

**THE ISOLATION AND IDENTIFICATION OF CARBONYL COMPOUNDS
ASSOCIATED WITH FEED FLAVORS IN MILK**

by

JOHN RAYMOND MILTON

B. S., Kansas State University, 1958

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

**KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE**

1959

KANSAS STATE UNIVERSITY LIBRARIES

LD
2668
T4
1959
M56
c.2
Documents

TABLE OF CONTENTS

INTRODUCTION	1
LITERATURE REVIEW	4
EXPERIMENTAL PROCEDURE	12
Preparation of Samples	12
Vacuum Treatment of the Milk	13
Preliminary Studies	14
Analysis of Carbonyl Compounds	16
Formation and Extraction of the Derivatives	17
Samples Analyzed for Carbonyl Compounds	19
Separation of the Hydrazones.	19
Determination of Absorption Spectra	20
Crystallization of Derivatives	20
EXPERIMENTAL RESULTS	21
Preliminary Studies of Reduced Pressure Steam Distillation of Milk in the DeLaval Vacu-Therm	21
Results of Analyses for Carbonyl Compounds in the Milk Distillate . .	23
Effect of Stage of Growth of Pasture upon Milk Flavors.	26
DISCUSSION	28
SUMMARY AND CONCLUSIONS	32
ACKNOWLEDGMENT	34
LITERATURE CITED	35
APPENDIX	39

INTRODUCTION

Feed flavors of milk can be defined as undesirable flavors that are present in freshly drawn milk as a result of some material taken into the body of the cow by eating or inhalation. They are different from flavors resulting from bacterial decomposition or chemical changes occurring in milk during storage. They also differ from those resulting from handling prior to, during or after processing. Disagreeable flavors in milks produced by cows that have eaten weeds are sometimes called "weed" flavors (38).

Feed flavors in milk were first reported in 1757 by Bradley (37) who observed that feeding beet and turnip tops and roots produced a bitter flavor in the milk produced in an area near London, England. Research work concerning the problem of feed flavors has been reported in the literature published since the early 1900's. In the past twenty years, with the advent of new methods of handling milk, there has been more interest in this problem (36).

Feed flavors in milk are important to the dairy industry because they limit consumption of dairy products (31). According to Trout (38), if milk does not have a pleasant flavor or if the flavor is not uniform from day to day the consumer fails to consume as much as he might otherwise. Adults and teenagers select foods that they like and drink milk for this reason. Young children drink milk because it is fed to them. Unfortunately, poor quality dairy products may create in children a dislike for these products which will adversely affect consumption of milk in the years to come.

The importance of the feed flavor problem in milk is apparent to the dairy farmer when milk is rejected at the processing plant or when Grade A milk is degraded and used for manufacturing purposes. The economic loss of both rejected and degraded milks represent a heavy burden to the farmer. No

data were available with respect to the annual economic loss to dairymen for these rejected milks but there is no doubt that this loss would be large.

The off-flavor problem makes it necessary for dairymen to manage carefully their roughage feeding programs to prevent, in so far as possible, the occurrence of taints in milk. Careful management practices include proper cultivation of pastures to remove weeds, removal of the cows from roughages at least four hours before milking, and feeding flavor-provoking feeds only after milking. Strobel et al. (37), reported in an extensive review of literature that roughages generally affect the flavor of milk more adversely than concentrates. Knott (22) stated that large quantities of roughages are needed for the nutrition of the dairy cow and economical milk production. This makes it necessary for the farmer to be mindful of the effects of feed upon the flavor of milk. Good management practices will help prevent off-flavors in milk but will not completely eliminate the problem (7). Furthermore, these practices require time, labor, equipment and supplies and may inconvenience the farmer during certain seasons of the year.

Because of the importance of feed flavors in milk and since there has been very little basic research in this area, a project was initiated through the Kansas Agricultural Experiment Station in 1957 to study this problem. The necessity of this research was based upon the following developments (21):

1. Greater emphasis and general acceptance by dairymen for the "Grassland Farming" program;
2. Nutritional demands for more milk in the diets of humans;
3. Economic need for greater markets and increased consumption of milk and dairy products;
4. Recent developments of milk processing equipment for flavor improvement;
5. The introduction of gas chromatography as an analytical tool to identify causative chemical compounds.

Conventional methods of flavor improvement have been directed toward management of dairy cattle. In general this includes reduction or elimination of the causative feed from the dairy ration. This practice is uneconomical, and at times inefficient and impractical. An inexpensive method of removal of the off-flavors at the processing plant would allow producers to produce milk more efficiently and increase the palatability and consumption of the products.

Roberts (33) stated that in the past several methods have been attempted to remove off-flavors from milk and cream. These include: washing with light mineral oil or water, aeration, application of vacuum without heat, and distillation with steam usually under reduced pressure with carefully controlled conditions. Aeration was found to be inefficient, and the addition of mineral oil was illegal. Reduced pressure methods employing either heat or heat with injected steam are presently being used to help eliminate some flavor problems and produce a uniform product. A degree of success has been attained by using this type of equipment (33). The flavor standardizing equipment in use today has been developed without the advantage of fundamental knowledge regarding the complete composition of feed flavors. With the advent of flavor standardizing equipment, the question of who should be responsible for the elimination of off-flavors has evolved. Since equipment for standardizing flavors is available, some producers have felt that the processor should be responsible for the removal of any feed flavors present in the milk. On the other hand, processors have felt that the producer was responsible for producing palatable milk (14).

A study of the effectiveness of various processing conditions for removal of rye pasture, alfalfa hay and silage off-flavors was made by Cotner at this station in 1958 (8). By using laboratory apparatus designed to simulate plant

equipment, he drew the following conclusions regarding optimum treatments for flavor removal: A preheat temperature of 174° F. under 19 inches vacuum with injected steam was necessary to remove alfalfa hay flavors; 140° F. under 19 inches vacuum with no injected steam was necessary to remove silage flavors; and 160° F. under 19 inches vacuum with injected steam was necessary to remove rye flavors. He further established that preheat temperatures in excess of 160° F. produced cooked flavors in the treated milk.

The research presented in this thesis was directed toward identification of chemical compounds associated with feed flavor defects. This was attempted by analyzing the vapors removed from milk by a commercial flavor standardizing apparatus. A DeLaval Vacu-Therm flavor standardization and high-temperature short-time pasteurization unit was adapted to the collection of volatile chemical compounds present in the vapors removed from normal and feed-flavored milks. Preliminary qualitative tests of these vapors indicated the presence of carbonyl compounds. Therefore emphasis was placed upon the isolation and identification of this group of compounds.

LITERATURE REVIEW

A review of the literature revealed that feed flavor problems are not limited to any specific geographical area (11, 13, 25, 37). Downs et al. (11) stated that 44 percent of all milk samples scored at Collegiate Student Dairy Products Judging Contests since World War II were criticized as having a feed flavor, either singly or in combination with other defects.

Dunkley (13) reported that 127 of 169 samples of milk secured in eight widely separated cities in the United States were criticized for having feed flavors. The samples were obtained at retail outlets and were scored by a panel of judges. Weed and feed flavor problems in Australia and New Zealand

were discussed in detail by McDowall (25). He made recommendations to dairy-men in these areas regarding the control of feed flavors. The United States Department of Agriculture (40) recently awarded a \$253,000 grant to the Bio-chemical Institute of Helsinki, Finland, for basic research on the transmission of flavor constituents and other compounds from the feed of dairy cattle to milk; this stresses the importance placed upon this problem by the Department of Agriculture.

Since as early as 1757 (37) many feeds have been studied to determine their ability to cause off-flavors in milk. In general, the roughages have been found to be more taint provoking than concentrates when fed to dairy cows (37). Alfalfa hay, alfalfa silage, corn silage, rye, grass, wheat, sweet clover, and other clovers grazed for pasture have all been found to cause undesirable flavors in milk (8, 19, 20, 37, 39). In this review, emphasis is placed upon the effect of rye and wheat pastures on the flavor of milk.

