⁂Homogeneous Catalytic Hydrogenation of Canola Oil Using a Ruthenium Catalyst

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ABSTRACT

Dichlorodicarbonylbis(triphenylphosphine)ruthenium(II), $RuCl_2(CO)_2(PPh_3)_2$, was investigated as a catalyst for edible oil hydrogenation in a preliminary screening of potential catalysts for producing partially hydrogenated fats with low *trans*-isomer content. Refined, bleached and deodorized canola oil was hydrogenated using $1.77 \times 10^{-5} - 6.64 \times 10^{-4}$ mol/kg-oil of ruthenium catalyst equivalent to $1.79 \times 10^{-4} - 6.71 \times 10^{-3}$ wt% Ru. The effects of temperature (50-180 C) and pressure (50-750 psig) on reaction rate, *trans*-isomer content and fatty acid composition were examined. The activities of $RuCl_2(CO)_2(PPh_3)_2$ and nickel (Nysel HK-4 and AOCS standard nickel catalyst) were compared on a molar basis. At 4.40×10^{-4} mol/kg-oil (0.0026 wt/ Ni or 0.0044 wt% Ru), 140 C and 50 psig, the nickel catalysts were completely inactive, but the ruthenium catalyst produced an IV drop of 40 units in 60 min. At 110 C, 750 psig and 1.34×10^{-4} mol/kg-oil (1.35×10^{-3} wt% Ru), a hydrogenation rate of 0.89 ΔIV/min and a maximum *trans*-isomer content of 10.4% (IV = 45.0) was obtained with the ruthenium catalyst.

INTRODUCTION

For over 80 years nickel has been the major catalyst for edible oil hydrogenation. It is active, stable, inexpensive and easily removed from hydrogenated oil. However, nickel does have well-defined limitations which have become the focus of considerable research (1). In particular, it catalyzes undesirable side-reactions such as *cis, trans* and positional isomerization of double bonds. Furthermore, nickel exhibits poor linolenate selectivity which is a definite disadvantage when processing oils such as soybean and canola (2).

In Canada, a study was commissioned by the Department of Health and Welfare Canada to review the relationship between *trans* isomers and human health. The report suggested that hydrogenated oil products should contain less than 1% *trans*, *trans*-octadecadienoic acid, less than 25% saturates, and that the total amount of saturated plus *trans*-fatty acids should not exceed 40% (3). The committee also recommended that further research by government, university and industry should be encouraged, and that a review of the field be conducted every five years.

Applewhite (4) reviewed the subject thoroughly from a different point of view. However, the issue is not settled. Thus, the FDA recently has commissioned the Federation of American Societies for Experimental Biology to undertake a study on *trans*-fatty acids (5). Based on this continuing controversy, we feel it would be prudent to develop alternative catalyst systems which would minimize the formation of *trans*-fatty acids.

The project formed a part of a larger program in the Food Engineering Group at the University of Toronto aimed at identifying potential catalysts that can hydrogenate oils to low or zero *trans*-isomer content, and to develop these into practical catalyst systems. The literature contains many references to catalysts that exhibit high *cis*-selectivity during hydrogenation (6-13). Gray and Russell (12) reviewed the field in 1979. For the present study we reviewed the catalytic activity of ruthenium catalysts that showed promise on the basis of their activity and selectivity in non-fat systems. Fahey (6) studied a number of different ruthenium compounds as potential hydrogenation catalysts, and found $RuCl_2(CO)_2(PPh_3)_2$ to be the most active and thermally stable. This catalyst, in the presence of a suitable Lewis base at 125-160 C and 200 psi, reduced cyclododecatriene to cyclododecene with 98.5% selectivity (selectivity defined as monoene/[monoene + saturate] × 100%). The catalyst was recovered and could be recycled repeatedly without appreciable loss in activity after the removal of the hydrogenated products by distillation.

In a subsequent study, Fahey (13) investigated the catalytic properties of $RuCl_2(CO)_2(PPh_3)_2$ in greater detail. By studying a variety of alkenes and alkadienes he found that reaction rates decreased in the following order: conjugated dienes > nonconjugated dienes > terminal alkenes > internal alkenes. Depending on the solvent used, polyenes could be reduced to monoenes with varying degrees of selectivity.

Based on the literature data, $RuCl_2(CO)_2(PPh_3)_2$ was selected for experimental investigation.

