undertaking but also has some shortcomings. These include the formation of large numbers of nondiagnostic ions (some of which interfere with the measurement of critical ions), calibration of a system to determine quantitatively compounds with differing degrees of unsaturation and the GC separation and transfer of wax esters to a mass spectrometer. Although capillary columns greatly enhance the GC separation of analogs by degree of unsaturation, they still are not particularly efficient when large numbers of positional isomers (such as are found in sperm whal oil) are present (12). Additional precautions must be taken to minimize adsorptive losses and degradation on the way to the mass spectrometer (4). These obstacles are not encountered in MS/MS.

A critical test of the results obtained by any MS method is to calculate, from these results, the alcohol and acid composition of the overall wax ester mixture. The calculated composition can then be compared to that found by hydrolysis which we consider the most accurate. These types of comparisons are made in Table III. Apparently, either MS technique gives reasonable answers, but the specificity, simplicity and sensitivity of the MS/MS procedure make it very attractive. As instruments become more widespread and less expensive (7), MS/MS analysis of wax ester mixtures should become routine.

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# Effect of Water Quality on Degumming and Stability of Soybean Oil

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## ABSTRACT

Solvent-extracted crude soybean oil was degummed with deionized distilled water containing various amounts of CaCO$_3$-MgCO$_3$, FeCl$_2$, and NaCl. The total phosphorus content remaining in the degummed oil was determined and the peroxide value of the degummed oil held at 98-101 C was measured daily for 10 days. The results were compared statistically with those from oil degummed with deionized distilled water as a control. It was found that 250 mg/L of CaCO$_3$-MgCO$_3$ significantly reduced the efficiency of the degumming process. FeCl$_2$ at concentrations of 150 and 250 µg/L and NaCl at 300 mg/L resulted in the removal of more phosphorus than the control at the 5% level of significance. Generally, the stability of the degummed oils decreased as the salt concentrations increased. The rate of oxidation was greater for oils degummed in the presence of FeCl$_2$ than of NaCl and CaCO$_3$-MgCO$_3$ under the same conditions.

## INTRODUCTION

Degumming is a process by which 1-3% water with or without degumming agents such as phosphoric acid is mixed with crude oil at 300-500 rpm for 30-60 min at 60-70 C to render fat-soluble impurities insoluble by hydration – these are then removed by centrifugation (1). Carr (2) reported that degumming removed 80-95% of the phosphorus present in soybean oil. Most of the hydratable phosphatides can be eliminated by degumming, but the nonohydratable phosphatides (Ca and Mg phosphatides) remain in the degummed oil. Letan and Yaron (3) reported that the presence of Ca and Mg in crude and degummed oil prevented complete elimination of phosphatides by hydration.

There is no available information on FeCl$_2$ or NaCl solutions as degumming agents for degumming vegetable oils. However, it has been reported that phosphatides have the ability to form combinations with salts of various heavy metals. Tompsett (4) indicated that Fe$^{+3}$ but not Fe$^{+2}$ salts formed complexes with phosphatides. According to Thurman (5), a 10% solution of NaCl aided the breaking of emulsions and he recommended that NaCl solutions be used for washing phosphatides or precipitates because of its solvent action.

The oxidation of lipids and oils and the decomposition of hydroperoxides are catalyzed by heavy metals, particularly those possessing two or more valency states (Fe$^{+2}$ and Fe$^{+3}$) with a suitable oxidation-reduction between them (6). According to List et al. (7), FeCl$_2$ at concentrations of 0.1, 0.5 and 1.5 ppm significantly lowered the flavor stability of soybean oil. Alkali and alkali earth metals possess catalytic acitivity in oil oxidation due to induced hydroperoxide decomposition of free radicals (8).

