☙Reverse Osmosis and Ultrafiltration of Stillage Solubles from Dry-Milled Corn Fractions[1]

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ABSTRACT

Grits, flour, degerminated meal and hominy feed from corn were fermented to make ethyl alcohol. The stillage, remaining after distillation of alcohol, was separated by screening and centrifuging into insoluble and soluble fractions. The stillage solubles contained 0.036 to 0.080% nitrogen and 1.4 to 7.2% total solids. Ultrafiltration (UF) membranes separated stillage solubles into permeate and concentrate fractions. Permeates from stillage solubles accounted for 85 to 95% of the original volume, 44 to 67% of the total solids and 40 to 75% of the total nitrogen. Reverse osmosis (RO) membranes separated the UF permeate into RO permeate and RO concentrate fractions. The RO permeate accounted for 70 to 92% of the original volume, 5 to 15% of the total solids and 5 to 21% of the total nitrogen in the UF permeate. Conductivity of some RO permeate fractions was lower than that of tap water. The combination of UF and RO processing of stillage solubles from corn dry-milled fractions appears to be an attractive method to recover most of the solids and nitrogen in small volumes of concentrate, and produces a final permeate with a low concentration of solids and nitrogen. The RO permeate may be reused as water, treated further or discharged.

INTRODUCTION

When cereal grain is fermented to make ethyl alcohol, a residue (stillage) that contains 5-10% solids remains after the alcohol is distilled off. Typically, stillage is screened or centrifuged to remove most suspended insoluble solids, and the remaining soluble fraction usually is evaporated to a syrup, which is marketed "as is" or co-dried with solids from the previous screening or centrifugation operation. Considerable energy is required to dry the soluble fraction, but discarding it would result in serious pollution.

Ultrafiltration (UF) is an efficient process for selectively separating solutions by convective solvent flow through a membrane. Solutes or particles larger than the specified membrane "cut-off" are quantitatively retained, whereas solutes smaller than the membrane pores pass through with the solvent. Reverse osmosis (RO) also can concentrate solutions by removing water by means of a membrane more permeable to it than to ions and other dissolved matter (1). In this technique, the solution is pumped at high pressure through the membrane to overcome the osmotic pressure that reverses the migration of water. Because no evaporation of water occurs in UF and RO, energy consumption is much lower than in concentration by heating. The total cost for equipment, power and labor for combined UF and RO is much lower per 1,000 gal of stillage treated than fuel costs alone for the evaporative route (2). Methods for handling traditional distillery waste water have been reviewed (3).

Reverse osmosis has been used to process the soluble fraction of stillage from whole corn (4). Dry-milled fractions of corn, including grits, flour, degerminated meal and hominy feeds, differ in composition from one another and from whole corn. Likewise, stillage solubles from these corn dry-milled fractions differ widely from whole corn stillage in nitrogen, solids and ash contents. This paper reports that UF and RO processing of stillage solubles from corn dry-milled fractions can produce a small volume of concentrate, having high solids and nitrogen contents, and a large volume of permeate with low solids and nitrogen contents. UF and RO operations provide an economical alternative for disposal of stillage liquids and recovery of potentially useful solids.

EXPERIMENTAL

Fermentation

Dry-mill processed yellow corn grits, flour, degerminated meal and hominy feed were obtained from Illinois Cereal Mills, Paris, Illinois. Each fermentation medium was prepared in duplicate to contain approximately 20% glucose equivalent. Each dry-milled fraction (1,821, 1,909, 2,069 and 2,568 g dry weights of grits, flour, degerminated meal and hominy feed, respectively) was dispersed in 5 liters of tap water in a 20-liter stainless steel jacketed fermentor, equipped with temperature-control and stirrers. The pH of each slurry was adjusted to 6.2, and 6 ml of Miles Taka-therm alpha-amylase (170,000 Modified Wohlgemuth Units/g, specific gravity 1.2 g/ml) was added. One Modified Wohlgemuth Unit is that amount of enzyme which will dextrinize 1 mg of soluble starch to a definite size dextrin in 30 min under the assay conditions. The fermentor temperature was held at 90 C for 1 hr to gelatinize and degrade starch to soluble dextrins. Tap water (1,309, 1,302, 1,229 and 1,209 ml for grits, flour, degerminated meal and hominy feed, respectively) was then added to the fermentor. The fermentor was cooled to 60 C; the pH was adjusted to 4.0 with 6N HCl; and 18 ml of Miles Diazyme L-100 glucoamylase (100 Diazyme Units/ml) was added to hydrolyze the dextrins. After incubation for 2 hr, the mixture was cooled to 30 C and adjusted to pH 4.5 with 12.5N NaOH. Then, 500 ml of yeast *(Saccharomyces cerevisiae)* broth in the logarithmic growth phase, containing an average of 5 million cells per ml (determined by dilution plating), was added. The yeast inoculum was made from 9 g of Fermivin dry yeast (G.B. Fermentation Industries, Des Plaines, Illinois), 0.3% yeast extract (Difco, Detroit, Michigan), 0.5% peptone (Difco) and 1.0% glucose in 500 ml of tap water. Each fermentation was stopped after 66 hr. Fermentation of corn for 48 hr is usually sufficient, and is complete at 66 hr.