MATERIALS AND METHODS

Refined, bleached and deodorized canola oil, commercially prepared by Canada Packers, Inc., Toronto, was used throughout this study. $RuCl_2(CO)_2(PPh_3)_2$ purchased from Strem Chemicals, Inc., Newburyport, Massachusetts, was used without pretreatment or additional purification. AOCS standard catalyst (Lot No. 1, April 1977 [25 wt% Ni]) was obtained from the American Oil Chemists' Society, and Nysel HK-4 was obtained from Harshaw/Filtrol Chemical Co., Cleveland, Ohio.

Hydrogenations were carried out in a 300 ml Parr Pressure Reactor Model 4521 (Parr Instrument Co., Moline, Illinois). In a typical run, 150 g of canola oil plus the appropriate amount of catalyst were placed in the reactor bomb. After degassing the oil-catalyst mixture in vacuo, nitrogen gas was introduced to 20 psig. When the oil reached reaction temperature, the nitrogen gas was evacuated and hydrogen gas was introduced to the required pressure. The stirrer was then turned on. Samples were taken at predetermined intervals without interrupting the hydrogenation process. During hydrogenation, catalyst activity was approximated by measuring the drop in refractive index as a function of time using a Bausch and Lomb Refractometer Model No. ABBE-3L. Iodine value was determined by the Wijs method (AOCS method Cd 1-25). The *trans*-isomer content was determined by the AOCS spectrophotometric method using a Beckman IR-9 or a Perkin-Elmer 598 double-beam spectrophotometer (AOCS method Cd 14-61) and elaidic acid, 99%+ pure, as the standard. The specific isomerization index, SII, defined as change in *trans* isomers × 100% divided by change in iodine value, was calculated as an indicator of *trans* selectivity. Fatty acid methyl esters were prepared using the procedure described in AOCS method Cc 2-66. A Hewlett-Packard Model 5840A gas chromatograph equipped with a 30-meter, SP-2330 coated, glass capillary column (Supelco, Inc., Bellefonte, Pennsylvania), and a flame ionization detector (FID) was used for separation and quantitation of these esters. Fatty acid methyl ester standards ranging from C-14 to C-24 were purchased from Nu Chek Prep, Inc., Elysian, Minnesota.

RESULTS AND DISCUSSION

Effect of Pressure

For hydrogenation runs using $RuCl_2(CO)_2(PPh_3)_2$, pressure had a pronounced effect on the hydrogenation rate and product distribution. The effect of pressure was examined

over the range 50 to 750 psig. Even though this catalyst performed best at higher pressures, lower pressure runs also were included so that the ruthenium and commercial nickel catalysts could be compared under temperature and pressure conditions typical of those used in the edible oil industry.

Runs 1, 2, 3 and 4 (Fig. 1) clearly illustrate the effect of pressure on activity. As the pressure increased from 50 to 600 psig, the activity increased from 0.55 to 4.00 ΔIV/min (averaged over the first 15 min of hydrogenation). The relationship between *trans*-isomer formation and degree of hydrogenation (iodine value) for runs 1 to 4 is shown in Figure 2. At low pressures (run 1), large amounts of *trans*-isomers were formed, with a corresponding high SII value of 157.1. As pressure increased, the level of *trans*-isomers steadily decreased. For example, at IV = 80 the *trans*-isomer content was reduced by over 40 percentage points from ~60% at 50 psig to ~20% at 600 psig. The SII for the same two runs dropped from 157.1 at 50 psig to 22.9 at 600 psig. In addition, a characteristic shifting of *trans*-isomer maxima to lower iodine values was observed.

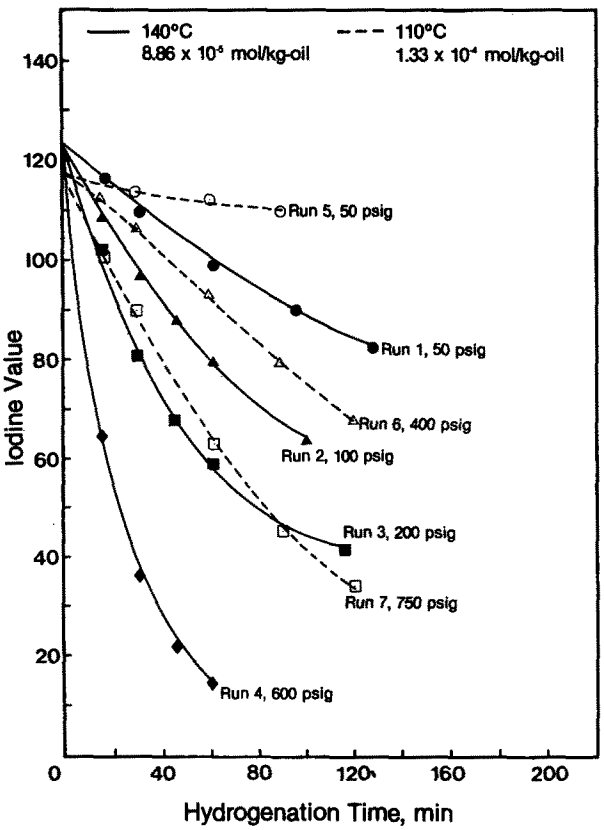

FIG. 1. Effect of pressure on hydrogenation rate.

