

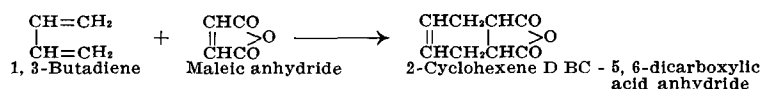
THE EFFECT OF HYDROXYL GROUPS AND ACETYLATION ON THE APPARENT DIENE VALUES OF SOYBEAN AND OTHER VEGETABLE OILS¹

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Abstract

During a study of the bodying of soybean oil under oxidative conditions it was observed that an increased hydroxyl number was accompanied by an increased diene value. The presumption was made that maleic anhydride had esterified the hydroxyl group and that the Diels-Alder reaction was not responsible for the increased diene value. It has been established that the hydroxyl group and oxidation products such as peroxides seriously interfere with the diene methods of Kaufmann and of Ellis. In the case of pure saturated hydroxy compounds and hydroxylated monoethylenic compounds the error can be avoided by acetylating the compound prior to carrying out the diene determination. However, on applying this procedure to such vegetable oils as soybean, linseed, and perilla, the diene value was increased. This point is being investigated more extensively.

DIELS AND ALDER (1) discovered that α , β -unsaturated carbonyl compounds enter into a 1, 4-addition with conjugated diene hydrocarbons as indicated by the reaction between maleic anhydride and 1, 3-butadiene.



Böeseken and Hoevers (2) applied the Diels-Alder reaction in investigating the dehydration product of ricinoleic acid and later Morrell and Samuels (3) studied the reaction with the α and β forms of eleostearic acid. During the course of their investigation they isolated and determined the structures of the maleic anhydride addition products. Morrell, Marks, and Samuels (4) employed the diene reaction to follow the changes that are produced during heat bodying in the conjugated trienoic system of tung oil.

Since the usual constants, such as iodine and thiocyanogen numbers, supply no evidence either for or against the presence of conjugation, it is obvious that a method based on

the Diels-Alder reaction would be of value in detecting both the presence and the extent of conjugation in fats and oils. In 1936 two methods based on this reaction were suggested, one of them by Kaufmann et al. (5, 6) and the other by Ellis and Jones (7). Kaufmann and co-workers referred to the values obtained by their method as diene numbers, while Ellis and Jones preferred the term maleic anhydride value.³ These investigators reported diene values for oils such as soybean, linseed, poppyseed, castor, and perilla, which theretofore had not been known to contain a conjugated diene system. Furthermore, Böhme and Steinke (8) obtained results by means of peracid oxidation which they interpreted as indicating the presence of conjugated systems in linseed, poppyseed, and sesame oils.

Kaufmann, Baltes, and Büter (6)

reported diene numbers of 5 to 13 for poppyseed, almond, cottonseed, soybean, and linseed oils.

In a study of the applicability of the two diene methods as a means of following changes in the polyethenoid linkages of soybean oil under various conditions of heat bodying, it was observed in this laboratory that oil heated at 100°C. in an atmosphere of oxygen showed an increase in diene values and hydroxyl numbers. Furthermore, the diene values appeared to be a nearly linear function of the hydroxyl numbers. A control sample under an atmosphere of carbon dioxide showed no such changes. It was therefore presumed that hydroxyl groups were combining with the maleic anhydride, and that 1, 4-addition was not entirely responsible for the observed increase in diene values.

While the authors (9) were en-

gaged in determining the apparent diene values of pure hydroxy compounds which did not contain a conjugated system, and in redetermining the value after blocking the hydroxyl group by acetylation, two papers appeared which lent support to their hypothesis. In the first communication reported by Pelikan and von Mikusch (10) the Ellis and Jones method was suspected of giving abnormal values in the presence of hydroxylated oils; however, the Kaufmann and Baltes method was considered wholly reliable since it was stated, "No indications have been observed in these experiments as well as in other routine determinations to support the statement that the Kaufmann method is unreliable. . . . His 'Diene Value' indicates exactly the quantitative evaluation of conjugated double bonds."