Fractionation of Stillage

Alcohol was distilled from the corn grits fermentor by admitting steam from a jet for 15 min. An increase in volume of the residual stillage occurred due to steam condensation. For the other dry-milled fractions, alcohol was distilled from the fermentor by circulating steam through the outer jacket. The residue (stillage) was filtered through cheesecloth under suction. The thin stillage that passed through the cheesecloth was centrifuged at 45,000 rpm (45,200 × g) in a Model T-1 Sharples continuous centrifuge with a 4.5-cm inside diameter bowl. Solution that passed through the continuous centrifuge was designated stillage solubles or soluble fraction of stillage. The solids that remained in the bowl of the centrifuge were termed centrifuge solids, and the materials that remained on the cheesecloth were distillers' grains.

Approximate Nitrogen Distribution of Components of Stillage Solubles

A Model 52 UF cell (Amicon Corp., Lexington, Massachu-

[1] Presented at the AOCS Annual Meeting, May 1984, Dallas, Texas.
[2] Deceased.

setts) with two 43-mm diameter membranes was used. The membranes tested had nominal molecular weight cut-offs of 500 daltons for UMO5 and 10,000 daltons for PM10; most solutes larger than these molecular weights are retained by the membranes. For each membrane, stillage solubles (10, 15 or 20 ml) were pipetted into the cell above the membrane, and distilled water was added under 50 lb/in.^{2} of nitrogen, so that the volume of solution retained above the membrane (concentrate) remained constant. About 4 volumes of permeate (solution passing through the membrane) were collected. Volumes of permeate and concentrate were measured, and nitrogen and solids content of each solution was determined.

UF and RO

An OSMO Econo-Pure RO apparatus (Osmonics, Inc., Minnetonka, Minnesota), equipped with OSMO-112 Sepralators (5.1 cm diameter, 66 cm long and 1.0 m^2 membrane spirally wound), having a holdup volume of about 600 ml, was used for UF and RO. For UF, a SEPA-0 membrane was used; this membrane has a molecular weight cut-off of approximately 1,000 for organic compounds, rejects 0-10% sodium chloride, and has a nominal pore size of 1.5 mμ. For RO, a SEPA-97 membrane having a molecular weight cut-off of 200 for organics, 94-97% sodium chloride rejection, and nominal pore size of 0.5 mμ was used. Solutions were pumped through these membranes under 100 psi for UF and 200 psi for RO. The solution passing through the membrane is termed permeate, and that which is retained is the concentrate. Since the rate of permeate flow was much slower than the concentrate flow, the concentrate stream was recirculated to the initial solution to reduce the concentrate volume. Samples of permeate and of concentrate plus initial solutions (subsequently designated as "concentrate") were taken for analyses.

The Osmonics apparatus used does not have a temperature control. Although both UF and RO instruments were at room temperature, solution temperatures increased during processing. To minimize evaporative loss, initial feed solutions at 4 C usually were used for UF and RO. The permeate flow rate of corn grits stillage solubles was 3.8 to 4.6 l/hr for RO and higher for UF.

Analyses

Nitrogen content was determined by micro-Kjeldahl and ash by heating to 600 C, as detailed in AACC methods (5). Solid content (dry matter) of solutions was determined gravimetrically by pipetting known volumes into previously weighed crucibles or flasks, drying overnight in an air oven at 100 C and for 3 days in a vacuum oven at 100 C, and weighing. Nitrogen determinations were made in triplicate or quadruplicate, and solids content and ash content determinations were made in duplicate. Conductivity measurements were performed on stillage fractions using a Radiometer type CDM 2e conductivity meter with a CDC 104 NS cell (Radiometer America, Westlake, Ohio). The temperature of each solution was recorded, and the conductivity value was corrected to 20 C. The relative conductivity of 0.1 N potassium chloride between 20 and 30 C was used as the temperature correction factor.

