**Title:** LOW-SATURATE EDIBLE OILS AND TRANSESTERIFICATION METHODS FOR PRODUCTION THEREOF

**Abstract**

Transesterified vegetable oils having a very low saturated fatty acid content and batch, cocurrent and countercurrent enzymatic transesterification methods of preparing such oils are disclosed. The edible fats and oils are a rich source of energy in the diet and are important in the synthesis of membranes and other cell components.
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LOW-SATURATE EDIBLE OILS AND TRANSESTERIFICATION METHODS FOR PRODUCTION THEREOF

Background of the Invention

The present invention is directed to methods for preparing edible triglyceride vegetable oils having a very low level of saturated fatty acid components, and to enzymatic transesterification methods for producing such low-saturate, edible oil products.

Edible oils and fats typically primarily comprise various fatty acid triesters of glycerol with the structure of the fatty acid moieties and their distribution on the glycerol backbone determining the physical characteristics of the oil or fat. The specific types of fatty acids also play an important role in diet and health. Fats and oils in general are a rich source of energy in the diet and are important in the synthesis of membranes and other essential cell components. Moreover, dietary fatty acid content may potentially be controlled to affect physiological characteristics such as serum cholesterol levels. For example, studies of normcholesterolemic men has shown that a dietary decrease in saturated fatty acids may have more of an effect in lowering serum cholesterol [Keys, "Prediction of Serum Cholesterol Response to Change in Fats in the Diet", Lancet, 2:959-962] than an increase in polyunsaturated fatty acids.

Natural vegetable oil triglycerides typically contain substantial amounts of esterified saturated fatty acids. For example, soybean oil may typically contain about 14-16 weight percent of esterified saturated fatty acids, and natural canola oil may contain about 5-8 weight percent of esterified saturated fatty acids. Intermediate carbon chain length (i.e., C₁₂-C₁₆) dietary saturated fatty acids, notably lauric, myristic and palmitic acids, have been reported in the medical literature as being a more significant factor in the increase of plasma cholesterol than stearic acid, which has been reported to
have minimal or even reducing effects on cholesterol levels ["Effect of Dietary Stearic Acid on Plasma Cholesterol and Lipoprotein Levels", Bonanome, et al., New England Journal of Medicine, Vol. 318, 1244-1271 (1988)]. Soybean oil and canola oil typically contain, respectively, over 10 percent and over 3 percent by weight of esterified intermediate chain length saturated fatty acids, primarily palmitic acid. Accordingly, it would be desirable to economically produce or manufacture triglyceride oils having very low levels of saturated fatty acids, and particularly low levels of lauric, myristic and palmitic acids. Most food products prepared from vegetable oils having less than about 3.5 weight percent of esterified saturated fatty acids may be regarded as substantially free of such fatty acids for regulatory purposes.

Potentially, the modification of vegetable oils to produce low-saturate oil products could be carried out by dehydrogenation, chemical transesterification, enzymatic transesterification or genetic selection and modification. However, dehydrogenation processes are not available for selectively dehydrogenating esterified saturated fatty acids of vegetable oils. The use of somaclonal variation as a means of selection for genetic variation in plant parts which may produce triglycerides with specific desirable fatty acids may be used, but problems may arise in the stabilization of the desired trait in future generations. Recombinant DNA techniques might be used to increase the production of an oil of predetermined composition, but this is a very complex task in difficult or presently unknown areas of plant lipid biosynthesis. [Stumpf, "Biosynthesis and Function of Plant Lipids", Am. Soc. Plant Physiol., pp. 1-15, 1983]. For example, fatty acid synthesis has many potential rate limiting enzymes as well as proteins, such as acetyl-CoA carboxylase, \( \text{ACP-acetyl transferase, 3-oxoacyl ACP synthase, ACP malonyl transferase and acyl carrier protein.} \) Modifications of specific triglyceride synthesis entail changes in fatty
acid composition dependent on acyl ACP thioesterase and various desaturase complexes, as well as acyltransferase enzymes which attach the fatty acid moieties to glycerol. Thus, to isolate the rate limiting enzymes/proteins and their corresponding genes to create transgenic plants which specifically express them in tissues for storage purposes poses substantial technical problems.

Chemical and enzymatic transesterification are well known for modifying the fatty acid composition or distribution of triglyceride oils. Chemical transesterification is based on the use of a chemical catalyst such as sodium methoxide or a sodium metal to promote the migration of the fatty acid moieties between glyceride molecules, to produce a random distribution of the fatty acid moieties.

Enzymatic transesterification of triglycerides may also be used to modify the characteristics and/or composition of triglycerides. Such processes may be used for selective interchange under relatively mild reaction conditions. For example, vegetable oils may be transesterified with a fatty acid or monooester to produce a variety of end products as described in U.S. Patents 4,268,527; 4,426,991; 4,275,011; 4,472,503 and U.K. Application 2,199,397.

Extracellular microbial lipases are generally of three types, depending upon their specificity. One group of lipases is generally nonspecific, both as regards the position on the glycerol molecule which is hydrolyzed or esterified, and the nature of the fatty acid released or esterified. Depending on the reaction conditions, such lipases catalyze the nonselective hydrolysis, alcoholysis and/or esterification (including transesterification) of fatty acid triglycerides. The lipases produced by Candida cylindraceae, also known as C. rugosa (Benzonana, G. and S. Esposito, Biochim. Biophys. Acta, 231:15 (1971)), Corynebacterium acnes, (Hassing, G.S., Ibid. 242:381

A second group of lipases preferentially acts on the primary, 1- and 3- positions of the glycerol or triglyceride molecule. When a 1-,3- positionally specific lipase is used to catalyze the transesterification of a mixture of triglycerides or a mixture of triglyceride plus free fatty acid or monoester, the action of the enzyme is substantially confined to the 1- and 3- positions of the glycerol. The lipases of *Rhizopus delemar* and *Mucor miehei* such as described in U.S. Patent 4,798,793, are examples of 1-,3- specific lipases.

A third group of lipases has substantial selectivity for certain long chain unsaturated fatty acids having a *cis* double bond at the 9- position from the carboxylate group of the fatty acid. Long chain saturated fatty acids, and unsaturated fatty acid esters without a double bond in the 9- position, are only slowly hydrolyzed in the presence of such lipases. Thus the esters of oleic, palmitoleic, linoleic and linolenic acids, all of which have a *cis* double bond in the 9- position, are preferentially hydrolyzed, esterified or transesterified. The presence of an additional double bond between the carboxyl group and the double bond in the 9- position makes fatty acid esters resistant to the action of this lipase. Triglycerides containing medium chain saturated C\textsubscript{10} and C\textsubscript{8} fatty acids may exhibit some, albeit reduced, reactivity with such enzymes. Examples of such delta-9 specific lipases which preferentially act on long-chain fatty acids containing a *cis* double bond in the 9 position are the lipase produced by the mold *Gottichum candidum* [Macrae, A.R., in *Microbial Enzymes and Biotechnology*, edited by W.M. Fogarty, Applied Science Publishers, London, 1983, p. 225, Jensen, R.G., *Lipids* 9:149 (1974), Jensen, R.G., and R.E. Pitas, in *Lipids*, edited by R. Paollette, G. Porcellati and G. Jacini, Raven Press, New York, 1976,
Vol. 1, p. 141], and the lipase produced by *Penicillium cyclopium* [Glyceride Synthesis by Four Kinds of Microbial Lipase, Tsujisaka, et al.; Biochim. Biophys. Acta 489; 415-422 (1977)]. Such lipases will activate transesterification of unsaturated delta-9 fatty acid groups of glyceride oils, but do not affect the saturated acid components of the oils.

Enzymatic methods which may be used to reduce the saturated fatty acid content of vegetable oils to levels below about 3.5 weight percent, and total levels of intermediate chain length fatty acids below about 2 weight percent, would be desirable, and it is an object of the present invention to provide such low-saturate vegetable oils. It is a further object to provide such methods which may be used to provide low-saturate oils having specific unsaturated fatty acid distribution, as well as low saturate edible oils having specific, nutritionally desirable properties of oleic, linoleic and linolenic acids. These and other objects will become apparent from the following detailed description and the accompanying drawings.

**Description of the Drawings**

FIGURE 1 is a process flow diagram for an embodiment of a single step batch or cocurrent continuous enzymatic transesterification reaction method for producing a low saturate triglyceride in accordance with the present invention having less than 3 weight percent esterified saturated fatty acids;

FIGURE 2 is a process flow diagram for a continuous countercurrent selective enzymatic reaction method utilizing a supercritical countercurrent gas stream for producing a low saturate triglyceride oil having less than 3 weight percent of esterified saturated fatty acids;

FIGURE 3 is a process flow diagram for another method of preparing a low-saturate edible oil by countercurrent enzymatic transesterification reaction, utilizing a countercurrent subcritical gas; and
FIGURE 4 is a process flow diagram of another embodiment of a transesterification method for transesterifying an unsaturated fatty acid monoester with saturated fatty acid-containing triglyceride vegetable oil to provide a triglyceride oil having very low saturated fatty acid content.

Description of the Invention

Generally in accordance with the present invention, methods are provided for manufacturing a triglyceride oil having less than 3.5 and preferably less than 3 weight percent of esterified saturated fatty acid, and desirably less than 2, and more preferably less than 1 weight percent of intermediate chain length esterified saturated fatty acid.

Various aspects of the present invention are also directed to low saturate liquid vegetable oil products having less than 3.5 weight percent, and preferably less than 3 weight percent saturated fatty acid moieties, and specific unsaturated fatty acid distribution, which liquid vegetable oil products have desirable properties for use in food products such as mayonnaise, margarine, table spreads or salad dressings. Such low saturate vegetable oil products will also desirably have less than about 2 and preferably less than 1 weight percent of intermediate chain saturated fatty acids. By "intermediate chain saturated fatty acid" is meant, lauric, myristic and palmitic acids having carbon chain lengths of C_{12}, C_{14} and C_{16}, respectively. As used herein, the weight percent of saturated or unsaturated fatty acid moieties in a margarine oil or vegetable oil glyceride composition is calculated based on the total weight of the fatty acids contained in the margarine oil. Also, as used herein, when referring to weight percent of one or more fatty acid moieties of a composition (such as the weight percentage of C_{12}-C_{16} fatty acids), the weight percent is calculated based on all of the fatty acids of the composition being fully
hydrolyzed to free fatty acid. The weight percent of one or more species of fatty acid is then calculated as the weight percent of such one or more species based on the total weight of free fatty acids. AOCS official method Ce 1-62 (81) may be used to determine the weight percent of respective fatty acids of an oil or fat composition.