The second of these papers was communicated by Bruce and Denley (11) in which they confined their remarks to the Ellis and Jones method. These observers recorded results on soybean oil, analogous to those of the present authors and also obtained appreciable maleic anhydride values with castor oil, blown oleic acid, linseed, and perilla oils. They likewise suspected that a reaction occurred between hydroxyl groups and maleic anhydride, and attempted to prove the point by a study of one hydroxy compound (dihydroxystearic acid) and blown oils which were found to have maleic anhydride values. The Ellis and Jones method was modified by substituting acetic anhydride for maleic anhydride, and as might be expected it was found that the acetic anhydride entered into combination with the dihydroxystearic acid. It was also observed that acetic anhydride reacted with the blown oils. The conclusion drawn from these observations was that maleic anhydride esterified hydroxyl groups present in the oils, although the only evidence presented was that both of the anhydrides reacted with the oils. These observations did not preclude the possibility of a Diels-Alder reaction with the maleic anhydride. In fact, Goswami and

¹Presented before Section C, American Association for the Advancement of Science, Indianapolis, Indiana, December 30, 1937.

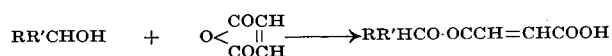
²A cooperative organization participated in by the Bureaus of Chemistry and Soils and Plant Industry of the U. S. Department of Agriculture and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

³Throughout this communication the terms "diene number" and "maleic anhydride value" are used as the original authors indicated, while "diene value" is used without reference to the method of determination.

Saha (12), prior to the communication of Bruce and Denley, noted that linseed oil blown in the presence of a catalyst had an increased Kaufmann diene number. This increase they attributed to the formation of compounds containing conjugated double bonds, and they postulated a mechanism for inducing conjugation through oxidation to hydroxy compounds with the subsequent formation of a conjugated ethylenic bond by elimination of water.

Simultaneously with the appearance of the authors' note to the editor (9) Sabetay and Naves (13) reported the results of a study of the effect of primary and secondary alcohols on the diene value of essential oils. Phenylethyl, benzyl, and octyl alcohols, geraniol, menthol, borneol, cholesterol, and castor oil were shown to give appreciable diene values under the conditions of the Sandermann (14) modification of the Kaufmann method. They also found that cyclohexanol, propylene glycol, and ethylene glycol when heated for two hours under reflux with maleic anhydride in benzene solution reacted to the extent of 56 to 78 percent, based on the formation of the half ester.

In view of the conflicting results reported by other workers and the anomalous results obtained in this laboratory it was deemed essential to investigate the nature and extent of the effect of hydroxyl groups on the course of the reaction with maleic anhydride under the conditions of the Ellis and the Kaufmann methods. The procedure which was followed in this investigation involved the determination of the apparent diene value on pure hydroxy compounds before and after acetylation. In all cases the apparent conjugation became practically zero after acetylation, thus indicating that maleic anhydride does react with hydroxyl groups under the conditions given for the quantitative determination of conjugation, probably with the formation of the half ester of the acid, as shown in the following equation:



In extending the acetylation procedure to oils it was found that castor, tung, and oiticica oils likewise exhibited a decrease in diene value following acetylation. However, the diene values of perilla, linseed, and soybean oils were found to increase after acetylation. Acetylation of all the oils examined resulted in a decrease in the iodine num-

ber and in most instances in a simultaneous decrease in the refractive index. All of the oils examined were found to have hydroxyl numbers prior to acetylation and most of them contained peroxides following, as well as prior to, acetylation.

EXPERIMENTAL

Diene values were determined by the Kaufmann 15-hour reflux and 20-hour sealed tube toluene methods and by the Ellis 3-hour reflux toluene method. The reagent specifications and the procedures given by the authors were followed without modification. The diene values are expressed as grams of iodine equivalent to maleic anhydride added by 100 grams of fat or oil. All hydroxyl numbers, expressed as the number of milligrams of KOH equivalent to the hydroxyl content of 1 gram of oil, were determined by the pyridine-acetic anhydride method of West, Hoagland, and Curtis (15). Except where otherwise stated, the peroxide numbers were determined by the method of Wheeler as modified by Lowen, Anderson, and Harrison (16). The values were calculated as milliliters of 1N $\text{Na}_2\text{S}_2\text{O}_3$ per 1,000 grams of fat. The Wijs method was used with a 30-minute reaction time in determining the iodine numbers while the thiocyanogen numbers were determined according to the directions given in the Report of the Committee on Analysis of Commercial Fats and Oils (17).