RESULTS AND DISCUSSION

Fractionation of Stillage Solubles

When the stillage solubles from dry-milled corn fractions were fractionated on the basis of molecular weight in the Amicon cell, only 12 to 30% of the total nitrogen was found to be above 10,000 molecular weight, and 34 to 65% of the total nitrogen below 500 molecular weight (Table I). Apparently, most of the nitrogenous materials in stillage

TABLE I

Nitrogen Distribution of Stillage Solubles from Dry-Milled Corn Fractions[a]

	UM05 (500 daltons)		PM10 (10,000 daltons)	
Fraction	Concentrate %	Permeate %	Concentrate %	Permeate %
Grits	35	65	17	83
Flour	56	44	30	70
Degerminated meal	66	34	17	83
Hominy feed	59	41	12	88

[a]The nominal molecular weight cut-offs of UM05 and PM10 are 500 and 10,000 daltons, respectively.

TABLE II

Ultrafiltration and Reverse Osmosis of Corn Grits Stillage Solubles[a]

	Volume, ml	Nitrogen, mg/ml	Solids, mg/ml	Ash, % of dry matter
Stillage solubles	9,500	0.801	14.45	7.19
Permeate (UF)	9,025	0.636	9.79	10.77
Concentrate (UF)	305	1.70	30.96	4.74
Permeate (RO), total	7,530	0.093	1.07	6.85
Permeate (RO), end	760	0.328	2.89	12.34
Concentrate (RO), total	1,100	1.45	24.69	10.31
Concentrate (RO), end	200	3.21	55.68	10.00

[a]Permeate from ultrafiltration (UF) accounts for 95% of original volume, 64% of total solids and 75% of total nitrogen. UF permeate was used as feed solution for reverse osmosis (RO) after about 100 ml was removed for analyses. RO permeate accounted for 85% of original volume, 9% total solids and 12% total nitrogen of UF permeate. In addition to the permeate and concentrate, holdup volume in the machine and water loss from evaporation during processing also accounted for the initial volume. The original volume of UF permeate for RO was 8,870 ml.

solubles of dry-milled corn fractions are amino acids and peptides rather than proteins, although other nitrogen-containing material also may be present. For comparison, the nitrogenous materials from stillage solubles of whole corn were all smaller than 10,000 molecular weight, and 48% of the total nitrogen was below 500 molecular weight (6).

UF and RO of Corn Grits Stillage Solubles

Table II shows the results of processing of corn grits stillage solubles by UF and RO. UF processing continued until almost all concentrate disappeared. The UF concentrate had about twice the nitrogen and solids, but lower ash contents compared with stillage solubles (Table II). The composition of the holdup solution in the apparatus at the end of UF was close to that of the UF concentrate (data not shown). After about 100 ml of UF permeate was removed for analysis, the remainder was used as a feed solution for RO until the RO concentrate was nearly exhausted. RO permeate was collected at 750-ml intervals; 100-ml RO concentrate also was removed at each interval. Ten fractions each of RO permeate and RO concentrate were collected. "Permeate, end" refers to the last permeate fraction and "permeate, total" is the sum or composite of all permeate fractions. Nitrogen and solids contents of RO permeate increased slowly at first, as the volume of the remaining concentrate decreased (Fig. 1). A rapid increase in solids and nitrogen contents of RO permeate occurred near the end of RO processing.

The change in solids content of RO permeate and RO concentrate during processing, as indicated by fraction number, is plotted in Figure 2. The initial volume of 8.9 l was reduced by 0.85 l each time a fraction was removed. The increase in solids content was slow initially, but increased rapidly near the end of RO processing. Table II shows that solid and nitrogen contents of RO permeate and concentrate at the end of RO processing were much higher than the average values for all permeate or concentrate fractions.

Solids and ash contents per ml of RO concentrate are nearly linearly related to conductivity at 20 C (Fig. 3). Conductivity measures the salt (ash) concentration. Both solids and ash in the same system can be represented by the same line, and conductivity can predict solids contents of RO concentrates. For RO permeates (Fig. 4), however, some curvature is evident for the ash or solids vs. conductivity data, and different curves are needed for solids and ash data.