Observations upon rye pasture have been somewhat contradictory. In 1925, Babcock, as quoted by Trout and Horwood (39), reported that feeding 25 to 30 pounds of green rye to cows one hour before milking produced an objectionable flavor in the milk. It was further reported (17) that when rye began to head, objectionable flavors were produced in the milk from cows consuming such a roughage. The odor and flavor of the milk was described as "fishy." Johnson, quoting Trout, (39) stated that rye was capable of imparting off-flavors to milk rendering it unfit for fluid milk or market cream consumption unless special herd management precautions were taken.

In 1933, a new variety of rye was introduced to the United States from Italy and named Balbo by the Tennessee Station (39). This crop found usefulness for spring pasture in the Midwest and was studied in 1944 by two stations,

Michigan State University and Missouri University, to determine its flavor provoking capabilities (16, 39). The Michigan workers (39) found that Balbo rye imparted a less intense flavor than common rye but an off-flavor was present in milk produced on either pasture. The flavor of milk produced by cows on Balbo rye was described as "grassy" while the milk obtained from cows on common rye was described as "soapy," "sodium hydroxide" or even "fishy." Both rye pastures were 42 inches tall when grazed. The flavor was present in the milk drawn two and one-half hours after the cows were removed from pasture (39).

Herman and Garrison (16) working at the Missouri Station reported that Balbo rye pasture did not impart an objectionable flavor to milk sampled one to two hours after the cows were removed from the pasture. The milk was scored 18 successive days by a panel of judges familiar with flavor defects in milk.

The Kansas Agricultural Experiment Station (19, 20) reported that rye produced a more offensive flavor than wheat when both pastures were grazed in the spring and fall. In comparing milk produced in the spring and fall it was found that the season of the year had no effect on the production of off-flavors from wheat or rye pastures.

At this same station, Cotner (8) in 1958 confirmed that milk from cows grazed on Balbo rye pasture had an objectionable flavor and odor. Five lots of milk, analyzed by an experienced panel of judges, using the standard score-card, were reported to have an average flavor score of 35.5.

Very little reference was made in the literature to the effect of wheat pasture on milk flavor. The Kansas Station (20) reported that an off-flavor was found in milk from cows grazed on wheat pasture but the flavor was not as objectionable as that produced by cows grazing rye. Linn, in correspondence with Trout (39) stated that based upon Kansas observations, wheat was as

acceptable as any of the cereal grains for pasturing dairy cows.

Several factors have been reported as influencing the intensity of feed flavors in milk. Babcock (2) stated that the intensity of the flavor depended upon the character of the feed, the quantity consumed and the time interval between consumption and milking. Other workers have verified these views (3, 13, 26, 36). Frye et al. (15) found that there was no statistically significant correlation between milk and fat production and the intensity of feed flavors. He also concluded that there was no agreement between the amount of silage fed and the occurrence of feed flavors. Babcock (2) observed that most weed flavors are more pronounced in the cream portion of the milk than in the milk from which the cream was separated. He found that bitterweed flavor, which was more concentrated in the skim portion, was an exception to this observation. MacCurdy and Trout (24) found that if a given amount of silage was fed to both high- and low-producing cows, the flavor was more intense in the milk from the low producers. They suggested that 0.79 pounds of corn silage or 0.40 pounds of alfalfa silage per pound of milk produced would cause an off-flavor in the milk drawn one hour after feeding.

According to most workers, the majority of feed flavors were not apparent in milk if a time interval of at least four hours was allowed to elapse between feeding and milking (36, 37, 38, 39). However, Babcock (1, 2) stated that some weed flavors persisted after a four-hour period. It was also reported that garlic flavor in milk reached a maximum intensity four hours after it was consumed. He further observed that the bitterweed flavor persisted in milk for as long as 12 hours after this weed was consumed. Trout and Horwood (39) reported that no rye off-flavor was detected in milk drawn three and one-half hours after the cows had been removed from Balbo rye pasture. These findings

have been used in the past for recommending roughage feeding practices to dairymen (1, 10, 17, 32, 36).

Most of the feed flavor studies in the past have involved feeding a given feed and noting the development of an off-flavor in the milk (1, 2, 3, 15, 16, 19, 20, 36, 39). In evaluating milk for flavor defects, organoleptic methods of analysis have been used even though such tests were criticized for lacking objectivity (33, 34, 38). Trout (38) stated that this was a practical method because small amounts of materials could be detected very quickly. He further stated that chemical tests in many cases would not detect the presence of the small amounts of materials and if detected, the test would take a much longer time to perform. In recent years, gas chromatography (42) and mass spectrometry (28) have been used to study the flavoring materials in milk and other foods. Niegisch and Stahl (28) reported that a mass spectrometer will detect five ten-millionths molar concentration of a compound.

Very little work has been done to determine the characteristic chemical compound(s) in feeds that cause off-flavors to be produced in milk (33). It was found that an alkaloid called betain was present in beet tops, and upon reaching a certain concentration in the milk, it was oxidized by heat to form trimethylamine producing a fishy flavor (38). Some weeds that produce off-flavored milk and the suspected chemical compounds are presented in Table 1.

Although there is little information available regarding the agents responsible for feed flavors in milk, some chemical compounds have been suspected as contributing to its natural flavor. Carbonyl compounds have been isolated from whole and skim milk (6, 9, 12, 23, 27, 30, 42). Day et al. (9) isolated acetone and acetaldehyde as their 2,4-dinitrophenylhydrazones (DNPH) derivatives from reduced pressure distillation of skim milk. Wynn and Brunner (42) found acetaldehyde, acetone, methyl sulfide and an unknown aldehyde or

Table 1. Weeds producing off-flavors in milk and their suspected causative chemical compounds¹

Name	Botanical Classification	Suspected Chemical Compounds
Land cress	<u>Coronopus didymus</u> or <u>Senebiera didyma</u>	Benzyl mercaptan
Penny cress Frenchweed or Stinkweed	<u>Thlaspe arvense</u>	Allyl isothiocyanate
Penny Royal	<u>Mentha pulegium</u>	Pulegone
Pepper grass	<u>Lepidium virginicum</u>	Indole
Onion and garlic		Di-n-propyl sulfide Isopropyl mercaptan Propionaldehyde

¹ Roberts (33).

ketone in increasing order of concentration in the expelled vapors from vacuum steam-injected pasteurized milk. These same components, they found, were absent or present only in trace quantities in the processed milk. Morgan *et al.* (27) found that most of the acetone in skim milk was removed by steam and vacuum treatment in a Vacreator. Patton *et al.* (29) stated that abnormally high concentrations of methyl sulfide could be responsible for certain "cowy" or feed-type off-flavors in milk.

Acetone bodies have been found in milk by workers studying ketosis (12, 23, 30). Knodt *et al.* (23), using the method of Barnes and Wick, found the following concentration of acetone type compounds in milk from normal cows: acetone and acetosuccinic acid, 1.00 mg. percent β -hydroxybutyric acid, 0.80 mg. percent. Duncan *et al.* (12) found that by using the Van Slyke method, acetone bodies in milk from cows suffering ketosis varied in concentration from a trace to 42.9

mg. percent. Potts et al. (30) using the Behre salicylaldehyde method, found the concentration of total acetone bodies in normal cows' milk to be 5.67 mg. percent for five samples of milk from cows analyzed eight times. The acetone body concentration of cows fed alfalfa silage before milking was found to be 6.00 mg. percent. Potts et al. (30) also stated that milk from ketotic cows had a slightly sweetish, sickening flavor.

Although little information is known about the chemical characteristics of feed flavors, various types of equipment have been designed to standardize the flavor of milk (32, 33, 34). Roberts (32) described three basic types of flavor standardizers available for commercial processing of milk. He classified them with respect to the severity of the steam treatment that they employed. According to Roberts' classification, a Type I machine injects no live steam into the milk and pasteurization does not occur in the machine itself. It is installed in combination with a high-temperature short-time (H.T.S.T.) pasteurizer. Milk passes through a regenerator where it is heated to approximately 138° F., and enters a vacuum chamber in which air and non-condensable gases are removed. After pasteurization, at temperatures varying from 164° F. to 200° F., depending upon the treatment desired, the milk enters a second vacuum chamber and boils rapidly or flashes. The flavor of the milk is thereby standardized by removing some of the volatile components. Cooling and final packaging follow the second chamber. This type of machine subjects the milk to the least severe heat and steam treatment.

