Oxidation and Quality of Soybean Oil: A Preliminary Study of the Anisidine Test[1]

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ABSTRACT

The anisidine test, a measure of secondary oxidation products in glyceride oils, was applied to a number of soybean salad oils processed from sound and damaged soybeans. A highly significant correlation (-0.68) was found between the anisidine values of salad oils from sound soybeans and their flavor scores. Multiple correlations between flavor scores, anisidine, and peroxide values yielded a correlation of 0.81 and provided a method for predicting the initial flavor scores of sound soybean salad oils. Similar data for oils from damaged beans gave a highly significant, but lower, correlation (-0.65). Comparative studies indicated that sound crude oils usually contain lower levels of oxidation products than damaged crude. Oxidation in both sound and damaged crudes increased roughly in proportion to iron content. Reproducibility of the test and the effects of hydrogenation, accelerated storage, and fluorescent light on anisidine values were studied. Analysis of damaged oils before and after deodorization showed that little, if any, reduction of anisidine value occurred. Deodorization of sound oils, however, lowered anisidine values. In comparison with damaged oils, the anisidine values of sound oils were lower at comparable stages of processing. The poor quality of damaged soybean oil was substantiated by organoleptic evaluations. Flavor scores of oils given special processing treatments increased as anisidine values decreased.

INTRODUCTION

Field and storage damage to soybeans has occurred intermittently over the years, particularly when mature beans remain in the field during prolonged wet weather and subsequently are stored with high moisture levels (1-3). Wet weather prevailed over much of the southeastern United States during the fall and winter of 1971, and private reports indicated that the quality of crude and finished oils from soybeans in this region was lower than usual.

Field and storage damage is known to increase free fatty acids (FFA) in crude soybean oil (4) and to cause poor flavor in refined salad oil (5). Because knowledge is limited of how to process oil from damaged beans, many inquiries came to the staff of the Northern Regional Research Laboratory. A preliminary investigation (Evans, et al., unpublished data) indicated that crude oils from damaged beans contained up to five times the amount of iron in oils from sound beans and that processing might not remove iron to levels acceptable for high quality salad oils. Possibly iron-catalyzed oxidation was contributing to the poor flavor of salad oils prepared from damaged beans.

In 1957 Holm, et al., (6) introduced the benzidine test to measure secondary oxidation products formed during processing of glyceride oils. The benzidine test demonstrated that high molecular carbonyl compounds, primarily aldehydic in nature, arise through oxidation of glyceride oils and that their removal during processing affected the shelf-life and quality of margarine and rapeseed and soybean oils (6,7). Owing to the carcinogenic properties of benzidine, an alternative but similar method was presented (8) based upon anisidine (*p*-methoxyaniline). We report here preliminary studies with the anisidine test to determine quality and oxidation of oil processed from sound and damaged soybeans.

MATERIALS AND METHODS

Soybean Oils

Crude and partially processed oils, as well as nonhydrogenated soybean salad oils, were collected from commercial processors located in the Midwest, Southeast, and Southwest. Partially hydrogenated-winterized salad oils were purchased in local retail markets or were prepared in the pilot plant of the Northern Laboratory.

Analyses and Oil Evaluations

Chromatographic refining of soybean oil was conducted according to Crossely, et al. (9). Iron was determined by atomic absorption spectroscopy (10). Peroxide values were determined by a modification of the Wheeler method (11). Organoleptic evaluations were conducted according to methods described by Moser, et al. (12). Flavor data were supplied by a 20 member taste panel. Accelerated 60 C aging and light exposure tests were carried out as described previously (13,14).

Anisidine Test

Anisidine (*p*-methoxyaniline) reagent grade was purchased from Aldrich Chemical Company, Milwaukee, Wis., and was recyrstallized from ethanol before use. Isooctane was spectroquality grade purchased from Matheson, Coleman, and Bell, Norwood, Ohio. Anisidine values were determined according to the method of Holm (8) on a Beckman DU spectrophotometer. Absorption readings were made at 350 nm in 1 cm cells. The anisidine test is similar to the benzidine test, except that isooctane is the fat solvent. The analytical reagent is 1 ml 0.25% anisidine in glacial acetic acid, and 10 min at room temperature is allowed for the reaction. The anisidine value (8) is defined by convention as 100 times the absorbance measured in a 1 cm cell of a solution resulting from the reaction of 1 g fat with 100 ml mixture of solvent and reagent.

