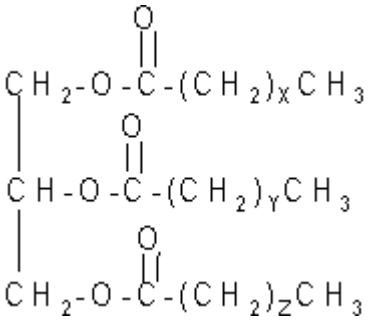
SALATRIM

Prepared at the 59th JECFA (2002) and published in FNP 52 Add 10 (2002), superseding specifications prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997). The safety evaluation of salatrim could not be completed at the 59th JECFA.

DEFINITION Salatrim is the acronym for short- and long-chain acyl triglyceride molecules. Salatrim is prepared by non-enzymatic inter-esterification of triacetin, tripropionin, tributyrin, or their mixtures with hydrogenated canola, soybean, cottonseed, or sunflower oil. Triglycerides with three short-chain fatty acids (SCFA) are removed in the process. Stearic acid is the predominant long-chain fatty acid (LCFA).

Structural formula



where 14 _ (x + y + z) _ 42 for those triglycerides with either one LCFA and two SCFA or two LCFA and one SCFA.

Assay Not less than 87% triglycerides. Not less than 90% of the triglycerides with a SCFA-to-LCFA mole ratio in the range 0.5-2.0. Not more than 70% by weight of saturated LCFA.

DESCRIPTION Clear, slightly amber liquid to a light-coloured waxy solid at room temperature. Free of particulate matter and of foreign or rancid odour.

FUNCTIONAL USES Reduced-energy fat and oil replacement.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in hexane, cyclohexane, acetone, ether, tetrahydrofuran, and liquid triglyceride oils. Insoluble in water

Melting range (Vol. 4) 16-71°, depending on triglyceride composition.

Infrared spectrum (Vol. 4) The infrared absorption spectrum of the sample, contained in a sodium chloride cell or between salt plates, corresponds to the infrared spectrum in the Appendix to this monograph.

PURITY

Water (Vol. 4) Not more than 0.3% (Karl Fischer method).

Sulfated ash (Vol. 4)

Not more than 0.1%
Test 2 g of the sample (Method I for solid samples; Method II for liquid samples).

Free fatty acids (Vol. 4)

Not more than 0.5% (as oleic acid). Use an equivalence factor (e) of 28.2.

Monoglycerides

Not more than 2%
See description under METHOD OF ASSAY.

Peroxide value

Not more than 2.0
See description under TESTS.

Unsaponifiable matter

Not more than 1.0%
See description under TESTS.

Lead (Vol. 4)

Not more than 0.1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
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TESTS

PURITY TESTS

Peroxide value

Reagents
Acetic acid-chloroform solution: Mix 3 volumes of acetic acid with 2 volumes of chloroform.
Potassium iodide solution, saturated: Dissolve excess potassium iodide in freshly boiled water. Excess solid must remain. Store in the dark. Test daily by adding 0.5 ml to 30 ml of the acetic acid-chloroform solution, then add 2 drops of starch TS. If the solution turns blue, requiring more than 1 drop of 0.1 N sodium thiosulfate to discharge the colour, prepare a fresh solution.

Procedure
Weigh accurately about 5 g of the sample into a 250-ml Erlenmeyer flask. Add 30 ml of the acetic acid-chloroform solution and swirl to dissolve. Add 0.5 ml of the saturated potassium iodide solution, allow to stand with occasional shaking for 1 min, and add 30 ml of water. Slowly titrate with 0.01 N sodium thiosulfate, swirling the flask vigorously until the yellow colour is almost gone. Add about 0.5 ml of starch TS and continue the titration while swirling the flask vigorously to release all the iodine from the chloroform layer until the blue colour disappears.

Perform a blank determination and make any necessary correction.

Peroxide value = (S x N x 1000)/W

where
S = ml of N sodium thiosulfate
N = normality of sodium thiosulfate

W = weight of the sample (g)

Unsaponifiable matter

Maxwell, R.J., Reimann, K.A., and Percell, K. (1981) Determination of the Unsaponifiable Matter in Fatty Acids by a Rapid Column Method, JAOCS 58:1002-1004.

Reagents

Calcium Chloride-Celite Mixture: Using a mortar and pestle, grind 1 part anhydrous calcium chloride with 1 part water; add 3 parts Celite 545 (grade: not acid-washed, C-212). Grind to a uniform consistency. The mixture may be stored in a covered amber jar for up to one month.
Potassium Hydroxide-Celite Mixture: For multiple analyses, prepare in lots of 75 g or more. Using a mortar and pestle, grind 2 parts potassium hydroxide pellets with 1 part water (CAUTION: considerable heat is generated; wear eye protection and gloves); add 4 parts Celite 545. Grind to a uniform consistency. The mixture may be stored in a covered amber jar for up to 10 days.

