that have been heated or stored for long periods remains to be investigated.

Because of the difficulty of obtaining a nonretrograded cane starch from a raw sugar to serve as a color standard, the authors have preferred to use an alcohol-precipitated formamide solubilized potato starch as a standard (Figure 1, *C*).

Acknowledgment

The authors express their appreciation to the management of The Colonial Sugar Refining Co., Ltd., Sydney, Australia, for permission to publish this paper.

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Received for review June 9, 1958. Accepted April 24, 1959.

BUTTERFAT OXIDATION

Evaluation of Lea's Aldehyde Determination Method

ARJEN TAMSMA[1] and ROBERT D. POWELL[2]

Dairy Industry Section, Iowa Agricultural Experiment Station, Ames, Iowa

Lea's method for determination of aldehyde in fats is excellent for *n*-heptanal. For normal aldehydes with more than seven carbon atoms, recovery decreased with increasing chain length and limiting values were reached with the C_9 and C_{10} aldehydes. For "aldehydes" from autoxidized milk fat the value found by Lea's method is arbitrary, because these carbonylic compounds do not behave like heptanal. Incomplete recovery can be caused by low solubility in water and low reactivity with bisulfite. Too high recovery may be obtained with unsaturated carbonyl compounds as a result of reaction of the double bonds. Reaction products and yields were examined by isolation of the aldehyde by solvent extraction after decomposition of the bisulfite complex. "Milk-fat aldehyde" was of ketonic character; the yield was about 1/3 to 1/10 as compared to synthetic aldehyde.

LEA'S METHOD for the determination of aldehyde involves an iodometric titration of bisulfite bound by the carbonyl group (*4*). A fat sample in benzene is shaken with a bisulfite solution. The excess of the bisulfite is removed from an aliquot of the aqueous layer by the reaction with iodide. The bound bisulfite is liberated with sodium bicarbonate and titrated with standard iodine. Lea claims quantitative recovery of *n*-heptanol.

As saturated aldehydes of medium molecular weight (heptanal) were at that time considered mainly responsible for the objectionable flavors and odor of oxidized fats, Lea proposed this method for estimating fat oxidation. Tamsma (*5*, *6*) and others have since shown that a wide variety of saturated and unsaturated carbonyl compounds, both aldehydes and ketones, are formed during fat oxidation. These may behave differently from heptanal, and recovery by Lea's bisulfite procedure is therefore not necessarily complete.

The present study was undertaken to investigate the extent and nature of aldehydes recovered by Lea's method from milk fat as compared with saturated normal aldehydes with 7 to 10 carbon atoms.

Since completion of this study, development of chromatographic methods has begun to make available more accurate knowledge of the nature of carbonylic compounds produced from autoxidized fats. Carbonylic oxidation products from butterfat appear to be largely ketonic rather than aldehydic, whereas in most fats they are very largely aldehydic. For this reason butterfat is hardly a fair choice of substrate on which to "evaluate" the Lea method, because ketones react more slowly and incompletely with bisulfite than do aldehydes. For this same reason, bisulfite extraction offers an interesting method for fractionation of the carbonyl compounds from butterfat.

Experimental Procedure

Synthetic Aldehydes. Commercial preparations (Givaudan-Delawanna, Inc., New York, N. Y.) of various aldehydes were purified by distillation under reduced pressure (about 12 mm.) to constant boiling point, density, and refractive index. The density was determined by the pycnometer method, and the refractive index (D line) with an Abbe refractometer, both at 20° C. Data for the purified aldehydes are presented in Table I.

Aldehydes were unstable at −15° C., as indicated by increases in density, refractive index, and melting point, and decrease in recovery by Lea's determination. In a dilute solution containing 1 to 6 μmoles per ml. of solution in a nitrogen atmosphere in the dark, purified aldehydes could be kept unchanged, at room temperature, for 2 months or more.

The ultraviolet absorption of the aldehydes in Skellysolve B was determined for solutions of approximately 4 μmoles of aldehyde per ml. Methods

[1] Present address, Dairy Products Laboratory, U. S. Department of Agriculture, Washington, D. C.
[2] Present address, Department of Botany, University of Florida, Gainesville, Fla.

were as described previously (*5*). All aldehydes had a maximum at 293 to 294 mμ with molar extinction of 23. This maximum is caused by the aldehyde group and agrees with Conrad-Billroth's results (*1*). The aldehydes with 8, 9, and 10 carbon atoms showed a second maximum at 215 to 216 mμ, indicating the presence of some α-β-unsaturated aldehyde (*2, 3*). A slight contamination with these compounds (molar extinction 10,000 to 20,000) shows clearly in the ultraviolet absorption.