In accordance with various method aspects of the present invention, a high-unsaturate vegetable oil, such as canola (low erucic acid rapeseed) oil, high oleic safflower oil, high oleic sunflower oil, high linoleic safflower oil, high linoleic sunflower, corn oil, olive oil, peanut oil, soybean oil or mixtures thereof, having from about 4 weight percent to about 18, weight percent of saturated fatty acids with respect to the total fatty acid content of the vegetable oil is provided for transesterification reaction. Canola oil is a particularly desirable starting material because it has a relatively low amount of saturated fatty acid content. The vegetable oil should best be refined and bleached oil which is substantially free of proteinaceous and other nonglyceride components which might poison or otherwise interfere with transesterification enzymes.

Canola oil typically comprises in the range of from about 5 to about 7 weight percent of saturated fatty acid moieties. However, the saturated fatty acids of canola oil (and other vegetable oils) is not randomly distributed with respect to the 1-, 2- and 3- positions of the glyceride molecules of the oil. A major portion of the saturated fatty acid content is located in the 1- and 3- primary positions of the glyceride molecules of the canola oil. In this regard, canola oil typically may have an overall stearic acid content of about 1.5 - 2 weight percent based on the total fatty acid content of the oil, with from about 2 to about 3 weight percent of the fatty acid moieties in the 1- and 3- positions being stearic acid and less than about 1 weight percent of the fatty acids in
the secondary 2- position being stearic acid. Similarly, canola oil may typically have an overall palmitic acid content of about 4 weight percent, with about 6 weight percent of the fatty acid groups in the 1- and 3- positions being palmitic acid and less than about 1 weight percent of the fatty acid groups in the 2- position being palmitic acid.

In accordance with the present methods, the saturated fatty acids of the primary 1- and 3- positions are selectively removed, and replaced with unsaturated fatty acids by selective transesterification reaction. This may be accomplished without substantially affecting the fatty acid distribution of the starting material oil in the 2- position. By "transesterification" is meant an exchange of fatty acid moiety or acyl radical at a glyceride oxygen or hydroxyl group, which includes interesterification and intraesterification.

In order to carry out such methods, the high unsaturate vegetable oil such as canola oil is combined with an unsaturated fatty acid transesterification component selected from the group consisting of free fatty acids, fatty acid monoesters of lower alkyl monohydric alcohols (e.g., methanol, ethanol and propane), and mixtures thereof, which has less than 2 weight percent, and preferably less than 1 weight percent of saturated fatty acids to provide a transesterification blend. The unsaturated fatty acid transesterification component should comprise at least about 98 weight percent of unsaturated fatty acids having a chain length of from 12 to about 22 carbon atoms, and less than 2 weight percent of saturated fatty acids having a carbon chain length in the range of from 12 to 18 carbon atoms based on the total fatty acid content of the transesterification component. Desirably, the fatty acid transesterification component comprises less than 0.75 weight percent and preferably less than 0.5 weight percent of intermediate chain saturated fatty acids,
by weight, based on the fatty acid content of the
transesterification component.

The high-unsaturate vegetable oil such as canola
oil and the unsaturated fatty acid are combined in a weight
ratio of vegetable oil to unsaturated fatty acid
transesterification component in the range of from about
1:10 to about 4:1 in batch reaction processes (which may
utilize multiple reaction steps), and typically in the
range of from about 1:2 to about 2:1 in single stage batch
or cocurrent reactions to provide a transesterification
mixture. The ratio of reactants may be selected to provide
a desired degree of substitution under the reaction
conditions, to provide a low-saturate glyceride product
having a selective level of saturated fatty acid content
below about 3.5 weight percent, and preferably below 3
weight percent. Moreover, the ratio of reactants and the
composition of the unsaturated fatty acid transesterifi-
cation component may be selected to provide specific
compositions of the transesterified low saturate oil, in
terms of unsaturated fatty acid composition. For example,
nutritionally desirable unsaturated fatty acids in
appropriate levels and ratios have been identified such
that an increase in the omega-3 to omega-6 ratio in the
average diet could yield distinct health benefits. Natural
triglycerides do not typically contain these unsaturated
acids in such proportions, but such compositions may be
provided in accordance with the present invention. Further
in accordance with methods of the present invention, the
transesterification mixture is contacted with a
transesterification enzyme, which is desirably a 1-, 3-
specific transesterification enzyme such as an immobilized
lipase from Mucor miehei as described in U.S. Patent
4,798,793 issued January 17, 1989, which is incorporated by
reference herein. The transesterification mixture is
contacted with the immobilized enzyme under time and
temperature conditions for substantially equilibrating the
fatty acid content in the 1- and 3- positions of the glyceride component, with the fatty acid transesterification components of the reaction mixture. The enzymatic transesterification reaction produces a transesterified triglyceride component and a transesterified fatty acid component. The reaction time may range from about 0.5 hour to about 100 hours, depending upon the concentration and activity of the lipase and the temperature of the reaction mixture. The transesterification reaction will desirably be carried out at a temperature in the range of from about 20° C. to about 80° C., and more preferably in the range of from about 40° C. to about 70° C. By "substantially equilibrate" is meant that the transesterification reaction is at least about 50 percent complete, and preferably at least 90 percent complete. Lower equilibrium transesterification conditions (e.g., 50-90 percent equilibrated) may be utilized to increase the reaction speed and or reduce the amount of enzyme used, but this increases the unsaturated fatty acid required and increases the separation step processing requirements.

Following enzymatic transesterification, a transesterified fatty acid component is separated from the transesterified glyceride component of the transesterification mixture. The transesterified glyceride component has less than 3 weight percent esterified saturated fatty acid content, based on the total weight of the glyceride. Depending upon the ratio of initial components and the extent of transesterification reaction, the transesterified glyceride component will comprise less than 3.5 weight percent of saturated fatty acids based on the total weight of fatty acids in the transesterified glyceride component, and may preferably have less than 3 weight percent of esterified saturated fatty acids, and for specific uses may desirably comprise less than two weight percent of saturated fatty acids.

Further, in accordance with the present invention, the transesterified fatty acid component separated from the
transesterification mixture is fractionated to separate the unsaturated fatty acids from saturated fatty acids, to provide a recycle unsaturated fatty acid source material comprising less than 2 weight percent and preferably less than 1 weight percent of saturated fatty acids, based on the total weight of fatty acids in the source material. The recycle fatty acid source material is combined in a recyclic manner with the high unsaturated vegetable oil such as canola oil as an unsaturated fatty acid transesterification component, as previously described, to produce low-saturate triglyceride oils in accordance with the present invention. The recyclic use of the unsaturated fatty acid components is important to the economics of the process.

The fractionation of saturated fatty acids from the fatty acid mixture to provide such a low level of saturated fatty acid content is a difficult fractionation step and may be carried out by a variety of procedures, such as vacuum distillation, selective urea adduction and/or selective adsorption chromatography. The saturated fatty acids are difficult to remove from unsaturated fatty acids at low concentration levels. Solvent crystallization at low temperatures may be used to remove at least a portion of the saturated fatty acid content, although typically such solvent crystallization procedures do not reduce the saturated fatty acid content to levels below about 2-3 weight percent of the fatty acid mixture. However, such solvent crystallization fractionation procedures may be used to reduce the saturated fatty acid content for subsequent procedures such as molecular distillation, urea adduction or other selective absorption fractionation procedures. It is important that the technique utilized ultimately provide a separation such that an unsaturated fatty acid recycle component having less than 2 weight percent by weight, based on the total weight of the fatty acids of the recycle component, and
preferably less than 1 weight percent, is provided for recyclic transesterification use. Desirably, at least about 90 weight percent of the unsaturated fatty acid component from the transesterification reaction will be recovered for recyclic use.

In accordance with the present methods, enzymatic transesterification is utilized to reduce the level of saturated fatty acids in a triglyceride vegetable oil by the addition of unsaturated fatty acids in a ratio (oil:fatty acid) such that at substantial equilibrium among the exchangeable fatty acids, the percentage of unsaturates on the glyceride backbone is reduced from that of the starting material. By selectively choosing the unsaturated fatty acids used for transesterification, the enzymatic transesterification reaction may be driven to a desired targeted fatty acid composition in accordance with the present invention.

In accordance with such methods, enzymatic transesterification processes may produce oils with less than 3.5 weight percent of saturated fatty acids and preferably less than 3 weight percent saturated fatty acids, based on the total fatty acid content. For example, by using an immobilized *Mucor miehei* lipase and canola oil, high oleic sunflower oil or high oleic safflower oil, respectively, as the respective starting feedstocks, interesterified oils having reduced levels of saturated fatty acid contents of 1.6%, 1.0% and 0.9%, respectively, may be readily provided. Moreover, such low saturate products may be provided having a substantially 1:1 ratio of monounsaturates to polyunsaturates. Such starting feedstock materials may also readily be converted to oils having a 2:1:1 weight ratio of omega-9:omega-6:omega-3 unsaturated fatty acid content. For example, canola oil may also be readily interesterified to produce an oil with a 2:1:1 weight ratio of omega-9:omega-6:omega-3 esterified unsaturated fatty acids, and a saturated fatty acid content of 2.1%.
In order to provide economical manufacturing methods, the free fatty acids which are used to drive the reaction are recycled by separation of saturated fatty acids from unsaturated fatty acids. Separation of triglycerides from free fatty acids and/or separation of oleic, linoleic and linolenic acids from each other may also be utilized in specific embodiments of such methods.

As previously discussed, the saturated fatty acid distribution of vegetable oils such as canola oil is non-random, and is predominantly distributed at the 1-, 3-positions of the vegetable oil glycerides. Accordingly, by utilizing a 1-, 3- specific lipase such as previously described, the transesterification reaction may be limited to the 1-, 3- positions containing the predominant amount of saturated acids in the vegetable oil, without substantially affecting (or having a substantially reduced effect on) the fatty acids at the 2-position. In this manner, the amount of unsaturated fatty acid component utilized to achieve saturate reduction is decreased and effective transesterification efficiency is increased.

While 1-, 3- specific lipases are preferred for use with oils such as canola oil in which the saturated fatty acids are concentrated in the 1- and 3- positions, other lipases may also be used, particularly in oils which do not significantly concentrate saturated fatty acids in the 1-, 3- positions or which nevertheless have excessive saturated fatty acid amounts at every glyceride position. For example, it may be more economical to use a non-specific lipase, such as the lipase from Candida rugosa to enable the reduction of stoichiometric amounts of free fatty acids in oils in which all three positions on the triglyceride will desirably be available for exchange.

