be noted that an iodine value drop of about 5% is required before there is a measurable decrease in the Coefficient of Digestibility of the oil. Hence it must be concluded that any change in digestibility attributable to the iodine value drop of about 1% found in the present study is beyond the realm of measurement and therefore is of no nutritional significance. This conclusion presupposes that the very small drop in iodine value, noted in the present survey of frying oils in commercial use, may have actually been due to polymer formation. Such however is not the case; in an extension of the present studies (21) the constancy in composition of the frying oils—heated as compared to fresh—and the results of physico-chemical studies have confirmed the absence of thermal polymers in the commercial oils.

The findings presented in this report cover only operations in the potato chip industry. Studies similar to this one should be conducted on oils employed in other frying operations, especially when limpid unhydrogenated oils are used. The frying of potato chips, insofar as heat abuse of the frying oil is concerned, is a relatively mild treatment. There is such a rapid turnover in oil, *i.e.*, constant replenishment with fresh oil to compensate for the oil absorbed by the potato chips, that undesirable by-products do not accumulate in the frying oils (21). The free fatty acid value seldom exceeds 0.5% and there is very seldom, if ever, the need to discard the frying oil. It is also worth remembering that the rapid and almost complete volatilization of the water from potato chips during frying is, in essence, continuous steam-deodorization and refining of the frying oil throughout its use.

Summary

The problem of thermal polymers of acceptable flavor in potato-chip-frying oils has been discussed from the standpoint of potentiality of such polymers forming during commercial frying operations. Publications on heat-abused oils have been critically reviewed, and many of these have been shown to yield findings irrelevant to practical operations. Conclusions based upon such studies should not be extended beyond the scope of the findings reported. The change in iodine value has been shown to constitute a simple and highly precise method to determine for survey purposes whether thermal polymers may have formed in the oils used in a given industry. Such a survey has now been completed covering the operations of 89 different potato chip manufacturers, using all types of frying oils. A 1% decrease in the iodine value of the oils in commercial use has been noted. Whereas this change in iodine value is statistically significant, it is shown to have no nutritional significance. The constancy in composition of the frying oils—heated as compared to fresh—and the results of physico-chemical studies, noted in a related study (21), support the present conclusion that thermal polymers are absent from the oils employed in the commercial manufacture of potato chips.

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Paper Chromatographic Separation of Aliphatic Lactones[1]

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GAMMA AND DELTA LACTONES, particularly those aliphatic lactones containing 8 to 12 carbon atoms, have strong persistent odors most often described as reminiscent either of peach or coconut. Gamma hendecalactone (so-called aldehyde C_{14}) is a common constituent of synthetic peach essence, and gamma nonalactone (aldehyde C_{18}) is often added to synthetic coconut flavor preparations. In addition to these two lactones, other lactones of both the gamma and delta series have been suggested for use in a variety of synthetic fruit, berry, and nut flavors. A recent patent application (1) covering both the synthesis and use of certain lactones in synthetic butter flavor indicates that these flavor compounds might eventually find wide use in margarine and shortenings.

Lactones have been implicated in the flavor deterioration of dry whole milk (6), and delta decalactone (the lactone of 5-hydroxy decanoic acid) subsequently was found to be present in butteroil (anhydrous milk fat), dry whole milk, dry cream, and evaporated milk

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(4, 5). While the precursor of delta decalactone in milk is not known, it has been established that its origin is in the fat phase and that lactone formation is initiated by the heat treatments used during processing of the above-mentioned products. Since practically all milk is subjected to heat processing, delta decalactone will be formed and, as a consequence, will contribute to the characteristic flavor of most fat-containing milk products, possibly including even fresh pasteurized milk. Whether the flavor will be described as normal or objectionable will depend upon the amount of lactone present.

The methods herein reported are based, in part, upon the paper chromatographic techniques used in identifying the coconut-like, off-flavor compound of milk fat (4). The lactones are converted into hydroxamic acids, which can be separated by paper chromatography. Their positions on the paper are revealed by spraying the chromatogram with a $FeCl_3$ solution. The work is extended to show how lactones can be separated and distinguished chromatographically from glyceride and simple ester material. This latter method is based upon the work of Goddu *et al.* (3), which showed that lactones and anhydrides could be converted to hydroxamic acids at a lower pH than is the case with esters. A demonstration of the practical use of these methods is illustrated by the analysis of three different synthetic peach extracts.

Experimental Procedures and Results

Reagents. These are a saturated solution of hydroxylamine hydrochloride in methanol; a 20% solution of potassium hydroxide in methanol (the precipitated potassium carbonate is removed by filtration); and an aqueous solution containing 1% $FeCl_3$ and 1% HCl.