Procedure

Saponification: Place 10 g Potassium Hydroxide-Celite mixture in a 400-ml mortar; accurately weigh 5 g of sample (W_S) and transfer to the mortar. Grind the mixture until the sample is uniformly distributed. Add another 10 g of Potassium Hydroxide-Celite mixture; grind to uniform consistency. Transfer the mixture to a jar. Using a pestle, transfer any residual sample by sweeping 5 g Celite 545 along the sides of the mortar and into the jar. Shake until the mixture is uniform. Heat for 20-30 min in a 130^{o} oven.
Gravimetric Extraction: Transfer the cooled mixture to the mortar; regrind (approximately 30 sec) to a uniform granular consistency. Loosely fit a plug of glass wool into the tip of a glass chromatography column (30 mm i.d.; 30 cm long, overall, with a drip tip 5 cm x 8 mm o.d.). Pack the column with 5 g of Calcium Chloride-Celite mixture. Transfer the contents of the mortar to the column. Pack to a total bed height of 50-60 mm. Place a 150-ml tared flask under the column. Qualitatively transfer residue from the mortar to the column using about 25 ml of dichloromethane. Once this solution has percolated into the column bed, add sufficient dichloromethane so that the column bed is wet and a few drops of eluate have been collected in the flask. Charge the column with 150 ml of dichloromethane, and collect the entire volume in the flask (approximately 25 min). Remove the solvent under a stream of nitrogen with gentle heating while the eluate is being collected. Take the contents of the flask to constant weight under vacuum. Determine the weight of the residue (W_R).

To check for completeness of extraction, add 20 ml of dichloromethane to the column and collect in a second tared flask. Evaporate the contents of the second flask to dryness and examine for residue. Determine the weight of the residue (W_{R1}), if present. If residue is observed, repeat the procedure using an additional 20 ml of dichloromethane.
The total residue weight and the weight of the original sample are used to calculate the percent unsaponifiable matter:

$[(W_R + W_{R1} + ...)/W_S] \times 100$

Lead (Vol. 4)

Determination by graphite furnace atomic absorption spectrophotometry. (Food Chemicals Codex, 4th Ed. (1996), National Academy of Sciences, Washington, DC, pp. 765-766).

Apparatus

An atomic absorption spectrophotometer (Perkin-Elmer Model 3100 or equivalent) fitted with a graphite furnace (Perkin-Elmer HGA 600 or equivalent), a lead hollow cathode lamp (Perkin-Elmer or equivalent) with argon as the carrier gas.

Follow the manufacturer's directions for setting the appropriate instrument parameters for lead determination. (NOTE: Use reagent-grade chemicals with as low a lead content as practicable; use high-purity water and gases.) Prior to this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid. Then rinse thoroughly with high-purity water, preferably obtained from a mixed-bed strong acid/strong base ion-exchange cartridge capable of producing water that has an electrical resistivity of 12 to 15 megohms.

Solutions

Hydrogen Peroxide-Nitric Acid Solution: Mix together equal volumes of 10% hydrogen peroxide and 10% nitric acid (NOTE: use extreme caution; wear gloves and protective eyeware).

Butanol-Nitric Acid Solution: Introduce approximately 500 ml of n-butanol in a 1000-ml volumetric flask. Slowly add 50 ml nitric acid. Dilute to volume with n-butanol and mix.

Lead Nitrate Stock Solution: Using glassware that is free of lead salts, dissolve 159.8 mg of reagent-grade lead nitrate in 100 ml of Hydrogen Peroxide-Nitric Acid Solution. Dilute to 1000.0 ml with Hydrogen Peroxide-Nitric Acid Solution and mix. Each ml of this solution contains the equivalent of 100 µg of lead ion.

Standard Lead Solution: On the day of use, pipet 10.0 ml of Lead Nitrate Stock Solution into a 100.0-ml volumetric flask, add Hydrogen Peroxide-Nitric Acid Solution to the mark and mix. Each ml of Standard Lead Solution contains the equivalent of 10 µg of lead ion.

Standard Solutions: Into separate 100-ml volumetric flasks, pipet 0.2, 0.5, 1, and 2 ml, respectively, of Standard Lead Solution; dilute to volume with Butanol-Nitric Acid Solution. These solutions contain 0.02, 0.05, 0.1, and 0.2 µg lead per ml, respectively.

Sample Solution: (NOTE: Perform this procedure in a fume hood). Accurately weigh 1 g of the sample, and transfer to a large test tube. Add 1 ml of nitric acid. Place the test tube in a rack in a boiling water bath. As soon as the rusty tint (reddish-brown colour) is gone, add 1 ml of 30% hydrogen peroxide dropwise (in order to avoid a vigorous reaction) and wait for bubbles to form. Stir with an acid-washed plastic spatula if necessary. Remove the test tube from the water bath and let it cool. Transfer the solution to a 10-ml volumetric flask and dilute to volume with Butanol-Nitric Acid Solution. Use this solution for analysis.

