

# Method for Determining the Contribution of Methyl Ketones to Flavors of Sterile Concentrated Milks

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## Abstract

A method for determining the concentrations of the  $C_5$  through  $C_{15}$  odd-carbon-numbered methyl ketones in fluid milks, based on the free  $C_{13}$  methyl ketone content and the methyl ketone potential remaining in the fat phase of the product, is presented. Application of the procedure to samples of commercial evaporated milk led to the conclusion that the role of methyl ketones in the off-flavor of this product is dependent on total methyl ketone potential of the milk fat, composition of the methyl ketone potential (especially the heptanone-2 potential), and degree of hydrolysis and decarboxylation of  $\beta$ -keto acids as determined by heat treatment and storage conditions.

Studies on the chemical compounds which develop during the manufacture and storage of sterile concentrated milks have been the subject of numerous investigations concerned with the off-flavors associated with this product. With few exceptions, these previous studies were concerned with compound identification or changes in concentration during manufacture and storage, with little attention to the relevancy of the compound or compounds in the off flavor of the product. Quantitative analysis on the concentration of a particular compound or group of compounds is, in many instances, a formidable task because of the nature of the product, chemical nature of flavor compound(s), or exceptionally small concentrations of the compound present in the product. In the methyl ketones, the situation is further complicated by the presence of an homologous series which act synergistically on the flavor threshold (3). Hence, quantitative data on all members of the homologous series are required to determine the significance of this class of compounds on off-flavor of the product.

Steam distillation or solvent extraction techniques, for various reasons, are not entirely suitable for quantitative studies on an homologous

series of compounds varying widely in boiling points, partition coefficients, or subject to formation by heat treatments. This report presents a different approach, based on the knowledge that a definite methyl ketone potential exists in milk fat, in determining the concentration of these compounds in evaporated milks and the role they play in the off-flavor of this product.

## Experimental Methods

To one liter of commercial 2:1 evaporated whole milk, purchased at local retail outlets and having flavors typical of these products, was added a 400-ml aqueous solution containing 11.34 g of sodium oxalate. Following overnight holding at 4.4 C, the mixture was centrifuged at  $6,300 \times g$  for 45 min at 4.4 C in a Sorvall<sup>1</sup> refrigerated centrifuge. The recovered cream was reconstituted with 200 ml of distilled water and freeze dried. Seven hundred and fifty milliliters of carbonyl-free hexane (8) were added to the freeze-dried cream in a three-liter, round-bottom flask and the mixture stirred vigorously with a magnetic stirrer. One milliliter of distilled water, in increments of 0.2 ml, was added to the dried cream-hexane mixture and stirring continued for 2 hr. The cream powder-hexane mixture was transferred to a Büchner funnel and vacuum filtered. The cream powder was rewashed with three additional 250-ml portions of carbonyl-free hexane and the combined filtrates were made up to two liters with additional carbonyl-free hexane.

*Determination of free methyl ketones in evaporated milk.* Duplicate 400-ml portions of the hexane-evaporated milk fat solution from the two-liter stock solution were dried with sodium sulfate and the free methyl ketone 2,4-dinitrophenylhydrazones<sup>2</sup> prepared and isolated as a class according to methods previously described (7, 9). The individual methyl ketone 2,4-DNP-hydrazones were obtained by partition chromatography on 50-g analytical grade Celite

<sup>1</sup> Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

<sup>2</sup> DNP hereafter refers to dinitrophenyl.

columns employing acetonitrile as the immobile phase and hexane saturated with acetonitrile as the mobile phase (2). Optical density determinations of the 2,4-DNPhydrazones were obtained in chloroform at 365  $m\mu$  and concentrations calculated on the basis of a molar extinction coefficient of 22,500.