In another set of experiments (Figs. 1 and 2, runs 5, 6 and 7), the temperature was lowered from 140 C to 110 C and the catalyst concentration was increased to 1.33×10^{-4} mol/kg-oil (1.34×10^{-3} wt % Ru). As expected, activity increased and *trans*-isomer formation decreased with increasing pressure. The lower temperature used resulted in lower overall activity and *trans*-isomer formation. Comparing runs 1 and 4b with runs 5 and 7, respectively, illustrates the effect of temperature on *trans*-isomer formation. As will be shown later, *trans*-isomer formation is relatively insensitive to changes in catalyst concentration in the range of concentration studied (Table I). In both cases, decreasing the temperature from 140 C to 110 C resulted in lower SII values. In the case of runs 4b and 7 the SII was lowered from 22.1 at 140 C to 14.4 at 110 C.

The reduction of hydrogenation rate may be counteracted by increasing the pressure. Fortunately, higher pressures reduce the formation of *trans*-isomers. Consequently, by balancing temperature and pressure, it is possible to optimize the hydrogenation in terms of *trans*-isomer content, final IV, and reaction rate. For example, hydrogenating at 750 psig, 110 C and 1.33×10^{-4} mol/kg-oil (run 7) produced less than 10% *trans*-isomer content (maximum) at a high catalyst activity.

Effect of Temperature

Temperature had an effect on the hydrogenation rate but, unlike pressure, the relationship was not uniform over the entire range studied. The temperature was varied from 50 to 180 C while the pressure and catalyst concentration were maintained constant at 50 psig and 8.86×10^{-5} mol/kg-oil (8.95×10^{-3} wt%), respectively. Figure 3 illustrates the relationship between temperature and hydrogenation rate.

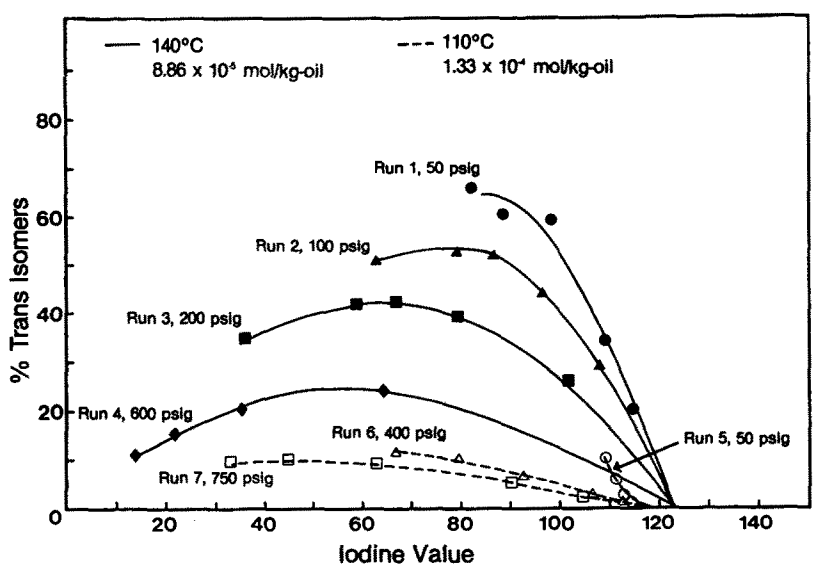

FIG. 2. Effect of pressure on *trans*-isomer formation.