EXPERIMENTAL RESULTS

The reproducibility of the anisidine test was determined

TABLE I

Reproducibility of Anisidine Values

Soybean oil	Anisidine value[a] Day 1	Day 2	Mean
Crude	0.33	0.72	0.52
Crude	0.30	0.59	0.45
Salad	1.66	1.74	1.70
Salad	1.94	2.02	1.98
Salad	2.84	3.14	2.99
Salad	6.74	6.96	6.85
Salad	4.42	4.45	4.43
Salad	3.80	3.92	3.86
Salad	6.06	6.23	6.15

[a] Standard deviation = 0.09 anisidine value units.

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[2] ARS, USDA.

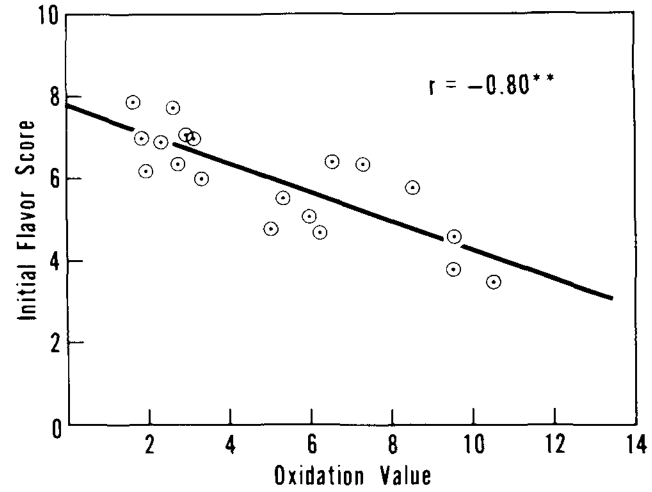

FIG. 1. Correlation between flavor scores and oxidation values of salad oil processed from sound soybeans.

by analyzing a number of soybean oils on different days. Anisidine values are summarized in Table I. The standard deviation for the method is 0.09 anisidine value units. In our experience, crude oils with low levels of oxidation products vary more than salad oils because the change in absorbance readings is small even at the maximum sample wt (4 g) recommended (8).

Oils from Sound Beans

Initial quality and oxidation: To orient our work with the anisidine test, we established levels of anisidine reactive materials in sound soybean salad oils and compared them with organoleptic evaluations to ascertain what, if any, relationship exists between quality and oxidation. During the early course of our work, a highly significant linear correlation (-0.82 significant at 99% level) was observed between anisidine value of 10 freshly deodorized nonhydrogenated soybean salad oils and their initial flavor scores, i.e. as anisidine values increased flavor scores decreased. As work progressed, some samples were evaluated that had comparatively low anisidine values, which suggested acceptable quality, but received low flavor scores. Invaribly these samples had measurable peroxide values at the time of testing.

Holm (8) has shown that a unit peroxide value corresponds to an increase of ca. 2 anisidine value units and has introduced the term oxidation value (OV) to describe the degree of oxidation. Oxidation value = anisidine value + 2 (peroxide value).

Figure 1 plots oxidation values vs. initial flavor score for 19 lots of commercially processed unhydrogenated soybean salad oils ranging in quality from good to poor. The linear correlation (-0.80) was significant at the 99% level.

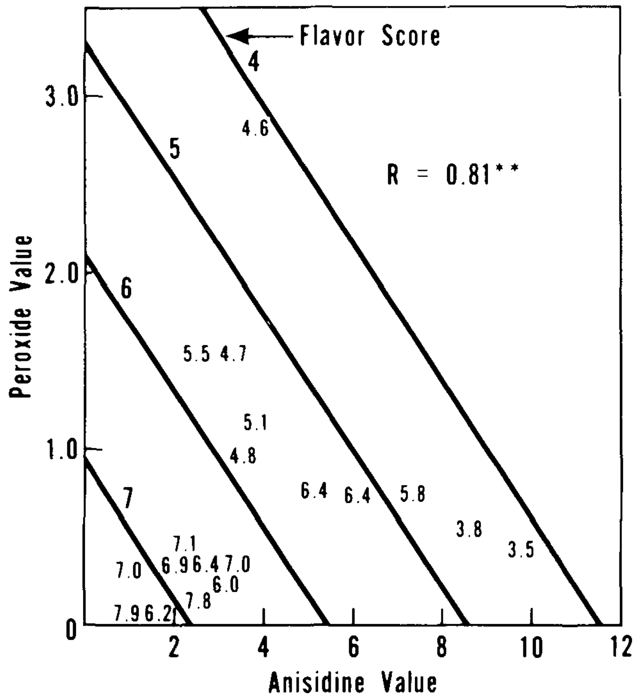

FIG. 2. Correlation contours for predicting the initial flavor scores of sound soybean salad oils from anisidine values and peroxide values. The numbers within the contours are flavor scores given by the panel.