A Type II machine differs from a Type I unit in that steam is injected directly into the milk and both vacuum chambers occur after the H.T.S.T. pasteurizer. The milk is first pasteurized, then enters a vacuum chamber where varying quantities of steam are injected raising the temperature to a maximum of 195° F. The milk then flows into a second chamber and the temperature of

the milk is reduced to about 165° F. The milk is then passed through the regenerator and cooler prior to packaging.

The Type III machine differs from Types I and II in that it injects excessive steam and may be used as a pasteurizer if the product temperature reaches a minimum of 194° F. This type of machine consists of three chambers and accessories for controlling the heat and vacuum treatment. The milk is warmed to 140° F. in a heat exchanger and enters the first vacuum chamber where steam is injected and pasteurization occurs. The milk is boiled at reduced pressure and cools in the second and third chambers. The vacuum may be regulated to adjust the temperature of each chamber. Off-flavors are removed in these chambers and the temperature reduced to about 140° F. The milk is then cooled and packaged. This type of deodorizer gives the most severe treatment for flavor standardization.

According to Roberts (32) the degree of flavor removal is directly related to the amount of steam injected or generated. He studied all of these types of equipment under commercial conditions in milk plants and reported that each plant manager was satisfied with his respective type of equipment. It was found, however, that stricter receiving room grading programs were necessary when using Types I and II machines for processing since the treatment in these types was less severe than in Type III. Even though all machines improved the flavor of milk, no machine removed hydrolytic rancidity, bitterweed or spoiled silage off-flavors.

One of the objections Roberts (33) raised to the Type III machine concerned the quality of the steam supply. Since steam comes into contact with the milk, no harmful boiler compounds or impurities may be present in the steam supply. To gain efficiency and control dilution of milk the steam should be dry and free from superheat. He stated that non-harmful boiler

compounds were available but some water supplies contained high chloride ion concentrations which produced medicinal flavors in the milk. He further reported that some milk processing codes would not allow direct injection of steam into milk.

Roberts (33) stated that the lack of knowledge about the chemical nature of flavor-producing substances complicates the problem of evaluating the means of removing them from milk and dairy products. He further stated that some milk treatments may cause chemical changes that cover up or mask the off-flavors rather than removing them.

The following experimental procedure was designed to identify some of the chemical compounds in milk and to determine if an association exists between these compounds and feed flavors.

EXPERIMENTAL PROCEDURE

Preparation of Samples

Samples of milk for this study were obtained from the Kansas State University herd. Normal milk, utilized as a control in this experiment consisted of that milk processed through the University creamery during the course of the routine commercial operation. The cows from which the control milk was drawn were on normal winter rations of hay and grain. The milk when sampled was free from any objectionable or severe feed flavors.

In order to create a feed flavor in the milk, a portion of the herd, consisting of from 50 to 70 Holstein, Guernsey, Jersey and Ayrshire cows, in various stages of lactation, was pastured on Balbo rye until a feedy flavor developed in the milk. This milk was segregated from the normal herd milk for a period of three days. A three-day supply of the feedy milk usually consisted of about 250 gal. This milk, to be referred to hereafter as "rye milk," was

analyzed in the manner to be described in the following pages.

In like manner, the same group of cows was pastured on young wheat until a flavor developed in the milk. This milk was collected in the same way as the rye milk and analyzed after an accumulation of three days. The milk obtained from cows on wheat pasture will be referred to hereafter as "wheat milk."

The stage of growth of these pastures was noted and the intensities of the milk flavors produced were recorded. Attempts were made to characterize the flavor that was produced under the different feeding programs.

Vacuum Treatment of the Milk

The milk in this study was analyzed with the aid of a DeLaval Vacu-Therm flavor standardizer (Type I)¹. This commercial equipment was designed to remove objectionable odors and flavors from milk. It consists of two vacuum chambers that are incorporated into the line of flow of milk in a high-temperature short-time (H.T.S.T.) pasteurization operation. One vacuum chamber receives milk from the H.T.S.T. regenerator at approximately 138° F. and the second chamber receives the milk after pasteurization at a temperature above 161° F. The flashing in these chambers when the hot milk is exposed to vacuum allows for volatile materials to be removed from the milk. The volatile materials are normally drawn through a vacuum pump and exhausted.

The DeLaval equipment was modified for this study by incorporating a series of traps between the first vacuum chamber and the vacuum pump so that all of the vapors removed from the milk could be directed into the trapping system.

¹ Type I, see p. 10 Literature Review.

Preliminary Studies

A sample of normal milk was analyzed with the aid of the modified Vacuum Therm equipment by directing the exhaust vapors through a series of cold traps. This system was designed after the one employed by Day et al. (9) and is shown in Figure 1. For ease of manipulation, all joints in the trapping system were of the ball and socket ground glass type. The connection between the stainless steel pipe from the vacuum chamber and the glass apparatus was made by first brazing the stainless steel pipe and machining the brazed area to fit the ball and socket joints. The collection system was comprised of the following traps. Trap 1, a 5-l. round-bottom two-neck flask, was used as a "splash trap" to prevent milk solids from passing into the system. The vapors leaving trap 1 were cooled with the aid of a 400 mm. Allihn condenser, cooled with sweet water, mounted in reflux position in the outlet of this flask. This trap was not cooled and its contents were discarded upon completion of a collection. A 600 mm. Allihn condenser in a vertical position cooled and directed the distillate into a 12-l. round-bottom two-necked flask, trap 2, which was immersed in ice water. Another 600 mm. condenser directing the vapors from trap 2 was in reflux position. Both 600 mm. condensers were cooled with sweet water. Most of the water in the vapors was condensed into trap 2. Trap 3, a 2-l. round-bottom two-neck flask, was immersed in a salt-ice water bath to effect further removal of water vapors. Traps 3 and 4, 1-l. round-bottom two-neck flasks, were immersed in dry ice-ethanol baths contained in Dewar flasks. It was intended that the more volatile compounds would be trapped in these flasks. The connections were made using appropriate pieces of glassware with 25/35 ball and socket joints. The apparatus was held securely

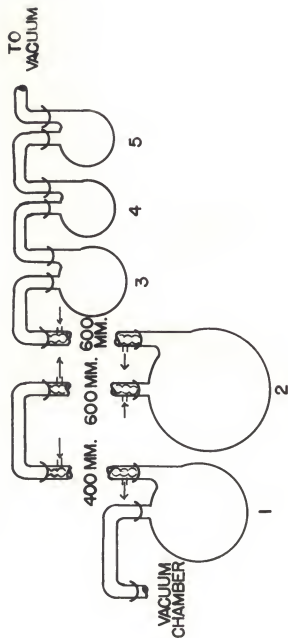


Figure 1. Distillate trapping system using cold traps.

1. 5-l., "splash" trap, no coolant bath.
2. 12-l., immersed in ice water bath.
3. 2-l., immersed in salt-ice water bath.
4. 1-l., immersed in dry ice-ethanol bath.
5. 1-l., immersed in dry ice-ethanol bath.

by attachment to aluminum alloy rods that were mounted on a 2" x 6" plank adjacent to the Vacu-Therm.

In the first trial approximately 530 gal. of milk was processed and the distillate collected. The odor of the distillate in each of the cold traps was observed after all of the milk was processed.