"Aldehyde" from Milk Fat. Milk fat was oxidized at 100° C. (*5*). Solutions were obtained by dissolving either the oxidized fat or the "volatile fraction" in Skellysolve B. This volatile fraction was obtained by steam distillation of the oxidized fat (*5*).

Evaluation of Lea's Method. Recovery was studied for the synthetic aldehydes by dissolving weighed quantities in known volumes of benzene or Skellysolve B. The theoretical value per determination (100% recovery) followed then from the volume of solution used, and recovery was expressed as percentage of the theoretical value.

The aldehyde liberated from the aldehyde-bisulfite complex was also examined. The liberated aldehyde was isolated by extraction with 2.5 ml. of Skellysolve B, immediately after the titration of bisulfite with 0.002*N* iodine. The extract was washed twice with 1.5 ml. of distilled water. Extracts from several single determinations were combined, and the aldehyde content was determined by Lea's method and by ultraviolet absorption. Completeness of the isolation was calculated on the basis of the original aldehyde content as determined by the same method. Quantitatively complete isolation of aldehyde was not attempted, but the procedure was carefully standardized to compare the amounts isolated with synthetic aldehydes or aldehyde from milk fat as a substance. Ultraviolet absorption characteristics provided information about the nature of the isolated aldehydes (*5*). Ultraviolet absorption was corrected with a blank value obtained by following the same procedure without aldehyde present.

Modifications of Lea's Method (*4*). Lea's method was modified by shaking in a nitrogen atmosphere and using Skellysolve B instead of benzene as a solvent. Skellysolve B is suitable for spectral analysis (*5*). Preliminary work indicated identical results for both solvents. Shaking in air rather than in nitrogen produced 20 to 30% higher results for oxidized milk fat, indicating that further oxidation occurred during shaking.

Results by Lea's method are obtained by titrating twice to the same faint color from starch with dilute iodine. Considerable skill and practice are required for good results. Titration has to be fast, if possible within half a minute. In the first step (removal of excess bisulfite) the color darkens with time, while in the second step (titration of liberated bisulfite) it fades. The color varies for different aldehydes, from reddish to brown or yellow, and is very difficult to observe. The abnormal color is caused by the high iodide concentration. Oxidation of 90% of the excess bisulfite in the first step with hydrogen peroxide (same titer as iodine), and only the last 10% with iodine, produced regular blue iodine-starch colors, which were much more stable with time. However, where benzene was used as a solvent for the sample, the color faded rapidly in the second step. This fading was prevented by complete removal of benzene (nitrogen flow through the solution) before the addition of hydrogen peroxide. Results were the same as those obtained from the regular method. The partial removal of the excess bisulfite with hydrogen peroxide makes it easier to obtain dependable values.

Table I. Physical Properties and Recovery by Lea's Method of Saturated Normal Aldehydes Purified by Distillation

No. of C Atoms	B.P., ° C. (Mm. Hg)	Melting Point, ° C.	Density, 20° C.	Refractive Index, 20° C.	μmoles per Detn.	Recovery, %
7	43.2–43.4 (12)	<−15	0.8178	1.4120–1.4125	2.5–12.3	95.4–98.9
8	61.4 (12)	<−15	0.8229	1.4187–1.4189	12.6	94.4
9	78.4–78.8 (12.5)	<−15	0.8243	1.4235–1.4240	12.6	63.1
10	94.2–94.4 (12)	−2	0.8260	1.4280	12.5	22.1–24.2

Results

Synthetic Aldehydes. Recovery by Lea's method decreases with increase in the chain length of the aldehyde (Table I). This trend probably results from a decreased solubility in water of the aldehyde with an increase in chain length. The effects of shaking period and position of the bottles for aldehydes with 9 and 10 carbon atoms (12.6 to 12.8 μmoles per determination) are presented in Figure 1. Recovery with aldehydes C_9 and C_{10} was increased to 90 and 74%, respectively, by increasing the time of shaking. Slightly higher recovery was obtained by placing the bottles horizontally, with the long axis in the shaking direction. This position induced a more effective mixing of the layers. Results indicate a decreased velocity of transfer between the layers and lower concentration in the water layer at equilibrium for aldehydes of increased chain length. Six hours of shaking was adopted for aldehydes with 9 or 10 carbon atoms.