Data plotted in Figure 1 suggest that initial flavor scores of soybean salad oil might be predicted from anisidine and peroxide values. Accordingly, a multiple regression computer program was used to compute equations for predicting flavor scores (F) with various combinations of anisidine value (AV) and peroxide value (PV). The relationship between F, AV, and PV using PV and AV as separate terms in the computation is F = 7.7 - 0.33 (2.6 PV + AV). When OV is defined as 2(PV) + AV, the equation is 7.7 - 0.35 (OV). There is no significant difference between the two equations.

Figure 2 shows contours for predicting the initial flavor score of commercially processed unhydrogenated soybean salad oils based upon the equation F = 7.7 - 0.33 (2.6 PV + AV) (data taken from oils plotted in Fig. 1). Such contours provide a fairly reliable method for predicting the initial flavor scores of soybean salad oils. The standard error of the estimated flavor score (1σ) was ±0.8. Thus, flavor scores can be predicted within ±1.6 flavor units (95% confidence limits). In taste trials, the observed standard deviation of the flavor score generally falls within the range 0.6-1.0 for our organoleptic panel. In general, the flavor scores indicated on Figure 2 fall into the predicted ranges.

Effects of fluorescent light and accelerated storage:

TABLE II

Effect of Accelerated Storage Test upon Quality and Oxidation of Soybean Salad Oil[a]

Test conditions	Flavor score	Peroxide value	Anisidine value	Oxidation value
Control	6.9	0.1	2.0	2.2
2 Days, 60 C	6.2	0.5	2.6	3.6
4 Days, 60 C	5.5	0.6	2.2	3.4
6 Days, 60 C	5.3	2.2	2.2	6.6
8 Days, 60 C	5.7	5.5	2.9	13.9
10 Days, 60 C	3.4	5.9	3.4	15.2
2 Hr light exposure	6.0	1.0	2.2	4.2
4 Hr light exposure	6.3	1.3	2.2	4.8
8 Hr light exposure	4.5	1.6	2.0	5.2
16 Hr light exposure	4.9	2.6	2.0	7.2

[a]Nonhydrogenated commercial salad oil.

TABLE III

Initial Quality and Anisidine Values of Hydrogenated-Winterized Soybean Salad Oils[a]

Catalyst	Source	Initial flavor score[b]	Anisidine value	Oxidation value
Nickel	Commercial	8.2 (0.0)	0.0	0.0
Nickel	Commercial	7.5 (0.0)	0.6	0.6
Nickel	Commercial	7.1 (0.1)	0.6	0.8
Nickel	Commercial	6.9 (0.0)	1.3	1.3
Nickel	Laboratory	8.4 (0.0)	0.1	0.1
Copper	Laboratory	8.3 (0.2)	0.2	0.6
Copper	Laboratory	8.4 (0.4)	0.1	0.9
Copper	Laboratory	8.3 (0.3)	0.6	1.2
Copper	Laboratory	8.5 (0.3)	0.8	1.4
Copper[c]	Laboratory	8.8 (0.4)	0.4	1.2

[a] All samples contained butylated hydroxyanisole, butylated hydroxytoluene, methyl silicone, and citric acid.

[b] Values in parentheses are peroxide values at the time of tasting.

[c] Not winterized.

Fluorescent light and storage at elevated temperatures are detrimental to the flavor stability of soybean oil (13,14). Their effect on anisidine values is of interest because such information might be useful when the quality of soybean oils of unknown history is considered. Effects of accelerated tests on the flavor stability and anisidine values of a commercially processed soybean salad oil are given in Table II. Storage at 60 C and exposure to fluorescent light show typical decreases in flavor score and increases in peroxide value. Oils exposed to 60 C aging increase little in anisidine value until after 8 days' storage. After 10 days, anisidine value increased from an initial 2.0-3.4. Exposure to fluorescent light increased anisidine values little, if any. However, a correlation of 0.66 (significant at the 95% level) was obtained between flavor scores and oxidation values in Table II. Although one erratic flavor score (8 days, 60 C) suggests that the correlation might be lowered by including it, an identical correlation was obtained from the flavor scores and oxidation values of 10 different oils aged 4 days at 60 C.