Separation of Homologous Series of Gamma and Delta Lactones. If no glyceride or ester material is present in the lactone-containing substance to be analyzed, hydroxamic acid derivatives can be easily formed by mixing with an alkaline hydroxylamine solution. However if such compounds are present, a slightly modified procedure (see below) must be used. Since pure lactone solutions were utilized in developing the optimum conditions for paper chromatographic separation of the compounds, the simpler method for derivative formation was used in obtaining the results expressed in Table I. Later work revealed that comparable results were obtained with either method.

Derivative Formation. Ten parts of saturated hydroxylamine solution are mixed with about 7 parts of 20% KOH solution, and the precipitated KCl is removed by filtration. The ratio between these two reacting solutions can be varied considerably so long as the resulting mixture is fairly alkaline in reaction (pH 10 or higher). One part of 3M. methanol solutions of the lactones to be studied are mixed with 2 parts of the alkaline hydroxylamine solution. The reaction is almost immediate, and no heat need be applied. An alternate method is first to spot the lactone solutions onto the chromatographic paper. After air-drying the alkaline hydroxylamine solution is spotted on the paper. This latter technique is of particular value when only minute quantities of lactone solutions are available.

Chromatographic Separation. The hydroxamic acids are spotted on Whatman No. 1 paper, which then is stapled in the shape of a cylinder and, after equilibration, developed by ascending techniques. Typical R_f values, using 3 different solvent systems, are given in Table I.

It was found that about 1 hr. of equilibration facilitated good resolution of the derivatives. Equilibrating for longer periods did not materially alter the results. The rate of development is such that the solvent front will have moved 8 to 10 in. in a 5-hr. period. When the solvent has moved the desired distance, the chromatogram is removed from the chamber, air-dried, and the positions of the derivatives revealed by spraying with a neutral or slightly acidic $FeCl_3$ solution. Red-brown to purple, round or oval-shaped spots will appear. As little as 0.05 mg. of the lactone is easily detected by these chromatographic methods.

The choice of solvent system to use in analyzing a flavor extract or other substance will be governed primarily by the particular lactone believed to be present. For lactones containing 8 to 12 carbon atoms solvent systems No. 1 and No. 2 will give the best separation. The more polar solvent system, No. 3, will give better separation of the lower molecular weight lactones.

Chromatographic Identification of Lactones in Mixtures Containing Glyceride or Other Ester Material. The hydroxylamine reaction is a classic test used in the detection of ester material (2). Thus as ordinarily carried out, using an alkaline hydroxylamine solution, derivatives will be formed not only from lactones (which are actually inner esters) but also from glycerides and other compounds containing an ester linkage. Consequently if a mixture containing lactones and simple esters or glycerides were reacted with alkaline hydroxylamine and then chromatographed, it would not be possible to determine whether the spots arose from lactones or from other ester material. Experience has shown that the chromatographic behavior of hydroxamic acids formed from esters and glycerides containing short chain fatty acids will be similar to that of the lactones used in this study (4). The work of Goddu *et al.* (3) suggested that this difficulty could be overcome by modifying the hydroxylamine reaction so that only lactone derivatives would be formed. Goddu's observations were confirmed, and further work was carried out to establish reaction conditions that would best serve the purpose of the work at hand. This led

TABLE I

Typical R_f Values for Hydroxamic Acid Derivatives of Several Lactones

Lactone	Solvent system[a]		
	No. 1	No. 2	No. 3
γ-Butyrolactone	.00	.02	.08
γ-Valerolactone	.00	.07	.15
γ-Hexalactone	.03	.15	.33
γ-Heptalactone	.06	.28	.54
γ-Octalactone	.14	.43	.68
γ-Nonalactone	.32	.54	.77
γ-Decalactone	.64	.64	.83
γ-Hendecalactone	.74	.71	.87
γ-Dodecalactone	.84	.83	.92
δ-Nonalactone	.18	.50	.73
δ-Decalactone	.33	.60	.79
δ Hendecalactone	.59	.69	.84
δ-Dodecalactone	.70	.78	.89

[a] No. 1. *Equilibrating solvent*—aqueous phase from a 5:5:1 by volume mixture of benzene, water, and glacial acetic acid.
Developing solvent—10% solution of glacial acetic acid in benzene.
No. 2. *Equilibrating solvent*—aqueous phase from a 5:2:5:1 by volume mixture of benzene, isopropyl alcohol, water, and glacial acetic acid.
Developing solvent—upper phase of the above.
No. 3. This solvent system was the same as No. 2 except that 3 parts of isopropyl alcohol was used.

to the development of the derivative formation procedure described below.