Tungsten Solution: Transfer 0.1 g of tungstic acid (H_2WO_4) and 5 g of sodium hydroxide pellets into a 50-ml plastic bottle. Add 5.0 ml of high-purity water (distilled water may be redistilled from an all-glass apparatus or may be passed down a column of cation exchange resin, e.g., Amberlite IR 120(H)), and mix. Heat the mixture in a hot water bath until completely dissolved. Cool and store at room temperature.

Procedure

Place the graphite tube in the furnace. Inject a 20-µl aliquot of Tungsten Solution into the graphite tube, using a 300-ml/min argon flow and the following sequence of conditions: dry at 110° for 20 sec, char at 700° to 900° for 20 sec and, with the argon flow stopped, atomize at 2700° for 10 sec; repeat using a second 20-µl aliquot. Clean the quartz windows.

(NOTE: The sample injection technique is the most crucial step in controlling the precision of the analysis; the volume of the sample must remain constant. Rinse the µl pipet tip (Eppendorf or equivalent) three times with either the Standard Solutions or Sample Solution before injection. Use a fresh pipet tip for each injection and start the atomization process immediately after injecting the sample. Between injections, flush the graphite tube to eliminate any residual lead by purging at a high temperature, as recommended by the manufacturer.)

With the hollow cathode lamp aligned for maximum absorbance, and the wavelength set at 283.3 nm, atomize 20-µl aliquots of the four Standard Solutions, using a 300-ml/min argon flow and the following sequence of conditions: dry at 110° for 30 sec (20 sec ramp period and 10 sec hold time), char at 700° for 42 sec (20 sec ramp period and 22 sec hold time) and, with the argon flow stopped, atomize at 2300° for 7 sec.
Plot a standard curve of concentration, in µg/ml, of each Standard Solution versus its maximum absorbance value compensated for background correction, as directed for the particular instrument. Atomize 20 µl of the Sample Solution under conditions identical to those for the Standard Solutions and measure its background-corrected maximum absorbance. From the standard curve, determine the concentration of lead in the Sample Solution, C, in µg/ml, Calculate the concentration of lead in the Salatrim sample, in mg/kg, using:

$$10C/W$$

where W is the mass, in g, of the sample.

METHOD OF ASSAY

[Characterization of Triacylglycerols in Saturated Lipid Mixtures with Application to Salatrim 23CA (Huang et al, J. Agric. Food Chem., (1994) 42, 453).]

I. Determination of monoacylglyceride and triacylglyceride content

Principle

This method permits the quantitation of monoglycerides (MAG) with one LCFA, and triglycerides (TAG) with the same acyl carbon number (ACN) in Salatrim by high-temperature capillary gas chromatography (HTCGC). The ACN is the sum of the number of carbons of each carboxylic acid sidechain of each TAG. (E.g., the ACN for tristearin is 54 (i.e., 3 x 18); the

ACNs for both dipropionylstearoylglycerol and diacetylarachidoylglycerol are 24 (i.e., [(2 x 3) + 18] and [(2 x 2) + 20], respectively). MAG and TAG are identified by comparison with standards. The weight percent of each MAG and TAG in Salatrim is determined from the peak areas and calibration curves constructed from data from analyses of standard solutions.

Materials

MAG Standard: Monopentadecanoin (mono-C15) and monostearin (mono-C18); purity: 99% minimum (available from Nu Check Prep., Inc., Elysian, MN, USA).
TAG Standards: Tricaproin (tri-C6), triheptanoin (tri-C7), tricaprylin (tri-C8), trinonanoin (tri-C9), tricaprin (tri-C10), triundecanoin (tri-C11), trilaurin (tri-C12), tritridecanoin (tri-C13), trimyristin (tri-C14), tripentadecanoin (tri-C15), tripalmitin (tri-C16), triheptadecanoin (tri-C17), and tristearin (tri-C18); purity: 99% minimum (available from Nu Check Prep., Inc., Elysian, MN, USA).

Sample Preparation

Internal Standard Stock Solution: Dissolve 200 mg of tri-C11 in 2 litres of undecane/toluene (95/5, v/v; spectroscopic grade).
Standard Solutions: Standard Solution (Group 1) - To each of twenty-two 10-ml volumetric flasks, add the thirteen TAG Standards so that each flask, respectively, will contain 1600, 800, 400, 200, 100, 50, 32, 25, 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, and 2 mg/l of each TAG when filled to the mark with the Internal Standard Stock Solution (tri-Cll; 100 mg/l).
Standard Solution (Group 2) - To each of seven 10-ml volumetric flasks, add the two MAG Standards so that each flask, respectively, will contain 500, 250, 125, 62.5, 31, 15.6, and 7.8 mg/l of each MAG when filled to the mark with the Internal Standard Stock Solution (tri-C11; 100 mg/l).
Salatrim Solution: Accurately weigh 2 g Salatrim into a 1-L volumetric flask. Dilute to volume with Internal Standard Stock Solution.

