation in the potential of different samples of butteroil to elaborate methyl ketones may, therefore, be expected to occur.

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