There is an increased interest in the use of vegetable oil as a replacement for or extender of diesel fuel, and an interest in processing the oil on the farm. The water used for degumming can be a factor in the quality of the final

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TABLE I

**Effect of $CaCO_3$ and $MgCO_3$, $FeCl_2$ and NaCl Solutions on Phosphorus Removal from Crude Soybean Oil During Degumming**

| Sample | Phosphorus (ppm) remaining in degummed oil[a] | % Phosphorus removal |
|---|---|---|
| Control | 33.1 ± 3.1 | 92.1 |
| 50 mg/L $CaCO_3$ + $MgCO_3$ | 35.4 ± 4.0 | 91.6 |
| 150 | 35.4 ± 1.4 | 91.6 |
| 250 | 42.1 ± 1.9 | 90.0[b] |
| 50 $\mu$g/L $FeCl_2$ | 38.5 ± 3.9 | 90.9 |
| 150 | 26.2 ± 3.5 | 93.8[b] |
| 250 | 28.3 ± 4.0 | 93.3[b] |
| 100 mg/L NaCl | 35.3 ± 1.4 | 91.6 |
| 300 | 25.2 ± 1.2 | 94.1[b] |
| 500 | 34.6 ± 2.0 | 91.8 |

[a] Average of duplicate analyses on duplicate columns of two samples.
[b] Significantly different from the control at the 5% level.

product. The purpose of this investigation was to study the effect of hardness of water ($CaCO_3$ and $MgCO_3$), $FeCl_2$ and NaCl on the efficiency of degumming crude soybean oil. These degumming agents were used at concentrations in the range of those found in the groundwater of Nebraska. The concentration of $CaCO_3$ and $MgCO_3$, Fe and Na in the groundwater of eastern Nebraska, where soybeans are grown, exceeds 360 mg/L, 300 $\mu$g/L and 50 mg/L, respectively (9). NaCl concentration is relatively low but it is important to know the effect of higher Na concentration when water is softened.

## EXPERIMENTAL PROCEDURES

### Sample Preparation and Degumming

Crude soybean oil was obtained from a commercial refiner. The $CaCO_3$-$MgCO_3$ solutions were prepared with a Ca:Mg ratio of 3:2. The crude oil samples were mixed with 1.5% of each solution as well as deionized distilled water (the control) individually. The mixtures were agitated mechanically at 400 rpm for 25 min at 60 C to hydrate the phosphatides and other colloidal impurities. They were then transferred to 250-mL bottles where the hydrated phosphatides and gums were separated by centrifugation at 4000 × g for 15 min.

### Total Phosphorus and Peroxide Value Determination

The phosphorus contents of the crude and degummed oils were determined by the method of Racicot and Handel (10). Degummed oil (100 mL) from each treatment was transferred to 120-mL glass jars (60 mm od × 70 mm) which were capped and stored at rest at 98-101 C. Peroxide value was determined daily on each sample in duplicate for 10 days (11).

### Statistical Procedures

Significant differences between means were determined at the 5% level using Duncan's Multiple Range Test. Differences in slopes of regression lines were assessed to determine differences in the increase of peroxide values.

## RESULTS AND DISCUSSION

### Effect of $CaCO_3$ and $MgCO_3$, $FeCl_2$ and NaCl Solutions on Removal of Phosphorus During Degumming

Table I shows the percentage of phosphorus removed from soybean oil during degumming with 50, 150 and 250 mg/L soultion of $CaCO_3$-$MgCO_3$. The efficiency of the degumming process, when 250 mg/L was used, was lower than that with the control at the 5% level of significance. Ca and Mg reduced the efficiency of degumming process by leaving some of the phosphorus in the degummed oil. Nonhydratable Ca and Mg phosphatides were formed and depressed the hydration of the phospholipids in the crude soybean oil (12). The depression resulted from the competition between water and Ca and Mg for the same sites on the phospholipids. Hvolby (13) reported that Ca and Mg reduced the hydration of the phospholipids by reducing the contact between the oil and the water phase.