Procedure

1. Instrumentation and Analysis Parameters
Instrument: Hewlett-Packard 5890 Series II GC equipped with flame-ionization detector (FID), pressure programmable on-column injector, HF 7673 auto-sampler, and HP Series II integrator; or equivalent.
GC Column: Chrompack SIM-DIST CB fused-silica column (Chrompack Inc., Raritan, NJ, USA) 5 m x 0.32 mm i.d.; 0.1 µm film thickness, or equivalent. A deactivated fused-silica pre-column (0.5 m x 0.53 mm i.d.) coupled to the analytical column via a butt connector (Quadrex Corp., New Haven, CT, USA) or equivalent.
Oven Temperature: 140^{o} to 350^{o} at 10^{o}/min; total run time 21.0 min.
Injector temperature: Track mode "ON" (injector temperature follows the oven temperature conditions).
Injection Mode: On-column injection.
Sample Size: Inject 0.5 µl.
FID Temperature: 375^{o}.
Flow Rate: Hydrogen gas constant flow mode "ON"; pressure: 5.5 psi (140^{o}).

2. Quantitation of MAG

Calibration Curve and Response Factor (RF) Determination for Mono-C15 and Mono-C18

Analyze each of the Standard Solutions of Group 2 using a sample injection volume of 0.5 µl. From each chromatogram, establish the response factors (RF_i) for the two MAG using the formula:

$RF_i = (C_{IS}/C_i) \times (A_i/A_{IS})$

where A_{IS} is the peak area of the Internal Standard (tri-C11); A_i is the peak area of a MAG; C_{IS} is the concentration of the Internal Standard (100 mg/l); and C_i is the concentration (mg/l) of a MAG.

For each MAG, construct a calibration curve by plotting the peak area ratios of (A_i/A_{IS}) (x-axis) versus the RF_i (y-axis) for each solution.

Weight Percent of MAG

Analyze the Salatrim sample using a sample injection of 0.5 µl. From the chromatograms, obtain the peak area of each MAG (A_i) and the Internal Standard (A_{IS}). Example chromatograms of Salatrim are provided in the Appendix to this monograph. Calculate the peak area ratio (A_i/A_{IS}) and determine the response factor for each MAG (RF_i) from the calibration curve. The concentration (µg/ml) of each MAG (C_i) in the Salatrim sample is:

$C_i = (C_{IS}/RF_i) \times (A_i/A_{IS})$

where C_{IS} is the concentration of the Internal Standard (tri-C11; 100 mg/l). The weight percent of each MAG in the Salatrim sample ($(W\%)_i$) is:

$(W\%)_i = (C_i/C_T) \times 100$

where C_T is the concentration of Salatrim Solution (2000 mg/l). The total weight percent of MAG in the Salatrim sample is:
Ó $_i$ $(W\%)_i$.

3. Quantitation of TAG with the Same ACN

Determination of the Response Factors for TAG Standards

Analyze each of the Standard Solutions of Group 1 using a sample injection volume of 0.5 µl. The response factor for each TAG standard, j, is:

$RF_{n,j} = (C_{IS}/C_{n,j}) \times (A_{n,j}/A_{IS})$

where A_{IS} is the peak area of the Internal Standard; $A_{n,j}$ is the peak area of the TAG standard with ACN = n (where n = 18-54 and the ACNs of the TAG standards are as follows: tri-C6, 18; tri-C7, 21; tri-C8, 24; tri-C9, 27; tri-C10, 30; tri-C11, 33; tri-C12, 36; tri-C13, 39; tri-C14, 42; tri-C15, 45; tri-C16, 48; tri-C-17, 51; and tri-C18, 54)and concentration $C_{n,j}$ (mg/l); C_{IS} is the concentration of the Internal Standard (100 mg/l).

