dation. Hence, by this measure their presence could account for the spectral behavior of the autoxidizing oleic acid. For reasons cited earlier neither satisfies all other conditions found in the oxidized fatty acids.

(6) Oxidostearic acid increases its enolization ratio upon autoxidation and in this regard is not at all like the autoxidized fatty acids

(7) Of the last four reference compounds in Table I acetonylacetone and diacetyl have low absorption in alcohol and high enolization ratios reacting very much like the ketol- and diketostearic acids. Acetylacetone, on the other hand, reacts wholly unlike oxidized fats or the above fatty acid derivatives.

Summary

1. The ultraviolet absorption spectra of lard in various stages of autoxidation are presented, showing that as oxidation proceeds the absorption increases, and in the region of 2750 Å. this increase is roughly proportional to the peroxide content. The increase in absorption is not due to peroxide.

2. The absorption spectra of partially oxidized oleic acid, ethyl oleate, and elaidic acid have been studied. The mild oxidation of these compounds increases their absorption only slightly, but prolonged oxidation causes marked increases in the absorption.

3. Dihydroxystearic acids and oxidostearic acid cannot account for the absorption bands observed in oxidized fatty acids.

4. Although the absorption of the diketostearic acid and ketolstearic acids show absorption bands in the region of 2750 Å. they do not account for the absorption of oxidized fats in this region.

5. Ketols and α-dicarbonyls do not accumulate in oxidized monoethenoic acids in more than trace amounts.

6. Alkaline treatment of autoxidized ketol- or diketostearic acids or alkaline treatment of mixtures of these compounds with oleic acid leads to products which are spectroscopically very similar.

7. It is suggested that the increased absorption found in oxidized fats is due in part to conjugated unsaturated systems containing carbonyl groups, or to conjugated polyenes formed by enolization of these systems.

8. During the oxidation monoethenoic acids show increased absorption at 2350 Å. indicating the probable formation of conjugated dienes.

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Acceleration by Electrolytes of Alkaline De-esterification of Pectin

BY HANS LINEWEAVER

The observation that electrolytes accelerate the alkaline de-esterification of pectin was reported in an abstract on the effect of electrolytes on the enzymic de-esterification of pectin.[1] This note gives the results of the non-enzymic, alkaline de-esterification studies.

Investigations of reactions between ions have shown that increasing the ionic strength causes an increase in the rate of reaction between ions of like sign.[2] In the hydrolysis of simple esters, where a reaction between a neutral molecule and an OH^- ion is involved, an increase in the salt concentration has only a very small accelerating effect (about 10% in 0.1 *M* solution[3]). In the case of pectin, however, a considerable acceleration due to a primary salt effect might be expected, since the reaction is between ions of like sign (that is, provided the negative charges in pectin are close enough to the ester bonds to interact with the OH^- ions as they approach the pectin molecule). The formation of soluble ion complexes, such as are formed between polyvalent cations and organic acids, also might result in an increased rate of hydrolysis greater than that due merely to change in ionic strength (*e. g.*, the increase in the rate of reaction of bromacetate and thiosulfate in the presence of polyvalent cations in aqueous media has been explained by the formation of complex ions[4]).

The results reported in Table I verify the expectation and show that electrolytes increase the rate of de-esterification of pectin as much as 400% at *p*H 9. Similar results were obtained at other *p*H's above 6. Also, McCready, *et al.*,[5] of this Laboratory reported that electrolytes accelerate the rate of de-esterification of pectin in ammonium hydroxide solution at *p*H 10.9.

Table I shows further that at equal ionic strengths calcium chloride causes a greater acceleration of hydrolysis than sodium chloride. This greater effectiveness of calcium may be attributed, of course, to ion complex formation between the divalent calcium cations and the pectin anions or to the inadequacy of the principle

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(1) H. Lineweaver and G. A. Ballou, *Proc. Fed. Am. Soc. Exptl. Biol.*, **2**, 66 (1943); *Arch. Biochem.*, **6**, 373 (1945).

(2) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 423.

(3) E. A. Moelwyn-Hughes, "The Kinetics of Reactions in Solution," Oxford Press, 1933, p. 184.

(4) Ref. 2, p. 429; V. K. LaMer and M. E. Kamner, THIS JOURNAL, **57**, 2662 (1935).

(5) R. M. McCready, H. S. Owens and W. D. Maclay, *Food Industries*, **16**, 794 (1944).