After being observed for odor, the milk distillate was subjected to several qualitative reagents. A few drops of the following reagents were added to 10 ml. of the distillate from each trap: (1) 5 percent H_2SO_4 ; (2) 5 percent NaOH; (3) Saturated $HgCl_2$ and (4) 0.1 percent 2,4-dinitrophenylhydrazine in 10N. H_2SO_4 . After each reagent was added to the distillate, the solution was evaluated for any change in odor.¹

Analysis of Carbonyl Compounds

After the observations in the preliminary study it was deemed advisable to analyze milk for carbonyl compounds. The trapping system was modified slightly by exchanging the alcohol-dry ice traps for carbonyl reagent traps and adding an extra trap. It was considered advisable to maintain the ice-water and salt-ice water traps in the reagent trapping system to prevent drastic dilution of the acid in the reagent traps. Acid conditions are necessary to form the carbonyl derivatives. The trapping system was identical to the one described in the section "Preliminary Study" with the following exceptions: Traps 4 and 5 were partially filled with 0.1 percent 2,4-dinitrophenylhydrazine in 2N. H_2SO_4 with the vapor inlet tube passing into this solution

¹ Basic compounds are decolorized by acid, acidic compounds are altered by base, sulfides are suppressed by $HgCl_2$ and carbonyl odors are destroyed by acidic 2,4-dinitrophenylhydrazine.

and another trap, number 6, a 5-l. round-bottom two-neck flask, was placed between trap 5 and the vacuum pump to protect the vacuum pump from any acid that might possibly splash from traps 4 and 5. This system is shown in Figure 2. With these manipulations any vapors that did not condense in the cold traps were directed through the reagent traps. This method has been used successfully as a trapping system for carbonyl derivatives (6). All of the milk vapors analyzed throughout the balance of this experiment were subjected to the carbonyl reagent traps.

Formation and Extraction of the Derivatives

All of the aqueous distillate from a single sample was poured together and additional 0.5 percent 2,4-dinitrophenylhydrazine in 10N. H_2SO_4 was added to adjust the normality of the final solution to one. This solution was allowed to react from two to four hours.

The 2,4-dinitrophenylhydrazones thus formed were extracted with n-hexane, hereafter called hexane in this thesis. The hexane utilized for the extraction of the hydrazones was treated and redistilled to render it as free of carbonyl compounds as possible. The solvent was treated with a solution composed of 0.4 g. of 2,4-dinitrophenylhydrazine, 2 ml. of concentrated sulfuric acid, 3 ml. of distilled water and 10 ml. of 95 percent ethanol. This mixture was partially soluble in the hexane and was added to about 10-l. just prior to distillation. The fraction boiling from 68 - 69° C. was collected.

To establish the concentration of carbonyl compounds in the hexane, an analysis of this solvent was made by extracting an aqueous solution of 0.1 percent 2,4-dinitrophenylhydrazine in 1N. H_2SO_4 . The hydrazones thus formed were washed with distilled water and taken to dryness.

The 2,4-DNPHs formed from the milk distillate were extracted with hexane.

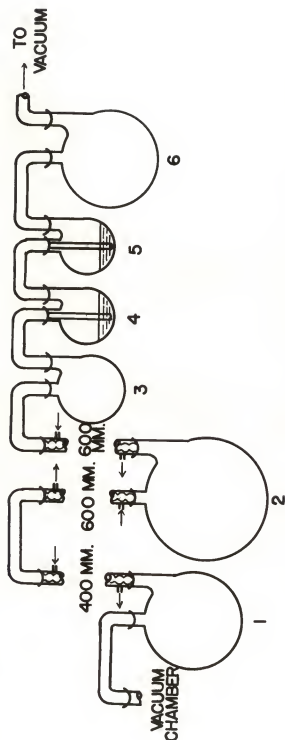


Figure 2. Distillate trapping system using cold and reagent traps.

1. 5-l., "splash" trap, no coolant bath.
2. 12-l., immersed in ice water bath.
3. 2-l., immersed in salt-ice water bath.
4. 1-l., containing 2,4-dinitrophenylhydrazine reagent in 2N sulfuric acid.
5. 1-l., containing 2,4-dinitrophenylhydrazine reagent in 2N sulfuric acid.
6. 5-l., no coolant or reagent; used to prevent entrance of any reagent from trap 5 into the vacuum pump.

The volume of hexane used was approximately five percent of the volume of the aqueous distillate. This extraction procedure was repeated and the hexane solutions were washed with about one-tenth of their volume with distilled water two times and evaporated under aspirator-reduced pressure over a boiling water bath.

While all carbonyl derivatives may not have been extracted by this method, it was felt that the most significant material from a flavor standpoint would be found in the hydrazones extracted by hexane.

Samples Analyzed for Carbonyl Compounds

In the course of this study, two samples of normal milk, two samples of rye milk and two samples of wheat milk were analyzed for carbonyl compounds. Attempts were made to use the same techniques in handling all samples so that some idea could be established with regard to the relative concentrations of the materials found. The solvent analysis was made in order to cancel out any material found in the solvent.

Separation of the Hydrazones

The hydrazones extracted from the distillates of each of the milks and the solvent were analyzed by column chromatography according to the method of Bassette et al. (5). The celite, nitromethane, hexane column employed was useful in separating the derivatives, but in addition to this the column was also valuable in providing a clue to the identity of the derivatives. A measure of the volume of solvent necessary to move a particular hydrazone through a gram of the packing material in the column (T.V. value) was useful in the identification of the derivative. This information along with melting points and/or absorption spectra has been used by others (6) for positive identification of

2,4-DNPH derivatives.

Determination of Absorption Spectra

All absorption spectra were obtained with the aid of a Beckman Model D.U. spectrophotometer using silica absorption cells and chloroform solvent. The spectra were obtained after initial separation by column chromatography and the molar concentrations calculated from these data. If the spectrum was atypical, i.e., not a symmetrical curve, a portion of the derivative was rechromatographed and a new spectrum obtained. This spectrum was not, however, used to calculate the molar concentration of the compounds. The molar concentration was calculated by using the following equation.

$$\frac{\text{optical density}}{23,000} = \frac{\text{absorbance} \times 1000}{x}$$

absorbance = number of moles

x = volume of solvent used for color density measurements

23,000 = molar absorptivity of 2,4-dinitrophenylhydrazones

Crystallization of Derivatives

The 2,4-DNPHs that were separated on the chromatographic column were dried under reduced pressure (aspirator) over a steam cone. They were redissolved in a minimum amount of petroleum ether (b.p. 38-42° C.) and cooled in a freezer cabinet. Crystals that formed were removed and melting points determined on a Fisher-John melting point apparatus. Mixed melting points were determined on samples from which sufficient crystals could be isolated to do so.

EXPERIMENTAL RESULTS

Preliminary Studies of Reduced Pressure Steam Distillation of Milk in the DeLaval Vacu-Therm

The milk used in this preliminary study was criticized for having a slight feed flavor. It was doubtful, however, that this flavor would have been recognized by other than experienced judges. Organoleptic analysis of this milk by a panel of five judges assigned it an average flavor score of 37.5.

Even though this milk was not produced from flavor-provoking rations, it was felt that some information could be obtained about normal milk. There was a slight possibility that a so-called normal flavor in high concentrations in milk might be classified as feed. Since rye and wheat pastures were not to become available for six months this milk was analyzed.

Approximately 9-l. of distillate was collected in the traps from 530 gal. of normal milk in this preliminary study. The DeLaval Vacu-Therm was used with the incorporation of cold traps as described in the procedure. This trial established that such a system of collecting milk volatiles was practical. The collection of these vapors did not interrupt the routine creamery operations and the odor of the materials found in the cold traps, when the equipment was dismantled, was quite similar to the original milk.

In order to characterize the odoriferous materials in the cold traps, the distillate was subjected to qualitative reagents. Among the qualitative reagents used was 2,4-dinitrophenylhydrazine. This reagent altered the character in some of the traps and thereby suggested that carbonyl compounds might be present. These data are presented in Table 2.