The isolation of aldehyde by solvent extraction after decomposition of the bisulfite complex was examined for aldehydes with 7 and 9 carbon atoms (20 μmoles per determination). Yields based on Lea's determination were 61% for C_7, and 65% for C_9; and based on ultraviolet absorption were at 293 to 294 mμ 67 and 64%, respectively. These isolated aldehydes showed only one maximum at 293 to 294 mμ. The C_9 aldehyde originally showed a maximum at 215 to 216 mμ, and 37% of this maximum remained in the Skellysolve B layer after extraction with bisulfite. This indicates that the α-β-unsaturated aldehyde has a longer chain length (probably dimer molecule).

Aldehyde from Milk Fat. Two solutions of the volatile fraction were examined. These solutions (according to Lea's determination) contained 38.1 and 100.4 μmoles of aldehyde per ml. These values increased to 40.6 and 104.8 μmoles, respectively, on increase of the shaking period to 6 hours. The small increase in recovery indicates that only small quantities of normal aldehyde with 9 or 10 carbon atoms can be present. Otherwise, a marked increase in recovery would be expected with an increase in shaking time. Yields of aldehyde isolated from the bisulfite complex were 19.1 and 21.5%, respectively, for the two solutions (based on Lea's determination). If it is assumed that as a limiting value this yield represents only heptanal, previously shown to give about 60% yield, the fraction of heptanal present in the aldehyde from milk fat would be 20/60 = 1/3. This represents a maximum value. Based on ultraviolet absorption at 293 to 294 mμ, the yield of aldehyde isolated by solvent extraction after decomposition of the bisulfite complex was 6% as compared with the original aldehyde. This figure, combined with 67% yield previously shown for heptanal, would give a heptanal fraction of approximately 1/10.

The ultraviolet absorption spectra of the volatile fraction and the recovered aldehyde from this fraction are shown in Figure 2. Curves were similar in shape, but the original maximum at 265 mμ shifted to 280 mμ for the recovered aldehyde, which indicates saturated ketone (*6*). The other maximum at 215 mμ is quantitatively unimportant because molar extinction of α-β-unsaturated carbonyls is about 1000 times that of saturated compounds. Unconjugated unsaturation is another possibility because it does not affect ultraviolet

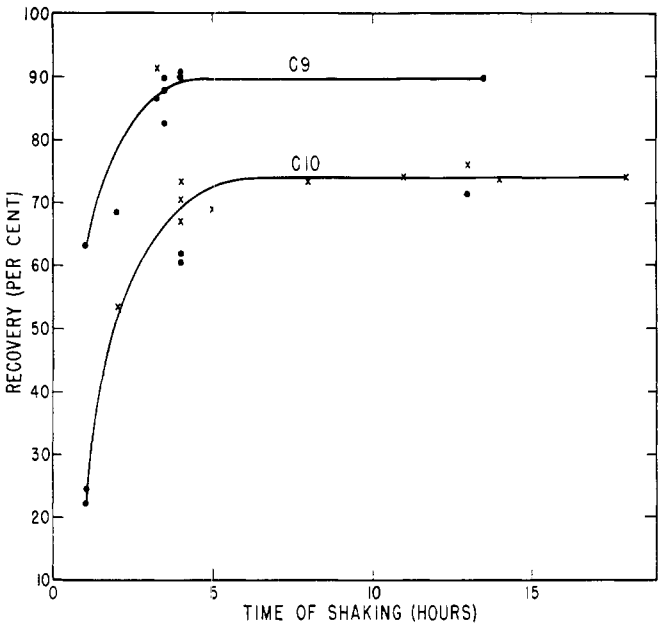

Figure 1. Recovery for aldehydes C_9 and C_{10} with varying time of shaking

X Bottles with long axis horizontal, in direction of shaking
● Bottles with long axis vertical