Ten parts of the saturated hydroxylamine hydrochloride solution are mixed with 3 parts of 20% KOH, and the precipitated potassium chloride is removed by filtration. This results in a solution that is only about 60% neutralized and will react only with lactones under the prescribed conditions of the test. The partially neutralized hydroxylamine reagent is added to the material to be tested and held for 15 min. at 65°C. The reaction mixture is then cooled, and an appropriate quantity is applied to the chromatographic paper. All of the ethyl esters of fatty acids from butyric through stearic, tributyrin, and glycerol mono-stearate and all of the lactones listed in Table I were subjected to the modified test as follows.

Approximately 10 mg. of lactone or ester were reacted under the above stated conditions with 0.2 ml. of hydroxylamine reagent, and then 2 λ (equivalent to about 0.1 mg. of lactone) of the mixture were applied to the paper. Solvent system No. 3 was used in developing the chromatogram. Spots appeared only from lactone derivatives when the developed chromatograms were sprayed with 1% $FeCl_3$ solution. The same results were obtained when lactones were first mixed with some of the ethyl esters or glyceride material and then reacted with the modified hydroxylamine reagent. When the same compounds were reacted with alkaline hydroxylamine and then chromatographed, spots appeared corresponding to each lactone and ester. These results indicated that besides being used as a method for the identification of the lactones present in an unknown mixture, the two chromatographic procedures could be helpful in identifying some of the esters that are present. Any spots appearing on the chromatogram developed from the alkaline hydroxylamine mixture that did not show up on the chromatogram developed from the partially neutralized, reagent mixture would have arisen from ester material.

Analysis of Synthetic Peach Extracts

An analysis of three synthetic peach extracts can be cited as a practical demonstration of the use of these chromatographic techniques. The peach extracts, suitable for fortifying peach ice cream, were obtained from different flavor manufacturers.

One ml. of partially neutralized hydroxylamine reagent was added to 1-ml. quantities of each flavor extract. To each extract in another series of tubes was added 1 ml. of the alkaline hydroxylamine solution described previously. Samples (50 mg.) of gamma decalactone, gamma hendecalactone (gamma undecalactone), ethyl acetate, and ethyl butyrate were treated in like manner. All tubes were placed in a 65°C. water bath for 15 min. After cooling, five λ quantities were spotted on paper and the chromatograms were developed, using solvent system No. 3. The data obtained from this study are found in Table II.

These chromatograms revealed that gamma hendecalactone was the only lactone present. The chromatogram developed from the alkaline hydroxylamine reaction revealed the presence of other ester material in two of the extracts. While no attempt was made to identify these esters, an acetate in peach extract A and a butyrate in B may be indicated. Peach extract C apparently contained neither lactone nor ester material since no spots appeared on the chromatogram. It was noted that extracts A and B were superior to extract C as flavor fortifiers in peach ice cream.

TABLE II
Chromatographic Analysis of Synthetic Peach Extracts

	R_f values of spots appearing on chromatograms	
	Using partially neutralized hydroxylamine reagent	Using alkaline hydroxylamine reagent
Ethyl acetate	No spot	0.19
Ethyl butyrate	No spot	0.72
γ-Decalactone	0.81	0.82
γ-Undecalactone	0.88	0.87
Peach extract A	0.87	0.05, 0.19, 0.44, 0.87
Peach extract B	0.87	0.88, 0.69
Peach extract C	No spots	No spots

Summary

Paper chromatographic procedures are described, whereby a homologous series of n-aliphatic gamma lactones from butyrolactone through dodecalactone can be resolved as hydroxamic acid derivatives. Similar resolutions can be obtained with a series of n-aliphatic delta lactones from nonalactone through dodecalactone. Procedures also are described, whereby lactones in the presence of ester or glyceride material can be identified using a modified hydroxylamine reagent. Analyses of commercial synthetic peach extracts demonstrate the practical use of these procedures.

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Benzamides, p-Nitrobenzamides, Benzenesulfonamides, p-Toluenesulfonamides, and Acetamides as Identification Derivatives of Long-Chain Amines

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ALTHOUGH LONG-CHAIN AMINES have been manufactured for several years, very few derivatives have been reported that are suitable for identification. Previous to this study substituted benzene sulfonamides and phenylthioureas of dodecylamine, tetradecylamine, hexadecylamine, and octadecylamine, as well as the substituted acetamide and the substituted benzamide derived from octadecylamine were the only derivatives which were synthesized from readily available reagents and could be useful as identification derivatives (1, 2).

In this study straight-forward, simple procedures