Effect of hydrogenation: Relationships between quality and anisidine values of hydrogenated soybean oils are important because such oils represent a major outlet of edible oil. Table III summarizes initial flavor scores and oxidative data for 10 lots of hydrogenated-winterized soybean salad oils. No significant correlations were found between anisidine, peroxide, and flavor data since the data cover too small a range to indicate trends. The extremely low anisidine and peroxide values are indicative of high initial flavor socres and quality. Our data suggest that high quality hydrogenated oils have lower anisidine values than do comparable unmodified oils. Apparently the anisidine reactive materials formed during processing are reduced greatly under the conditions of deodorization rather than being saturated during hydrogenation. (This statement is supported by data taken on an oil during processing but not reported here.)

At 60 C (2, 4, 6, 8, and 10 days) a single lot of hydrogenated-winterized soybean salad oil increased little in anisidine value if at all. Oxidation values increased with storage time and correlated well with flavor scores.

Oils from Damaged Beans

Initial quality and oxidation: Initial flavor scores of salad oils from damaged beans ranged from 5-6, typical of poor quality. Flavor descriptions were predominantly rancid, grassy, and painty—all of which are characteristic of oxidized soybean oil. In addition, some damaged oils were described as having a rubbery flavor, and many panel members scored them low.

To avoid extensive tabulation of data, the relationship between anisidine value and oil quality for hydrogenated and unhydrogenated oils from sound beans, as well as for unhydrogenated oils from damaged beans, is shown graphically in Figure 3. Since freshly deodorized damaged oils usually have low peroxide values, anisidine values approximate their oxidation values. Figure 3 confirms that the poor quality of damaged oil can be attributed, at least partly, to oxidation, since the low initial flavor scores are accompanied by high anisidine values.

Correlation coefficients for some variables with flavor scores of 16 damaged oils are shown in Table IV. Included for comparison are data from sound oils. The data for

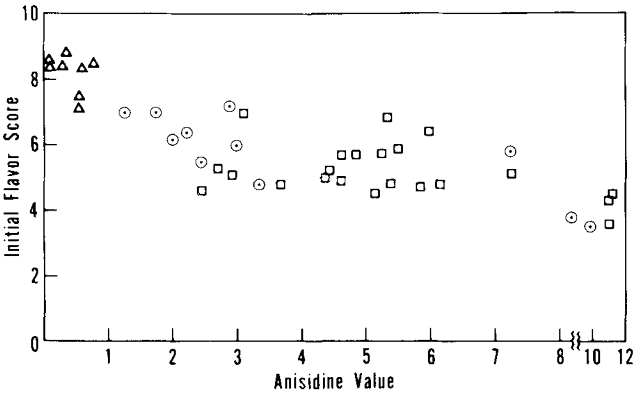

FIG. 3. Relationship between anisidine value and flavor scores of hydrogenated and unhydrogenated salad oils prepared from sound and damaged soybeans. Δ hydrogenated, sound, ⊙ sound, and □ damaged.

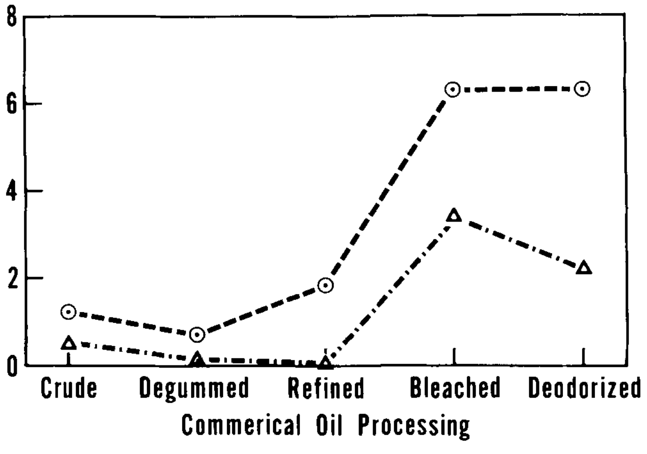

FIG. 4. Anisidine values of oils from damaged and sound soybeans at different stages during processing. Soybean oils: ⊙ damaged and Δ sound.

TABLE IV

Correlation of Variables with Initial Flavor Scores

Variable	Correlation coefficient (r) and significance[a]		
	Sound oil[b]	Damaged oil[c]	Pooled data
Flavor and peroxide	-0.52*	0.13	-0.24
Flavor and anisidine	-0.68**	-0.02	-0.61**
Flavor, peroxide, and anisidine	0.81**	0.01	0.65**
Flavor and oxidation value	-0.80**	0.13	-0.65**

[a] * = significant at 95% level; ** = significant at 99% level.