Having generally described various aspects of the present invention, the invention will be more particularly described with the specific embodiment of FIGURE 1. Illustrated in FIGURE 1 is a schematic diagram of a system
for producing edible low saturate triglyceride oils in accordance with the present invention. As shown in FIGURE 1, a variety of triglycerides may serve as fatty acid source materials for various unsaturated fatty acids, to produce a desired end product. In this regard, the system 100 comprises a linseed oil storage vessel 102 to provide a source high in linolenic acid, a sunflower, safflower, corn or soybean oil storage vessel 104 to provide a source high in linoleic acid, and a high oleic sunflower, high oleic safflower or olive oil storage vessel 106 to provide a source high in oleic acid.

Depending on the desired fatty acid content for the transesterification product, one or more of these high linolenic, high linoleic or high oleic source triglycerides is conducted through a conduit 108 to a hydrolysis reactor such as membrane reactor 110 for hydrolysis of the fatty acids of the oil. The reactor 110 may utilize a basic hydrolysis catalysts such as sodium hydroxide, sodium methoxide or an immobilized non-specific lipase to fully hydrolyze the triglyceride source material, or may utilize an immobilized unsaturated fatty acid-specific lipase, as previously described, to hydrolyze substantially only the unsaturated fatty acid components of the source triglyceride.

The fatty acid components, which may include saturated fatty acid components from the source triglyceride, are separated from the glycerol component and conducted to a saturated fatty acid separator 120. The fatty acid separator 120 may utilize any appropriate separation technology, and, for example may be a separator such as a low temperature molecular distillation column, a urea addition separator, ["Fatty Acids, Their Chemistry, Properties, Production and Uses", Part 3, K.S. Markley, Ed., Interscience Publishers, 1964], or a selective absorption elution column [Sorbex Separations, A Key to New Product Opportunities, Gembicki, et al., World Conference
on Emerging Technologies in the Fats and Oils Industry, A.R. Baldwin, Ed., American Oil Chemists Society (1985)], which is suitable for separating substantially all of the saturated fatty acids from the unsaturated fatty acid components. The fatty acids may be separated in a pretreatment step by low temperature solvent crystallization procedures to remove a substantial portion of the saturated fatty acid content prior to introduction into the separator 120, in order to reduce the cost of the separation step, if desired.

The separation step performed by the separator 120 is an important step in the method and, as will be described in more detail hereinafter, is also used to process recycled fatty acids and optionally diglycerides produced by the transesterification reaction. An unsaturated fatty acid transesterification mixture 122 comprising less than about 2 weight percent of saturated fatty acids, and preferably less than about 1 weight percent fatty acids is produced by the separator 120. A saturated fatty acid stream 118 is discharged from the separator, and may be utilized for other purposes such as the preparation of hard fats, soaps or chemical synthesis.

In the illustrated embodiment 100, the unsaturated transesterification fatty acid mixture 122 is combined with refined canola oil from storage vessel 130. The canola oil may typically have a saturated fatty acid content which is concentrated in the 1-, 3- position, as shown in the following table:

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<th>Fatty Acid</th>
<th>Overall</th>
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<td>6.06</td>
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<td>1.81</td>
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<tr>
<td>Oleic</td>
<td>56.74</td>
<td>59.64</td>
<td>50.96</td>
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<tr>
<td>Linoleic</td>
<td>19.97</td>
<td>13.42</td>
<td>33.09</td>
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<tr>
<td>Linolenic</td>
<td>7.85</td>
<td>3.96</td>
<td>15.64</td>
</tr>
<tr>
<td>Other</td>
<td>9.48</td>
<td>14.20</td>
<td>less than 0.1</td>
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The unsaturated fatty acid and canola oil are combined in a weight ratio in the range of from about 0.4 to about 3 of fatty acid to canola oil, to provide a canola oil-fatty acid transesterification reaction mixture 132 which is saturated or supersaturated with water by heat exchanger 134, and water saturator 136, as shown in FIGURE 1. The heat exchanger 134 heats the mixture 132 of canola oil and unsaturated fatty acids to approximately the desired reaction temperature of the transesterification reaction, which will preferably be in the range of from about 40° C. to about 70° C.

The heated transesterification mixture 135 is conducted into the water saturator 136, which desirably is a column of anionic resin such as the AmberLite IRA-900 anionic resin product of Rohm and Haas, which is saturated with water. The anionic resin, in addition to rapidly saturating the canola oil-unsaturated fatty acid mixture with water, may also function to remove impurities which may poison or adversely affect the enzyme in the subsequent enzymatic transesterification reaction step.

The reaction mixture 138 which is discharged from the water saturator 136 is saturated or supersaturated with water, and in this regard, may typically comprise from about 0.2 to about 0.8 weight percent of water. The mixture 138 may be supersaturated by slightly cooling a water-saturated reaction mixture. The saturation or supersaturation of the mixture 138 with water is necessary to maintain the utility of the resin-bound transesterification enzyme in the transesterification reaction, which will now be described in more detail.

The water-saturated or supersaturated reaction mixture 138 is introduced into an transesterification reactor 140, which in the illustrated embodiment 100 contains an immobilized 1-, 3- positionally specific transesterification lipase immobilized on or within an organopolymeric resin, such as the Novo 3A lipase product.
of Novo Industries, which is an immobilized 1-, 3-
positionally specific lipase derived from *Mucor miehei*, as
described herein. The fatty acids of the fatty acid source
and the fatty acids of the 1-, 3- positions of the canola
oil are substantially equilibrated in the reactor 140 to
provide an transesterification reaction product mixture 142
which is conducted to a triglyceride/fatty acid membrane
separator 150. The mixture 142 generally contains from
about 2 to about 8 weight percent of diglycerides produced
by the action of the enzyme and water, but may contain up
to about 15 weight percent of diglycerides, depending on
factors including water content and reaction conditions.
The life time of the immobilized lipase system is important
in commercial production, and may be monitored by means of
an assay system to determine the half-life of the
immobilized lipase in the canola oil reactor 140. As
previously indicated, impurities in both the fatty acids
and canola oil may accumulate on the resin and affect the
enzymatic activity, and accordingly, pure materials should
best be used in the reaction.

The illustrated triglyceride/fatty acid separator
150 may be a separator apparatus which separates the fatty
acid components including those produced in the reaction
from the di- and triglyceride components by any appropriate
methods, such as by selective absorption fractionation
processes, subcritical liquified gas (e.g., propane)
countercurrent extraction ["Liquid-Liquid Extraction
Employing Solvents in the Region of their Critical
Temperatures", Hixson, et al., American Institute of
Chemical Engineers, Boston, MA meeting May, 1942, pp. 929],
water washing, and/or vacuum deodorization, etc.
Optionally, at least a portion of the diglyceride
components may be separated with the fatty acid component
if desired by selective adsorption fractionation processes,
countercurrent selective solvent treatment processes, or
other suitable procedures, depending upon desired
composition and food product utilization of the transesterified vegetable oil 160.

The transesterified di- and triglyceride product 160 has an esterified saturated fatty acid content of less than 3.5 weight percent, and may be used in a wide variety of food products, such as liquid margarine or cooking oils, mayonnaise and salad dressings, to provide products having extremely low levels of saturated fat. High levels of diglycerides together with very low levels of monoglycerides which may be provided in the process may be particularly desirable in the manufacture of certain emulsified food products containing such low saturate content oils.

The free fatty acids in product stream 162 are separated from the fractionation solvent (if used) by appropriate recovery apparatus 170, which is recycled to separator 150, to provide a recovered fatty acid stream 172 and recovered solvent 174. The recovered fatty acid stream 172 contains saturated fatty acids from the canola oil 130, and from the unsaturated fatty acid stream 122 as a result of the transesterification reaction. This fatty acid recycle stream, as previously indicated, is conducted to the separator 120 for separation of the fatty acid components and removal of saturated fatty acid components to permit recycle use of the unsaturated fatty acid components. Amounts of saturated fatty acid recovered, and unsaturated fatty acids lost by the separator 120 or in other processing are made up by fatty acids from the source tanks 102, 104, 106 as needed to provide the proper proportion of fatty acid components, in the desired ratio, for the transesterification reaction.

Accordingly, it will be appreciated that low saturated oil may be produced by batch or continuous transesterification reaction methods to produce low-saturate oils with desired fatty acid compositions. Such oils may be selected for fatty acid composition based
on the fatty acid composition of the starting oil and the fatty acid composition and relative proportions of the fatty acids used in the reaction.

While the production of low saturated edible vegetable oils using batch or continuous cocurrent reaction has been described, countercurrent systems may also be utilized to manufacture such products. Countercurrent processes utilizing countercurrent supercritical or subcritical fluids which selectively extract and transport the fatty acid may desirably be utilized to provide efficient transesterification of the recycled stearic acid components. Countercurrent transesterification procedures may not only provide the reaction efficiencies of countercurrent operation, but also may facilitate separation of reaction products.

In supercritical fluids (solvents above their critical temperature and critical pressure) such as supercritical carbon dioxide, hydrocarbons such as ethane and ethane/propane mixtures, and fluorocarbons such as hexafluorothane, solubility of fatty acid esters such as fatty acid methyl and ethyl esters are typically an inverse function of molecular weight of the fatty acid monoester under various conditions. Similarly, the solubility of fatty acids is inversely proportional to molecular weight of the fatty acid, although fatty acids are typically less soluble in supercritical gases, than corresponding fatty acid lower alkyl monoesters of corresponding molecular weight because of the associative or hydrogen bonding characteristics of the fatty acids.

The respective solubilities of fatty acids, fatty acid esters and triglycerides in carbon dioxide are also a function of temperature and partial pressure of CO₂ at relatively low supercritical pressures.

An embodiment of continuous transesterification process which moves a fatty acid or fatty acid monoester component countercurrent to triglyceride flow, and which
also removes such fatty acid transesterification reaction
components from the transesterified glyceride, is
illustrated in FIGURE 2.

As shown in FIGURE 2, a source of canola oil 212
or other vegetable oil having a low (e.g., preferably less
than 10 weight percent, and more preferably less than 7
weight percent) esterified saturated fatty acid content is
provided as a starting material.

An unsaturated fatty acid (or fatty acid low alkyl
monoester such as a mixture of unsaturated fatty acid
methyl or ethyl esters) derived from canola oil having less
than 2 weight percent and preferably less than 1 weight
percent of saturated fatty acids is provided as a
transesterification reaction by selective enzymatic
alcoholysis or esterification of unsaturated canola oil
fatty acids components. Such methyl or ethyl esters may be
provided by appropriate purification procedures such as
fractional crystallization, solvent/solvent extraction,
urea adduct fractionation, selective absorption
fractionation, and/or sub- or supercritical solvent
extraction, to separate the unsaturated fatty acid or
alcohol ester components from saturated components.