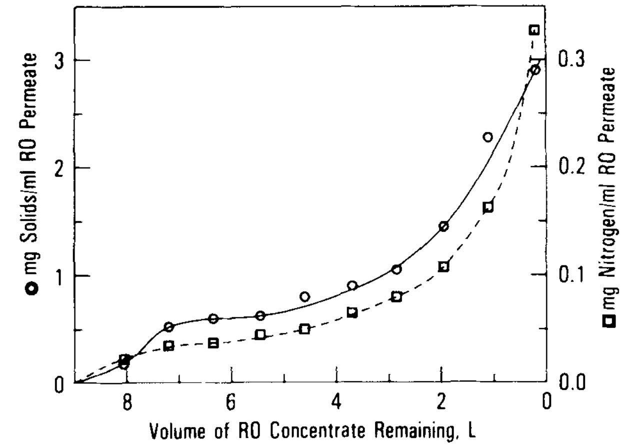

FIG. 1. Solids (—o—) and nitrogen (—□—) contents of RO concentrate during RO of UF permeate from corn grits stillage solubles.

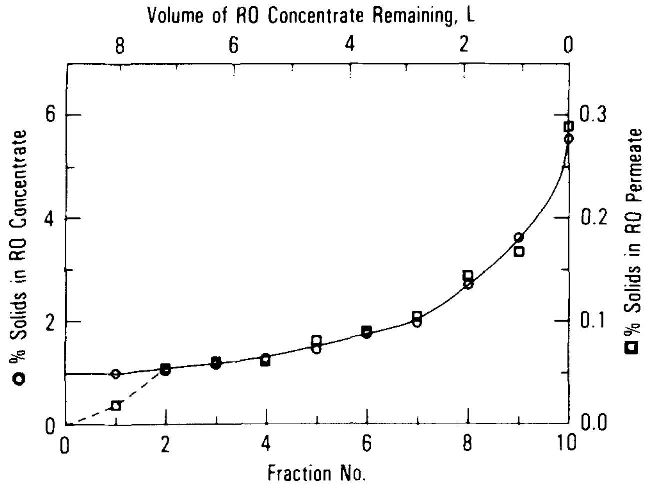

FIG. 2. Solids content of concentrate (—o—) and permeate (—□—) during RO of UF permeate from corn grits stillage solubles. Solids content of concentrate is about 20 times higher than permeate.

UF and RO of Corn Flour Stillage Solubles

Results of UF and RO processing of corn flour stillage solubles are summarized in Table III. UF of the stillage solubles continued until the concentrate solution was almost consumed. The UF concentrate had more than twice the nitrogen

TABLE III

Ultrafiltration and Reverse Osmosis of Corn Flour Stillage Solubles[a]

	Volume, ml	Nitrogen, mg/ml	Solids, mg/ml	Ash, % of dry matter
Stillage solubles	4,490	0.358	71.53	3.06
Permeate (UF)	4,118	0.161	52.28	3.60
Concentrate (UF)	192	0.870	79.28	2.40
Permeate (RO), total	3,390	0.039	9.32	5.09
Permeate (RO), end	1,630	0.052	17.11	5.21
Concentrate (RO), total	550	0.291	108.5	3.35
Concentrate (RO), end	450	0.316	117.7	3.37

[a]Permeate from ultrafiltration (UF) accounts for 92% of original volume, 67% of total solids and 41% of total nitrogen. Permeate (UF) was used as feed solution for reverse osmosis (RO) after about 100 ml was removed for analyses. Permeate (RO) accounts for 85% of original volume, 15% of total solids and 21% of total nitrogen of permeate (UF). In addition to the permeate and concentrate, holdup volume in the machine and water loss from evaporation during processing also accounted for the initial volume. The original volume of UF permeate for RO was 4,000 ml.

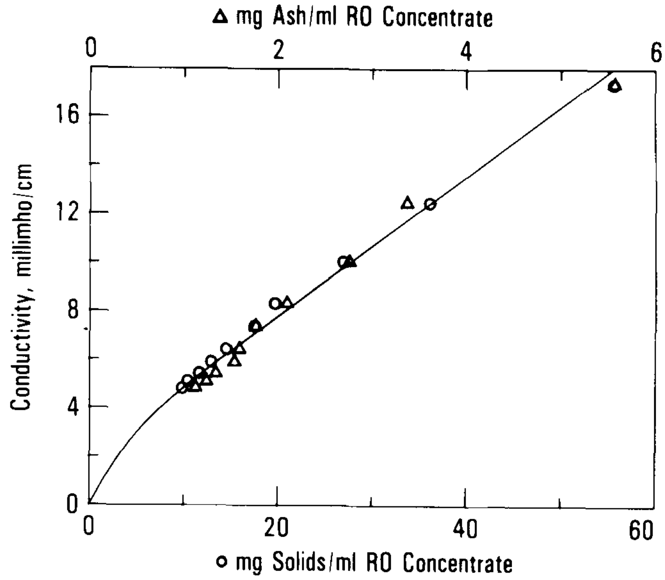

FIG. 3. Conductivity of concentrate fractions from RO of UF permeate from corn grits stillage solubles at 20 C as a function of solids (—○—) and ash (—△—) concentrations. Solids per ml of concentrate is about 10 times that of ash.

