CODEX STANDARD FOR OLIVE OIL, VIRGIN AND REFINED, AND FOR REFINED OLIVE-POMACE OIL

CODEX STAN 33-1981 (Rev. 1-1989) [1]

1. SCOPE

This Standard applies to virgin olive oil, refined olive oil, refined olive-pomace oil, blends of refined olive oil and virgin olive oil and blends of refined olive-pomace oil and virgin olive oil.

2. DESCRIPTION

2.1 ***Olive oil*** is the oil obtained from the fruit of the olive tree (*0lea europaea sativa* Hoffm. et Link) without having been subjected to manipulation or any treatment not authorized by sub-sections 2.2 and 2.3 of this Standard.

2.2 ***Virgin olive oil*** is the oil obtained from the fruit of the olive tree by mechanical or other physical means under conditions, particularly thermal, which do not lead to alteration of the oil. Virgin olive oil is an oil which is suitable for consumption in the natural state.

2.3 ***Refined olive oil*** is the oil obtained from virgin olive oil, the acid content and/or organoleptic characteristics of which render it unsuitable for consumption in the natural state, by means of refining methods which do not lead to alterations in the initial glyceridic structure.

2.4 ***Refined olive-pomace oil*** is the oil obtained from "olive pomace" by extraction by means of solvents and made edible by means of refining methods which do not lead to alteration in the initial glyceridic structure.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS [2]

3.1 Identity Characteristics (under normal ecological conditions) [3]

3.1.1 GLC ranges of fatty acid composition (% m/m of methyl esters)

Lauric acid	(C 12:0)	Not present in discernible amounts
Myristic acid	(C 14:0)	< 0.1
Palmitic acid	(C 16:0)	7.5 – 20.0
Palmitoleic acid	(C 16:1)	0.3 - 3.5
Heptadecanoic acid	(C 17:0)	< 0.5
Heptadecenoic acid	(C 17:1)	< 0.6
Stearic acid	(C 18:0)	0.5 - 5.0

---

1 Formerly CAC/RS 33-1970.

2 The limits of essential composition and quality factors of virgin olive oils show very widely spaced minimum and maximum values, since they take account of the oil characteristics of all producing countries. Characteristics and limits of physical and chemical indices and values, and of fatty acid composition for the various grades of virgin olive oils produced in each olive-growing area, determined at the outset and close of the olive oil production year, are published yearly in each producing country's "National 0live 0il Index File".

3 Samples falling outside the GLC fatty acid ranges are not in compliance with the Standard. Supplementary non-mandatory criteria may be employed if it is considered necessary to confirm that a sample is in compliance with the standard.

0leic acid	(C 18:1)	55.0 – 83.0
Linoleic acid	(C 18:2)	3.5 – 21.0
Linolenic acid	(C 18:3)	< 1.5
Arachidic acid	(C 20:0)	< 0.8
Behenic acid	(C 22:0)	< 0.3
Erucic acid	(C 22:1)	Not present in discernible amounts
Lignoceric acid	(C 24:0)	< 1.0

3.1.2 Physical and chemical indices

3.1.2.1 Relative density (20^oC/water at 20^oC)

Virgin olive oil	)	
Refined olive oil	)	0.910 - 0.916
Refined olive-pomace oil	)	

3.1.2.2 Refractive index (n_D^{20})

Virgin olive oil	)	1.4677 - 1.4705
Refined olive oil	)	
Refined olive-pomace oil		1.4680 - 1.4707

3.1.2.3 Saponification value (mg KOH/g oil)

Virgin olive oil	)	184 – 196
Refined olive oil	)	
Refined olive-pomace oil		182 – 193

3.1.2.4 Iodine value (Wijs)

Virgin olive oil	)	75 – 94
Refined olive oil	)	
Refined olive-pomace oil		75 – 92

3.1.2.5 Unsaponifiable matter (using light petroleum)

Virgin olive oil	)	not more than 15 g/kg [4]
Refined olive oil	)	
Refined olive-pomace oil		not more than 30 g/kg [5]

[4] A characteristic feature of the unsaponifiable matter in olive oil is its content of squalene, which is higher than that of the other vegetable oils. Another distinctive feature is that its sterols are composed of practically pure beta-sitosterol.

[5] The unsaponifiable matter of olive-pomace oil contains more alcoholic compounds than that of virgin or refined olive oils, and its iodine value is therefore lower than that normally noted in virgin or refined olive oils, and its melting point is higher.

3.1.2.6 Bellier index

Virgin olive oil	not more than 17 [6]
Refined olive oil	
Refined olive-pomace oil	not applicable

3.1.2.7 Semi-siccative oil test

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.8 0live-pomace oil test

Virgin olive oil	)	negative
Refined olive oil	)	
Refined olive-pomace oil		not relevant

3.1.2.9 Cottonseed oil test

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.10 Teaseed oil test

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.11 Sesameseed oil tests

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.12 Sterol content (% of the sum of beta-sitosterol, campesterol and stigmasterol) [7]

		Beta-sitosterol	Campesterol	Cholesterol
Virgin olive oil	)			
Refined olive oil	)	= 93	= 4.0	= 0.5
Refined olive-pomace oil	)			

3.1.2.13 Saturated fatty acids at position 2

	Maximum level
Virgin olive oil	1.5% m/m
Refined olive oil	1.8% m/m

---

[6] Should this index be higher than 17, the content of arachidic, behenic and lignoceric acid shall be given.

[7] Beta-sitosterol as determined by the method of analysis specified by the Standard includes Δ-5 avenasterol since this is not separated from beta-sitosterol by the column packing material SE30.