*Determination of methyl ketone potential remaining in fat of evaporated milk.* Two hundred milliliters of the hexane-evaporated milk fat solution were added in 50-ml increments to a 250-ml round-bottom flask and the hexane removed by evaporation under vacuum at 30 C. A definite weight (4 to 6 g) of the evaporated milk fat was added to a 10-ml glass vial, one to two drops of distilled water added, nitrogen bubbled through the fat for 7 min, the vial sealed under nitrogen, and the fat heated 16 hr at 140 C (3). The vial was cooled to room temperature and broken under 200 ml of carbonyl-free hexane, the fat-hexane solution dried with sodium sulfate and the methyl ketone 2,4-DNPhydrazones prepared, isolated, and concentrations determined spectrophotometrically as described.

*Recovery of methyl ketones added to milk fat homogenized into fresh skim milk.* Approximately 3 mg each of the  $C_{11}$ ,  $C_{13}$ , and  $C_{15}$  methyl ketones (99.5% purity<sup>3</sup>) were added to 200 ml of fresh butteroil and stirred thoroughly with a magnetic stirrer. The actual concentrations of the methyl ketones added were determined on 9 g of the butteroil by formation, isolation, and spectrophotometric determination of the 2,4-DNPhydrazones according to the procedure outlined. Fresh butteroil free of added methyl ketones was employed as the control sample.

Thirty-six grams of the methyl ketone-enriched butteroil were homogenized into 1,000 g of fresh skim milk at 140.6 kg/cm<sup>2</sup> and 60 C in a pilot plant-size homogenizer. The initially homogenized product was passed through the homogenizer a second time under the same conditions. Recombined milk containing 36 g of fresh butteroil homogenized into 1,000 g of fresh skim milk was employed as the control. The homogenized samples were cooled and held at 4.4 C for 24 hr, then 400 ml of distilled water containing 11.34 g of sodium oxalate were added, and the samples stored an additional 16 hr at 4.4 C. The methyl ketone 2,4-DNPhydrazones were determined in the ketone-enriched and control samples according to procedures previously described. Recovery of individual methyl ketones was calculated in two

ways: 1) on the basis of the concentration of methyl ketone per gram of fat recovered relative to the concentration per gram of fat added, and 2) quantity recovered relative to the quantity added to the product.

*Flavor studies.* Weighed samples of the  $C_5$  through  $C_{15}$  odd-carbon number methyl ketones (99.5% purity) were added singly to 36-g portions of fresh butteroil at concentrations equal to the original potential concentration of each methyl ketone in the fat of the individual evaporated milks analyzed. The methyl ketone-enriched butteroils were homogenized individually into 1,000-g portions of fresh skim milk at 49 C and 140.6 kg/cm<sup>2</sup>. Dilution of the ketone-enriched samples to levels equal to 75%, 50%, and the free methyl ketone levels in the evaporated milks studied was accomplished with reconstituted butteroil-fresh skim milk. The samples were presented to a six- to eight-member taste panel who were asked to indicate the absence or presence (and to what degree) of methyl ketones in the samples. Each sample was replicated a minimum of three times.

## Discussion

The quantitative recovery of milk fat from a homogenized product is difficult and is further complicated by the need for solvents which can be made carbonyl free and suitable to the quantitative methods available for analyzing carbonyl compounds.

The procedure employed for recovering milk fat from evaporated milk, when applied to fresh recombined whole milks, resulted in the recovery of  $36.1 \pm 2.3$  g of freeze-dried cream plug and  $78 \pm 7\%$  of the original milk fat added. The fat recoveries, although not quantitative, were consistent and hence suitable for studies on the recovery of added methyl ketones. The amount of freeze-dried cream and percentage of fat recovered from the commercial evaporated milks are not as consistent (Table 1) as for the recombined whole milks; this was to be expected, since we were examining five different commercial samples. It can only be speculated that the wide discrepancies in some samples (especially Sample 135) are the result of variations in the nature and extent of the fat-protein complex in these products, a complex dependent to a certain degree on the age of the product. These differences did not appear to influence to any extent the results of the free methyl ketone analyses, the results of which are elaborated on later.