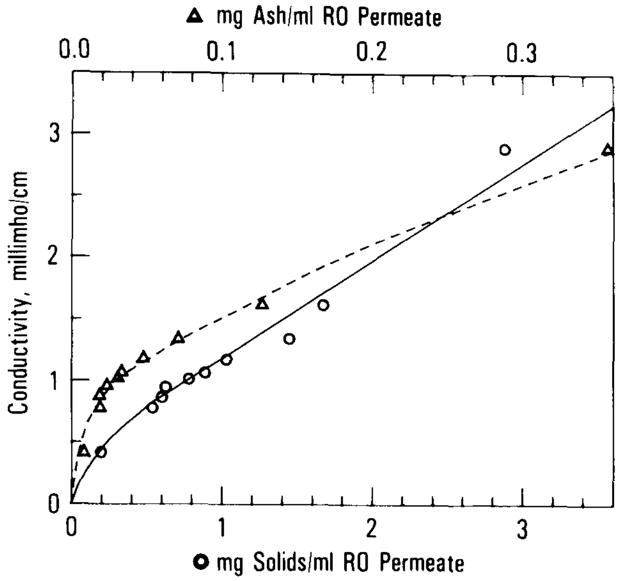

FIG. 4. Conductivity of permeate fractions from RO of UF permeate from corn grits stillage solubles at 20 C as a function of solids (—○—) and ash (—△—) concentrations.

content of the starting stillage solubles. After 100 ml of pooled UF permeate was set aside for analysis, the remaining UF permeate was used as feed solution for RO. Two fractions, each of RO permeate and RO concentrate, were collected. "Total" refers to the sum or composite value, and "end" refers to the final fractions of permeate and concentrate in Table III. The RO permeate of corn flour stillage solubles had much lower nitrogen and solids contents than the UF permeate, but the percentage reductions were not quite as great as for corn grits stillage solubles. The end RO concentrate volume from corn flour stillage solubles (450 ml) (Table III) was considerably higher than that for corn grits stillage solubles (200 ml) because the permeate flow rate for corn flour stillage solubles decreased drastically and RO was stopped earlier. Apparently, high osmotic pressure or viscosity, due to higher solids in the solution, counteracted most of the pressure applied by the pump.

UF and RO of Degerminated Corn Meal Stillage Solubles

Table IV summarizes UF and RO results for degerminated corn meal stillage solubles, which had a much higher ash content (as per cent of dry matter) than did stillage solubles from corn grits or flour. UF of degerminated corn meal stillage solubles resulted in a permeate having approximately half the original nitrogen and solids contents, and a concentrate having about twice the nitrogen and solids contents of the stillage solubles. Two fractions each of RO permeate and RO concentrate were collected. "Total" refers to the sum or composite value, and "end" refers to the final fraction in Table IV for permeate and concentrate. When the UF permeate was subjected to RO, the resulting RO permeate (total) had a fivefold decrease in nitrogen and a tenfold decrease in solids contents. The RO concentrate (total) had a threefold increase in nitrogen and solids contents compared with UF permeate.

UF and RO of Corn Hominy Feed Stillage Solubles

The nitrogen, solids and ash contents of corn hominy feed stillage solubles (Table V) are similar to those of degerminated corn meal stillage solubles. After UF of hominy feed stillage solubles, the permeate had half the nitrogen and solids contents, and the concentrate had higher nitrogen and solids contents, compared with the stillage solubles. Nine fractions each of RO permeate and RO concentrate were collected. "End" means the final fraction and "total" means the sum or composite of all fractions. Fractions 1 through 8 were 200 ml for RO permeate and 50 ml for RO concentrate. When the UF permeate was subjected to RO, fifteenfold decreases in nitrogen and solids contents for RO

TABLE IV

Ultrafiltration and Reverse Osmosis of Degerminated Corn Meal Stillage Solubles[a]