The relative peak area of each TAG standard with ACN = n and concentration $C_{n,j}$ to that of the Internal Standard is given by:

$RA_{n,j} = A_{n,j}/A_{IS}$

The response factors and relative peak areas for each TAG with ACN = (n + 1) and with ACN = (n + 2) are calculated from the measured response

factors and relative peak areas for TAG standards with ACN = n and (n + 3) at the same concentration, $C_{n,j}$, according to the following:

$RF_{n+1,j} = RF_{n,j} + (RF_{n+3,j} - RF_{n,j}) \times 1/3$
$RA_{n+1,j} = RA_{n,j} + (RA_{n+3,j} - RA_{n,j}) \times 1/3$
$RF_{n+2,j} = RF_{n,j} + (RF_{n+3,j} - RF_{n,j}) \times 2/3$
$RA_{n+2,j} = RA_{n,j} + (RA_{n+3,j} - RA_{n,j}) \times 2/3$

Determination of the Response Factors for Salatrim
For the Salatrim Sample, the response factor for a TAG with a relative peak area $RA_{n,j}$ is calculated from:

$RF_{n,j} = RF_{n,j-1} + (RF_{n,j+1} - RF_{n,j-1}) \times [(RA_{n,j} - RA_{n,j-1})/(RA_{n,j+1} - RA_{n,j-1})]$
where $RF_{n,j+1}$ and $RF_{n,j-1}$ are the response factors and $RA_{n,j+1}$ and $RA_{n,j-1}$ are the relative peak areas, respectively, of the TAG standards with the same ACN value. $RA_{n,j+1}$ and $RA_{n,j-1}$ must meet the following condition:
$RA_{n,j-1} < RA_{n,j} < RA_{n,j+1}$

where $RA_{n,j}$ is the ratio of the peak area of the TAG in Salatrim with ACN = n, ($A_{n,j}$), to the peak area for the Internal Standard (A_{IS}). (NOTE: The notation "j+1", “j”, and "j-1" denotes consecutive concentration values in the series of standard solutions. For example, for a TAG standard with ACN = n and concentrations of 100, 200, and 400 mg/L, the relative peak areas $RA_{n,j+1}$ and $RA_{n,j-1}$ correspond to data for standards with concentrations of 400 mg/l and 100 mg/l, respectively.)
Weight Percent Determination of Salatrim TAG Components
The concentrations (mg/l) of TAG in the Salatrim Sample with ACN = n are given by

$C_{n,j} = (C_{IS}/RF_{n,j}) \times (A_{n,j}/A_{IS})$

where C_{IS} is the concentration of the Internal Standard (tri-C11; 100 mg/l).
The weight percent of a TAG with ACN = n in Salatrim is:
$(W\%)_n = (C_{n,j}/C_T) \times 100$

where C_T is the concentration of the Salatrim sample (2000 mg/l).
The total weight percent of TAG in the Salatrim sample is:
$Ó_n (W\%)_n$

II. Determination of the SCFA/LCFA mole ratio

Principle
Triglycerides are converted to fatty acid butyl esters and determined by capillary column FID gas chromatography.

Apparatus
Gas chromatograph with FID (Hewlett Packard HP 5890 capillary columns, equipped with 5-µl syringe for 0.32 mm i.d. columns. Automatic sampler (HP 7673, or equivalent). Chromatographic data system or integrator (HP 3365 Series II software, or equivalent). Retention gap, deactivated fused silica, 1 mm x 0.32 mm i.d. with capillary column connectors. DB 5-HT, 15 m x 0.32 mm i.d. fused silica capillary column (J&W Scientific, Inc., 91 Blue Ravine Road, Folsom, California 95630-4714, USA; catalogue no. 123-5711 or equivalent). Crimp caps and vials (HP 5181-3375 or equivalent) for on-line autosampler.

Reagents

Sodium butoxide (50% in butanol); hexane (HPLC grade, 95%); hydrochloric acid (0.5 N volumetric standard); butyl butyrate (98%); n-butyl acetate (99%); butyl propionate; butyl palmitate; butyl stearate (93%); 1-butanol (anhydrous, 99%).

Solutions

0.5 N Sodium Butoxide: Transfer 9.62 g of 50% sodium butoxide solution into a 100-ml volumetric flask, dilute to the mark with 1-butanol, and mix.
Standard Reference Solution: Weigh accurately approximately 50 mg of each butyl ester standard (5 standards) into a single 100-ml volumetric flask. Dilute to volume with hexane and mix. More than one Standard Reference Solution may be necessary if impurities co-elute with standard peaks. (NOTE: Melt butyl stearate standard before sampling.)
Sample Solution: Weigh accurately approximately 50 mg of Salatrim (melt first if sample is solid) into a 100-ml volumetric flask. Dilute to volume with hexane and mix.

Butyl Ester Conversion: For each sample to be assayed (hexane blank, Standard Reference Solutions, Sample Solutions), pipet 5.0 ml of solution into a clean 2 dram (8 ml) clear glass vial. Add 0.5 ml of 0.5 N Sodium Butoxide, seal and shake vigorously (solution will turn yellow). Allow the solution to stand for 2 minutes (for hexane blank and Sample Solutions, only); then, neutralize by pipetting 1.0 ml of 0.5 N hydrochloric acid into the solution. Seal and shake well until the solution is clear. Check the pH using pH paper. The solution should be acidic. If it is not, the column will degrade. (NOTE: For the Standard Reference Solution, 1-butanol and water may be substituted for 0.5 N Sodium Butoxide and 0.5 N hydrochloric acid, respectively.)