TABLE I

Hydrogenation of Canola Oil with $RuCl_2(CO)_2(PPh_3)_2$

Run[a]	Time[b] (min)	Pressure (psig)	Temp. (C)	Catalyst conc. (mol/kg-oil X 10^5)	Ru (wt% X 10^4)	Final IV[c]	Maximum *trans* isomers[d], (%)	IV[e]	SII[f]
				Effect of pressure					
1	128	50	140	8.8	8.9	82.3	62.5	82.3	157.1
2	100	100	140	8.9	9.0	63.3	52.3	78.9	116.0
3	115	200	140	8.9	9.0	40.9	42.4	67.2	74.6
4	60	600	140	8.9	9.0	14.5	20.2	35.7	22.9
4b	60	750	140	8.8	8.9	26.4	18.9	38.5	22.1
5	90	50	110	13.0	13.1	110.1	10.5	110.1	152.2
6	120	400	110	13.2	13.3	67.0	12.5	67.0	25.0
7	120	750	110	13.4	13.5	33.3	10.4	45.0	14.4
				Effect of temperature					
8	120	50	50	8.8	8.9	114.5	0.0	114.5	0.0
9	120	50	100	8.9	9.0	112.4	4.1	112.4	89.1
10	121	50	140	8.9	9.0	80.0	55.2	80.0	149.2
11	90	50	180	8.8	8.9	80.4	73.7	81.8	209.4
12	120	750	110	1.8	1.8	109.0	7.0	109.0	46.7
13	90	750	110	4.6	4.6	62.5	9.7	62.5	15.8
14	90	750	110	66.4	67.1	31.6	9.8	47.2	14.0

[a]The IV of unhydrogenated oil for runs 5-11 and 14 is 117. For the remaining runs, the IV is 124.
[b]Time required to complete the hydrogenation run.
[c]Wijs iodine value at the end of hydrogenation.
[d]Maximum amount of *trans* isomers formed during the hydrogenation run.
[e]Wijs iodine value at the maximum *trans*-isomer content.
[f]Specific isomerization index, SII, is defined as: % *trans* isomers X 100/ΔIV.

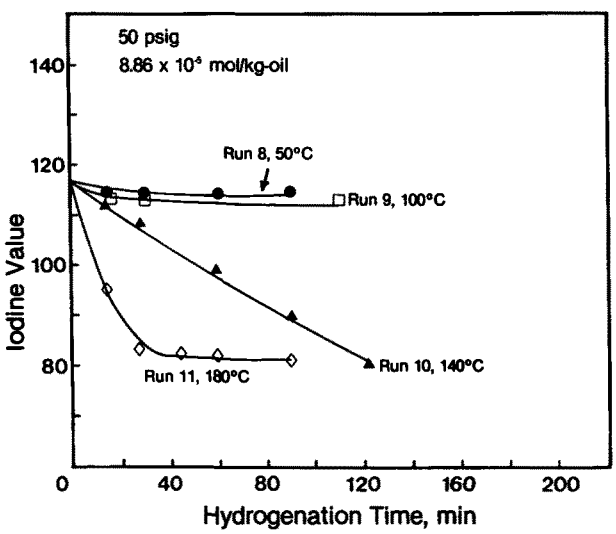

FIG. 3. Effect of temperature on hydrogenation rate.

Above a relatively narrow temperature range, activity increased rapidly. While there was no measurable change in activity when the temperature was increased from 50 to 100 C, the change from 100 to 140 C resulted in a dramatic increase in activity. The exact temperature where the activity changes abruptly was not determined. Fahey (6) reported similar behavior when 1,5,9-cyclododecatriene was hydrogenated using $RuCl_2(CO)_2(PPh_3)_2$. He noted that below 135 C hydrogenation occurred very slowly, and below 125 C the reaction did not occur at all.

Runs 8 to 11 in Figure 4 show that *trans*-isomer formation increases with increasing temperature. A combination of low pressure (50 psig) and high temperature (180 C) was highly selective for the formation of *trans*-isomers, as shown by the observed SII value of 209.4 in run 11 (also see Table I). The maximum *trans*-isomer concentration in this run was 73% at IV = 81.8.

Higher temperatures produced a dramatic change in the activity and *trans*-isomer profiles. For example, at 180 C (run 11, Fig. 3) the hydrogenation rate, during the initial portion of the run, remained constant at 1.35 ΔIV/min. However, at an iodine value near 83, the hydrogenation rate abruptly leveled off to zero. For the same run (run 11, Fig. 4) the *trans*-isomer content increased rapidly to over 70% until an iodine value of 83.5 was reached. After this point the *trans*-isomer content did not change, with increasing hydrogenation time providing additional evidence that activity had decreased to zero.

Catalysts such as methyl benzoate-tricarbonylchromium-(O) exhibit infinite linoleate selectivity, because no saturates are formed during hydrogenation (7-9). Once the monoene stage is reached no further hydrogenation occurs. In our system, at higher temperatures (180 C, run 11) the hydrogenation also leveled off at an IV of 80-82 (Fig. 3). However, substantial amounts of saturates are produced (Table II, run 11), indicating a mechanism different from methyl benzoate-tricarbonylchromium(O). The reaction stops abruptly, probably because of decomposition of the catalyst. Catalyst poisoning also may be suspect; however, additional work is required.