Table 2. Preliminary study of normal milk distillate collected October 17, 1958

Sample	Reagent	Judges' comments on odor			
		1	2	3	4
Trap 2 (ice water)	control	no odor	no odor	no odor	sweet
	A ¹	no odor	no odor	no odor	alcoholic, sweet
	B ²	sl. acrid	fresh milk	no odor	sheep's wool
	C ³	v. sl. odor	moldy	no characteristic odor	
	D ⁴	almost no odor	v. s. sweet	no odor	sl. sweet
Trap 3 (salt-ice water)	control	Methyl sulfide	no odor	warm milk, cow	artificial vanilla
	A	like Methyl sulfide	no odor	warm milk	feed, hay, alfalfa
	B	Methyl sulfide	odor, not identified	strong warm milk	Methyl sulfide
	C	oyster, Methyl sulfide	sharp, rotten, unsanitary	strong tallowy	animal-like odor
	D	Methyl sulfide	faint sweet, banana	no odor	unidentifiable
Trops 4 & 5 (ethanol-dry ice)	control	sweet, heated, cooked	musty grain	sweet	feed hay
	A	pungent, soapy	musty grain	sl. musty	feed, hay very intense
	B	pungent, fruit juice	odor, not identified	sweet, sticky	chemical
	C	tallowy	musty hay	no characteristic odor	smothered animal
	D	v. sl. soapy	soft, dead air	warm milky	feed

A. 5 percent H_2SO_4

B. 0.5 percent 2,4-dinitrophenylhydrazine in 10N. H_2SO_4

C. 5 percent NaOH

D. Saturated aqueous $HgCl_2$

Results of Analyses for Carbonyl Compounds
in the Milk Distillate

From the results of the preliminary study, it was decided to analyze the remaining milks for carbonyl compounds. The trapping system was modified as described previously and the analyses made. Two samples of normal milk¹ were processed during the winter, on December 15 and February 16. The rye milks were processed on April 2, 6 and 17, and the wheat milks on April 10 and 13.

The hydrazone derivatives formed from each of these trials were separated by column chromatography. The volume of solvent necessary to move the derivatives through 1 g. of packing material gave clues to the identification of these compounds. This value was referred to as the T.V. value, volume of solvent necessary to move a particular hydrazone through a gram of packing material (5). Melting points, mixed melting points and absorption spectra were used to identify the derivatives. A summary of these data is presented in Table 3.

The hydrazone derivatives from one sample of rye milk, April 6, appeared to be contaminated with a considerable amount of greasy material. As the greasy residue could not be separated from the derivative, this sample had to be discarded.

Acetone was found in each of the milks analyzed. The concentration of acetone, presented in Table 3, was found to vary between 2.56×10^{-10} and 4.23×10^{-8} moles per liter of milk. There did not appear to be any relationship between flavor or specific feed and acetone concentration. Melting points and mixed melting points of the acetone derivative as well as T.V. values and spectrophotometric data conclusively identified this compound.

¹ Free from any objectionable off-flavor.

Table 3. Results of analyses of milk distillates and solvent for carbonyl compounds.

Date	Sample : Suspected : vol.(gal.): Compounds	Chromatography : Absorption peak(m μ) ² : Melting points (corr.): Concentration		Moles/l. milk	
		Obs.: Authentic:	Obs. : Rptd. :	Obs. : Rptd. :	Mixed :
Milk Distillate					
Dec. 15	Normal	22	--	--	--
	500 acetone	30	32.5	366	364
	acetaldehyde	40.1	43.4	357	354
Feb. 16	Normal	26.0	--	--	--
	550 acetone	33	32.5	382	364
	acetaldehyde	43	43.4	357	354
April 2	Eye	17.6	--	--	--
	270 acetone	33	32.5	366	364
	acetaldehyde	47.3	43.4	357	354
April 17	Eye	17.0	--	--	--
	260 acetone	28.8	32.5	362-364	364
				363	125.5
April 10	Wheat	20.6	--	--	--
	250 acetone	32.0	32.5	362	364
				363	124.5
April 13	Wheat	24	--	--	--
	250 acetone	33	32.5	362-364	364
				363	124.5
Solvent					
June 15	1 l.	22	--	--	--
	acetone	32	32.5	365	364
	acetaldehyde	41.9	43.4	360-362	354
Moles/l. solvent					
June 15	1 l.	22	--	--	--
	acetone	32	32.5	2.74 x 10 ⁻⁶	2.74 x 10 ⁻⁶
	acetaldehyde	41.9	43.4	3.48 x 10 ⁻⁶	3.48 x 10 ⁻⁶
Moles/l. solvent					
June 15	1 l.	22	--	--	--
	acetone	32	32.5	5.82 x 10 ⁻⁶	5.82 x 10 ⁻⁶
	acetaldehyde	41.9	43.4	5.82 x 10 ⁻⁶	5.82 x 10 ⁻⁶

1. Known samples of these derivatives were run concurrently with the unknowns.

2. Spectrophotometric raw data Tables 6 through 10 in the Appendix.

3. Jones et al. (18).

4. Shriner, Fuson, Curtin (35).

5. Corrected by subtracting concentration of carbonyl compounds found in solvent to obtain these values.

At least two other hydrazone derivatives were found from this analysis, but it was established that they originated in the solvent used for extraction. One of these two derivatives appeared to be acetaldehyde. The T.V. value of the suspected compound agreed with the authentic T.V. value and the absorption spectrum was similar to the reported acetaldehyde spectrum. Attempts to crystallize this derivative were unsuccessful.

The other hydrazone was not separated in pure enough form to yield reliable data for identification. Absorption peaks of this compound varied from 361 to 382 m μ and T.V. values from 17.0 to 26.0. Melting points of 218 and 219° C. were observed.

The negative values appearing in Table 3 indicate that there was more of a compound recovered from the solvent than was found in the milk sample. Although considerable effort was exerted to remove carbonyl compounds from the solvent, a rather high concentration persisted.

Solvent Analysis. The solvent used to extract the hydrazone derivatives formed, was found to contain three compounds. These derivatives appeared to be the same compounds that were isolated from the analysis of the sample. The solvent analysis with evidence for compound identification is presented in Table 3.

Quantitative Studies. For each of the analyses, attempts were made to measure the concentration of the particular hydrazone derivative in question by obtaining the optical density of a measured solution at the absorption peaks. A molecular extinction of 23,000 was assumed for all 2,4-DNPH, and the concentrations were calculated and expressed as moles of carbonyl per liter of milk. The concentration of 2,4-DNPH found in the solvent was subtracted from the corresponding concentration of the sample analysis to give a corrected concentration. These results are summarized in Table 3.

Effect of Stage of Growth of Pasture upon Milk Flavors

It was observed that when pastured on young rye cows did not show an objectionable feed flavor in their milk. After one week a mild feed flavor did appear, but it was not considered to be strong enough to be criticized by consumers after processing.¹ This milk after processing was sold with normal milk to University creamery patrons. The first rye milk (March 31 through April 2) was judged for flavor. The results of this study are presented in Table 4.

Table 4. Effect of types of pastures and stages of growth upon milk flavor.²

Rye Pasture			:	Wheat Pasture		
Date of Sampling	Height of Plants (in.)	Raw Milk Flavor Score	:	Date of Sampling	Height of Plants (in.)	Raw Milk Flavor Score
March 31 - April 2	5 - 7	37		April 7-10	4 - 6	36.5
April 14-17	10-12	35.2		April 10-13	3 - 5	36.4

² Mean flavor scores from Table 5.

Milk samples obtained from the same cows two weeks later on the same pasture were found to possess a severe feed flavor after the cows had grazed only a few days on this more mature pasture.

There was little difference in the stages of growth of the wheat pasture when cows were grazed as evidenced by the height of the wheat and the dates that the milk was sampled. The flavor scores were almost identical and the criticisms were similar. (Tables 4 and 5).

¹ Whenever a severe feed flavor appeared in the University creamery milk, there were numerous complaints from customers.

Table 5. Flavor scores of milk samples analyzed.

