

DIRECT RECOVERY OF ADDED KETONES FROM FOAM-DRIED WHOLE MILK

F. E. KURTZ

Dairy Products Laboratory, Eastern Utilization Research and Development Division, ARS
USDA, Washington, D. C.

ABSTRACT

To facilitate the development of a method for collecting flavor compounds directly from dried whole milk a study has been made of the recovery of ketones incorporated into dried milks. 2-Butanone, 2-heptanone, and 2-tridecanone were added to separate batches of butteroil. The butteroils with added ketones were homogenized with skimmilk. These whole milks were foam-dried by a standard method. The procedure for recovering the ketones involved a short-path migration under a temperature differential at about 1 μ pressure. A liquid nitrogen-cooled condenser, powder temperature of 40 C and 60 C, and heating periods of from 3 to 6 hr were employed. The use of conditions more severe than 40 C for 3 hr increased the yield of tridecanone only slightly, if at all. The recoveries from unaltered dry milks were: butanone, with the powder heated 4 hr at 60 C—7.4%; heptanone, 3 hr at 40 C—9.0%; tridecanone, 3 hr at 40 C—7.3%. Under the same heating conditions, the following increased yields of ketones were obtained from powders in which the fat had been freed prior to collection: butanone—57%, heptanone—82%, and tridecanone—90%. Earlier findings that the critical moisture content for freeing the fat is in the region of 9% were confirmed. A procedure is described for freeing the fat by hydrating a dried milk within the evacuated collection system.

In a projected study of the flavor components of dried whole milk it was desired to have the isolated flavor compounds not only readily accessible to chemical study, but also to have both them and the residual dried milk in a condition suitable for taste panel evaluation. While none of the available methods seemed to satisfy all of these conditions it was felt that they would be satisfied if the flavor compounds could be volatilized directly from dried milk by relatively mild heating. The success of such a method, however, appeared to be doubtful, since it had already been found that flavor compounds known to be present in dried skimmilk could not be removed directly from the dried milk even under rather severe conditions of heating (2). Under the circumstances, it was felt that a study of the recovery of known compounds, incorporated into dried milk in substantial quantities, would enhance the likelihood of finding conditions which would lead to the successful recovery of flavor compounds. The selected compounds were chosen for their range of volatility and the availability of satisfactory analytical methods.

MATERIALS

Chemicals. 2-Tridecanone, 2-heptanone, and 2-butanone, Eastman grade, were used as received. 2,4-Dinitrophenylhydrazine, Eastman grade, was crystallized from ethyl acetate. Methanol, Fisher reagent grade, and ethanol, Commercial Solvents Corporation, 200 proof, were treated to remove carbonyls by the method of Claborn and Patterson (1), modified by the inclusion of an additional distillation. Hexane, Phillips' high-purity grade, was treated to remove carbonyls by the method of Schwartz and Parks (5).

Dried whole milks with added ketones. A weighed quantity of a ketone was added to 1,634 g of melted butteroil with which it was thoroughly mixed by stirring. The butteroil with added ketone was mixed with 24 lb of 42% total solids skimmilk concentrate and the mixture was homogenized and then foam-dried by a method described elsewhere (6). The butteroil containing tridecanone, 5.33 g, was incorporated into skimmilk by three homogenizations at 4,500 lb pressure. Those containing heptanone, 10.0 g, and butanone, 15.0 g, were incorporated into skimmilk by two homogenizations in two stages of 500—500, and

Received for publication October 31, 1965.

4,000—500 lb pressure. The dried milks were packaged in polyethylene bags and stored at 0 C.