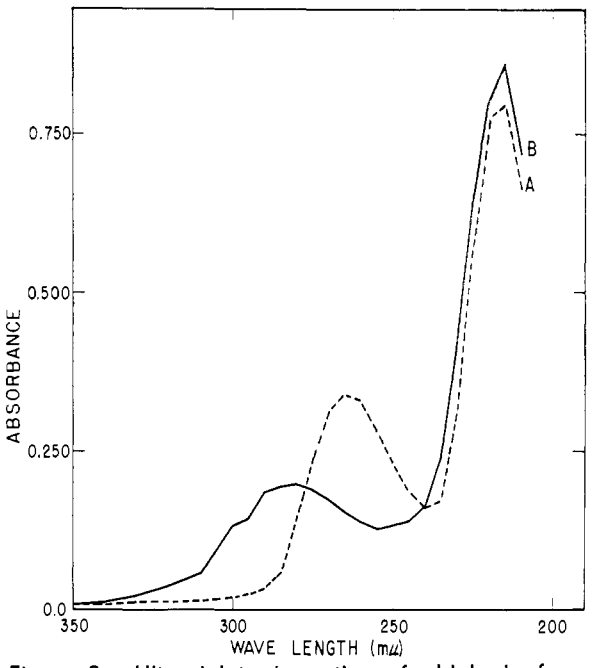

Figure 2. Ultraviolet absorption of aldehyde from milk fat recovered by Lea determination

A. Origional solution of volatile fraction, diluted 1:1000 (0.073 μmole of aldehyde per ml.)
B. Solution of recovered aldehyde at original volume, diluted 1:10 (1.51 μmoles of aldehyde per ml.)

absorption. After extraction with bisulfite, 30% of the original maximum at 215 mμ and 6% of that at 265 mμ were still present in the Skellysolve layer. This shows that part of the unsaturated carbonyl compounds did not react to the bisulfite complex.

Starting from solutions of oxidized milk fat, ultraviolet absorption of the aldehyde isolated from the bisulfite complex was similar with maxima at 215 and 270 to 280 mμ. The yield was 7 to 11%. (Based on Lea determinations, the original level was 36 μmoles per determination.)

The data for the yield of "isolated aldehyde" are summarized in Table II.

Table II. Yield of Aldehyde Isolated by Solvent Extraction after Decomposition of Bisulfite Complex

Type of Aldehyde	μmoles per Ml. Soln., Lea Detn.	% Yield of Isolated Aldehyde	
		Lea detn.	U.V., 293 mμ
Synthetic C_7	20.0	61	67
Synthetic C_9	20.0	65	64
Volatile milk fat aldehyde	40.6	19.1	6
	104.8	21.5	6
Total milk fat aldehyde	36.0	7–11	—

Discussion

Three steps are involved in recovery of aldehyde by Lea's method.

1. Transfer of aldehyde from organic solvent to the water phase. The solubility in both media determines the ratio of the aldehyde concentrations in water phase and organic solvent.
2. Formation of complex in the reaction: aldehyde + bisulfite ⇌ complex, completed as much as possible by use of a large excess of bisulfite. This also maintains a low concentration of aldehyde in the water phase, providing the driving force for step 1.
3. Determination of the amount of aldehyde by titrating the bisulfite liberated from the complex.

Recovery is limited by step 1 for aldehydes with very low solubility in water. The resulting low aldehyde level maintained by step 2 in the water phase then corresponds with the appreciable quantities of aldehyde left in the organic solvent layer at equilibrium, and explains the decrease in recovery with increasing chain length of normal aldehydes with 7 to 10 carbon atoms. It also explains why in the case of nonanal and aldehyde from the volatile fraction of oxidized milk fat, part of the conjugated unsaturated fraction remained in the Skellysolve layer after extraction with bisulfite. Part of the "saturated" fraction of "aldehyde" from milk fat may remain in the Skellysolve layer, but this does not show in ultraviolet absorption (low molar extinction). These saturated aldehydes would be expected to appear after reaction with semicarbazide (*6*), because semicarbazones of saturated carbonyl compounds have maximum absorption at 230 mμ with high molar extinction. Such data were not obtained in this study.

Step 2 is for aldehydes as well as ketones; however, ketones are much less reactive. Only methyl ketones undergo this addition with difficulty. The equilibrium may differ for different carbonyl compounds. The yield of the carbonyl compounds isolated from milk fat is lower than from aldehyde solutions. This is true also for the volatile—relatively short-chained and water-soluble—compounds and may be caused by a ketonic character of the carbonyls from milk fat (formed at 100° C.).