Effect of Catalyst Concentration

The effect of catalyst concentration ($1.77 \times 10^{-5} - 6.64 \times 10^{-4}$ mol/kg-oil [$1.79 \times 10^{-4} - 6.71 \times 10^{-3}$ wt% Ru]) on activity and *trans*-isomer formation was examined (Table I). It already has been shown that high pressures (750 psig) and moderate temperatures (110 C) promote low *trans*-isomer formation. Hence, these conditions were selected for this comparison.

Canola oil can be hydrogenated at ruthenium catalyst concentrations as low as 1.77×10^{-5} mol/kg-oil. Based on equal metal molarity, the equivalent concentration of nickel is only 1.0×10^{-4} wt% Ni. At these concentration levels,

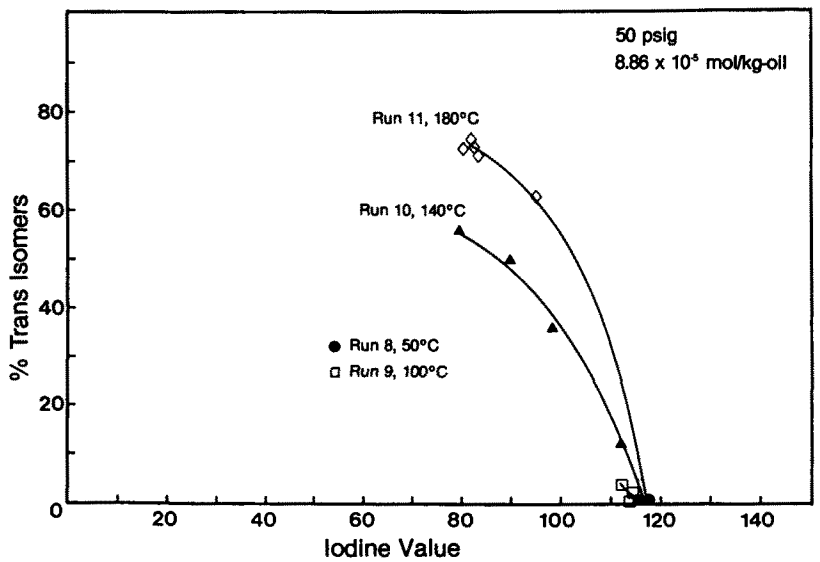

FIG. 4. Effect of temperature on *trans*-isomer formation.

TABLE II

GC Analysis of Canola Oil Hydrogenated with $RuCl_2(CO)_2(PPh_3)_2$

Run[a]	GC Analysis[b], (%)				Selectivity[c]		IV[d]
	S	M	D	T	Ln	L	
1	7.4	66.5	20.0	6.0	1.8	8.9	115.8
2	10.5	69.9	17.3	2.3	2.3	8.1	109.2
3	14.6	66.9	16.8	1.8	2.5	5.0	102.8
4	44.8	53.7	2.4	0.0	–	–	64.4
4b	–	–	–	–	–	–	–
5	6.5	63.9	23.8	5.8	1.3	–	115.2
6	–	–	–	–	–	–	–
7	12.5	63.6	20.3	3.6	1.8	3.2	105
8	6.5	62.8	24.5	6.2	–	–	114.6
9	6.3	63.3	24.3	6.1	–	–	114.3
10	8.2	66.1	21.6	4.1	1.9	8.4	112.7
11	13.6	83.8	2.6	0.0	–	–	95.8
11[e]	22.4	77.6	0.0	0.0	–	–	80.4
12	–	–	–	–	–	–	–
13	17.0	62.6	16.8	3.6	1.7	3.5	98.9
14	14.2	65.3	18.1	2.4	2.0	4.0	99.8

[a]The analysis of unhydrogenated oil for runs 5-11 and 14 is as follows: IV=117; S=6.5%; M=62.7%; D=24.6%; T=6.2%. For the remaining runs: IV=124; S=4.6%; M=60.9%; D=22.5%; T=11.9%.

[b]S=saturate; M=monoene; D=diene; T=triene. Values are reported as weight % of the corresponding fatty acid methyl ester.

[c]Ln=linolenate selectivity; L=linoleate selectivity. Selectivities were calculated using the simplified kinetic model: Triene→Diene→Monoene→Saturate.