The canola oil 212 may be conducted through a
column containing a water-saturated anionic exchange resin
to remove non-triglyceride impurities which might poison
the enzyme, and condition the oil 212 for the reaction.
The rate of introduction corresponds to the
transesterification reaction rate permitted by the activity
of the immobilized enzyme in the column 214. In this
regard, the column is packed with an immobilized lipase
enzyme, which is immobilized on an organic or inorganic,
high surface area substrate such as porous ceramic rings or
pellets, organic substrates such as crosslinked ion
exchange or phenolic resins (e.g., Novo 3A lipase product
as described herein) which are insoluble in the
supercritical fluid, or diatomaceous earth (e.g., Calite).
The surface area of the column packing is very large in order to promote interesterification reaction (e.g., more than 750 square meters of surface area per cubic meter), and to promote equilibrium dissolution of the low molecular weight components in the supercritical fluid.

The unsaturated fatty acid (or preferably lower alkyl monoester) component 220 is introduced into a transesterification reactor 214 together with canola oil 212, for transesterification to produce a low saturate triglyceride. An immobilized 1-, 3- specific enzyme 222 such as previously described may be used for transesterification of canola oil, because the saturated acid content of canola oil is concentrated in the 1- and 3- positions. However, an immobilized nonspecific transesterification lipase such as the nonspecific enzyme of Candida cylindracea or Candida rugosa, may be used.

Moreover, dried lipase-containing microorganism cells and mycelia may also be used as a transesterification enzyme, either retained in the reaction zone, or conducted therethrough with the triglyceride liquid phase flow [e.g., see Gancet, et al., "Catalysis by a Lipase-Bearing Rhizopus Arrhizus Mycelium in (Halogeno)Fluorinated Hydrocarbons", Ann. N.Y. Acad. Sci., Vol. 542, pp. 213-218 (1988)].

The unsaturated fatty acid or preferably a lower alkyl unsaturated fatty acid monoester 220, such as a methyl or ethyl ester of e.g., ethyl oleate, ethyl linoleate, ethyl linolenate mixture, which is desired to be transesterified with the triglyceride 212, which may be saturated with water is introduced into the column 214 at a point 222 between the point 224 of introduction of triglyceride, and the lower outlet 218 at a rate which maximizes the desired transesterification reaction. Because this transesterification reaction is conducted in a countercurrent manner, a lower ratio of unsaturated acid esters or acids 220 to canola oil 212 may be used.

In operation, supercritical carbon dioxide (or another supercritical fluid such as an ethane-propane
mixture of a fluorocarbon gas having a supercritical
temperature for example in the range of from about 30° C.
to about 80° C.), is introduced at the bottom of the column
214 under pressure and temperature conditions at which
relatively low molecular weight fatty acids or fatty acid
esters are significantly dissolved, but at which the high
molecular weight triglycerides are relatively not
substantially dissolved. For example, carbon dioxide
pressures in the range of from about 1100 psi to about 4500
(e.g., 2000-3000 psi for ethyl esters of oleic, linoleic
and linolenic acids), at a reaction temperature in the
range of, for example, from about 30° C. to about 70° C.,
are particularly preferred to provide relatively high fatty
acid and/or fatty acid monoester solubility, while
providing relatively low triglyceride solubility in the
upwardly moving supercritical carbon dioxide stream. The
supercritical fluid may contain a small amount of water
vapor to maintain the catalyst and to facilitate fatty acid
solubility. The temperature, of course, cannot exceed the
operating temperature of the enzyme, which will be damaged
at high temperatures. In this regard, at lower
supercritical pressures, the solubility of the fatty esters
and triglycerides is higher at lower temperatures. A
reaction temperature should be selected (e.g., 35° -
55° C.) which maximizes throughput rate for countercurrent
transport of the fatty acid monoester, and the
transesterification reaction rate which is provided by the
enzyme to achieve transesterification of the triglyceride
and the fatty acid or fatty acid monoester. Fatty acid
lower alkyl monoesters are substantially more soluble in
the supercritical fluid than the corresponding acids, and
accordingly are preferred reactants. The supercritical gas
also serves as a diluent of the triglyceride phase to
increase the reaction rate. If it is desired to operate
the countercurrent column at a temperature higher than the
lipase enzyme can tolerate, a plurality of enzyme reaction
zones 215 may be distributed along the column 214 which may
be operated at a lower temperature, with appropriate
heating and cooling means for fluid pumped therebetween.
In this manner, the transesterification reaction and
countercurrent gradient functions may be separately
maximized.

The supercritical carbon dioxide is less dense
than the downwardly moving canola oil stream at pressures
and temperatures used in the system of FIGURE 2 (e.g.,
35-70° C., 1500-3500 psia). The pressure, temperature,
column distances and flow rates of fatty acid or fatty acid
monoester and carbon dioxide are selected so that in the
zone 228 between the point of introduction of the carbon
dioxide and the point 222 of introduction of the
unsaturated fatty acid or monoester, the fatty acid or
fatty acid monoester is progressively dissolved from the
triglyceride into the upwardly moving supercritical CO₂
stream; the acid or fatty acid monoester components
(including the transesterified components) are
substantially completely removed from the triglyceride
stream 226 before it is discharged from the column at
outlet 218. In this regard, the weight ratio of the flow
rate of the carbon dioxide to the flow rate of the
unsaturated acid or monoester component 220 introduced in
the column 214 may desirably be selected to be in the range
of from about 5:1 to about 50:1, under conditions to
maximize solubility of the fatty acid or preferably fatty
acid monoester component while minimizing the solubility of
the triglyceride component phase. In the zone 224, during
the time of transit of the canola oil (e.g., .25 - 6
hours), the unsaturated acid component 220 undergoes transesterification with the triglyceride component.
Because the flow of triglyceride, and acid or monoester is
cocurrent in this stripping zone, the enzymatic
transesterification reaction will tend to approach the
equilibrium condition of the unsaturated acid
monoester-triglyceride blend at the point 222 of introduction of the monoester 220. Accordingly, the composition of the fatty acid or fatty acid monoester which enters the countercurrent transesterification zone 226 from the monoester stripping zone 224 will be different from the composition of the fatty acid or monoester 220 introduced into the column 214 at least in part because of the transesterification which occurs in the stripping zone 224. The transesterified triglyceride product, which may have substantially all fatty acid and fatty acid monoester components removed therefrom, is withdrawn from outlet 218.

The ratio of triglyceride components to the unsaturated acid or monoester component 220 to achieve a desired degree of transesterification of the canola oil triglyceride is substantially greater in the system of FIGURE 2 than the ratio of triglyceride to fatty acid or monoester utilized to achieve an equivalent degree of transesterification in a one or two step batch reaction. In this regard, the unsaturated acid or monoester 220 is introduced into the bottom of the column at a rate compared to the rate of introduction of canola oil which may, for example, be about half the proportion used in a batch reaction (e.g., 1:3 to 1:1 weight ratio of unsaturated acid component to canola oil).

The fatty acid or monoester component is dissolved in the upwardly moving supercritical CO₂ gas stream and carried into the transesterification zone 226, where it approaches equilibrium with the countercurrent oil flow through the action of the immobilized enzyme in the column. It is further transesterified in a countercurrent manner with the liquid triglyceride stream as it is conducted from its point of introduction 224 to the point 220 of introduction of the fatty acid monoester.

The triglyceride phase mixture continuously undergoes transesterification reaction as it moves
downwardly in the zone 226 containing lipase enzyme
countercurrent to the flow of supercritical gas, such that
the mixture has an increasing concentration of the desired
triglyceride components as it moves down the column. There
is also an increasing concentration of transesterified
fatty acid or monooester having fatty acid or monooester
components derived from the triglyceride in the upwardly
moving supercritical gas stream, in the direction toward
the point of introduction of the triglyceride. Water vapor
may be included in the carbon dioxide flow, the fatty acid
ester flow and/or the triglyceride flow to accommodate the
transesterification reaction, which may exceed the
solubility of water in the triglyceride component, and to
produce a desired level of diglycerides, if desired. Fatty
acid components produced by hydrolysis reactions in the
column 214 may also be removed by the supercritical carbon
dioxide flow.

The transesterified fatty monooester or fatty acids
dissolved in the supercritical CO₂ gas stream is carried
from the column at outlet 216, through a pressure let-down
system, where dissolved fatty acids or monooesters are taken
out of supercritical solution as a result of pressure
reduction. The tank 232 may alternatively be heated to
further reduce the solubility of the fatty acid monooester.
The solubility reduction may also be accomplished by a
combination of a limited pressure reduction (e.g., by
500-1000 psi) and a temperature increase (e.g., to
70-100° C.) so that the work to recompress the CO₂ for
recycle use may be reduced. The energy recovery system may
comprise a piston or turbine engine 232 in which the
dissolved fatty acid or monooester 234 are collected in the
recovery system, so that the energy may be recovered for
recompression of the carbon dioxide upon recyclc
operation. The lower pressure CO₂, which is depleted in
or free of dissolved fatty acids or monooesters, is
recompressed by compressor 238.
The carbon dioxide 236 which is separated from the fatty acid or monoester is conducted to compressor/thermal conditioner 238 where it is recompressed and reintroduced at the preselected operating temperature as previously discussed. A heat-pump or other thermal connector 240 may be used to transfer heat between the compressor 238 and the decompression engine 232. A portion of the extracted fatty acid components 234 may be recycled for reflux purposes to increase selectivity. The flow rate of supercritical carbon dioxide (or other supercritical gas solvent) through the column 214 is correlated with the flow rate of fatty acid ester 220 so that it is adequate to transport and dissolve substantially all of the fatty acid monoester under the operating conditions, but dissolves a minimal amount of the initial canola oil and other triglyceride components. The solubility of the fatty acid or fatty monoester components will desirably be greater than 1 weight percent, and preferably greater than 2 weight percent, while the solubility of triglycerides will be less than 0.5 weight percent and preferably dissolved triglycerides will be less than 10 percent of the dissolved fatty acid or monoester in the extracted product 234.

The separated fatty acid or monoester 234 may be purified in fractionation system 250 to separate purified unsaturated fatty acids 220 from other materials 252 in any suitable manner, and the unsaturated transesterification component 220 may be recycled for transesterification use. Another embodiment of a continuous countercurrent transesterification process is illustrated in FIGURE 3, which moves a fatty acid or fatty acid monoester component countercurrent to triglyceride flow, utilizing a subcritical liquified gas solvent.