APPARATUS

The collection system shown in Figure 1 was designed for greater versatility than was needed

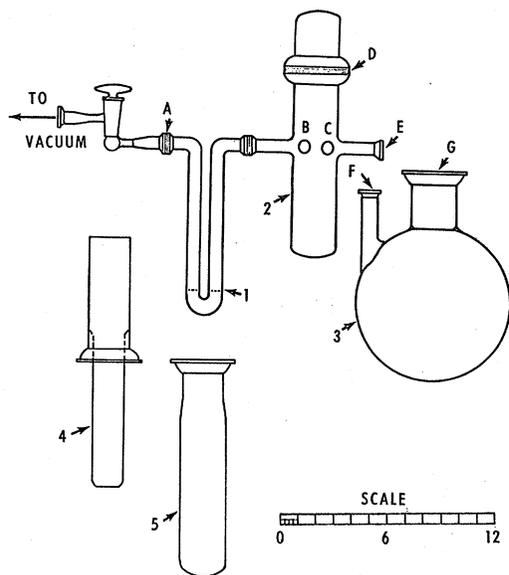


FIG. 1. Apparatus for collecting volatiles directly from dried milk.

in this particular investigation. It leads through a cold trap to a McLeod gauge, a vacuum pump, and a cylinder of nitrogen. All attachments are made through O-ring connectors, sizes 15 and 50. The U-tube, Piece 1, was packed to the indicated depth with $\frac{1}{8}$ -in. stainless steel balls. The outlets B and C on the manifold, Piece 2, were capped during this investigation. They provide a means, for instance, of connecting condensed volatiles to a desiccant.

A sample of dried milk, or other material, may be placed in any round-bottomed flask which has been provided with a Size 15 O-ring connector and attached directly to the manifold at E. If desired, the manifold may be omitted and the flask attached to the U-tube or to a special U-tube (not shown) provided with vacuum valves (Kopp Scientific Company, New York City) that can be closed at the end of a collection. Alternatively, the sample may be placed in the five-liter flask, Piece 3, which is attached by a right-angle connector from F to E, and the volatiles collected on the cold finger, Piece 4. This provides the shortest, and least obstructed, migration path of any of the

alternatives. If the cold finger becomes overloaded, some of the condensate may drop off during a collection or while the cold finger is being removed from the flask. This may be obviated, when solvent or excess moisture is present, by a preliminary condensation into the manifold well, followed by a condensation of the least volatile materials onto the cold finger.

At the end of a collection, condensate on the cold finger may be taken up in a solvent in the special flask, Piece 5, or the connector at E may be capped and the cold finger with its condensate inserted into the manifold at D for further manipulation.

EXPERIMENTAL PROCEDURES

Analytical methods. Free fat, except for the use of a 5-g sample and the removal of solvent on a steam bath, was determined by the method of Tamsma et al. (7), and calculated as a percentage of the total fat. Total fat was determined by the Mojonnier method, and moisture by the toluene-distillation method. Both were calculated as a percentage of the whole powder, including its moisture.

Ketones were determined by one of two methods. The first method is a modification of that described by Claborn and Patterson (1). The modifications are: the use of a reaction temperature of 60 C, a reaction time of 1 hr, a reagent containing 5 mg of 2,4-dinitrophenylhydrazine per milliliter of methanolic HCl, the measurement of optical density at 432 $m\mu$, and the removal, by suction, of most of the aqueous layer prior to its separation from the ethylene dichloride layer. This last modification prevents an otherwise troublesome contamination of the solution of hydrazones with aqueous phase. The second method for determining ketones is essentially that of Schwartz et al. (4). It was modified to the extent of determining the absorbance of the hydrazones in an alcoholic potassium hydroxide solution at 432 $m\mu$, as in the modified Claborn and Patterson method. Although this is more laborious than the direct measurement used in the reference procedure, it is more selective.

Collection of ketones directly from dried milks. Ketones were collected from each dried milk, both in its normal state and after hydration to free its fat. The former is referred to as a low-free-fat dried milk and the latter as a high-free-fat dried milk. Recovered ketones were determined by the modified method of Claborn and Patterson.