Date	Judge	Score : Raw	: Criticism	Score : Processed	: Criticism
----- Normal -----					
Dec. 15	1	37.5	sl. feed	38.0	
	2	38.5		38.5	
	3	38.5		38.5	
	4	38.0	sl. feed	38.0	
	5	<u>38.5</u>		<u>39.0</u>	
	mean	38.2		38.4	
Feb. 16	1	37.5		39.0	
	2	37.5	sl. feed	38.5	
	3	37.0	feed	38.5	
	4	<u>37.0</u>	feed	<u>38.5</u>	
	mean	37.25		38.5	
----- Rye -----					
April 2	1	36.5	dirty, unclean	37.0	cowy
	2	36.5	rancid	37.0	sl. rancid
	3	37.5	feed	36.0	cook, feed
	4	37.5	feed, cook	36.0	feed
	5	<u>37.0</u>	feed	<u>37.0</u>	feed
	mean	37.0		36.6	
April 17	1	34.0	feed, fish	35.5	feed, unclean
	2	35.5	feed, unclean	36.5	feed
	3	35.5	feed	36.0	feed, cook
	4	36.0	feed	38.0	feed, unclean
	5	<u>35.0</u>	unclean, feed	<u>35.5</u>	feed, bitter
	mean	35.2		36.3	
----- Wheat -----					
April 10	1	37.0	feed, cowy	37.5	sl. feed, cook
	2	37.5	feed	38.0	
	3	36.5	feed	38.0	sl. feed, sl. cook
	4	<u>35.0</u>	feed, unclean	<u>37.5</u>	sl. feed, sl. cook
	mean	36.5		37.75	
April 13	1	36.0	unclean, feed	37.0	feed, cook
	2	37.0	feed	37.5	feed
	3	36.0	feed	38.0	feed
	4	<u>36.5</u>	dirty, feed	<u>37.5</u>	cook, feed
	mean	36.4		37.5	

Although processing improved three of the four feed flavored milks by 1.1 units, on the standard milk scorecard, rye milk from the early sample was not improved. The milk obtained from cows pastured on the mature rye although improved was still poor quality. The other milks after processing were all acceptable by the University creamery patrons. The milk produced on young rye pasture was of fair quality, flavor score 37.0. After processing the flavor score was reduced to 36.6 (Table 5).

DISCUSSION

The results obtained from this study indicate that carbonyl compounds do not play a significant role in the appearance of rye or wheat pasture induced feed flavors in milk. While acetone was isolated and studied as its 2,4-DNPH derivative from the vapors of the feed flavored milks, it was also found in the normal milks in approximately the same concentration. The amounts of this derivative recovered in the study was low considering the volume of milk analyzed. Bassette (6) found 1.19×10^{-5} g. of acetone per liter of raw milk or 12 ppm. In his study, a laboratory scale distillation was conducted with much lower temperature and pressure, with more controllable conditions. If the concentrations of acetone found by Bassette had been isolated in this study, approximately 20 g. would have been removed from 500 gal. of milk sample. Obviously this was not accomplished. The poor recovery of acetone would also account for the failure to find acetaldehyde and formaldehyde which was recovered by the Maryland workers in concentrations of less than one percent of the acetone levels.

The low concentration of acetone recovered in this study may have been the result of the inability of the Vacu-Therm to remove this compound from milk or it may have been due to the inefficiency of the trapping or extraction systems.

Roberts (33) stated that the removal of a material from milk by commercial flavor standardizing equipment depends upon the volatility of the substance and its solubility in water. The solubility in water influences the degree of steam distillation that will occur. Acetone has a boiling point of 56.5°C . and is infinitely soluble in water. The latter property would make it much less steam distillable than if it were insoluble. Under reduced pressure, however, acetone would be more volatile and more easily removed due to the decreased boiling point. The forces, if any, holding acetone in solution in milk are not known. From what is known about the properties of acetone in a strictly aqueous system, it would be expected that it would be removed by the Vacu-Therm treatment. Interaction between various milk components and acetone may influence its properties. The same arguments are valid for acetaldehyde and formaldehyde. It is felt that the reasons that poor recoveries of acetone were obtained also explain the failure to detect the more volatile carbonyl compounds.

Numerous investigators (4, 27, 41) concluded that the longer chain aldehydes and ketones are more important in producing off-flavors in milk products than the shorter chain carbonyl compounds. Bassette (4) concluded that acetone, acetaldehyde and formaldehyde in the concentration found in normal milk did not contribute to its flavor.

Assuming that the longer chain carbonyl compounds were present in the feed flavored milks, it was felt that they would have been removed by the Vacu-Therm and trapped from the vapors. Surely the components that were removed to improve the flavor of the milk, if long chain carbonyl compounds, would have been trapped by the trapping system. In addition to these longer chain compounds being removed and trapped more easily, the hydrazone derivatives once formed would be soluble in hexane and extracted from the aqueous solution with no difficulty. Other extracting solvents might have yielded different

quantitative results, but it was felt that any derivative of a carbonyl compound normally associated with off-flavors would be extracted from the aqueous phase by hexane. A more polar solvent that would have recovered more of the shorter chain carbonyl derivatives would also have extracted the hydrazine reagent which would have interfered somewhat with the chromatographic separation.

It was observed that acetone and acetaldehyde 2,4-DNPHs were soluble to some extent in water. The shorter the carbonyl compound chain length the more soluble it was found to be in water and the less soluble in hexane. Formaldehyde 2,4-DNPH was found to be only slightly soluble in hexane. This might explain the negative values for some derivatives reported in Table 3. An error in technique was committed when the solvent blank was determined. Only 100 ml. of aqueous 2,4-dinitrophenylhydrazine reagent was used to treat a liter of hexane to determine hydrazones that might be formed during the extraction step. During the extraction of hydrazones from the distillate, 1-l. of hexane was used to extract 10 l. of the reagent and 2,4-DNPH distillate solution. It is probable that more water soluble or polar hydrazones would remain in the 10-l. of solution than would remain in 100 ml. of solution. The negative values indicate that there was more material found in the solvent than was found in the sample. Since these short chain carbonyl compounds were not felt to be important as flavor compounds, the correction in the solvent analysis was not made.

One derivative fraction was not identified. It appeared consistently in the analyses and probably came from the solvent. The I.V. values were fairly constant and the appearance of the band on the column was characteristic. The absorption peaks varied from 363 μ to 382 μ . The melting points obtained in two cases were 218° and 219° C. It was felt that a greasy material that

contaminated some of the bands might have altered some of the constants used for identification.

A greasy residue on the 2,4-DNPH derivatives was encountered during the chromatographic analyses. Melting points could not be obtained in some cases because of this problem. Bassette (4) was troubled by this difficulty, but found that thorough purification of the hexane minimized the oily residue. He purified this hexane by passing it through a three foot long column of concentrated sulfuric acid and subsequently distilling it over sodium hydroxide pellets. Facilities were not available in this laboratory to treat the hexane as the Maryland workers had done. An attempt to make the hexane free of carbonyl compounds by mixing it with an alcohol-water solution of 2,4-dinitrophenylhydrazine reagent may have added to the grease problem. In retrospect it may have been that a small amount of alcohol co-distilled with the hexane that was used as the mobile solvent in the chromatographic procedure.

The qualitative studies of the milk distillate referred to in Table 2 indicated that compounds other than carbonyl compounds were present in the distillate. Acid tended to diminish the odor of the distillate when added to the material in the traps. On the other hand, a basic reagent enhanced the odor of the distillate. Based upon this observation, amines could be present and contribute to the odor of the distillate. There are reports in the literature that amines in milk may cause off-flavors (33, 38). There is a possibility that at least some of the effect of the 2,4-dinitrophenylhydrazine reagent observed was due to the acid present. The cowy odor observed consistently in the distillate was probably due to methyl sulfide as reported by Patton *et al.* (29).

The results of this study suggested that carbonyl compounds are not the cause of wheat and rye flavors in milk. Acetone was identified as a component

of normal, rye and wheat milks, but no relationship was found between the concentration of this compound and the occurrence of wheat and rye pasture induced off-flavors.

A suggested approach to the problem of isolating and identifying feed induced flavors in milk is the use of vapor phase chromatography. Only small amounts of materials are needed for analyses by this method and analyses are rapid. Gas chromatography used for fractionation followed by mass spectrometry could conceivably solve this problem. A few simple modifications in the trapping system would allow the use of liquid nitrogen as a coolant or the collection of samples for analysis by gas chromatography.

SUMMARY AND CONCLUSIONS

1. The results from this study indicate that carbonyl compounds do not play a significant role in causing either wheat- or rye-induced off-flavors in milk. No carbonyl compounds were found in rye- or wheat-flavored milks that were not found in normal milk.
2. A trapping system was designed to collect the vapors removed by the DeLaval Vacu-Therm commercial flavor standardizing equipment. This was done by replacing the vapor removal pipe of the Vacu-Therm with a series of either cold or cold and reagent traps. In this study 0.1 percent 2,4-dinitrophenylhydrazine in 2N. sulfuric acid was used in the reagent traps. This modification of the Vacu-Therm did not necessitate alteration of the normal operating procedure.
3. Acetone was isolated from all samples of milk and identified conclusively by the agreement of T.V. values, melting points, mixed melting points and absorption spectra of its 2,4-DNPH derivative with known acetone 2,4-dinitrophenylhydrazone.