As shown in FIGURE 3, the vegetable canola oil 312 to be transesterified is introduced into a high pressure column 314 at a point 324 intermediate the upper outlet 316 and the lower outlet 318. The rate of introduction
corresponds to the transesterification reaction rate permitted by the activity of the immobilized enzyme in the column 314. The column may be packed with an immobilized lipase enzyme as previously described, which is immobilized on an inorganic, high surface area substrate such as porous ceramic rings or pellets, or diatomaceous earth (e.g., Celite), or a suitable organic substrate such as an ion exchange resin substrate (e.g., Novo 3A lipase). Such packing may be in particulate film or fiber form, on plates, etc. Alternatively, as shown in FIGURE 3, the column 314 may be used to establish concentration gradients and countercurrent flow in stages, and the immobilized enzyme 313 may be located in corresponding stages in a separate pressure vessel 315. The surface area of the column packing should be very large in order to promote transesterification reaction (e.g., more than 750 square meters of surface area per cubic meter), and to promote equilibrium dissolution of the low molecular weight components in the supercritical fluid.

A fatty acid or lower alkyl fatty acid monoester 320, such as a methyl or ethyl ester of an unsaturated fatty acid component (e.g., a mixture of unsaturated fatty acids or unsaturated fatty acid esters such as methyl or ethyl esters), which is desired to be transesterified with the triglyceride 312, is introduced into the column 314 at a point 322 intermediate the point 324 of introduction of triglyceride, and the lower outlet 318 at a rate which maximizes the desired transesterification reaction. For manufacture of the low saturated fat vegetable oil, a mixture of unsaturated fatty acids or monoesters comprising less than 2 weight percent of saturated fatty acids is used. The rate of introduction is about 0.25 to 1.0 the rate of vegetable oil input, lower ratios being used for higher purity (e.g., less than 0.5 or 0.25 weight percent saturated fatty acid content). The transesterification reaction is conducted in a countercurrent manner, so that
it is substantially more efficient than the batch reaction of the fatty acid and triglyceride component at the selected reaction ratios.

In operation, liquified propane 309 is introduced at a position 319 near the bottom of the column 314 under pressure, flow rate and temperature conditions at which relatively low molecular weight fatty acids and monooesters are significantly dissolved, but at which the high molecular weight triglycerides are less soluble and form a separate phase. The temperature, of course, cannot exceed the operating temperature of the enzyme, which will be damaged at high temperatures. In the illustrated embodiment, the propane may be introduced at a pressure of about 600 psi and a temperature of 70° C. at a rate of about 10 times the rate of introduction of fatty acid 320 on a weight ratio basis. If a lower temperature is necessary, ethane or ethane/propane mixtures may be used. The liquified propane gas also dissolves to some extent in the triglyceride phase and serves as a diluent to increase the reaction rate.

The liquified propane phase is less dense than the downwardly moving vegetable oil stream, having a density of 0.25-0.4 g/cm³, depending primarily on composition and temperature. The pressure, temperature, column distances and flow rates of fatty acids (or monooesters) and liquid propane are selected so that in the zone 324 between the point 319 of introduction of the pure liquified propane and the point 322 of introduction of the unsaturated fatty acid or monooester 320, the fatty acid or monooester components are progressively dissolved from the triglyceride into the upwardly moving liquid propane phase; the fatty acid or monooester is substantially completely removed from the triglyceride stream 326 before it is discharged from the column at outlet 318. Because of countercurrent transesterification which occurs in the transesterification zones 313 (or in the column 314 in
embodiments having lipase enzyme therein), the fatty acids or monoesters in the upwardly rising liquid propane droplets will be different from the composition of the fatty acids 320 introduced into the column 314 because of the transesterification. The transesterified triglyceride product is withdrawn from outlet 318. It contains an amount of propane which is an inverse function of temperature (e.g., 30-60 weight percent propane), which is removed in propane stripping column 327 and returned for recycle use.

The ratio of triglyceride component 312 to the unsaturated fatty acid component 320 to achieve a desired degree of transesterification of the triglyceride will be substantially greater in the countercurrent system than the ratio of triglyceride to fatty monoester necessary to achieve an equivalent degree of transesterification in a batch or cocurrent reaction, as previously discussed. Moreover, high levels of transesterification substitution may be obtained with the proposed countercurrent process, which could otherwise only be achieved with multiple batch reaction, and multiple component separation.

The fatty acid component is dissolved in the upwardly moving propane phase, and is continuously exchanged with the downwardly moving vegetable oil phase, creating a gradient along the column, and carried into the transesterification zone, where it tends toward dynamic equilibrium with the countercurrent oil flow. It is transesterified in a countercurrent manner with the liquid triglyceride stream as it is conducted from the point of introduction 324 to the point 320 of introduction of the fatty acid monoester.

A portion of the mixture is pumped from mixing stages in the column 314 to transesterification zones 313 containing a suitable transesterification enzyme. The mixture continuously undergoes transesterification reaction in the separate zones 313, and is returned to the
respective phase separation zones of the column 314, such that the mixture has an increasing concentration of the desired triglyceride as it moves down the column. It is noted that the columns 314 may be maintained at a higher temperature (e.g., 70-90° C.) at which phase separation is enhanced, while the lipase reaction zones 313 may be maintained at a lower temperature (e.g., 50-60° C.) at which the enzyme longevity and reaction are maximized, by appropriate heating and cooling of the streams conducted there between. It is also noted that by cooling the liquid phases which increase mutual solubility (toward and including miscibility), and reheating them, which causes phase separation, extraction efficiency may be increased. Accordingly, there is also an increasing concentration of transesterified fatty acid components derived from the triglyceride in the upwardly moving liquified gas stream, in the direction toward the point of introduction of the triglyceride. Water vapor may be included in the liquified gas phase flow, the fatty acid flow and/or the triglyceride flow to accommodate the transesterification reaction, and which may equal or exceed the solubility of water in the triglyceride component, if desired, as previously discussed. Any fatty acid components, as well as monoglycerides (and diglycerides) produced by hydrolysis reactions in the column 314 may also be (at least partially) removed by the upwardly moving liquified propane phase (which may be facilitated by a small amount of water or ethanol in the propane phase).

The transesterified fatty acid component which is preferentially dissolved in the upwardly moving liquid propane phase, is carried from the column at outlet 316 at e.g., 70-85° C., through heater/evaporator 330 where it is heated to 93° C. to evaporate pure propane 331 and then into separation tank 332, where dissolved fatty acid components 335 are taken out of solution as a result propane evaporation and temperature increase. In this
regard, the exiting propane phase is heated to further reduce the solubility of the fatty acid (and any triglycerides), without deactivating the enzyme, which is not present in the tank 332 or the upper reflux portion of the column 314. The liquid propane exiting the column may be heated to 94–95°C, to create two phases in the separation tank or column 332. Of course, all of the propane may be evaporated to recover the fatty acid components, but this is not necessary, particularly in view of the amount of propane utilized. A portion (e.g., 20–50 weight percent) of the separated fatty acid component may be reintroduced into the top of the column 314 into a reflux zone to enhance the selectivity of the countercurrent transesterification reaction component separation. The remaining portion of the fatty acid material 335, which contains 30–60 weight percent propane as well as some di and triglycerides (and perhaps very small amounts of monoglycerides) in addition to the fatty acid or monoester components, is conducted to a propane stripper 350 and from there the propane-free fatty acid components 352 (which may optionally first be hydrolyzed as previously described) are conducted to an appropriate separation system 352 for separation of the unsaturated fatty acid components in relatively pure form for recycle use. The saturated fatty acid components 354 are separated from the unsaturated fatty acid components in a suitable separator 355. The fatty acids (and any mono, di- and triglycerides) may be further utilized as desired. The unsaturated fatty acid components may be esterified with ethanol and recycled.

The liquid propane which is separated from the fatty acids in the tank 332 is conducted to preheat the canola oil 312 and then to pump/thermal conditioner 334 where it is cooled to 70° – 75°C and reintroduced at the preselected operating temperature. Because it has a very small amount of product fatty acids, it is introduced at an
intermediate position in the column 314. A portion of the propane is evaporated at heater/propane boiler 330 and recompressed to produce a pure propane stream for fully stripping the fatty acids and lower molecular weight components upon introduction at the bottom 319 of the reactor 314. The dissolved fatty acid or monoester components may also be separated by heating the propane stream in heater 330 to a temperature above the critical temperature (96-98° C.) of the propane (e.g., 100-110° C.) while maintaining the system at a pressure slightly above the critical pressure (42 atmospheres), such as 44-50 atmospheres. In this way, a substantial portion of the components 335 are separated in column 332, and the supercritical propane gas may be reliquified by cooling to a temperature below the critical temperature for reintroduction into the column 314. Such a substantially isobaric procedure minimizes heat and pumping expense.

The flow rate of liquified gas solvent through the column 314 is correlated with the flow rate of fatty acid ester 320 so that it is adequate to dissolve substantially all of the fatty acid or monoester under the operating conditions and remove it from the transesterified triglyceride 321.

As previously discussed, urea adduction may be utilized to separate saturated fatty acid components from unsaturated fatty acids or monoesters to provide a substantially pure unsaturated fatty acid reaction component. Although urea typically crystallizes in a tetragonal form, it forms complexes with straight chain fatty acids and lower alkyl monoesters in which the straight chain fatty compound is included within a hexagonal crystalline urea framework to form urea inclusion compounds having a weight ratio of approximately 3:1 of urea to the included compound. In general, saturated fatty acids form more stable urea complexes than unsaturated fatty acids of the same carbon chain length. The stability constants decrease by an order of magnitude in the series
stearic, oleic and linoleic acids, respectively [Chapter XX Techniques of Separation E. Urea Complexes, p. 2309, et seq., K.S. Markley, Ed., supra]. Saturated monoesters may have greatly increased stability. The stability of fatty acid complexes also increases with the carbon chain length of the fatty acid, and saturated monoesters of lower alkyl alcohols may have enhanced stability.

The urea complexes may be readily decomposed by adding water or other solvent to dissolve the urea, leaving the saturated fatty acid inclusion compounds as an oil or solid, depending upon the temperature of decomposition. A small quantity of an acid such as hydrochloric acid may be utilized to prevent formation of emulsions by traces of ammonia soaps. Conversely, heating the urea complex with a solvent such as hydrocarbon solvent in which the urea is insoluble may also be used to extract the included saturated fatty acid compound.

In order to separate saturated fatty acids (or monoesters) from unsaturated fatty acids (or monoesters), insufficient urea to combine with all the complex forming components of a mixture is added, such that the saturated component will combine with the urea and preferentially precipitate with respect to the less stable unsaturated fatty acid complexes. Utilizing differences in complex-forming capacity, highly purified unsaturated fatty acids may be isolated from various natural sources for use in transesterification processes of the present invention as previously described.