Low-free-fat dried milks. For the recovery of tridecanone and heptanone, 30.0-g samples of powder were placed in the five-liter flask

and the volatiles condensed on the cold finger. A three-liter flask containing 200.0 g of the dried milk with added butanone was attached at E and the volatiles condensed in the manifold well. Prior to evacuation, each powder was cooled for 1 hr with a dry ice-ethanol bath. Then the U-tube, manifold well, and cold finger, when present, were cooled with liquid nitrogen, and the volatiles collected at about 1μ pressure under the heating conditions shown in the tables. At the end of a collection the vacuum was broken with nitrogen gas. Condensates were taken up in methanol—10 ml for the U-tube, and 25 ml each for the manifold well and cold finger. Because of condensed moisture, the total volume in each fraction had to be measured.

High-free-fat dried milks. Except for the use of a 30.0-g sample of the dried milk containing butanone, and the variations connected with hydrating the dried milks, the collection of volatiles was conducted as with the low-free-fat powders. A 1.5-in. Teflon-clad magnet was added to each powder charge. Water was added to the manifold well. The amount needed depends upon the composition of the dried milk. In this instance, 3.00 ml was sufficient. After the 1-hr cooling period, the collection system was evacuated for 2 min, isolated from the pump, and all cooling baths removed. The following morning, the water having all evaporated from the manifold, the powder was thoroughly mixed with the aid of an external magnet and held 1 hr to insure freeing of its fat. Then the manifold well and U-tube were cooled with liquid nitrogen for 1 hr, after which the cold finger, if being used, was also cooled for 1 hr. The system was opened to continuous evacuation and the ketones collected at 1μ pressure under the desired heating conditions.

Determination of ketones in fat solutions extracted from dried milks. The following methods were used in assaying the concentration of ketone present in each of the three dried milks:

Method A. 50.0 g of dried milk and 150 ml of hexane were mixed in a Waring Blender. While stirring at moderate speed, 7.5 ml of water was slowly added. After stirring 1 hr, the mixture was filtered and the carbonyls determined by the modified Claborn and Patterson procedure, but using ethanolic HCl to obtain a homogeneous solution. A fat blank was determined on the fat used in making the dried milks with added butanone and heptanone.

Method B-1. The dried milks containing tridecanone and heptanone were hydrated by spraying a thin layer of 50.0 g of the powder

in an open tray with a mist of water until its weight had increased 4.0 g. The powder was placed in a one-liter stoppered flask, thoroughly mixed, held 30 min, then to it was added 200 ml of hexane. The mixture was shaken for 5 hr on a Burrel wrist-action shaker at an intensity setting of 5, and filtered.

Method B-2. The dried milk with added butanone was hydrated similarly to the powders used for the direct recovery of ketones. A vacuum valve (Kopp Scientific Company, New York City) with Teflon gaskets was attached to the five-liter flask at F. The flask was charged with 50.0 g of powder and 4.0 g ice suspended in a crucible from a hook in a cap attached at G and the flask evacuated. After the fat was freed, 245 ml of hexane was sucked into the flask. The mixture was stirred magnetically for 5 hr, and filtered.

The hexane solutions obtained by Methods B-1 and B-2 were analyzed for ketones by the method of Schwartz et al., modified as described. Fat samples for the blanks were obtained by taking part of each extract, removing the solvent on a steam bath, and the ketones by heating the fat, while stirring, for 5.5 hr at 85 C and 1μ pressure in the collection system of Figure 1. It had already been shown that tridecanone added to butteroil could be completely removed, with other conditions the same, by heating 2 hr at 60 C.

Method C. A 50.0-g sample of each dried milk was extracted for 3 hr at 60 C with a weighed volume of 200 ml of absolute ethanol. Using glassware prewarmed to 60 C, the mixture was filtered, a nominal 5-ml aliquot was taken for assay of fat and alcohol, and a 50-ml aliquot transferred to a one-liter flask which was attached to the manifold for collection of ketones. With the U-tube and manifold well cooled with liquid nitrogen, the alcohol was distilled, at atmospheric pressure and 85 C, into the manifold well. The 85 C bath was replaced with a 60 C bath and, after holding 1 hr for condensing vapors, the system was evacuated to 1μ and the collection of ketones continued for 2 hr. The U-tube was washed with 10 ml of ethanol and the two ethanol solutions analyzed by the modified Claborn and Patterson method.