[d]Wijs iodine value of sample used for GC analysis.

[e]GC analysis of sample taken at end of hydrogenation run (time=90 min).

nickel catalysts are completely inactive. In fact, nickel must be used at concentrations two orders of magnitude higher in order to obtain rates suitable for commercial edible oil hydrogenation.

The relationship between catalyst concentration and activity was not linear. For example, increasing the catalyst concentration by a factor of 2.6 (i.e., runs 12 and 13 in Table I) resulted in an increase in activity from 0.20 to 0.84 ΔIV/min (a factor of 4.2). At higher levels of activity the effect of catalyst concentration on activity was less pronounced. Comparing run 7 (0.89 ΔIV/min) with run 14 (1.38 ΔIV/min) revealed that a five-fold increase in concentration resulted in an increase in activity by a factor of 1.6.

Catalyst concentration had an insignificant effect on *trans*-isomer formation over most of the range studied (compare runs 7, 13 and 14 in Table I). For example, increasing the catalyst concentration by a factor of 14.4 (runs 13 and 14) produced only a marginal change in the *trans*-isomer profiles. At the low end of the range (i.e., 1.77×10^{-5} to 4.60×10^{-5} mol/kg-oil) there was a significant decrease in SII with increasing catalyst concentration. SII decreased from a high of 46.7 at 1.77×10^{-5} mol/kg-oil (1.79×10^{-4} wt% Ru) to 15.8 at 4.60×10^{-5} mol/kg-oil (4.65×10^{-4} wt% Ru).

It is important to note that the more active runs (runs 13, 14 and 7) produced low *trans*-isomer levels (less than 10% maximum) and SII values less than 16.0, illustrating one of the major advantages of using $RuCl_2(CO)_2(PPh_3)_2$ as an edible oil hydrogenation catalyst.

Effect of Process Variables on Selectivity and Fatty Acid Composition

When canola oil was hydrogenated at 750 psig, 110 C and 1.34×10^{-4} mol/kg-oil or 1.35×10^{-3} wt% Ru (run 7), large amounts of saturates were formed (Table II). Saturates formed immediately and increased rapidly to almost 60% (IV=33.0), while at the same time dienes were reduced to 1%. The linolenate and linoleate selectivities were only 1.9 and 3.2, respectively. Unfortunately, low selectivity (linolenate and linoleate) was typical for $RuCl_2(CO)_2(PPh_3)_2$ regardless of the conditions used. For example, the linolenate selectivity ranged from 1.8 to 2.5 while the linoleate selectivity varied from approximately 3 to 9. In comparison, nickel offers the same range of linolenate selectivity but is far superior in terms of linoleate selectivity, with values as large as 100 reported in the literature (1).

Comparison of $RuCl_2(CO)_2(PPh_3)_2$ with Commercial Nickel Catalysts

$RuCl_2(CO)_2(PPh_3)_2$ is an extremely active catalyst. In one of the first runs attempted, the conditions used were 140 C, 750 psig and 8.86×10^{-3} mol/kg-oil (8.95×10^{-2} wt% Ru), 1 g of catalyst in 150 g of canola oil. After only 10 min of

TABLE III

Comparison of Catalytic Hydrogenation Properties Between $RuCl_2(CO)_2(PPh_3)_2$ and Commercial Nickel Catalysts

Run[a]	Catalyst type	Time[b] (min)	Pressure (psig)	Catalyst conc. (mol/kg-oil kg × 10^5)	Ru (wt% × 10^4)	Final IV[c]	Maximum *trans* isomers[d], (%)	IV[e]	SII[f]
15	Nysel HK-4	125	50	44.2	44.7	121.9	1.4	121.9	66.7
16	AOCS catalyst	90	50	44.9	45.4	121.9	1.8	121.9	85.7
17	$RuCl_2(CO)_2(PPh_3)_2$	90	50	44.7	45.2	73.9	66.5	84.2	167.1
18	AOCS catalyst	150	750	178.0	179.9	95.4	26.2	95.4	91.6
19	Nysel HK-4	120	750	178.0	179.9	92.6	26.1	92.6	83.1
20	$RuCl_2(CO)_2(PPh_3)_2$	15	750	178.0	179.9	20.5	27.4	58.6	41.9

[a]For all runs, T=140 C and IV of unhydrogenated oil is 124.
[b]Time required to complete the hydrogenation run.
[c]Wijs iodine value at the end of hydrogenation.
[d]Maximum amount of *trans* isomers formed during the hydrogenation run.
[e]Wijs iodine value at the maximum *trans*-isomer content.
[f]Specific isomerization index, SII, is defined as: % *trans* isomers × 100/ΔIV.