Initial studies on the fat recovered from evaporated milk revealed the presence of the

<sup>3</sup> Lachat Chemicals Inc., Chicago, Illinois.

TABLE 1. Recovery data on five samples of commercial evaporated milk.

	Sample no.:				
	135	137	139	141	147
Weight of freeze-dried cream (g)	140.0	103.0	100.0	100.5	100.9
Fat recovered (%) <sup>a</sup>	89.7	61.0	59.8	79.3	60.9

<sup>a</sup> Based on 84.27 g of fat/liter of 2:1 evaporated milk.

C<sub>15</sub>, C<sub>13</sub>, C<sub>11</sub> and traces of the C<sub>7</sub> methyl ketones. Previous evidence (1, 3, 10, 11) indicates that the rate of hydrolysis and decarboxylation of β-keto acids to methyl ketones is not dependent on chain length. Hence, it is concluded that the absence of the C<sub>9</sub> and C<sub>5</sub> methyl ketones and the presence of only traces of the C<sub>7</sub> compound were the result of these compounds partitioning to a greater extent away from the fat portion of milk, losses as a result of freeze-drying, or some other undetermined reasons.

The recoveries on the C<sub>15</sub>, C<sub>13</sub>, and C<sub>11</sub> methyl ketones from recombined milks, reported in Table 2, demonstrate the expected lowering of the recovery rates with decreasing chain length. The recovery values based on the micromoles of methyl ketone per gram of fat recovered from the product are higher than those reported on the basis of the actual amount recovered from the product. The results suggest that either a portion of the methyl ketones exists in the cream plug independent of the fat phase, making them readily available to extraction procedures, or are extracted from the residual fat-protein complex in a manner similar to that demonstrated by Patton (5). These results, substantiated by a greater than 100% recovery (on a fat basis) of the C<sub>15</sub> methyl ketone, indicate that recoveries based on the actual amount recovered relative to the quantities added to the product are more accurate when applied to a fluid product. The reasons for large variations in the recovery of the C<sub>15</sub> methyl ketone are unexplainable, because of the consistent re-

coveries of the C<sub>13</sub> and C<sub>11</sub> methyl ketones. Whatever the reason(s) for these variations, the C<sub>15</sub> methyl ketone is unsuitable as the basis for the calculations to follow.

As noted previously, quantitative data on the complete homologous series of methyl ketones generated during manufacture and storage of evaporated milk are required for accurate flavor evaluations. Results of our initial analyses indicated that direct quantitative measurement of the methyl ketones by our procedure was inadequate. However, by determining the total methyl ketone potential (precursor) remaining in the isolated fat and having the capability of determining the free concentration of one or more higher members of the homologous series, it is possible by calculation to determine adequately the free concentration of all members of the homologous series. The following formulae, utilizing the results of the recovery studies on the C<sub>13</sub> methyl ketone, were employed for this purpose:

$A_{cx} - B_{cx} = C_{cx}$  where A is the concentration of the methyl ketone in the fat of evaporated milk heated 16 hr at 140 C; B is the concentration of the free methyl ketone determined in the hexane extract of the freeze-dried cream; and cx is the methyl ketone carbon number. When the formula is applied to the C<sub>5</sub> through C<sub>15</sub> odd-carbon-number methyl ketones, the potential (precursor) concentration (C<sub>cx</sub>) of each methyl ketone remaining in the fat of evaporated milk is determined.

$B_{c13} \div 0.625 = D_{c13}$  The concentration of the free C<sub>13</sub> methyl ketone (B<sub>c13</sub>) determined, divided by the recovery factor for the C<sub>13</sub> methyl

TABLE 2. Recovery data on methyl ketones added to recombined whole milk.