RESULTS AND DISCUSSION

Free fat. In the present study it soon became evident that only a small proportion of ketones incorporated into dried whole milk could be recovered directly from the unaltered dried milk. Presumably, the structure of a foam-dried milk presents an effective barrier to the diffusion of a ketone from its interior to its

surface. Lampitt and Bushill (3) had found that when a spray-dried whole milk was stored in a thin layer in a humid atmosphere it took up moisture until, in the region of 9% moisture, its lactose crystallized. Prior to lactose crystallization, the addition of moisture had little or no effect on the free-fat content of the powder; with crystallization, however, the fat became almost completely accessible to organic solvents.

It seemed likely that the structural disruption associated with lactose crystallization would also greatly facilitate the diffusion of volatile molecules out of the particle. The data of Table 1 indicate that the structural changes

TABLE 1
Effect of various conditions on direct recovery of tridecanone from dried whole milk

Temperature	Heating time	Free fat	Recovered ketone
(C)	(hr)	(%)	(moles/100 g of fat)
40	3	7.5	9.4×10^{-5}
40	3	85	1.10×10^{-3}
40	3	85	1.23×10^{-3}
40	5	85	1.22×10^{-3}
60	3	85	1.25×10^{-3}
60	3	85	1.33×10^{-3}
60	5	85	1.24×10^{-3}
60	6	85	1.26×10^{-3}

associated with freeing the fat do, indeed, facilitate the recovery of tridecanone. Consequently, the free fat of dried milks was determined routinely after recovering ketones. To insure a proper interpretation of these values, a preliminary study was made of the effect of moisture, and of the conditions used in recovering ketones, on the free fat.

This study confirmed the findings of Lampitt and Bushill that the critical moisture content for freeing the fat is in the region of 9%. However, increasing the moisture, whether up to this region or beyond it, decreases the free fat of a dried milk. These conclusions are illustrated by the following experiment: In an evacuated vessel, 60.0 g of a dried milk having 2.0% moisture, 29.1% total fat, and 20% free fat, was exposed to 5.5 ml of water. Hydration proceeded with the following results: at 17 hr, 8.0% moisture and 11% free fat; at 21.5 hr, undetermined moisture and 11% free fat; at 41 hr, undetermined moisture and 12% free fat; at 161 hr, 9.0% moisture and 93% free fat. The sample was then exposed to additional moisture. At 12.0% moisture, the free fat was 75%; at 15% moisture, it was 74%. It seems

that water not combined with lactose, perhaps due to its polar nature, lowers the accessibility of a nonpolar solvent to the fat.

Excess moisture would not be expected, however, to affect the recovery of volatiles, since it is removed in the recovery process—the residual moisture in a dried milk in which the fat has been freed being about 2%. The recovery process showed no significant effect on the free-fat content of a dried milk, other than that resulting from this lowered moisture.

Direct recovery of ketones. Except for variations of free fat the recovery of butanone was investigated under only one set of conditions. With high-free-fat powders, the recovery of tridecanone at 25 C for 3 hr was about 80% of that at 40 C for 3 hr, but as the data of Table 1 show, more severe heating increased the yield only slightly, if at all. The results with heptanone were similar—the yield at 60 C for 3.5 hr was about 2% lower than at 40 C for 3 hr. Low-free-fat dried milks behaved similarly. In contrast, when their fat was freed the yields of ketones were increased dramatically.

Assay of dried milks for ketones. To calculate the percentage yields of ketones obtained in direct-recovery experiments, it was necessary to determine what portion of the ketones added to the original butteroils had survived during manufacture and subsequent storage of the dried milks. The dried milks were assayed for ketones at three storage periods, using the best method available at the time. Results are reported in Table 2.