4. An unidentified substance(s) with T.V. values ranging from 17.0 to 26.0 was found in the analysis. There was some evidence to indicate that this material came from the solvent. Melting points were obtained twice and found to be 218 and 219° C.
5. Qualitative studies conducted in this experiment suggested that an odor neutralized by an acidic and intensified by a basic reagent was present in the distillate from normal milk. An amine-type compound was suggested by this observation. A strong cowy odor resembling methyl sulfide was detected in the distillate when smelled immediately after collection.
6. The results of this analysis indicate that there is a need for additional research work in the area of feed flavors in milk. Gas chromatography and mass spectrometry may prove to be useful tools in future investigations.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. Richard Bassette for his advice, encouragement and criticisms throughout the course of this study and the preparation of this manuscript.

Appreciation is also expressed to Prof. W. H. Martin, Dr. T. J. Claydon, and Instr. Ross Mickelsen who in addition to Doctor Bassette helped conduct the organoleptic analyses.

The author wishes to thank Dr. C. L. Morton, Head, Department of Dairy Husbandry, for a critical review of this manuscript. Appreciation is further expressed to Dr. F. C. Fountains, Acting Head of the department who offered encouragement to the author.

Sincere thanks go to the employees of the University Creamery who helped obtain the distillate samples.

Appreciation goes to the graduate students in the Dairy Department who gave the author valuable advice and encouragement throughout this study.

LITERATURE CITED

1. Babcock, G. J.
Common causes of off-flavored milk and their control. J. Milk and Food Tech. 14(2):68. 1951.
2. Babcock, G. J.
Feed flavors in milk and milk products. J. Dairy Sci. 21(10):661. 1938.
3. Bartley, E. E., J. W. F. Chin, C. Y. Cannon and E. W. Bird.
The influence of cracked soybeans, soybean hay and various kinds of containers on the flavor of milk. J. Dairy Sci. 22(1):28. 1950.
4. Bassette, R.
The identification of some flavor compounds from stored instant non-fat dry milk. University of Maryland, Ph.D. thesis. 1958.
5. Bassette, R., E. A. Day and M. Keeney.
Column partition of 2,4-dinitrophenylhydrazones. J. Dairy Sci. (in press).
6. Bassette, R. and M. Keeney.
Identification of some compounds affecting the flavor of non-fat dry milk. J. Dairy Sci. (in press).
7. Bradfield, Alec.
Putting flavor into the quality program. J. Dairy Sci. 39(7): 944. 1956 (abs.)
8. Cotner, E. C.
Effectiveness of various vacuum, temperature, and steam treatments in reducing feed flavors in milk. Kansas State University Master's Thesis, 1958.
9. Day, E. A., D. A. Forss and S. Patton.
Flavor and odor defects of gamma-irradiated skim milk: I. preliminary observations and the role of volatile carbonyl compounds. J. Dairy Sci. 40(8):922. 1957.
10. Dodge, W. A.
Making dairymen milk flavor conscious. J. Dairy Sci. 39(7):944. 1956 (abs.)
11. Downs, P. A., E. O. Anderson, C. J. Babcock, F. H. Nerzer and G. M. Trout.
Evaluation of collegiate student dairy products judging contests since World War II. J. Dairy Sci. 37(8):1021. 1954.
12. Duncan, C. W., C. F. Huffman and H. A. Tobias.
A chemical study of ketosis in a dairy herd. J. Am. Vet. Med. Ass'n. 95(12):690. 1939.

13. Dunkley, W. L.
Off-flavors can be prevented. *Hoard's Dairyman*. 100:400. Apr. 25, 1953.
14. Flagg, F.
Eliminating feed flavors during the grassy season. *Milk Plant Monthly*. 39(7):34. 1950.
15. Frye, J. B. Jr., C. Y. Cannon and E. W. Bird.
The influence of cracked soybeans and other factors upon the flavors of milk and the iodine value of milk fat. *J. Dairy Sci.* 33(4):205. 1950.
16. Herman, H. A. and E. R. Garrison.
Balbo rye and Missouri Early Beardless barley do not produce objectionable flavors in milk. *Missouri Ag. Exp. Sta. Bul.* 477. 1944.
17. *Hoard's Dairyman*.
Editorial. *Hoard's Dairyman*. 65:322. Mar. 16, 1923.
18. Jones, L. A., J. C. Holmes, and R. B. Seligman.
Spectrophotometric studies of some 2,4-dinitrophenylhydrazones. *Anal. Chem.* 28(2):191. 1956.
19. Kansas Agricultural Experiment Station.
Dairy manufacturing investigations. *Kans. Ag. Expt. Sta. Biennial Report*. 1936-38. p. 84.
20. Kansas Agricultural Experiment Station.
Dairy manufacturing investigations. *Kans. Ag. Expt. Sta. Biennial Report*. 1938-40. p. 82.
21. Kansas Agricultural Experiment Station.
Station project no. 509. (unpublished communication).
22. Knott, C. B.
Successful dairying. New York: McGraw Hill. 1954.
23. Knott, C. B., J. C. Shaw, and G. C. White.
Studies of ketosis in dairy cattle: II. blood and urinary acetone bodies of dairy cattle in relation to parturition, lactation, gestation, and breed. *J. Dairy Sci.* 25(10):851. 1942.
24. Mac Curdy, R. D. and G. M. Trout.
The effect of holder and flash pasteurization on some flavors of milk. II. the effect on corn and alfalfa silage flavors. *J. Dairy Sci.* 23(9):855. 1940.
25. McDowall, F. H.
The buttermaker's manual, Vol. I. Wellington, New Zealand: New Zealand University Press. 1953.

26. Mead, S. W. and W. L. Dunkley.
Influence of cane molasses on feed flavors in milk. *J. Dairy Sci.* 41(10):1482. 1958. (abs.)
27. Morgan, M. E., D. A. Forss and S. Patton.
Volatile carbonyl compounds produced in skim milk by high heat treatment. *J. Dairy Sci.* 40(5):571. 1957.
28. Niegisch, W. D. and W. R. Stahl.
The onion: gaseous emanation products. *Food Res.* 21(6):657. Nov. Dec., 1956.
29. Patton, S., D. A. Forss and E. A. Day.
Methyl sulfide and the flavor of milk. *J. Dairy Sci.* 39(10):1469. 1956.
30. Potts, R. B. and E. M. Kesler.
Effect of grass silage on milk flavor and blood and milk acetone bodies. *J. Dairy Sci.* 40(11):1466. 1957.
31. Roehen, Dan C. and M. L. Mitten, Jr.
Milk flavor uniformity is possible. *J. Dairy Sci.* 39(10):1328. 1956.
32. Roberts, W. M.
The practical application of steam injection systems to fluid milk and cream operations. *Am. Milk Rev.* 19(12):44. 1957.
33. Roberts, W. M.
Problems involved in flavor removal. *J. Dairy Sci.* 42(3):560. 1959.
34. Roberts, W. M.
Removing feed flavors in the plant. *Milk Ind. Foundation Convention Proceedings.* 1956.
35. Shriner, R. L., R. C. Fuson, and D. Y. Curtin.
The systematic identification of organic compounds. New York. John Wiley and Sons. 1956.
36. Strobel, D. R. and C. J. Babcock.
An analysis of flavor research on milk. *Milk Dealer.* 43(7):80. 1954.
37. Strobel, D. R., W. G. Bryan and C. J. Babcock.
Flavors of milk: a review of literature. U.S.D.A. Washington, 1953.
38. Trout, G. M.
Have we properly emphasized flavor in the quality yardstick? *J. Dairy Sci.* 39(5):613. 1956.
39. Trout, G. M., and R. E. Norwood.
Influence of Balbo rye pasture on the odor of milk. *Mich. Agr. Exp. Sta. Quarterly Bul.* 27(1):39. 1944.