Chromatography

Carrier gas/flow: Helium/2.0 psi (constant flow)
Injection volume: 0.5 µl
Injection temperature: Temp. track mode (3^{o} above oven temp.)
Oven temperature: 40^{o} (6 min), 15^{o}/min to 280^{o} (5 min)
Detector temperature: 380^{o}
Allow the butyl ester sample phases to separate (centrifugation may be used to hasten the separation). Transfer approximately 1 ml of the hexane layer into an autosampler vial. Run the gas chromatography program.

Calculations

Response Factors for Butyl Ester Standards (RF_i):
$RF_i = A_i/(W_i \times (\%P)_i/100)$
where A_i is the average peak area counts for the ith standard; W_i is the weight (µg) of the ith standard in the reference solution; and $(\% P)_i$ is the purity of the ith standard expressed as a percentage.
Weights of Butyl Esters in the Sample:
$W_i = A_i/RF_i$
where W_i is the weight (µg) of the ith ester in the sample; A_i is the peak area counts for the ith ester in the sample; RF_i is the response factor for the ith ester standard (average area counts/µg).
Weights of Fatty Acids in the Sample:

$(W_i)_{fatty\ acid} = (W_i)_{butyl\ ester} \times (MW_i)_{fatty\ acid}/(MW_i)_{butyl\ ester}$
where $(W_i)_{fatty\ acid}$ and $(W_i)_{butyl\ ester}$ are, respectively, the weights (µg) of the ith fatty acid and its butyl ester in the sample; and $(MW_i)_{fatty\ acid}$ and $(MW_i)_{butyl\ ester}$ are their respective molecular weights.

Calculation of Short/Long (S/L) Mole Ratio:
$(mmoles_i)_{fatty\ acid} = (W_i)_{fatty\ acid}/ (1000 \times (MW_i)_{fatty\ acid})$
S/L mole ratio = $Ó_i(mmoles_i)_{SCFA}/Ó_i\ (mmoles_i)_{LCFA}$
where $(W_i)_{fatty\ acid}$ is in µg, (MW_i) is in mg/mmole, and $Ó_i(mmoles_i)_{SCFA}$ and $Ó_i(mmoles_i)_{LCFA}$ are the respective sums of the millimoles of the short-chain fatty acids (C_2 - C_4) and the long-chain fatty acids (C_{14} -C_{18}).

III. Calculation of the weight percent of saturated LCFA

Based on the weights of stearic acid and palmitic acid determined in II, above, (i.e., $(W_i)_{fatty\ acid}$ where i = palmitic or stearic acid), the weight percent of saturated LCFA in the sample is
$\%W_{LCFA} = 100 \times [(W_{stearic})_{fatty\ acid} + (W_{palmitic})_{fatty\ acid}]/W$
where W is the sample weight and all weights are in µg.

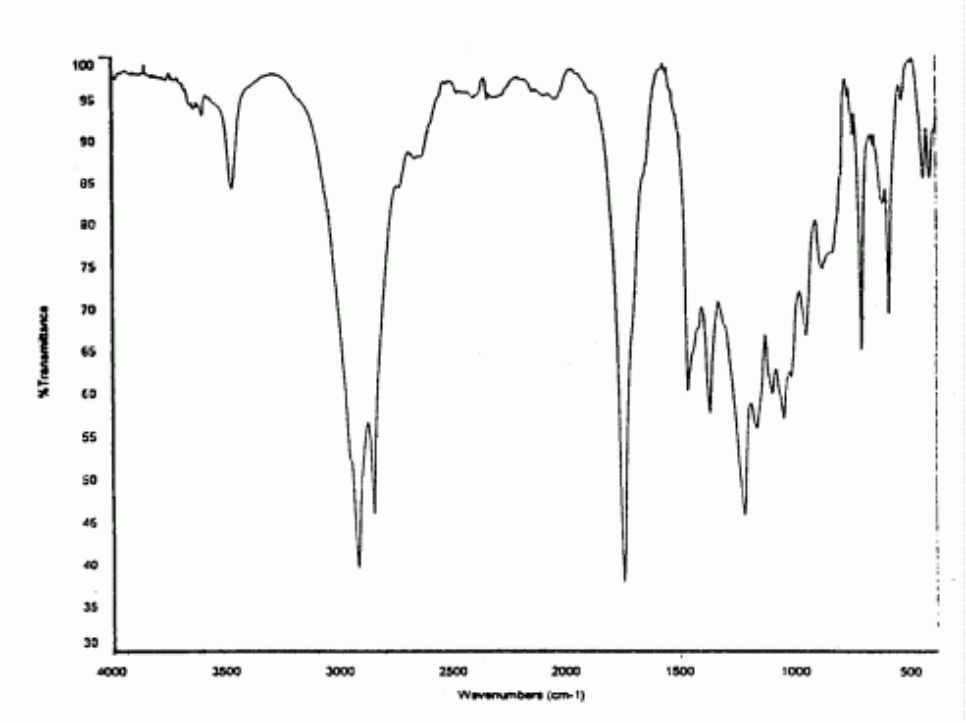

Figure 1: Representative infrared absorption spectrum of Salatrim
Representative HTCGC chromatograms of Salatrim (approximately 2000 mg/l; figures 2, 3 and 4).