The total phosphorus content of the degummed oil and phosphorus removed when 50, 150 and 250 $\mu$g/L solutions of $FeCl_2$ were used is shown in Table I. The higher concentrations of $FeCl_2$ resulted in the removal of more phosphorus than the control at the 5% level of significance. It is not clear why $FeCl_2$ might aid in removal of phosphorus compounds from crude soybean oil, since the use of $FeCl_2$ solutions for degumming vegetable oils has not been reported. However, it has been reported that phosphatides can form a complex with various heavy metals. Tompsett (4) found that $FeCl_3$ formed insoluble complexes with phosphatides, which may explain the greater phosphorus removal in this study. In addition, $FeCl_2$ may also cause precipitation of phytic acid which would also reduce the phosphorus content (14). Phytic acid is a significant source of phosphorus in soybeans: however, it has not been reported to be present in crude soybean oil.

The effect of 100, 300 and 500 mg/L solutions of NaCl on the phosphorus removed from the crude soybean oil during the degumming process is shown in Table I. The 300 mg/L concentration showed a significantly higher level of phosphorus removal during degumming than did the control. This confirms Thurman's (5) report that NaCl was satisfactory for the purpose of precipitating phosphatides. In this study, there was poor separation at 500 mg/L, as evidenced by a cloudy oil phase after centrifugation. The poor separation would lead to higher phosphorus values. The poor separation may be due to the formation of greater amounts of Na soaps at higher concentration. Beal et al. (15) faced similar problems when using high concentration of NaOh for refining. They explained it by saying that there was unsatisfactory separation.

In conclusion, higher concentrations of $CaCO_3$-$MgCO_3$

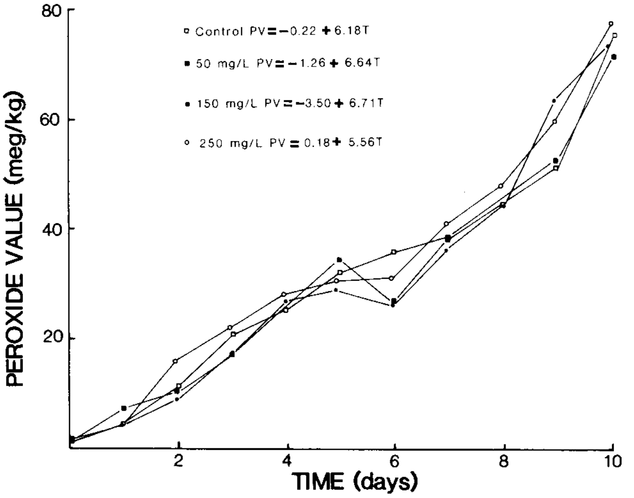

**FIG. 1. Stability of soybean oil degummed with $CaCO_3$ and $MgCO_3$ solutions.**

(250 mg/L) reduced the efficiency of the degumming process significantly when compared to the control. In contrast, $FeCl_2$ at 150 and 250 µg/L removed significantly more phosphorus than the control at the 5% level. A solution of 300 mg/L NaCl gave the most satisfactory results in removing more phosphorus than the control.

### Effect of $CaCO_3$ and $MgCO_3$, $FeCl_2$ and NaCl Solutions on Degummed Oil Stability

The increase in peroxide values over time of the oil degummed with $CaCO_3$-$MgCO_3$ solutions is shown in Figure 1. Linear regression effectively described the relationship between the peroxide value and time at the 5% level of significance for the control and 50, 150 and 250 mg/L $CaCO_3$-$MgCO_3$. The slope of each curve was compared to that of the control and there was no difference at the 5% level of significance.

It was expected that oil degummed with 250 mg/L $CaCO_3$-$MgCO_3$ would be more stable to oxidation because of greater amount of phosphorus remaining in the degummed oil. Lakshminarayana et al. (16) reported that the development of oxidation as measured by peroxide value was faster in degummed oil than that of crude oil, due to higher phosphorus content in crude oil. The expected stability caused by the higher level of phospholipids in degummed oil might have been balanced by an increased rate of oxidation due to $CaCO_3$-$MgCO_3$ which might have induced hydroperoxide decomposition of the free radicals. Ingold (8) mentioned that the alkali and alkali earth metals possessed catalytic activity due to induced hydroperoxide decomposition of free radicals.