Although urea complexes have the melting point of urea, which is approximately 133° C., urea adducts become less stable with increasing temperatures and decompose at a temperature below the melting point of urea, which is characteristic for a specific complex. The dissociation temperature for stearic acid/urea adduct in the absence of any solvent is about 126° C. The dissociation temperature of the palmitic acid/urea adduct in the absence of any
solvent is about 114° C., and the dissociation temperature of the oleic acid/urea compound in the absence of any solvent is about 110° C. These differences may be utilized in refining and separation processes.

The preferential activity of saturated fatty acids to form urea complexes may be used to remove substantially all of the stearic acid, and a substantial portion of the palmitic acid content of a saturated fatty acid stream in a liquid/solid countercurrent distribution method of separation. For example, the fatty acids and urea dissolved in appropriate solvent may be provided as a moving liquid phase, while the precipitated reaction products may serve as the stationary solid phase. The character of the distribution curve obtained for a given mixture of fatty acids depends on the differences in the distribution coefficients for the individual acids when they are distributed between solid inclusion compounds and the organic solvent [W.N. Sumerwell, J. Am. Chem. Soc., 79, 3411-3415 (1957)].

Illustrated in FIGURE 4, a continuous system for economically providing a transesterified low saturate vegetable oil in which the saturated fatty acids are removed by urea/unsaturated fatty acid inclusion reservoir compounds concomitantly with enzymatic acyl transfer reaction. In this regard, an unsaturated fatty acid/urea reservoir complex may be prepared which serves as an exchange reservoir for removal of saturated fatty acids. Such unsaturated exchange reservoir urea inclusion compounds may be prepared in a suitable manner such as shown in FIGURE 4 by dissolving canola oil fatty acids or a fatty acid lower alkyl monoesters 402, (e.g., methyl or ethyl esters) in a suitable solvent. Methyl and ethyl esters are preferred because urea adducts of palmitic and stearic monoesters may have substantially higher stability constants than oleic acid or oleic acid monoesters, thereby facilitating saturated component removal.
The exchange reservoir inclusion compound crystals are formed by dissolving the canola fatty acids or monoesters which comprise about 5-8 weight percent palmitic and stearic acids (or monoesters), in a suitable amount of a solvent such as ethanol or methanol 404 (and hexane if necessary), together with about 30-50 weight percent of urea 406, based on the weight of the fatty acids. The solution is cooled in a crystallization vessel 408 to produce a first crop of urea inclusion crystals which comprise about 10-12 weight percent of the fatty acid component. The first crop of urea inclusion crystals are separated as first crop product 410. The first crop urea inclusion crystals are predominantly urea stearate and palmitate compounds, because of the tendency for the saturated fatty acid (or preferably monoester) to form urea inclusion compounds, leaving in solution at least 98-99% pure unsaturated fatty acids or monoesters in the crystallization vessel solution 408. An excess of urea, e.g., a 7:1 or more weight ratio of urea to remaining canola unsaturated fatty acids in the vessel 408, is then added and dissolved at elevated temperature. Upon cooling, a second crop of urea inclusion compounds is formed which are predominantly urea oleate and linoleate inclusion compound crystals, which form an exchange reservoir inclusion compound material 420. These exchange reservoir crystals 420 may be dried to remove solvent and used with an immobilized lipase enzyme 422 such as 1-,3- specific lipase from *Mucor miehei* immobilized on an ion exchange resin, such as the Novo 3A Lipase product of Novo described in U.S. Patent 4,798,793, in a weight ratio of 2:1 to about 10:1 unsaturated fatty acid urea inclusion reservoir crystals to immobilized enzyme/ionic resin component to form the packing 424 of an intraesterification reaction column 426. The reservoir crystals are also placed, without an immobilized lipase component, as the packing into a column 430. The urea reservoir crystals may be
blended with, or alternated in layers with the transesterification lipase. Canola oil fatty acids or preferably lower alkyl monoesters 402 are conducted through the column 430 at a temperature in the range of 20-110°C, preferably in the range of 40-60°C.

A temperature selected for maximum relative stability of the palmitic acid complex over the oleic acid complex may be selected if desired to maximize removal of the palmitic acid component. Because the urea complexes of stearic and palmitic acid or preferably monoester components of the mixture 402 have a higher stability than the oleic or linoleic complexes, the stearic and palmitic acids or monoesters exchange with these unsaturated fatty acids to form more stable inclusion compounds, thereby producing an unsaturated fatty acid or monoester stream 432 which is substantially free of saturated fatty acids or monoesters. The unsaturated discharge stream 432 may be blended with refined and bleached canola oil 440 in a weight ratio of from about 30:1 to about 1:1 canola oil to fatty acid component, and preferably in a range of from about 10:1 to about 3:1, to produce a transesterification stream 436. The canola oil transesterification stream 436 may be conducted through a water saturated ionic exchange resin column 434 (e.g., at a temperature of 40-70°C) to saturate the oil/fatty acid (or monoester) blend 436 with water and remove impurities which might deactivate the enzyme.

The saturated intraesterification stream 436 accordingly may have a limited amount of fatty acid component and will require reduced separation treatment after transesterification.

A suitable solvent such as butane, pentane, hexane or pressurized propane 440 may be used to reduce the viscosity of the transesterification stream 436 which is introduced into the interesterification column 426. The stream 436 is conducted through the transesterification
column 424 where the 1-, 3- fatty acid moieties of the canola oil are progressively released and exchanged with unsaturated fatty acid components of the stream 436 by the acyl transfer activity of the immobilized enzyme. Such unsaturated fatty acid components may be initially present in the stream 436, may be produced by a small amount of hydrolysis from the water content of the stream, or may be derived from the unsaturated fatty acid urea adduct reservoir material 420. In this regard, as the saturated fatty acid moieties are released by the transesterification reaction, they undergo equilibration reaction with the reservoir urea inclusion compound crystals of the packing 424 and are exchanged with the unsaturated fatty acid components of the inclusion crystals. The efficiency of the overall production process is enhanced by close proximity of the enzyme and the inclusion compounds. In this manner, the saturated fatty components of the canola oil are progressively removed as the stream 436 is conducted through the column 426. An increase in diglyceride and fatty acid content will occur as a result of the small water content of the stream 432. When using unsaturated fatty monoesters, a vacuum may be periodically or continuously applied to the column 426 with a slow nitrogen bleed to remove monohydrate alcohol, increase the triglyceride content and decrease the diglyceride content of the stream, if desired.

The stream 436 is conducted through the column 426 at a rate, combined with the transesterification rate and inclusion compound exchange rate, which produces an output stream 450 comprising less than 3 weight percent of saturated fatty acid content based on the total weight of the stream. The processing may be continued until the saturated fatty acid exchange capacity of the urea inclusion reservoir component 424 or the component 420 of the column 430 are exhausted. The output stream 450 may be refined in any appropriate manner such as countercurrent
solvent processing, to remove the fatty acid or monoester and any small amounts of urea. The low saturate triglyceride mixture may alternatively, or subsequently, be deodorized by conventional steam deodorization or supercritical carbon dioxide deodorization to produce a very low saturate vegetable oil product 460.

The urea inclusion reservoir component column packings 420, 424 may be replaced or regenerated periodically. Such replacement and regeneration may be accomplished by redissolving the urea in a hydroxy solvent such as methanol to release and separate the saturated fatty acid inclusion compound, and recycling the urea in solvent-reformed unsaturated fatty acid reservoir crystals. However, it is preferred to regenerate the hexagonal urea compound without dissolving it, using solvent or fatty acid or monoester components which are compatible with and/or easily separable from, the adduct. In this regard, for example, the packing 420 may be regenerated by passing therethrough a stream of a solvent such as hexane, liquefied propane and/or unsaturated fatty acids 402 (which preferably may have had the saturated fatty acids removed therefrom) at a temperature sufficient to release the saturated fatty acid inclusion components, which may be up to a temperature above the decomposition temperature of the stearate and palmitate inclusion compounds (e.g., about 126° C.), but below the melting temperature of the urea (133° C.). An output stream high in stearic and palmitic acids is produced, which may be utilized or reprocessed as desired. The thermal stability limit for the enzyme should not be exceeded by such regeneration treatment if the enzyme is mixed or treated with the urea reservoir material. Layering as previously described permits separate regeneration treatment of the reservoir material. After releasing the saturated inclusion compounds from the packing 420 in this manner, it may be cooled in the presence of unsaturated fatty acids.
compounds, thereby reforming the reservoir material as an unsaturated fatty acid complex. Low molecular weight hydrocarbons such as hexane or petroleum ether, which form relatively unstable inclusion compounds, may be used to extract the saturated fatty acid component in such regeneration, or solvents which do not form an inclusion compound, such as liquified propane may be used. It may be desirable to remove all such solvent following regeneration by methods such as vacuum treatment. Regeneration may be carried out on a continuous countercurrent basis with the "spent" inclusion compound packing 424 being continuously or periodically withdrawn from the bottom (countercurrent inlet) and regenerated material added at the top transesterified oil outlet.

While described for urea/unsaturated fatty acid adducts, other suitable adsorbent or adduct forming materials may be used which preferentially absorb saturated fatty acids or monoesters over unsaturated fatty acids or monoesters. For example, X and Y type zeolites and/or silicalite having appropriate pore structures which preferentially absorb saturated fatty acids may be regenerated or "recharged" with purified unsaturated fatty acids or monoesters, and used in admixture with the immobilized enzyme. Countercurrent moving bed systems or simulated moving countercurrent bed systems may be used to facilitate continuous operation.

While the countercurrent methods disclosed herein have been specifically described with respect to low-saturate vegetable oil manufacture, such methods may be generally applied to enzymatic and base-catalyzed transesterification reactions of vegetable, marine and animal fats and oils.

Having generally described various aspects of the present low saturate vegetable oil products and methods which may be utilized to prepare such products, the invention will now be more particularly described with respect to the following specific examples.
Example 1

Runs 1 and 2 - Two different lots of canola oil were transesterified with oleic and linoleic acids. Transesterification reactions were carried out in 2.5 ml of hexane, per gram of reactants. Novo 3A Lipase, an immobilized 1-, 3- positionally specific lipase from *Mucor miehei* as previously described, was used at a level of 0.375 gram, per gram of oil. The transesterification reactions were run in a water bath at 40° C., under 250 rpm agitation for 6 hours. The reactions were stopped by removing the immobilized lipase by filtration. The hexane was removed by distillation. The reaction mixture at this point was deodorized under a vacuum of 0.1mm Hg to a maximum temperature of 500° F. The product was then analyzed for fatty acid distribution (FAD).

The FAD may be resolved into the five major fatty acids, palmitic (P), stearic (S), oleic (O), linoleic (L), linolenic (Ln) and all the remaining fatty acids designated as "other". A typical FAD of the fatty acid components is set forth in the following Table 1.