40. United States Department of Agriculture.
Newsletter, July 12, 1959, no. 1539. U.S.D.A., Washington, July 12,
1959.
41. Wong, W. P., S. Patton and D. A. Forss.
Methyl ketones in evaporated milk. J. Dairy Sci. 41(12):699. 1958.
42. Wynn, J. D. and J. R. Brunner.
Removal of feed flavors from milk by vacuum pasteurization. J. Dairy
Sci. 42(5):896. 1959. (abs.)

APPENDIX

Table 6. Spectrophotometric raw data for December 15.

Wave-length (mμ)	T. V. of fraction					
	22 ¹	34	32	44	40	
	Volume of solvent used for dilution (ml.)					
	300	10,600		200		
	crude	crude	purified	crude	purified	
Optical Density						
330	.236	.205	.280	.191		.047
340	.369	.345	.417	.202		.053
341				.204		
342				.205		
343				.204		
344				.206		
345				.210		
346				.207		
347				.210		
348				.210		
349				.209		
350	.309	.495	.544	.204		.061
355	.561	.544	.588	.206		.063
357	.574	.562	.601	.202		.064
358	.583	.576	.608	.202		
359				.204		.063
360	.595	.581	.615	.203		.062
361						
362	.606	.583	.620	.197		
363			.624	.195		
364	.613	.592	.624	.194		
365	.614	.595	.621	.196		.062
366	.616	.597		.196		
367	.614	.590	.610	.195		
368	.612	.585		.191		
369				.188		
370	.602	.570	.595	.187		.060
375	.570	.541		.172		
380	.517	.480	.488	.158		.054
390	.389	.355	.351	.131		.044
400	.272	.226	.231	.106		

¹ T.V. value of 24 upon rechromatographing.

Table 7. Spectrophotometric raw data for February 16.

Wave-length (m μ)	T. V. of fraction					
	24	26	33	33	43	43
	Volume of solvent used for dilution (ml.)					
	2000		4000		400	
	crude	purified	crude	purified	crude	purified
<u>Optical Density</u>						
330	.041	.073	.084	.047	.210	
335						.613
340	.058	.109	.105	.065	.250	.691
345						.741
350	.073	.157	.132	.085	.279	.783
355	.082	.182	.140	.093	.284	.809
357	.085	.193	.143	.096	.282	.815
358						.814
359	.088		.144		.280	.813
360	.089	.205	.145	.099	.279	.812
361	.091		.146		.278	.810
362		.214	.146	.101	.276	.807
363	.088	.219	.146	.102	.274	.804
364		.223	.146	.102	.272	.800
365	.091	.227	.145	.101	.269	.795
370	.099	.244	.141	.096	.240	.762
375	.099	.249				.700
380	.099	.254	.121	.085	.205	.631
382		.255				
384		.252				
385	.096	.251				
390	.098	.242	.093	.074	.158	.490
400	.076	.205	.071	.057	.123	

Table 8. Spectrophotometric raw data for rye milk distillates

Wave-length (m μ)	I. V. values of fraction					
	April 2			April 17		
	17.6	33	47.3	17.0	28.8	
	Volume of solvent used for dilution (ml.)					
	15	400	200	10	2000	
<u>Optical Density</u>						
330	.204	.166	.111	.124	.197	
340	.230	.242	.136	.151	.285	
350	.255	.312	.153	.173	.368	
355	.267	.335	.157	.180	.399	
357	.275	.342	.163	.182	.408	
358				.184	.411	
359			.158			
360	.278	.349	.157	.185	.416	
362	.281	.351		.186	.419	
363	.282	.350		.186	.420	
364	.283	.349		.186	.419	
365	.284	.348	.155	.184	.418	
366	.285					
370	.283	.332	.149	.180	.400	
380	.265	.275	.132	.164	.327	
390	.238	.198	.110	.139	.236	
400	.204	.141		.118	.167	

Table 9. Spectrophotometric raw data for wheat milk distillates.

Wave-length (mμ)	<u>T. V. values of fraction</u>					
	<u>April 10</u>			<u>April 13</u>		
	20.6	32.0	32.0 ¹	24	33	
	<u>Volume of solvent used for dilution (ml.)</u>					
	20	800		10	800	

<u>Optical Density</u>					
330	.187	.330	.302	.091	.236
340	.275	.484	.362	.125	.343
350	.355	.629	.467	.155	.447
355	.385	.678	.508	.166	.484
356	.391				
357	.395	.690	.519	.168	.491
358	.399	.698	.521	.169	
359	.402		.526		
360	.404	.702	.530	.171	.500
362	.409	.707	.532	.172	.503
363	.408	.710	.537	.172	.504
364	.407	.707	.533	.172	.503
365	.405	.704	.532	.170	.500
370	.390	.675	.512	.162	.478
380	.326	.558	.425	.134	.392
390	.240	.398	.322	.098	.282
400	.169	.280		.072	.196

¹ Purified by rechromatographing.

Table 10. Spectrophotometric raw data for n-hexane control.

Wave-length (m μ)	<u>T. V. value of fraction</u>		
	22.0	32.0	41.9
	<u>Volume of solvent used for dilution (ml.)</u>		
	100	400	125
<u>Optical Density</u>			
330	.270	.246	.660
340	.406	.366	.865
350	.538	.461	.990
355	.587	.490	.992
357	.591	.496	.995
358	.606	.497	.985
360	.616	.498	.967
362	.622	.498	.942
364	.628	.496	.925
365	.631	.495	.918
370	.608	.463	.820
375		.417	.715
380	.515	.368	.606
390	.376	.263	.422
400	.265	.187	.327

**THE ISOLATION AND IDENTIFICATION OF CARBONYL COMPOUNDS
ASSOCIATED WITH FEED FLAVORS IN MILK**

by

JOHN RAYMOND MILTON

B. S., Kansas State University, 1958

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

**KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE**

1959

The purpose of this study was to identify carbonyl compounds responsible for certain feed-induced flavors in milk. Attempts were made to trap the volatile materials removed by commercial flavor standardizing equipment. This was accomplished by passing the odor-laden vapors through a series of either cold traps or cold and reagent traps.

A preliminary study was directed toward characterizing the distillate for its odor and response to certain qualitative reagents. Normal milk was used for the preliminary studies since feed flavored milk was not available. Qualitative tests employing acid, base, 2,4-dinitrophenylhydrazine and mercuric chloride reagents were used in an attempt to gain information regarding the types of compounds responsible for the odor of the distillate. It was found that carbonyl compounds contributed to this odor.

Analyses for carbonyl compounds were performed, after the observations in the preliminary study, by reacting the distillate from the milk with acidic 2,4-dinitrophenylhydrazine reagent and separating and identifying the 2,4-dinitrophenylhydrazones. Once formed, these derivatives were extracted with n-hexane and separated by column partition chromatography. Concentrations of each derivative were calculated by measuring the optical density at the corresponding absorption peak. Identification of these compounds was based upon the agreement of absorption spectre, melting points, mixed melting points and the behaviour of the derivatives on the chromatographic column with authentic compounds.

Samples of milk were obtained from 50 to 70 cows grazed on wheat and rye pastures. Two collections of the milk from these cows on each pasture were analyzed as described above. Two control samples of normal flavored milk were analysed to determine if either the composition or concentration of carbonyl compounds in normal milk vapors was different from those recovered

from wheat or rye milks.

An analysis was made of the n-hexane used in the procedure to determine if any carbonyl compounds were present which would confound the results of the study.

By the described procedure, acetone was isolated and positively identified in the vapors from all milks studied. An unidentified substance(s) was also found in some samples. The absorption peaks of this unidentified compound(s) varied from 362 to 382 μ . and the melting points, obtained only twice, were 218 and 219° C. There was some evidence to suggest that this unknown compound(s) was obtained from the solvent during the analyses.

The results of these analyses indicate that the carbonyl compounds identified do not play a significant role in the occurrence of either wheat or rye induced feed flavors in milk.

A trapping system was described which did not necessitate alteration of normal operating procedures for a DeLaval Vacu-Therm deodorizer. This trapping system could be modified easily to collect samples for analysis by gas chromatography.

Based upon organoleptic analyses of the distillate odors following the use of qualitative reagents, a study of the amines in these vapors might yield valuable information in further investigations.

A distinct covey odor was present in the distillate. This odor was probably due to methyl sulfide which has been described as having a characteristic covey odor when in small concentrations.