Method A. The earliest assay method suffered in two respects. Since 50% or less of the total fat in the dried milk was extracted by this method it is not certain that the ketone content of the extracted fat was entirely representative of that in the powder. Then, too, the method had high fat blanks. Despite these limitations, the data are generally consistent with those of the later, improved assays and, accordingly, are included for their interest in connection with the other two.

Method B. The second assay method was typified by more complete fat extractions. The variation used for extracting butanone gave a nearly theoretical yield of fat, and precluded any possibility of losing ketone by volatilization from the powder. In this method the fat blanks, while relatively high, were no more than 6% of the values for tridecanone and heptanone, but because of the low concentration of butanone the fat blank was more than 20 times the net value of the ketone, so this assay was unusable.

RECOVERY OF KETONES FROM MILK

TABLE 2
Retention of ketones in dried whole milk during manufacture and storage

Ketone	Time after manufacture (yr)	Portion of total fat in extract (%)	Assay method	Ketones in dried milk	
				(moles/100 g of fat)	(% of added ketone)
Tridecanone	2	44	A	1.56×10^{-3}	95
	4.2	92	B-1	1.40×10^{-3}	85
	5	101	C	1.29×10^{-3}	79
Heptanone	0.2	30	A	2.44×10^{-3}	46
	2.4	87	B-1	1.06×10^{-3}	20
	3.2	102	C	8.14×10^{-4}	15
Butanone	0.2	50	A	1.68×10^{-4}	1.3
	2.4	97	B-2	3.06×10^{-6}	0.02
	3.2	101	C	9.06×10^{-5}	0.7

Method C. In this last assay method there is no fat blank, and the conditions most closely parallel those used in the direct recovery of the added ketones. Also, it was applied to the dried milks within a short time of the direct recovery determinations reported in this paper. Accordingly, the data obtained by this method are considered the most applicable for calculating the percentage recoveries of ketones.

Percentage recovery of ketones. The assays by Method B were made nine to ten months before those by Method C, and to the concurrent direct recoveries reported in this paper. To the extent that ketones were lost from the powders during this period, percentage recoveries based on the earlier assay would be lower than actually obtained. Considering this, and the great dissimilarity of the two methods, the recoveries of tridecanone, as shown in Table 3, are quite similar; those of heptanone are less so. The 90% recovery of tridecanone shown in this table was calculated from the average of two recoveries obtained by heating the powder at 40 C for 3 hr; the recovery calculated from the average of all of the high free-fat values listed in Table 1 is 95%.

It is notable that the percentage recoveries from high free-fat dried milks, Table 3, show a decrease with increased volatility of the ketone, whereas there is no indication of such a relationship between the low-free-fat dried milks. The reason for this is not clear. However, during the hydration of a powder, the release of adsorbed air sometimes increases the internal pressure from 1 μ to 100 μ or more. If volatilized ketones were not completely condensed during the scavenging period before opening the system to continuous evacuation, some of their vapors might be swept through the system and lost when the pressure was suddenly reduced.

It is conjectural whether the more volatile compounds would suffer the greater loss under these conditions. If they did, the trends in Table 3 would be explained, and the path to more complete recoveries of the more volatile ketones indicated. This would involve one or a combination of the following: a longer time for condensing vaporized compounds before opening the system to evacuation, a more gradual reduction of the internal pressure, and a more efficient packing in the U-tube.

TABLE 3
Percentage of ketones recovered directly from dried whole milk

Ketone	Temperature (C)	Heating time (hr)	Free fat (%)	% Recovery of ketone ^a	
				(Assay Method B)	(Assay Method C)
Tridecanone	40	3	85	83 ^b	90 ^b
	40	3	7.5	6.7	7.3
Heptanone	40	3	85	63	82
	40	3	8.2	6.9	9.0
Butanone	60	4	85 ^c	57
	60	4	7.9 ^d	7.4

^a (Ketones recovered directly) \times 100/(ketones found by assay of fat solution).

^b Average values of recoveries made under these conditions.

^c About 16 times ketones found in assay of fat solution.

^d About two times ketones found in assay of fat solution.

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