Methyl ketone	$\frac{\mu\text{moles recovered}}{\mu\text{Moles added}} \times 100$	$\frac{\mu\text{moles/g of fat recovered}}{\mu\text{moles/g of fat added}} \times 100$
	(Average per cent recovery)	
C <sub>15</sub>	84.5 ± 20.7	112.7 ± 32.6
C <sub>13</sub>	62.5 ± 5.0	80.2 ± 1.9
C <sub>11</sub>	30.3 ± 2.9	39.7 ± 3.8

TABLE 3. Calculated free methyl ketone content of five samples of commercial evaporated milk.

Methyl ketone	$\mu\text{moles/liter of reconstituted milk}$				
	135	137	139	141	147
	( $\mu\text{moles of reconstituted milk}$ )				
C <sub>15</sub>	1.801	1.476	3.427	1.877	1.246
C <sub>13</sub>	0.982	1.152	1.458	1.472	1.500
C <sub>11</sub>	0.605	0.805	0.966	0.588	0.603
C <sub>9</sub>	0.773	0.467	0.884	0.583	0.694
C <sub>7</sub>	2.250	2.706	2.884	3.565	1.880
C <sub>5</sub>	0.835	0.754	0.646	1.138	0.622
Total	7.246	7.360	10.265	9.223	6.545
Percentage of original potential	37.0	29.7	37.7	29.2	37.0

ketone from the data in Table 2, results in the actual concentration ( $D_{c_{13}}$ ) of the free C<sub>13</sub> methyl ketone present in the evaporated milk sample.

$C_{13c} + D_{c_{13}} = E_{c_{13}}$ , the sum of the potential C<sub>13</sub> methyl ketone remaining as the precursor in the fat of evaporated milk and the actual free C<sub>13</sub> concentration in the sample, equals the original C<sub>13</sub> methyl ketone potential ( $E_{c_{13}}$ ) in the milk sample prior to concentration and sterilization.

$C_{c_{13}} \div E_{c_{13}} = F$ , where F is the proportion of the original methyl ketone potential remaining in the fat of the evaporated milk sample. F is used to determine the original methyl ketones in the fat of the milk sample by the following formula:

$C_{cx}/F = E_{cx}$ . Subtracting the potential concentration of each methyl ketone remaining in the fat ( $C_{cx}$ ) from its original potential ( $E_{cx}$ ) results in the calculated free concentration of each methyl ketone ( $D_{cx}$ ) in the evaporated milk sample:  $E_{cx} - C_{cx} = D_{cx}$ .

The free methyl ketone concentrations, determined by previously outlined methods and calculations, of five samples of commercial evaporated milks, are presented in Table 3, along with percentage of the original methyl ketone

potential in each sample present as free methyl ketones. The total methyl ketone potential of the evaporated milk fat samples, ranging from 0.42  $\mu\text{moles/gram}$  in Sample 135 to 0.75  $\mu\text{moles/gram}$  in Sample 141, is relatively low compared to the total potential of other milk fats examined (3, 10); however, such concentrations are not unusual (4, 10). The relative molar concentrations of the free methyl ketones in each sample are, furthermore, similar to those previously reported (3, 4) in other milk fat samples heated in the presence of moisture.

Table 4 serves as a final check on the accuracy of our methods and calculations when applied to samples of commercial evaporated milk. With the exception of the C<sub>15</sub> in Sample 139 and the C<sub>11</sub> methyl ketone in Sample 141, the percentage of free methyl ketone recovered from the evaporated milk (based on the calculated amount present in the sample) compares favorably with recovery studies on known concentrations of these compounds added to fresh recombined whole milk (Table 2). Discrepancies in the two determinations cited can only be attributed to experimental errors as a result of the minute quantity of methyl ketones in the products and the rather involved experimental procedure.

TABLE 4. Recovery data on free C<sub>15</sub> and C<sub>11</sub> methyl ketones from samples of commercial evaporated milk.