Table 1

<table>
<thead>
<tr>
<th>Acid</th>
<th>Oleic</th>
<th>Linoleic</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.51</td>
<td>---</td>
</tr>
<tr>
<td>S</td>
<td>0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>O</td>
<td>98.46</td>
<td>0.67</td>
</tr>
<tr>
<td>L</td>
<td>0.27</td>
<td>98.85</td>
</tr>
<tr>
<td>Ln</td>
<td>---</td>
<td>0.06</td>
</tr>
<tr>
<td>Other</td>
<td>0.59</td>
<td>0.38</td>
</tr>
</tbody>
</table>

The weight of reactants used in these runs was as follows:

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Weight of Reactants (g)</th>
<th>Percent by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola oil</td>
<td>157.2</td>
<td>13.58</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>600.0</td>
<td>51.85</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>400.0</td>
<td>34.57</td>
</tr>
</tbody>
</table>

These amounts were selected based on a "target" transesterified composition having a weight ratio of...
esterified monounsaturated fatty acids to polyunsaturated fatty acids of about 1:1. Following the reactions, the fatty acid distribution (FAD) of the products was determined, as set forth in Table 3:

Table 3

<table>
<thead>
<tr>
<th></th>
<th>Starting Material Canola Oil</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Target for Run 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>4.15</td>
<td>2.78</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>1.81</td>
<td>1.39</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>56.74</td>
<td>53.78</td>
<td>45.71</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>19.97</td>
<td>30.83</td>
<td>45.53</td>
<td></td>
</tr>
<tr>
<td>Ln</td>
<td>7.85</td>
<td>6.73</td>
<td>5.67</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>9.39</td>
<td>4.49</td>
<td>1.82</td>
<td></td>
</tr>
<tr>
<td>Total Sats (C₁₂⁻¹₈)</td>
<td>6.03</td>
<td>4.26</td>
<td>1.26</td>
<td></td>
</tr>
</tbody>
</table>

* Starting FAD and targeted FAD are data from one experiment, but are representative of all three trials.

Neither reaction reached its targeted equilibrium. These results suggest that there is some property of canola oil which is affecting the catalytic properties of the immobilized lipase.

Run 3 - A further reaction was conducted in the same manner with 0.02% TBHQ added as an antioxidant to prevent the formation of peroxides during the reaction which might interfere with the transesterification. Also, the lipase enzyme product concentration was doubled (0.750
grams lipase per gram of oil) to compensate for any other
inhibition which might be occurring. Table 3 also
illustrates the FAD of the product triglycerides. Again,
the targeted equilibrium was not achieved and only a slight
decrease in the saturates was observed compared to Runs 1
and 2.

Run 4 - Runs 1-3 indicated that there was some
contaminant in the canola oil which affected the lipase
activity in such a way that the reaction could not reach
equilibrium under those conditions. Canola oil
triglycerides were purified by Florisil column
chromatography. Canola oil was purified on activated
Florisil (dried at 100° C. for 18 hours, then equilibrated
to 3 weight percent water at room temperature by the
addition of distilled water). A 125 ml volume of activated
Florisil was equilibrated in hexane in a column of 2.5 X 50
centimeters. Fifty-five grams of canola oil were eluted
with 3 column volumes of hexane. This fraction was
collected and was found to be the triglyceride fraction.
This fraction was distilled to remove the hexane and used
as a source of canola oil triglycerides. These purified
triglycerides were used to determine initial reaction
conditions in Run 4. Run 4 was carried out in a manner
substantially identical to Run 3 using the Florisil
purified canola oil. Table 4 illustrates the FAD of the
product triglycerides:

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Canola Oil (%)</th>
<th>Run #4</th>
<th>Run #5</th>
<th>Target for 4 &amp; 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>4.15</td>
<td>1.05</td>
<td>1.02</td>
<td>0.60</td>
</tr>
<tr>
<td>S</td>
<td>1.81</td>
<td>0.58</td>
<td>0.63</td>
<td>0.22</td>
</tr>
<tr>
<td>O</td>
<td>56.74</td>
<td>48.16</td>
<td>47.33</td>
<td>44.71</td>
</tr>
<tr>
<td>L</td>
<td>19.97</td>
<td>43.48</td>
<td>42.95</td>
<td>47.83</td>
</tr>
<tr>
<td>Ln</td>
<td>7.85</td>
<td>4.98</td>
<td>5.67</td>
<td>5.47</td>
</tr>
<tr>
<td>Other</td>
<td>9.39</td>
<td>1.75</td>
<td>2.40</td>
<td>1.14</td>
</tr>
<tr>
<td>Total Sats</td>
<td>6.03</td>
<td>1.64</td>
<td>1.69</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Monounsat
to
Polyunsat  2:1  1:1  1:1  1:1

* See Table 3

Run 4 Florisil purified canola triglycerides with 0.02% TBHQ - Run 5 canola oil with 0.02% TBHQ

Run 5 - Another lot of canola oil was also transesterified under substantially identical parameters as described for Run 3, including .02 weight percent TBHQ as an antioxidant. Table 4 also illustrates the FAD of this product. This lot did not demonstrate any lipase inhibition since the targeted equilibrium was nearly attained. Thus, the contaminant causing this inhibition is variable from lot to lot.

Example 2

High oleic sunflower oil and high oleic safflower oil having low levels of saturated fatty acids were transesterified with substantially pure oleic and linoleic acids, substantially as described in Run 4 of Example 1. Table 5 illustrates the starting FAD, and the FAD of the transesterified products:

Table 5
High Oleic Sunflower Oil

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Transesterified Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>3.71</td>
</tr>
<tr>
<td>S</td>
<td>4.15</td>
</tr>
<tr>
<td>O</td>
<td>80.86</td>
</tr>
<tr>
<td>L</td>
<td>8.92</td>
</tr>
<tr>
<td>Ln</td>
<td>0.10</td>
</tr>
<tr>
<td>Other</td>
<td>2.26</td>
</tr>
<tr>
<td>Total Sats</td>
<td>7.94</td>
</tr>
</tbody>
</table>

High Oleic Safflower Oil

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Transesterified Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>5.35</td>
</tr>
<tr>
<td>S</td>
<td>2.26</td>
</tr>
<tr>
<td>O</td>
<td>73.15</td>
</tr>
<tr>
<td>L</td>
<td>17.25</td>
</tr>
<tr>
<td>Ln</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Neither oil appeared to demonstrate any inhibition of the lipase, and oils with <1 weight percent saturates were produced.

Example 3
An oil having very low saturated fatty acid content and a 2:1:1 weight ratio of omega-9:omega-6:omega-3 fatty acids is a nutritionally desirable product which is not naturally available. Canola oil was transesterified with purified oleic, linoleic and linolenic acids as previously described for Run 4 of Example 1, with the addition of linolenic acid to the reaction mixture to prepare such a product. Table 6 illustrates these results:

<table>
<thead>
<tr>
<th>$%$</th>
<th>Starting Material</th>
<th>Transesterified Product</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>4.15</td>
<td>1.31</td>
<td>0.59</td>
</tr>
<tr>
<td>S</td>
<td>1.81</td>
<td>0.79</td>
<td>0.20</td>
</tr>
<tr>
<td>O</td>
<td>56.74</td>
<td>49.14</td>
<td>48.02</td>
</tr>
<tr>
<td>L</td>
<td>19.97</td>
<td>21.81</td>
<td>24.49</td>
</tr>
<tr>
<td>Ln</td>
<td>7.85</td>
<td>22.05</td>
<td>25.31</td>
</tr>
<tr>
<td>Other</td>
<td>9.39</td>
<td>4.90</td>
<td>1.38</td>
</tr>
<tr>
<td>Total Sats</td>
<td>6.03</td>
<td>2.10</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Monounsats to Polyunsats: 2:1

<table>
<thead>
<tr>
<th>$%$</th>
<th>Starting Material</th>
<th>Transesterified Product</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>omega-9:omega-6:omega-3</td>
<td>7:2:1</td>
<td>2:1:1</td>
<td>2:1:1</td>
</tr>
</tbody>
</table>

Example 4
In the previously described runs, the transesterified oils had a very low target amount of less than 1.3 weight percent saturated fats. To achieve this level of unsaturation in the final product a ratio of fatty acids: oil of 6.4:1 in the reaction mixture was utilized, in which the reaction mixture contained only 13.5% oil. Transesterified canola oils having about 3 weight percent saturated fats may be more economically produced using...
lower ratios of unsaturated fatty acids to oil in the reaction mixture. The calculations used to produce a canola oil with close to 3% saturates are shown in Table 7:

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Total Amount of Exchangeable Fatty Acids</th>
<th>Targeted Fatty Acids at Equilibrium, Weight Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola Oil</td>
<td>157.2 g</td>
<td>90 g</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>90 g</td>
<td></td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>90 g</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>59.64 + 0.036 + 0.15 = 6.52</td>
<td>2.33 1.67</td>
</tr>
<tr>
<td>L</td>
<td>13.42 + 88.97 + 0.24 = 148.85</td>
<td>53.16 52.42</td>
</tr>
<tr>
<td>Ln</td>
<td>3.96 + 0.06 + 0.53 = 4.02</td>
<td>36.66 35.46</td>
</tr>
<tr>
<td>Other</td>
<td>14.09 + 0.14 + 0.53 = 14.96</td>
<td>1.44 6.17</td>
</tr>
<tr>
<td>Total</td>
<td>280.00</td>
<td>100.00 100.00</td>
</tr>
</tbody>
</table>

Product at equilibrium: 2.38% saturates (theoretical) 1.15:1 Fatty acids:Oil

Theoretically, it should be possible to produce a canola oil having less than or about 3 weight percent saturates with a fatty acid:oil ratio of 1.15:1, assuming the fatty acid feedstock has a saturate level of 0.72 weight percent or less.