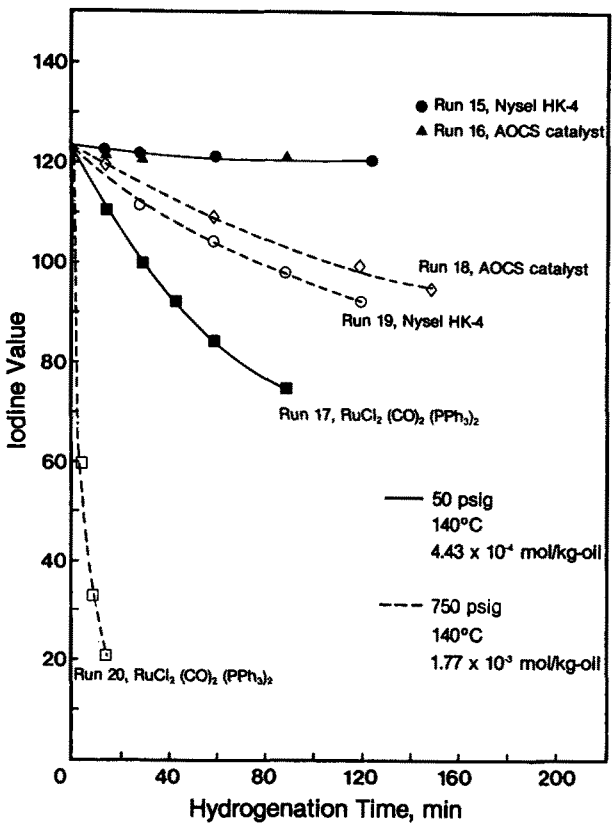

FIG. 5. Comparison of the activities of $RuCl_2(CO)_2(PPh_3)_2$ and commercial nickel catalysts.

hydrogenation, the sampling valve became completely clogged with hardened oil. Later experiments revealed that the hardened oil was completely saturated, indicating that the ruthenium catalyst was capable of hydrogenating at rates as high as 16.6 ΔIV/min. Based on the results of this experiment, it became necessary to reduce the catalyst concentration significantly. In fact, it was found that reducing the concentration by a factor of approximately 100 (0.010-0.015 g of catalyst in 150 g of canola oil) resulted in activities comparable to typical rates observed in commercial edible oil hydrogenation.

In Figure 5, the activity of two types of nickel catalysts and $RuCl_2(CO)_2(PPh_3)_2$ were compared at 140 C, 50 psig and ~4.43×10^{-4} mol/kg-oil (2.6×10^{-3} wt% Ni) (runs 15, 16 and 17). The temperature and pressure selected are typical of the values used in the edible oil industry. As expected, both nickel catalysts (AOCS and Nysel HK-4) proved to be virtually inactive due to low catalyst concentrations (Table III). On the other hand, $RuCl_2(CO)_2(PPh_3)_2$ displayed excellent activity with a hydrogenation rate of over 0.90 ΔIV/min.

It would be more informative to select conditions where both $RuCl_2(CO)_2(PPh_3)_2$ and commercial nickel show activity. In a subsequent comparison (runs 18, 19 and 20 in Fig. 5), the molar catalyst concentration was increased by a factor of 4 to 1.77×10^{-3} mol/kg-oil (1.79×10^{-2} wt% Ru), and the pressure was increased to 750 psig (Table II).

As expected, the increased pressure and catalyst concentration increased the activity of all three catalysts. The initial hydrogenation rates were 13.1, 0.41 and 0.20 ΔIV/min for the $RuCl_2(CO)_2(PPh_3)_2$, Nysel HK-4 and AOCS catalysts, respectively. Nysel HK-4 is designed specifically for oils such as canola, while the AOCS catalyst is suitable for edible oil hydrogenation in general. This could explain why Nysel HK-4 performed better than the AOCS catalyst in this comparison.

The preliminary results presented here demonstrate that $RuCl_2(CO)_2(PPh_3)_2$ is a very active catalyst with the potential for producing partially hydrogenated oils with low *trans*-isomer concentrations. To realize its potential, the catalyst will have to be reused, and a technique for its recovery from the oil must be developed. The immobilization of the catalyst is an approach that will be investigated.