[b] n = 19.

[c] n = 16.

damaged oils showed no significant correlation between flavor and oxidative data; however, flavor scores were low and limited in range. The correlation after pooling sound and damaged oil data was still significant although somewhat lower than that with sound oils alone.

To gain some insight into the higher levels of oxidative products associated with damaged oils, comparative studies were undertaken to determine the effects of processing upon anisidine reactive material in sound and field-damaged oils (Fig. 4). Comparatively little increase in anisidine value occurs during degumming or refining of either sound or field-damaged soybean oils. Bleaching raised anisidine values in both oils but to a lesser extent in sound oil. A marked difference in effect of processing sound and damaged oils appears to be in the deodorization step, because little, if any, reduction occurred with field-damaged oils. In studies with other damaged oils (data not shown), little reduction in anisidine values occurred upon deodorization, and sometimes they actually increased. On the other hand, deodorization of sound oils always lowered anisidine values.

Further evidence that anisidine reactive materials contribute to the poor quality of damaged oils comes from organoleptic evaluation of oils subjected to additional treatment, including hydrogenation, carbon bleaching, and chromatographic refining (Table V). Oils A (crude) and B (salad) represent commercial oils processed from damaged beans. A comparison of their anisidine, peroxide, and oxidation values shows that conventional oil processing methods failed to remove oxidative products; their oxidation values increased from 3.8 in the crude oil to 4.4 in the finished salad oil. A highly improved flavor score (significant at 99% level) and a reduction in oxidation products resulted through hydrogenation-deodroization, carbon bleaching, and chromatographic refining (oils C, D, and E). Apparently, chromatographic refining completely removes anisidine reactive materials. Removal of oxidation products by chromatographic refining lends support to the concept that anisidine reactive materials are highly polar aldehydic materials which contribute to the flavor of soybean oil (6-8).

Iron and oxidation: Many of the damaged crude soybean

TABLE V

Effects of Additional Processing upon
Anisidine Values and Quality of Damaged Oils

Oil	Treatment	Flavor score[a]	Anisidine value	Oxidation value
A	Control crude oil	--- (0.2)	3.4	3.8
B	Salad oil commercially processed from oil A	5.3 (0.7)	3.0	4.4
C	Hydrogenated-winterized from oil B (redeodorized)	6.9 (0.2)	1.1	1.4
D	Carbon bleached from oil B (redeodorized)	7.0 (0.2)	2.2	2.6
E	Chromatographically[b] refined oil B redeodorized in laboratory	8.0 (0.3)	0.1	0.7

[a] Values in parentheses are peroxide values at the time of tasting.

[b] Equal volumes (350 ml) of soybean oil and pentane-hexane passed through a column containing 300 g 80/200 mesh alumina.

TABLE VI

Effect of Iron upon Oxidation of Crude Soybean Oil

Bean condition	Iron content, ppm	Peroxide value	Anisidine value	Oxidation value
Sound	1.2	0.5	0.4	1.4
Sound	1.5	0.1	0.5	0.7
Sound	2.8	0.0	0.4	0.4
Sound	2.8	0.8	0.5	2.1
Damaged	2.0	0.2	3.4	3.8
Damaged	3.9	1.2	0.4	2.8
Damaged	4.0	1.0	3.8	5.8
Damaged	4.6	0.1	0.5	0.7
Damaged	6.0	1.5	1.8	4.8

oils had iron contents up to five times that of crude oils processed from sound beans. Therefore, we made some analyses to establish whether oxidation of the oil and its iron content were related (Table VI). Only slight oxidation occurred in sound crude oil as is evidenced by low peroxide and anisidine values. Damaged crudes generally, but not always, contained higher peroxides or anisidine reactive materials or both. Generally oxidation values increased with an increase in the iron content. We believe from our prelininary data that iron may catalyze formation of secondary oxidation products in crude oil which are not removed satisfactorily during conventional processing.

The extent to which iron contributes to quality and oxidation of damaged salad oils also remains somewhat uncertain. In our experience, the iron content of soybean oils needs to be 0.1-0.2 ppm or less for acceptable quality (15). Some damaged salad oils we examined contained up to 0.7 ppm iron, a figure that implicates iron as a factor in the damaged oil problem. On the other hand, other damaged salad oils had normal iron contents but were of poor quality. Further work is required to delineate the effects of iron in field-damaged soybean oil.

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