The peroxide values of the oils degummed with $FeCl_2$ solutions over the 10-day period are shown in Figure 2. In general, these data show that the higher concentrations of $FeCl_2$ led to higher peroxide value changed as the concentration changed. For 50 and 250 µg/L concentrations, a quadratic regression best described the relationship between the peroxide value of the degummed oil and time, whereas cubic regression was best for 150 µg/L $FeCl_2$.

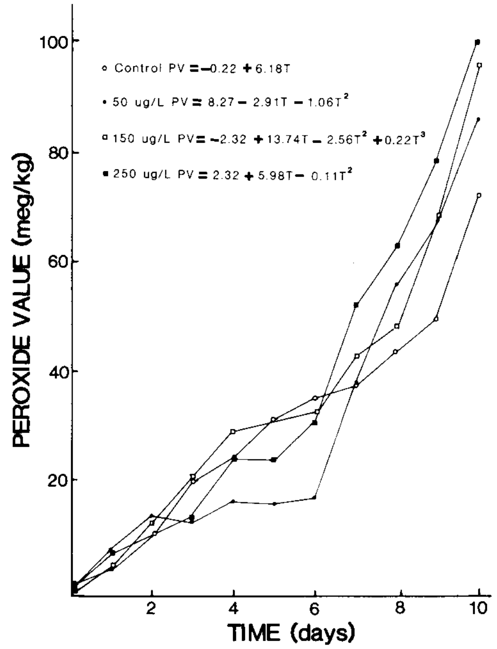

**FIG. 2. Stability of soybean oil degummed with $FeCl_2$ solutions.**

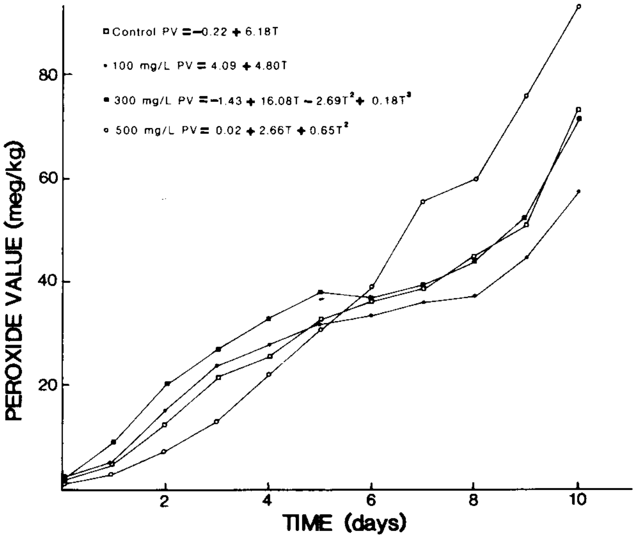

**FIG. 3. Stability of soybean oil degummed with NaCl solutions.**

$FeCl_2$ has been shown to increase the rate of oxidative deterioration of degummed oil by reducing the length of the induction period, by increasing the rate of oxidation, and by affecting hydroperoxide decomposition (6). Since at 50, 150 and 250 µg/L $FeCl_2$ the peroxide value was a quadratic or cubic function with time, the mechanism of oxidation was significantly different from that of the control, the regression of which was linear. This probably occurred because of the various reactions which $FeCl_2$ affects during the initiation, propagation and termination stages (17).