TABLE I

THE EFFECT OF ELECTROLYTES ON THE RATE OF DE-ESTERIFICATION OF PURIFIED PECTIN AT *p*H 9.0 (*p*OH = 4.8) AND 29.5°

Ionic strength of added electrolyte[a]	Second order reaction constant, *k*, in presence of indicated salt[b] NaCl	CaCl$_2$	MgCl$_2$[c]
None	0.8	0.8	0.8
0.05	1.5	1.7	1.6
0.15	2.0	2.6	
0.30	2.3	3.4	
2.5	3.5		

[a] The contribution of pectin at *p*H 9 to the ionic strength is not known. If it behaves as an acid with a valence of thirty the ionic strength in the absence of added salt would be about 0.1. If it is considered that the carboxyl ions on a single pectin molecule are far enough apart so that they can be considered as individual ions, the ionic strength would be 0.005. Similarly, were one pectin molecule considered to simulate ten trivalent molecules, the ionic strength would be 0.01; and likewise for divalency it would be 0.0075. The ionic strength due to the ash is estimated at not more than 0.001. [b] The values of *k* were calculated from the initial rate of de-esterification (less than 8% hydrolysis) according to the equation: *k* = (initial rate in equivalents per second)/(equivalents of ester bonds in solution) × ($10^{-4.8}$). The initial rate was obtained from frequent recordings of elapsed time and amount of alkali used. Constant *p*H (±0.1 unit) was maintained by addition with stirring of 0.02 *N* sodium hydroxide to the reaction mixture (20 ml. of 0.5% pectin having a CH$_3$O content of *ca.* 10%). The *p*H was measured continuously with a Beckman *p*H meter. [c] Mg^{++} is soluble at *p*H 9 only to the extent of about 4×10^{-4} *M*, but the low turbidity and the acceleration of hydrolysis make it appear that most of the Mg^{++} had combined in some way with the pectin.

of ionic strength for high valence ions[6] or for these salt concentrations.

The acceleration of the rates of reactions between ions due to the primary salt effect reflects largely a change in the entropy of activation rather than in the energy of activation, although the energy of activation may change to some extent.[7] The data in Table II show that this is also the case for pectin de-esterification. The values of the energy of activation at zero and 0.2 *M* NaCl are the same within experimental error, whereas the values of the entropy of activation, ΔS, are significantly different. The similarity of the effect of salt on pectin de-esterification to the effect of salt on reactions between simple ions indicates that the negatively charged carboxyl groups are close enough to the ester groups so that interaction with OH$^-$ ions occurs as they approach the ester bonds. For comparison it may be noted that the energy of activation for the alkaline hydrolysis of simple esters (ethyl, propyl, isopropyl, butyl, isobutyl, secondary butyl, and isoamyl acetates) is about 11.3 ± 0.3 kcal.,[8] but the entropy of activation for these esters varies from about −27 to −31 entropy units.

TABLE II

THE EFFECT OF SALT ON THE EXPERIMENTAL ENERGY OF ACTIVATION AND ENTROPY OF ACTIVATION AT CONSTANT OH$^-$ ION ACTIVITY (*p*OH = 4.46, *p*H = 9.5 at 26° AND 9.3 AT 32°)

Concn. of NaCl	*k*[a] 26.2°	32.1°	Energy of activation, *E* kcal./mole	ΔS at 32°[b] E. U./mole
None	0.63	0.95	10.7	−25.6
0.2	1.92	2.96	10.9	−22.8

[a] A different sample of purified pectin was used to obtain these data than was used to obtain the data in Table I. In this case *k* = (initial rate in equivalents per second)/(equivalents of ester bonds in solution) × ($10^{-4.46}$). [b] Ref. 2, p. 199, equation 178.

This study has shown that the effect of salt on alkali de-esterification is large enough to be of importance in the preparation of pectic and pectinic acids. A lower *p*H, lower temperature, or shorter time might be used conveniently in the presence of salt. Also, the results of unpublished tests showed that the stability of pectin materials in neutral or slightly acid solution is enhanced if the salt concentration is held to a minimum and multivalent cations are avoided.

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Summary

The effect of sodium chloride, calcium chloride and magnesium chloride on the rate of alkaline de-esterification of pectin in aqueous solution has been studied. These electrolytes, and in particular cations, have been shown to accelerate the rate of alkaline de-esterification of pectin as much as 400%. This acceleration contrasts with the small effect of electrolytes on the rate of hydrolysis of simple esters.

The results lead to the interpretation that the negatively charged carboxyl groups of pectin are close enough to the ester bonds to interact with the hydroxyl ions as they approach the ester bonds; salt decreases the interaction (repulsion). As has been found for the primary salt effect in other systems, the acceleration of de-esterification of pectin by salt reflects largely a change in the entropy of activation rather than in the energy of activation.

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(6) V. K. La Mer and K. Sandved, THIS JOURNAL, **50**, 2656 (1928).

(7) V. K. La Mer, *J. Franklin Inst.*, **225**, 709 (1938).

(8) Ref. 3, p. 87.