The oils and other products were acetylated by mixing with an equal weight of acetic anhydride, and refluxing for 2 hours. After cooling, the acetylated product was poured into distilled water and washed several times, first with water, then with a 2 percent sodium bicarbonate solution, and finally with distilled water until the wash water became neutral. The oils were then dried with anhydrous magnesium sulfate. Pouring the acetylated oil into water and shaking out with ether, followed by washing and drying of the ethereal solution and subsequent recovery

pared or purified in this laboratory.⁴ Recognized procedures were followed in the preparation and purification of the various hydroxylated compounds with the exception of methyl-12-hydroxystearate which was prepared as follows:

Two hundred fifty grams of castor oil was saponified with alcoholic potassium hydroxide (50 g. KOH in 500 ml. 95 percent alcohol). After diluting with water and acidifying, the ricinoleic acid obtained was purified by crystallization from alcohol in the form of the barium soap. The recovered ricinoleic acid was then esterified with a 3 percent solution of sulfuric acid in absolute methanol. The methyl ricinoleate was further purified by vacuum distillation. The fraction which was used for hydrogenation boiled at 168-170°/1 mm. and had a hydroxyl number of 177.8, calculated 179.6. Eighteen grams of methyl ricinoleate was dissolved in 120 ml. of absolute methanol and 1 g. of Raney nickel catalyst was added. Hydrogen was admitted at 44 pounds' pressure at room temperature. Hydrogenation in the Parr apparatus was complete in 1 hour. The catalyst was removed by filtration, and the solution placed in the refrigerator. After an hour 11.0 g. of methyl-12-hydroxystearate melting at 57-57.5°C. was deposited. Grün and Woldenberg (18) report a melting point of 58°, after many recrystallizations from ether. Evaporation of the alcoholic solution to a volume of 50 ml. and cooling in the refrigerator yielded 5 g. of a less pure product melting from 54-57°C. The corresponding acetyl derivative was prepared from the ester by acetylation with acetic anhydride.

RESULTS AND DISCUSSION

It is evident from the data recorded in Table I that hydroxy compounds, closely related to or identical with those associated with vegetable oils, do react with maleic anhydride to produce apparent diene values. The corresponding acetylated derivatives, however, show only negligible diene values. Although the reaction with maleic anhydride occurs irrespective of the method employed, the highest values are always observed by the Ellis method while the lowest values are obtained by the Kaufmann method.

It is interesting to note that the hydroxyl group in the β -position of the α, α' -distearin appears to be incapable of reacting with maleic anhydride even when heated in a sealed tube of 100°C. for 20 hours.

of the oil, led to the same final results.

With the exception of glyceryl monoricinoleate all the products shown in Table I were either pre-

⁴The authors wish to express their thanks and appreciation to L. A. Bedford for his assistance in preparing and purifying a number of the products used in this investigation and to P. Krauczunas for assistance in carrying out a number of the analytical determinations.

TABLE I.—THE APPARENT DIENE VALUES OF ANTHRACENE AND HYDROXY COMPOUNDS AND THEIR ACETATES

Substance	Ellis 3 hour reflux- Maleic anhydride value	Kaufmann toluene 15 hour reflux- Diene number	Kaufmann toluene 20 hour sealed tube- Diene number
Anthracene (Calc'd D. No. 142.5).....	140.1 140.5	130.8 131.5	139.4 141.1
Acetone-glycerol (Hydroxyl No. 421)...	18.8 19.3	8.2 8.3	8.6 8.7
Ethylene-glycol (B. P. 195-7°).....	80.2 94.8	57.5 58.1
Glycerol	77.9 80.2	40.0 48.6	51.8 55.3
Methyl ricinoleate (Hydroxyl No. 177.8) .	16.5 16.7	3.4 3.5	3.1 3.2
Methyl acetylricinoleate	2.7 2.9	0.1 0.3
Methyl-12-hydroxystearate (M. P. 57.5°)	24.5 26.0	3.6 4.0
Methyl-12-acetoxystearate	0.4 0.9	0.0 0.0
α -Mono-palmitin (M. P. 77°).....	19.6 21.3	6.1 6.3
Glyceryl monoricinoleate (Commercial grade)	77.0 77.7	36.3 40.0
α -12-Acetylricinoleo- α , β -diacetin	0.8 1.2	0.0 0.0
α , α' -Distearin (M. P. 78°)	0.0 0.0

In view of the demonstrated reactivity of maleic anhydride with pure hydroxy compounds it might be presumed that the diene values observed with oils having appreciable hydroxyl numbers could result from the presence of hydroxylated compounds in these oils. Consequently, the diene values of several oils exhibiting hydroxyl numbers were determined before and after acetylation with the results shown in Table II.