Methyl ketone	% of Calculated concentration				
	135	137	139	141	147
	(% of Calculated concentration recovered)				
C <sub>15</sub>	67.1	85.1	39.6	70.3	92.9
C <sub>11</sub>	29.5	28.6	28.4	54.3	40.1

TABLE 5. Response of taste panel to various fractions of the total methyl ketone potential of five samples of evaporated milk.

Sample	Total methyl ketone potential	Positive responses
	(%)	(%)
135	100.0	50
	75.0	28
	50.0	25
	37.0	6
137	100.0	78
	75.0	40
	50.0	33
	29.7	15
139	100.0	78
	75.0	61
	50.0	11
	37.7	17
141	100.0	85
	75.0	85
	50.0	60
	29.2	40
147	100.0	53
	75.0	39
	50.0	17
	37.0	6

Table 5 summarizes the results of flavor studies on the free methyl ketone levels found in five commercial samples of evaporated milks in addition to several levels of these compounds which can exist under the proper heating or storage conditions, or both, of the product. Applying the threshold criterion of Patton and Josephson (6), the free methyl ketone levels in the five samples analyzed, with the possible exception of Sample 141, do not contribute to the flavor of these particular products. This conclusion, however, is applicable only to these particular samples. We conclude from analysis of the results in Table 3, Table 5, and previously published data (1, 3, 9, 11) that the role of methyl ketones in the off flavors of sterile concentrated milks is dependent on a) the total methyl ketone poten-

tials of the sample, b) composition of the methyl ketone potential (especially the heptanone-2 potential), and c) the extent of hydrolysis and decarboxylation of the  $\beta$ -keto acids as governed by the initial heat treatment of the product, storage temperature, and storage time.

#### References

- (1) Arnold, R. G., and R. C. Lindsay. 1967. Flavor deterioration of stored sterilized concentrated milk. *J. Dairy Sci.*, 50: 957.
- (2) Corbin, E. A., D. P. Schwartz, and M. Keeney. 1960. Liquid-liquid partition chromatography. Separation of the 2,4-dinitrophenylhydrazones of saturated aldehydes, methyl ketones, 2-enals and 2,4-dienals. *J. Chromatog.*, 3: 322.
- (3) Langler, J. E., and E. A. Day. 1964. Development and flavor properties of methyl ketones in milk fat. *J. Dairy Sci.*, 47: 1291.
- (4) Parks, O. W., M. Keeney, I. Katz, and D. P. Schwartz. 1964. Isolation and characterization of the methyl ketone precursor in butterfat. *J. Lipid Res.*, 5: 232.
- (5) Patton, Stuart. 1961. Gas chromatographic analysis of flavor in processed milks. *J. Dairy Sci.*, 44: 207.
- (6) Patton, S., and D. V. Josephson. 1957. A method for determining significance of volatile flavor compounds in foods. *J. Food Res.*, 22: 316.
- (7) Schwartz, D. P., H. S. Haller, and M. Keeney. 1963. Direct quantitative isolation of monocarbonyl compounds from fats and oils. *Anal. Chem.*, 35: 2191.
- (8) Schwartz, D. P., and O. W. Parks. 1961. Preparation of carbonyl-free solvents. *Anal. Chem.*, 33: 1396.
- (9) Schwartz, D. P., O. W. Parks, and M. Keeney. 1962. Separation of 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes on magnesia. *Anal. Chem.*, 34: 669.
- (10) Schwartz, D. P., O. W. Parks, and R. A. Yoncoskie. 1966. Quantitative studies on methyl ketone formation in butteroil: effect of temperature. *J. Amer. Oil Chem. Soc.*, 43: 128.
- (11) Schwartz, D. P., P. S. Spiegler, and O. W. Parks. 1965. Effect of water on methyl ketone formation in butteroil. *J. Dairy Sci.*, 48: 1387.
- (12) Wong, N. P., S. Patton, and D. A. Forss. 1958. Methyl ketones in evaporated milk. *J. Dairy Sci.*, 41: 1699.