Step 3 for saturated aldehydes involves only the bisulfite linked to the aldehyde group. Double bonds (particularly if conjugated) in aldehyde from milk fat may also react with bisulfite and—after splitting of the complex—again with iodine, increasing the titration value. This would explain the loss of unsaturation in the Lea determination and low recovery of isolated aldehyde (based on too high a titration value). The reason for the different yields of isolated carbonyl compounds from the volatile fraction and the oxidized milk fat itself may be that the latter involves all the carbonyl compounds, whereas the volatile fraction does not contain the carbonyl compounds remaining in the fat and distillate (*5*).

Acknowledgment

The reviews and constructive criticism of this paper by C. H. Lea and another anonymous reviewer are highly appreciated.

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Received for review March 6, 1959. Accepted June 10, 1959. Journal paper J-3039 of the Iowa Agricultural Experiment Station, Project 1128. Work supported in part by the Quartermaster Food and Container Institute for the Armed Forces.

HERRING STICKWATER VISCOSITY

Identity of the Gel Factor in Herring Solubles and Means of Overcoming Its Effect

J. R. McBRIDE, ROBERT A. MacLEOD, and D. R. IDLER

Fisheries Research Board of Canada, Technological Station, Vancouver 2, B. C., Canada

Herring solubles, prepared by concentrating herring stickwater after acid treatment, varied in viscosity and capacity to gel depending on the season when the herring were caught. Solubles from herring caught in the late fall and winter had a much higher viscosity and tendency to gel than the product from fish caught in the summer and early fall. This change in viscosity correlated with an increase in the gelatin content of the solubles and paralleled the development of milt and roe in the fish. Proteolytic enzyme treatment of stickwater prior to its condensation to solubles was effective in overcoming the action of the gelatin. The capacities of various commercially available proteolytic enzyme preparations to produce solubles with desirable physical characteristics were compared.

HERRING STICKWATER is the water phase of the press juice obtained when whole herring are "reduced" to fish meal. To prepare a fluid concentrate of this by-product, referred to as solubles, suspended and dissolved proteins must first be either removed or destroyed. In one process, the proteins are coagulated by acidulation of the stickwater and then removed by centrifugation (*10*). In another, the protein molecules are reduced to smaller fragments by the action of proteolytic enzymes (*5*).

The acidulation process has been used exclusively in this area of the Pacific coast for some years. Even though the same process was applied to the stickwater throughout the year, the physical properties of the resulting solubles varied with the season. While solubles prepared from summer herring was fluid, that produced from late fall and winter herring was viscous and tended to gel upon standing.

The purpose of this study was to investigate the reasons for the seasonal change in viscosity of herring solubles and to determine how a product with satisfactory physical characteristics could be produced throughout the year.

Experimental

Special Apparatus. The centrifuge used for all pilot plant operations was a Centriwesta Model NWA 205 (Merco Centrifuge Co., San Francisco, Calif.). This centrifuge has a continuous flow arrangement and applies a force of approximately 7500 × gravity. It can be operated with either a chamber or a disk bowl.

Source of Materials. Fresh whole Pacific herring (*Clupea pallasii*) were obtained from local fishing companies and stored at 10° F. until used.

When large volumes of stickwater, representative of a particular season were required, arrangements were made to obtain primary stickwater from a local plant. These samples were held at 10° F. until needed. As a further precaution against bacterial deterioration of the stickwater, Aureomycin was added to all samples as a preservative, before freezing, at a level of 2 p.p.m. (*7*).

A pilot plant was designed to approximate commercial plant conditions as closely as possible (*3*). There whole herring were steam cooked and pressed. The expressed fluid was heated to 212° F. and maintained there for 15 minutes to achieve maximum oil separation. After oil removal by centrifugation, the primary stickwater was treated with either acid or enzyme.

The acid treatment consisted of lowering the pH of the primary stickwater to 5.0 with concentrated sulfuric acid and heating to 170° F. for 15 minutes. The temperature of the stickwater was then raised to 212° F. and centrifuged twice, first with the chamber bowl of the Centriwesta centrifuge to remove coagulated protein and then with the disk bowl to separate oil released by the acid and heat.

For enzyme treatment the temperature and pH of the stickwater were adjusted to the optimum for the enzyme under investigation. Following the addition to the stickwater, of a slurry of enzyme in water, digestion was allowed to proceed for the required length of time. At the completion of each enzyme digestion period the stickwater substrate was boiled for five minutes at a pH of 3.0 to insure enzyme deactivation. The stickwater was then cooled and the pH was readjusted to the desired level.

The treated stickwater was concentrated in vacuo by one of two methods: If a large quantity of solubles was required, the pilot plant concentrator was