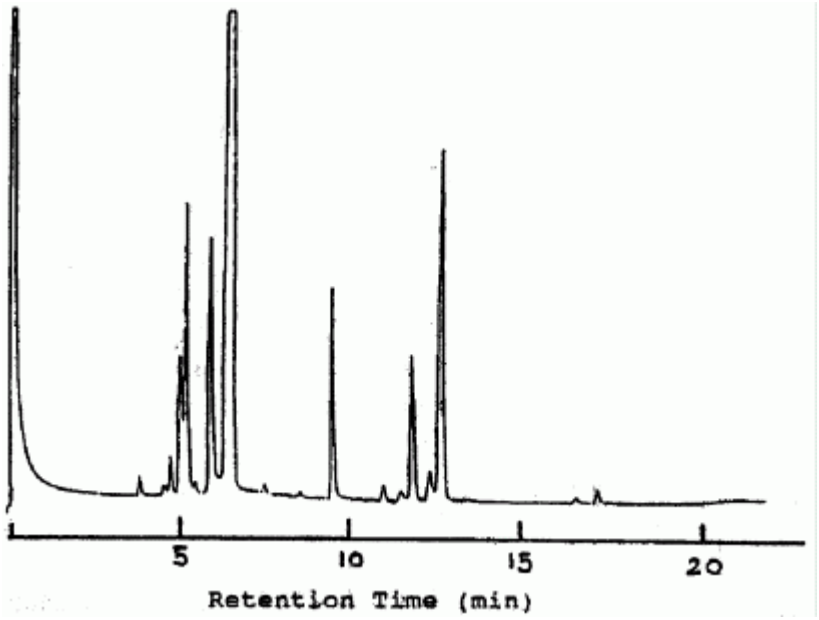

Figure 2: Salatrim derived from tributyrin, tripropionin, and hydrogenated soybean oil - 54% dibutyrylstearoylglycerol (ACN 26); 12% butyryldistearoylglycerol (ACN 40); 8.5% butyrylpropionylstearoylglycerol (ACN 25); 7.3% dibutyrylpalmitoylglycerol (ACN 24); 5.1% butyrylstearoylglycerol (ACN 22); and 4.4% butyrylpalmitoylstearoylglycerol (ACN 38). Note: The percentages are only representative and will vary from batch to batch.

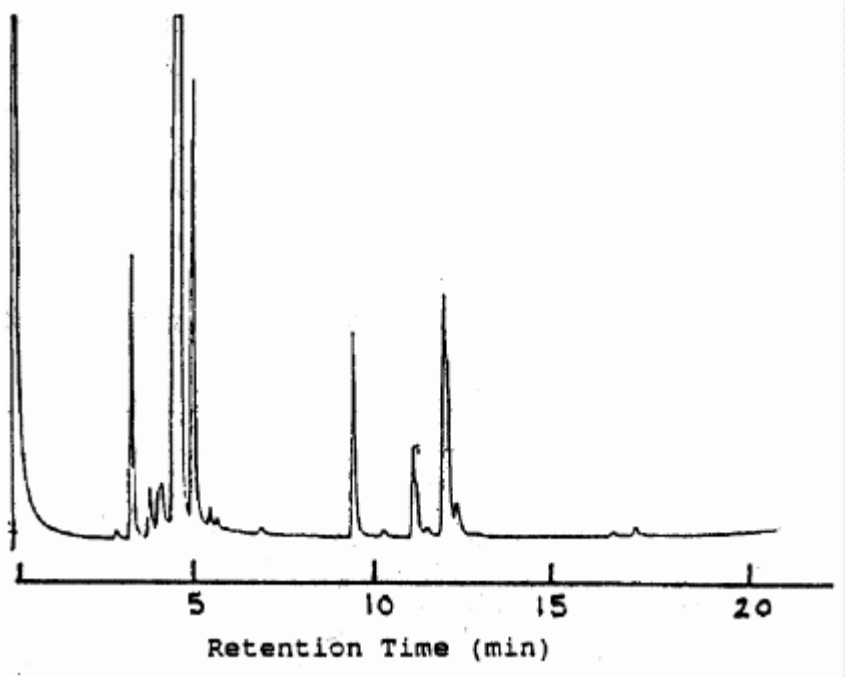

Figure 3: Salatrim derived from triacetin, tripropionin, and hydrogenated soybean oil - 60% diacetylstearoylglycerol (ACN 22); 11% acetylpropionylstearoyl- glycerol (ACN 23); 9.2% acetyldistearoylglycerol (ACN 38); 6.8% diacetylpalmitoylglycerol (ACN 20); and 2.3% acetylstearoylglycerol (ACN 20). Note: The percentages are only representative and will vary from batch to batch.