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The Isolation of Omega-3 Polyunsaturated Fatty Acids and Methyl Esters of Fish Oils by Silver Resin Chromatography

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ABSTRACT

Multigram quantities of the highly unsaturated ω3 component from samples of fish oil fatty acids and esters were isolated by silver resin chromatography. An XN1010 resin column saturated with silver ions was utilized. Polyunsaturated fatty acid (PUFA) esters from fish oil concentrate (FOC) were fractionated based on the number of double bonds by using solvent programming (acetonitrile in methanol). Larger samples (4-9 g) of FOC acids and esters and menhaden acids and esters were enriched in ω3 polyunsaturates to 82-99% (95-99% total PUFA) by use of a larger 100% silver resin column and isocratic elution with 30, 35 or 45% acetonitrile in acetone.

INTRODUCTION

During the last decade, interest in the dietary effects of PUFAs and specifically of ω3 polyunsaturated fatty acids has increased. A number of studies have correlated the effects of PUFAs on tumors (1), atherosclerotic diseases, membrane function and blood lipid composition (2-5). Samples enriched in these fatty acids are needed to further investigate their nutritional, health and biochemical effects and to serve as secondary analytical standards. For these reasons, we have investigated the fractionation and isolation of PUFAs from relatively large samples of fish oil by silver resin chromatography.

Separation of analytical quantities of PUFA methyl esters and other FA derivatives by reverse phase chromatography (6-10) and silver impregnated silica (11) have been reported previously. While PUFA-enriched oils can be produced by low temperature crystallization (12), molecular distillation (13) and supercritical fluid extraction (Krokonis, V.J., unpublished results, Phasex Corp., Nashua, New Hampshire, 1984), these techniques resulted in enrichments to only 70 to 85% of total FA. We found that silver resin chromatography could be used to provide more highly enriched PUFA fractions and also to isolate specific fatty acid methyl esters based on the number of double bonds.

EXPERIMENTAL

Materials

XN1010 sulfonic acid resin (16/50 mesh) was obtained from Rohm and Haas. A 50% ω3 fish oil concentrate (FOC) (Jahres Frabriker, Sandefjord, Norway) was esterified by sulfuric acid/methanol. Menhaden oil (Zapata Haynie Corp., Reedville, Virginia) was transesterified (sodium metal in methanol) to obtain the fatty acid methyl esters (FAMEs) and saponified (alcoholic potassium hydroxide) to prepare the fatty acids.

Methods

The preparation (grinding, sieving, sodium and silver ion incorporation) and nomenclature of the XN1010 resin have been described previously (14-17). Two columns were prepared. Column A (2.7 × 60 cm) was slurry packed with ca. 180 ml (65 g) of 200/270 mesh, 100% Ag^+/Na^+ resin and was used for sample sizes up to 500 mg of fatty acids or methyl esters. 100% Ag^+/Na^+ means that the sulfonic acid protons of the resin were first replaced by Na^+ ions and then 100% of these Na^+ ions were replaced by Ag^+ ions (17). Solvent programming was accomplished with two 6000A HPLC pumps and a Model 660 Solvent Programmer (all Waters Associates). The eluants were monitored by an ultraviolet detector (ISCO, Model 1840) at 210 nm. Column B, a slurry packed 4.7 × 45 cm Michel-Miller column (Ace Glass, Vineland, New Jersey), contained ca. 750 ml (250 g) of 100/200 mesh, 100% Ag^+/Na^+ XN1010 resin and had a fatty acid capacity of ca. 10 g. Compounds applied to column B were eluted isocratically. The mixed solvents were pumped by a metering pump (Metering Pumps Ltd., London) and products were monitored by a refractive index detector (Waters Associates).

Solvents were removed from the eluted fractions by rotary evaporation. Fatty acids were methylated with diazomethane. The FAMEs were analyzed in a Packard Model 428 gas chromatograph equipped with a 100 m × 0.25 mm (0.2 μm coating) SP 2560 fused silica capillary column (Supelco Inc., Bellefonte, Pennsylvania). Helium carrier gas and a flame ionization detector were utilized. The oven temperature was programmed from 190 to 215 C at 10 C/min with an initial hold of 30 min. Both FAME standards and gas chromatography/mass spectroscopy (GC/MS) were used to identify the various FAMEs. GC/MS analyses were made with a Finnigan gas chromatograph/EI-CI MS system equipped with a 30 m × .319 mm DB-1 capillary column. The GC was programmed from 176 to 221 C at 2.3 C/min and then 221 to 250 C at 8 C/min with helium as carrier gas.

RESULTS

The columns employed, solvents, sample sizes and other parameters are summarized in Table I. The compositions of the starting materials are tabulated in Table II and of the

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