	Volume, ml	Nitrogen, mg/ml	Solids, mg/ml	Ash, % of dry matter
Stillage solubles	4,268	0.691	29.60	20.82
Permeate (UF)	4,040	0.337	15.38	30.37
Concentrate (UF)	248	1.40	49.95	13.60
Permeate (RO), total	3,598	0.070	1.50	24.44
Permeate (RO), end	1,500	0.073	2.19	32.3
Concentrate (RO), total	345	0.910	40.87	28.8
Concentrate (RO), end	245	1.08	48.62	28.6

[a]Permeate from ultrafiltration (UF) accounts for 95% of original volume, 49% of total solids and 46% of total nitrogen. Permeate (UF) was used as feed solution for reverse osmosis (RO) after about 100 ml was removed for analyses. Permeate (RO) accounts for 92% of original volume, 9% of total solids and 19% of total nitrogen of permeate (UF). The original volume of UF permeate for RO was 3,920 ml. In addition to the permeate and concentrate, holdup volume in the machine and water loss from evaporation during processing also accounted for the initial volume.

TABLE V

Ultrafiltration and Reverse Osmosis of Corn Hominy Feed Stillage Solubles[a]

	Volume, ml	Nitrogen, mg/ml	Solids, mg/ml	Ash, % of dry matter
Stillage solubles	3,040	0.759	32.08	18.67
Permeate (UF)	2,580	0.355	16.50	28.62
Concentrate (UF)	106	1.04	37.44	16.56
Permeate (RO), total	1,699	0.023	1.11	20.2
Permeate (RO), end	85	0.063	2.23	23.9
Concentrate (RO), total	634	0.513	21.91	27.9
Concentrate (RO), end	234	0.691	27.92	28.1

[a]Permeate from ultrafiltration (UF) accounts for 85% of original volume, 44% of total solids and 40% of total nitrogen. Permeate (UF) was used as feed solution for reverse osmosis (RO) after about 100 ml was removed for analyses. Permeate (RO) accounts for 70% of original volume, 5% total solids and 5% total nitrogen of permeate (UF). In addition to the permeate and concentrate, holdup volume in the machine and water loss from evaporation during processing also accounted for the initial volume. The original volume of UF permeate for RO was 2,430 ml.

permeate and higher nitrogen and solids contents for RO concentrate were experienced. Reduction in nitrogen and solids contents of RO permeate was the largest, on a percentage basis, among all dry-milled corn fractions stillage solubles studied.

CONCLUSION

The combination of UF and RO processing of stillage solubles from corn grits, flour, degerminated meal and hominy feed appears to be an attractive method to separate stillage solubles into a large volume of permeate having low nitrogen and solids content and a small volume of concentrate with high nitrogen and solids contents. The RO permeate may be reused as process water, further treated, or discharged.

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✤The Structure of Rosmariquinone - A New Antioxidant Isolated from *Rosmarinus officinalis L.*

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ABSTRACT

A new diterpene, named rosmariquinone, was isolated from the leaves of *Rosmarinus officinalis L.* The leaves were first extracted using methanol and, upon further purification, this extract yielded rosmariquinone. Structure elucidation of the antioxidant was done using IR, MS, ^{1}H-NMR and ^{13}C-NMR.

INTRODUCTION

Work by Chipault et al. (1,2) described the antioxidant properties of more than 30 spices. They reported that rosemary *(Rosmarinus officinalis L.)* and sage *(Salvia officinalis L.)* had the greatest antioxidant properties of all the spices tested. The antioxidant findings concerning rosemary triggered several investigations aimed at isolating and identifying the active compounds in this spice.

The first important antioxidant compound isolated from *Rosmarinus Officinalis L.* was a phenolic diterpene named carnosol. The structure of carnosol was determined correctly by Brieskorn et al. (3). Wu et al. (4), using a different isolation method, also identified carnosol from rosemary leaves. Inatani et al. (5) isolated and characterized a new antioxidant, rosmanol, from rosemary leaves. Rosmanol is also a phenolic diterpene with a structure similar to that of carnosol. Recently, we reported (6) a novel antioxidant compound, rosmaridiphenol, from rosemary leaves. In addition to these specific compounds, several investigators (7-10) have reported preparing various extracts of rosemary which contained antioxidant activity.

This paper reports the isolation and characterization of a new antioxidant, rosmariquinone, from rosemary leaves.

EXPERIMENTAL

Extraction and Fractionation of Rosemary Leaves

A rosemary antioxidant product was prepared from ground rosemary leaves following a procedure described by Wu et al. (4). This antioxidant was fractionated by using a

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