It is observed that castor oil which contains about 80 percent of the hydroxy acids, ricinoleic and dihydroxystearic, does in fact exhibit a marked reduction in diene value after acetylation. Tung oil, containing the conjugated triethenoid elaeostearic acid, also exhibits a decrease in diene value, which is also the case with oiticica oil containing the keto-triethenoid, licanic acid. The decrease in diene value was accompanied by a decrease in

TABLE II.—THE EFFECT OF ACETYLATION (2 HOURS) ON THE APPARENT DIENE VALUES OF VEGETABLE OILS

Substance	Iodine number	n_D^{25}	Peroxide number	Hydroxyl number	Maleic anhydride value ^a	Diene number ^a
Castor oil	84.6	1.4775	10	165.2 166.6	9.9 10.2	3.2 3.2
Acetylated castor oil	75.3	1.4684	16	0.9 1.1	0.2 0.2
Oiticica oil	140.1	1.5190	9	38	56.0	52.9
Acetylated oiticica oil	112.7	1.5118	29	38	56.1 51.5	55.8 45.9
Tung oil	148.9	1.5170	4	7.2	51.1 66.7	43.9 63.6
Acetylated tung oil	128.8	1.5160	12	9.3	67.4 63.0	64.9 56.3
Perilla oil	203.8	1.4823	0	5.7 5.7	1.3 1.5	2.8 2.9
Acetylated perilla oil	200.4	1.4815	23	3.2 3.4	8.8 8.9
Raw linseed oil A.....	177.6	1.4790	21	9.7 10.3	1.8 1.9	1.3 1.3
Acetylated linseed oil A.....	174.1	1.4790	52	8.3 8.3	7.6 8.0
Extracted linseed oil B.....	173.2	1.4780	15	6.1 6.5	1.0 1.8	3.3 3.8
Acetylated linseed oil B	165.8	1.4780	212	12.2 12.8	9.9 10.6
Extracted soybean oil A	132.8	1.4732	0	6.6 6.8	1.7 1.8	0.7 0.8
Acetylated soybean oil A.....	122.0	248	16.1 16.5	8.2 8.3
Nonbreak soybean oil B	131.0	1.4731	47	4.0 4.8	1.2 1.8	1.8 2.0
Acetylated soybean oil B.....	129.9	1.4729	25	1.5 1.7	2.8 4.1
Edible soybean oil C.....	130.0	1.4730	11	4.6 6.5	0.7 0.8	1.9 2.0
Acetylated soybean oil C.....	128.8	1.4726	18	1.8 1.8	5.8 6.3

^aEllis 3 hour reflux method.

^aKaufmann toluene 20 hour sealed tube method.

iodine value and refractive index which might be expected as a result of the enhancement of the molecular weight through acetylation. It was also noted that the viscosity of both the tung and oiticica oils markedly increased following acetylation, and rapid gelatinization ensued even though they were stored under carbon dioxide. It appears from these results that the decrease in diene value following acetylation may result from the presence of hydroxylated compounds in both tung and oiticica oils, although the possibility that polymerization may be responsible for the observed lowering of the diene value is not precluded (19).

When the same procedures were followed with perilla, linseed, and soybean oils, surprisingly enough, the diene values were found to increase instead of decrease as a result of acetylation. This increase (3 to 10 fold) in diene value was especially marked in the case of extracted soybean and linseed oils. However, the iodine numbers of both oils were observed to decrease after acetylation.

Since the diene value is supposed to be a measure of the amount of conjugation, the enhancement which followed acetylation of perilla, linseed, and soybean oils might be presumed to arise from a shift of a portion of the double bonds of these oils to a conjugated position. Also it might be further presumed that the magnitude of the shift had been greater than indicated by the diene value since, as shown above, a simultaneous lowering in this value would be expected to occur as a result of the inactivation of any hydroxyl groups present in the original oil. However, the demonstrated reactivity of maleic anhydride with hydroxyl groups suggested the possibility that other unsuspected groups might also be reacting with the diene reagent. The possibility that peroxides or other oxidation products might be responsible for the observed diene values was investigated and this assumption was verified.

In order to determine the possible effect of the presence of peroxides and other oxidation products on the magnitude of the diene number, air was passed through samples of refined soybean oil maintained at 98°C. in a standard stability apparatus (20). Duplicate samples were removed at intervals and examined with respect to diene, peroxide, and other values as indicated in Table III. A positive Kreis test for the presence of aldehydes was obtained

after three hours' aeration, and between the fifth and sixth hours volatile acids were evolved in amounts sufficient to decolorize 0.01 N NaOH and phenolphthalein solution placed at the outlet side of the gas stream.