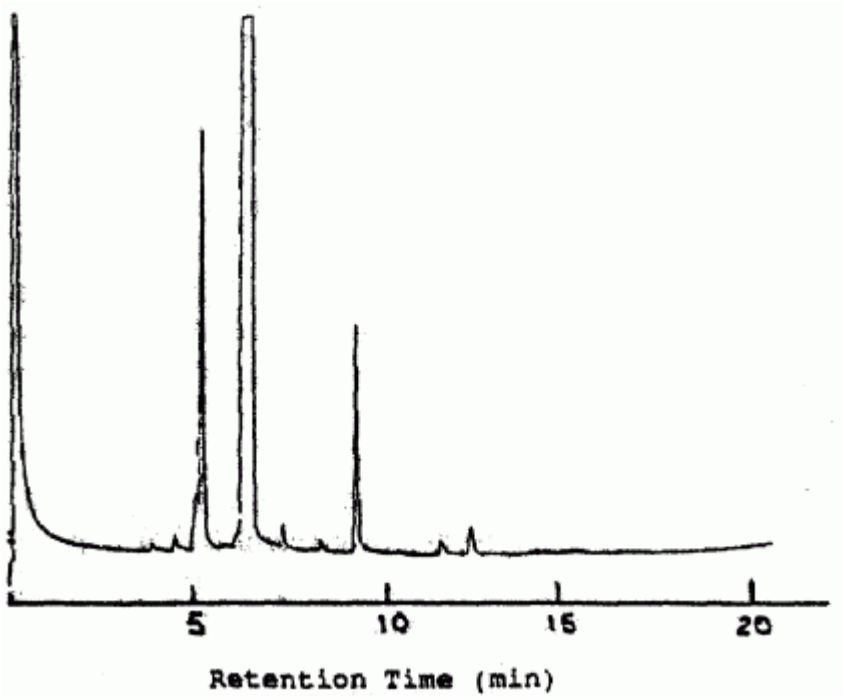

Figure 4: Salatrim derived from tributyrin and hydrogenated soybean oil - 86% dibutyrylstearoylglycerol (ACN 26); 10% dibutyrylpalmitoylglycerol (ACN 24); and 1.9% butyrylstearoylglycerol (ACN 22). Note: The percentages are only representative and will vary from batch to batch.

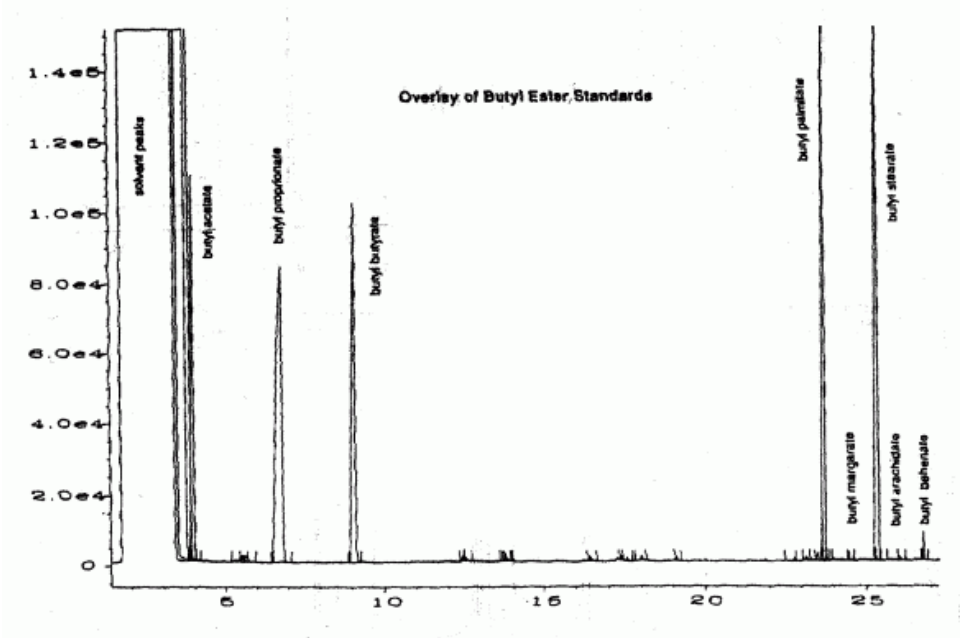

Figure 5: Representative capillary gas chromatograms of butyl esters of Salatrim fatty acids for determination of SCFA/LCFA mole ratio.