Canola oil (Kraft Food Ingredients Group) was transesterified using the procedure of Run 3, Example 1, with the proportion of reaction components as described hereinabove. The results from two trials were tabulated in Table 8:
<table>
<thead>
<tr>
<th></th>
<th>Starting Material Canola Oil</th>
<th>Run #1</th>
<th>Run #2</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>4.15</td>
<td>1.86</td>
<td>1.71</td>
<td>1.67</td>
</tr>
<tr>
<td>P</td>
<td>1.81</td>
<td>1.14</td>
<td>1.07</td>
<td>0.69</td>
</tr>
<tr>
<td>O</td>
<td>56.74</td>
<td>53.12</td>
<td>52.58</td>
<td>52.42</td>
</tr>
<tr>
<td>L</td>
<td>19.97</td>
<td>33.35</td>
<td>34.27</td>
<td>35.46</td>
</tr>
<tr>
<td>Ln</td>
<td>7.85</td>
<td>5.58</td>
<td>5.03</td>
<td>6.17</td>
</tr>
<tr>
<td>Other</td>
<td>9.39</td>
<td>4.95</td>
<td>5.34</td>
<td>3.59</td>
</tr>
<tr>
<td>Total Sats</td>
<td>6.03</td>
<td>3.00</td>
<td>2.78</td>
<td>2.38</td>
</tr>
</tbody>
</table>

The FAD results show that the reactions approached their targeted equilibria, to produce a canola oil with less than or about 3 weight percent saturates using a fatty acid:oil reaction weight ratio of 1.15:1 in a batch reaction. The reaction mixture of this Example 4 contains 46.5% oil. Instead of using a mixture of oleic and linoleic acids canola oil may be transesterified with a fatty acid mixture derived from canola oil, from which saturated fatty acids have been removed. The transesterification reaction may be carried out using a canola oil unsaturated fatty acid:canola oil reaction weight ratio of 1.15:1 as described in Runs 1 and 2 of this Example, to provide a transesterified canola oil having less than 3 weight percent esterified saturated fatty acids, and an omega-9:omega-6:omega-3 esterified unsaturated fatty acid weight ratio of about 5-7:2-3:1.

Accordingly, it will be appreciated that in accordance with the present invention, novel low-saturate vegetable oils and methods for manufacturing such oils have been provided. Such methods can be used to produce oils with preselected unsaturated fatty acid compositions as a function of the composition of the starting oil and the fatty acids in the reactions.

While the invention has been described with respect to certain specific embodiments, various modifications and adoptions will be apparent from the present disclosure, which are intended to be within the scope of the following claims.
WHAT IS CLAIMED IS:

1. A transesterified low-saturate liquid vegetable oil comprising less than 3.5 weight percent esterified saturated C₁₂-C₁₈ fatty acids and at least about 96 weight percent esterified unsaturated C₁₂-C₂₂ fatty acids, based on the total fatty acid content of said oil, said esterified C₁₂-C₂₂ fatty acids having a weight ratio of esterified monounsaturated fatty acids to esterified polyunsaturated fatty acids in the range of from about 10:1 to about 1:2.

2. A low-saturate liquid vegetable oil in accordance with claim 1 wherein said oil comprises less than about 2 weight percent of esterified intermediate C₁₂-C₁₆ saturated fatty acids.

3. A low-saturate vegetable oil in accordance with claim 1 wherein said oil comprises from about 2 to about 15 weight percent of diglycerides and from about 85 to about 98 weight percent of triglycerides based on the total weight of the oil.

4. A low-saturate vegetable oil in accordance with claim 1 wherein said oil has a weight ratio of esterified omega-9 unsaturated fatty acids to omega-3 unsaturated fatty acids in the range of from about 9:1 to about 1:1, a weight ratio of omega-9 unsaturated fatty acids to omega-6 unsaturated fatty acids in the range of from about 4:1 to about 1:1 and a weight ratio of omega-6 unsaturated fatty acids to omega-3 fatty acids in the range of from about 1:3 to about 3:1.

5. A low-saturate vegetable oil in accordance with claim 2 having a weight ratio of esterified omega-9;omega-6;omega-3 unsaturated fatty acids of about 5-7:2-3:1.

6. A low-saturate vegetable oil in accordance with claim 2 having a weight ratio of esterified

7. A low-saturate vegetable oil in accordance with claim 1 wherein said oil is a transesterified canola oil, high oleic sunflower oil, corn oil, olive oil, peanut oil, high oleic safflower oil, soybean oil or mixtures thereof.

8. An enzymatic transesterification method for preparing a low-saturate vegetable oil in accordance with claim 1, comprising the steps of providing an unsaturated fatty acid source material selected from the group consisting of unsaturated C₁₂-C₂₂ fatty acids, unsaturated C₁₂-C₂₂ fatty acid monooesters of low molecular weights monohydric alcohols, and mixtures thereof, comprising less than about 2 weight percent of saturated C₁₂-C₁₈ fatty acids, based on the total weight of fatty acids in said source material, providing an edible liquid vegetable oil selected from the group consisting of canola oil, corn oil, olive oil, peanut oil, high oleic safflower oil, high oleic sunflower oil and mixtures thereof, comprising at least about 92 weight percent of esterified unsaturated C₁₂-C₂₂ fatty acids and less than about 8 weight percent of saturated C₁₂-C₁₈ fatty acids, based on the total fatty acids content of the oil,

    transesterifying said unsaturated fatty acid source material and said liquid vegetable oil with a transesterification enzyme at a weight ratio of unsaturated fatty acid source material to liquid vegetable oil in the range of from about 10:1 to about 1:3 to provide a transesterification mixture,

    separating transesterified free fatty acid components from the glyceride components of the transesterification mixture to provide a transesterified liquid vegetable oil oil product comprising less than 3.5 weight percent of esterified C₁₂-C₁₈ saturated fatty
acids and a fatty acid component mixture comprising unsaturated fatty acids, unsaturated fatty acid monoesters or mixtures thereof released from said vegetable oil, and

separating the fatty acid mixture by removing saturated fatty acid components therefrom to provide an unsaturated fatty acid source material comprising less than 2 weigh percent of saturated C_{12}-C_{18} fatty acids based on the total fatty acid content thereof for recyclical reaction with said vegetable oil triglyceride.

9. A countercurrent method for preparing a low-saturate liquid vegetable oil comprising the steps of providing a transesterification reaction zone containing a lipase transesterification enzyme,

introducing a vegetable oil into the transesterification reaction zone to provide a triglyceride reaction stream through the reaction zone,

introducing an unsaturated fatty acid source material selected from the group consisting of unsaturated C_{12}-C_{22} fatty acids, unsaturated C_{12}-C_{22} fatty acid lower alkyl monoesters, and mixtures thereof into the transesterification reaction zone to provide a fatty acid or fatty acid monoester reaction stream,

conducting a supercritical gas or subcritical liquified gas which preferentially dissolves fatty acids and fatty acid monoesters over triglycerides under two-phase conditions through said zone countercurrent to the flow of the triglyceride reaction stream, at a rate and under pressure and temperature conditions to maintain a separate phase of countercurrent fluid containing fatty acid or fatty acid monoester through the reaction zone in intimate contact with the triglyceride reaction stream,

carrying out transesterification reaction of the triglyceride stream with the fatty acid or fatty acid monoester stream in the reaction zone,
withdrawing a transesterified low-saturate liquid vegetable oil stream which has been transesterified with the unsaturated fatty acid source material from the reaction zone,

withdrawing a countercurrent fluid phasee from said reaction zone countercurrent to the triglyceride oil reaction stream having dissolved therein transesterified fatty acids or fatty acid monoesters produced by transesterification of the unsaturated fatty acid source material with the liquid vegetable oil,

separating saturated fatty acid components from the transesterified fatty acids or fatty acid monoesters to provide a recycle unsaturated fatty acid source material, and

introducing the recycle source material into the reaction zone.

10. An enzymatic transesterification method for preparing a low-saturate vegetable oil comprising the steps of

providing an unsaturated fatty acid source material selected from the group consisting of unsaturated C_{12}-C_{22} fatty acids, unsaturated C_{12}-C_{22} fatty acid monoesters of low molecular weight monohydric alcohols, and mixtures thereof,

providing an edible liquid vegetable oil comprising at least about 85 weight percent of esterified unsaturated C_{12}-C_{22} fatty acids, based on the total fatty acids content of the oil,

transesterifying said unsaturated fatty acid source material and said liquid vegetable oil with a transesterification enzyme in a transesterification reaction mixture while selectively adducting saturated fatty acid components from said reaction mixture into a fatty acid reservoir material which preferentially adducts saturated fatty acid components at a weight ratio
of unsaturated fatty acid source material to liquid vegetable oil in the range of from about 1:50 to about 1:1 to provide a transesterified mixture, and separating transesterified free fatty acid components from the glyceride components of the transesterified mixture to provide a transesterified liquid vegetable oil oil transesterification product comprising less than 3.5 weight percent of esterified C_{12-22} saturated fatty acids.

11. A method in accordance with claim 10 wherein said reservoir material is a urea adduct of an unsaturated fatty acid or fatty acid lower alkyl monoester.
### INTERNATIONAL SEARCH REPORT

**International Application No:** PCT/US 90/07336

### I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)  
According to International Patent Classification (IPC) or to both National Classification and IPC  
IPC (5): A23D 7/00; C12P 7/64; C12 N 9/20  
U.S. CL.: 426/601; 435/134,198

### II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
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<tr>
<td>U.S.</td>
<td>426/601; 435/134,198</td>
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</table>

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

### III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of Document, 14 with indication, where appropriate, of the relevant passages 17</th>
<th>Relevant to Claim No. 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>EP A 0,286,106 (Spinnler et al.) 02 August 1989.</td>
<td>1-7</td>
</tr>
<tr>
<td>Y</td>
<td>US A 4,275,081 (Coleman et al.) 23 June 1981. See entire document.</td>
<td>8-11</td>
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<td>&quot;Production of a fatty acid urea adduct and its applicability in feeding of ruminants&quot;.</td>
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<td></td>
<td>Acts Aliment. 8(1), 357-71.</td>
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<td></td>
<td>modification of oils and fats&quot;. Pages 207-223. See entire document.</td>
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</table>

* Special categories of cited documents: 13
  * "A" document defining the general state of the art which is not considered to be of particular relevance.
  * "E" earlier document but published on or after the international filing date.
  * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).
  * "O" document referring to an oral disclosure, use, exhibition or other means.
  * "P" document published prior to the international filing date but later than the priority date claimed.
  * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention.
  * "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step.
  * "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
  * "Z" document member of the same patent family.
V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claim numbers ..., because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-6, 10 and 11 drawn to a product and process of making classifiable in class 426, subclass 601 and class 435, subclass 134.

II. Claim 9 drawn to a process of making classifiable in class 435, subclass 134.

The claims of these two groups are directed to different inventions which are not linked as to form a single general inventive concept. The inventions are not linked in operation and perform completely different operations.

The International Search Report covers the claims of the international application.

As only some of the required additional search fees were timely paid by the applicant, the international search report covers only those claims of the international application for which fees were paid. Specifically:

3. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is not limited to the invention first mentioned in the claims. It is covered by claim numbers.

Remark on Protest

- The additional search fees were accommodated by applicant.
- No request accompanied the payment of additional search fees.
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<td>Agricultural and Biological Chemistry. Volume 52. issued 1988. Chi et al. &quot;Enzymatic Interesterification in Supercritical Carbon Dioxide&quot;. pages 1541-1550. see entire document.</td>
<td>9</td>
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