Inspection of the data in Table III indicates that the diene number increases with increased values for

with 1.3 for the untreated oil. These values would seem to indicate that aldehydes in amounts in excess of those normally found in natural oils and fats do not affect the magnitude of the diene number.

SUMMARY

An investigation of certain factors affecting the determination of diene values of soybean and other

no diene value after acetylation. However, soybean, perilla, and linseed oils having low initial diene values were found to have increased diene values following acetylation. Further work pertaining to the effect and significance of acetylation, as well as other chemical treatments, on the diene value and drying properties of soybean oil will be reported in a separate communication.

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TABLE III.—EFFECT OF ACCELERATED OXIDATION ON THE PROPERTIES OF REFINED SOYBEAN OIL

Aeration time Hours	Peroxide number ¹	Iodine number	Diene number	Hydroxyl number	Acid number	Refractive index n _D ²⁰ /D
0.....	18.5 (10) ⁸	130.4 (5)	0.7 (18)	3.9 (2)	0.6 (2)	1.4730 (1)
1.....	31.4 (6)	130.5 (6)	1.1 (3)	1.4730 (3)
2.....	45.1 (6)	130.1 (6)	1.2 (4)	1.4730 (3)
3.....	60.5 (6)	130.3 (6)	1.1 (4)	4.1 (2)	1.1 (1)	1.4730 (3)
4.....	75.9 (6)	130.1 (6)	1.4 (4)	1.4731 (3)
5.....	92.4 (6)	129.8 (6)	1.3 (3)	1.4732 (3)
6.....	111.7 (6)	129.5 (6)	2.3 (3)	4.2 (2)	1.2 (2)	1.4733 (3)
7.....	137.8 (4)	128.8 (4)	3.0 (2)	1.4734 (2)
8.....	164.5 (5)	128.4 (5)	4.2 (2)	6.1 (2)	2.2 (2)	1.4737 (3)
10.....	341.8 (4)	125.7 (5)	7.9 (2)	11.5 (1)	4.3 (1)	1.4743 (1)

¹See reference (21).

⁸Values in parenthesis indicate the number of determinations which were made and averaged.

peroxides and other oxidation products. In fact, the data given in Table III may be related within the limits of experimental error by the equation: D. No. = 0.226 Hydroxyl No. + 0.0127 Peroxide No.

In order to determine the effect of the presence of aldehydes on the magnitude of the diene number butyraldehyde, crotonaldehyde, and octylaldehyde were added in a concentration of 5 percent to samples of refined soybean oils after which the diene numbers were determined in the usual manner. The values found for diene number of the three aldehyde-treated oils were, respectively, 0.8, 1.2, and 1.2, compared

vegetable oils by the Kaufmann and Ellis methods indicates that these methods do not necessarily measure the true extent of conjugation in oils of low diene value.

It has been established that the presence in fats and oils of hydroxyl groups, peroxides, and possibly other oxidation products influences the magnitude of the diene value apparently as a result of the reactivity of these substances with maleic anhydride under the conditions of the Kaufmann and Ellis methods.

Pure hydroxylated compounds having appreciable initial diene values were found to have little or

AN INVESTIGATION OF INVISIBLE LOSSES IN EXPELLER OPERATION

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Abstract

Description and results of a test run on a single Expeller mill set up for cotton seed crushing to determine losses. A material balance is included showing that the unaccounted for loss in the process was very small.

RECENTLY there has been considerable discussion centering on the question of whether or not there were so-called invisible losses in Expeller operation in cotton seed milling, and, if so, what has caused them and where they have been located in the course of operation.

In order to ascertain the facts in

this matter, a test was arranged and run at one of the larger cotton oil mills in the South.

In order that data obtained might be unbiased and fair to all concerned, all testing, sampling, weighing and analysis was placed in the hands of the Barrow-Agee laboratories of Memphis, Tennessee, Mr. Mays of that organization overseeing all operations.

The test was run over a twenty-four-hour period from eight o'clock of one morning until eight o'clock of the following morning.

Little need be said concerning the equipment. The Expeller was of the type known as the Anderson

Super Duo with three high tempering apparatus, extra long vertical drainage barrel and standard main drainage barrel, powered with a forty-horsepower motor. It was equipped to have oil cooling over both barrels. In other words cooled oil was circulated over the barrels to control their temperature to a predetermined point. Auxiliary equipment included a vibrating screen through which all oil produced was strained and from which the separated foots fed back automatically into the lower trough of the tempering apparatus, a recessed plate filter press, and an Anderson gravity scraper meats dryer. The