The peroxide values of oils degummed with NaCl solutions over the 10-day period are given in Figure 3. Higher concentrations of NaCl led to higher peroxide values compared to the control. Linear, cubic and quadratic regression described the oxidation with 100, 300 and 500 mg/L NaCl solutions, respectively. Since at 300 and 500 mg/L the peroxide values were cubic or quadratic functions, respectively, with time, the development and the mechanism of oxidation are significantly different from that of the control. However, Chang and Watts (18) concluded that concentrations of NaCl below 5% inhibited the oxidation of lard, and above 15% accelerated it. In this study, the oxidation of the oil degummed with 100 mg/L was lower than that of the control but that of the oil degummed with 500 mg/L was higher than the control.

In conclusion, the oxidative stability of degummed oil decreased as the metal concentration increased. The development of oxidation was greater for $FeCl_2$ concentrations than for NaCl and $CaCO_3$-$MgCO_3$ concentrations under the same conditions.

The authors recommend that when degumming soybean oil, hard water with levels of $CaCO_3$ and $MgCO_3$ higher than 150 mg/L should not be used because of the undesirable effect of Ca and Mg on the effectiveness of the degumming process. In spite of the ability of high concentrations of $FeCl_2$ to remove more phosphorus during the degumming process, water with more than 100 µg/L $FeCl_2$ should not be used because of its deleterious effect on the oxidative stability of the degummed oil. Finally, deionized distilled water or softened water with 300 mg/L NaCl should be used for degumming, depending on availability and cost.

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# Genetic Alteration of Soybean Oil Composition by a Chemical Mutagen[1]

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## ABSTRACT

Soybean (*Glycine max* [L.] Merr. cv. Century) seeds were treated with ethylmethanesulfonate (EMS) and the $M_2$ progeny were evaluated for fatty acid composition of the oil. Treatment with EMS significantly increased the variability in content of each of the fatty acids in comparison with those of the Century control. There was a strong inverse relationship between oleic and linoleic acids among seeds from $M_2$ plants. This supports the hypothesis of sequential desaturation as the method of formation of unsaturated fatty acids in soybean oil. A genetically stable mutant with 3.4% linolenic acid was identified that was similar to the cultivar Century in days to maturity, plant height and resistance to lodging.

## INTRODUCTION

Soybean oil is the major edible vegetable oil produced and consumed in the USA. The high linolenic acid content of the oil, 7-9%, has been associated with objectionable flavors and poor stability (1,2). Industrial processes have been developed to hydrogenate and deodorize soybean oil to improve flavor and stability, but the processes are expensive and result in formation of *cis* and *trans* positional monoenes and *cis-trans* 9-12 dienes. The latter unnatural fatty acids have questionable nutritive value (3-5).

Efforts to identify soybean strains with low linolenic acid contents, 3.5% or less, have been only partially successful (6,7). The US soybean germplasm collection contains strains with minimum linolenic acid contents of ca. 4.2% (7-9). Related species in the genus *Glycine* have higher linolenic acid contents than the cultivated soybean (10).

Breeding programs have been initiated to develop soybean strains with inherently low levels of linolenic acid. White et al. (11) intercrossed low linolenic acid lines of soybeans and from their progeny identified an $F_2$ plant with 3.35% linolenic acid. However, this work was terminated because in succeeding generations the low linolenic acid value of the line was not maintained and environmental effects markedly influenced the linolenic acid content of the line.

Hammond and Fehr (6) used combinations of recurrent selection and both X-rays and chemical mutagens to produce soybean strains with low linolenic acid contents. By crossing strains with the lowest linolenic acid content available, they were able to produce strains with amounts of linolenic acid 1-1.5% lower than the best parental strain. Treatment of one low linolenic acid breeding line with ethylmethanesulfonate (EMS) resulted in a line, designated A5, with linolenic acid contents of 2.9-4.1%, depending on the environment where the line was grown. In that research, selection was only for low linolenic acid content and not for agronomic characteristics. Selected strains would have to be crossed with lines that possess good agronomic characteristics to develop low linolenic acid cultivars that would be competitive with currently grown cultivars.

Wilson et al. (12,13) used recurrent selection for high

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