Blends of refined olive oil and virgin olive oil	1.8% m/m
Refined olive-pomace oil	2.2% m/m
Blends of refined olive-pomace oil and virgin olive oil	2.0% m/m

The saturated fatty acids at position 2 means the sum of the palmitic (16:0) and stearic (18:0) acids expressed as a percentage (m/m) of the total fatty acids at position 2.

3.2 Quality Characteristics

3.2.1 Colour, odour and taste

Virgin olive oil: Clear oil, of a yellow to green colour, with specific odour and taste, free from odours or tastes indicating alteration or pollution of oil.

Refined olive oil: Clear oil, limpid without sediment, of clear yellow colour without specific odour or taste and free from odours or tastes indicating alteration or pollution of the oil.

Refined olive-pomace oil: Clear oil, limpid, without sediment, of a yellow to yellow-brown colour, without specific odour or taste and free from odours or tastes indicating alteration or pollution of the oil.

Blends: The colour, odour and taste shall be intermediate between those of two types blended.

3.2.2 Free acidity

	Acidity maximum	Acid value maximum
	% m/m expressed as oleic acid	mg KOH/g oil
Virgin olive oil	3.3	6.6
Refined olive oil	0.3	0.6
Refined olive-pomace oil	0.3	0.6
Blends	l.5	3.0

3.2.3 Peroxide value (in milliequivalents peroxide oxygen/kg oil)

Virgin olive oil	= 20
Refined olive oil	= 10
Refined olive-pomace oil	= 10
Blends	= 20

3.2.4 Specific extinction in ultra-violet

	Extinction (E) maximum at 232 nm	Extinction (E) maximum at 270 nm	Δ E maximum variation at near 270 nm
Virgin olive oil	3.50	0.30	[8]
Refined olive oil	-	1.10	0.16
Refined olive-pomace oil	6.00	2.00	0.20
Blends of refined olive oil and virgin olive oil	-	0.90	0.15
Blends of refined olive-pomace oil and virgin olive oil	5.50	1.70	0.18

4. FOOD ADDITIVES

			Maximum level
4.1	Virgin olive oil	None permitted	
4.2	Refined olive oil Refined olive-pomace oil Blends	Alpha-tocopherol for the purpose of restoring natural tocopherol lost in processing	200 mg/kg total alpha-tocopherol in the final product

5. CONTAMINANTS

5.1 Matter volatile at 105°C

Virgin olive oil	≤ 0.2% m/m
Refined olive oil	≤ 0.1% m/m
Refined olive-pomace oil	≤ 0.1% m/m
Blends	≤ 0.1% m/m

5.2 Insoluble impurities

Virgin olive oil	≤ 0.1% m/m
Refined olive oil	≤ 0.05% m/m
Refined olive-pomace oil	≤ 0.05% m/m
Blends	≤ 0.05% m/m

5.3 Soap Test

Refined olive oil Refined olive-pomace oil Virgin olive oil	negative
Virgin olive oil Blends	not applicable

[8] 0ils having a specific extinction at 270 nm exceeding 0.30 may still be regarded as virgin oils if, after passage of the sample through activated alumina, their specific extinction at 270 nm is less than 0.11.

6. HYGIENE

It is recommended that the product covered by the provisions of this Standard be prepared in accordance with the appropriate Sections of the General Principle of Food Hygiene recommended by the Codex Alimentarius Commission (CAC/RCP 1-1969, Rev. 3-1997).

7. LABELLING

In addition to the provisions of the General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985, Rev. 1-1991), the following provisions shall apply.

7.1 Name of the Food

7.1.1 All products designated as "olive oil" shall conform to the provisions of this Standard for virgin olive or refined olive oil and shall be either virgin olive oil, refined olive oil or a blend of refined olive oil and virgin olive oil.

7.1.2 All products designated as "virgin olive oil" shall conform to the provisions for virgin olive oil.

7.1.3 All products designated as "refined olive oil" shall conform to the provisions for refined olive oil.

7.1.4 All products designated as "refined olive-pomace oil" shall conform to the provisions for refined-pomace oil.

7.1.5 Refined olive-pomace oil shall in no case be described as "olive oil" but shall always be designated as "refined olive-pomace oil".

7.1.6 Blends of refined olive-pomace oil and virgin olive oil shall be described as "olive-pomace oil".

7.2 Labelling of Non-retail Containers

In addition to the provisions of the General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985, Rev. 1-1991), the following provisions shall apply to outer containers of a number of prepackaged containers of the products covered by the Standard.

Information on the above labelling requirements shall be given either on the container or in accompanying documents, except that the name of the food, lot identification and the name and address of the manufacturer or packer shall appear on the container.

However, lot identification and the name and address of the manufacturer or packer may be replaced by an identification mark, provided that such a mark is clearly identifiable with the accompanying documents.

8. METHODS OF ANALYSIS AND SAMPLING

8.1 Determination of Fatty Acid Composition

According to IUPAC 2.301, 2.302 and 2.304 or ISO 5508: 1990/5509: 1999.

8.2 Determination of Relative Density

According to IUPAC 2.101, with the appropriate conversion factor. Results are expressed as relative density at 20°C/water at 20°C.

8.3 Determination of Refractive Index

According to IUPAC 2.102.

Results are expressed as the refractive index relative to the sodium D-line at 20ºC.

8.4 Determination of Saponification Value

According to IUPAC 2.202.

Results are expressed in mg KOH/g oil.

8.5 Determination of Iodine Value

According to IUPAC 2.205/1, Wijs method, or ISO 3961:1996

Results are expressed as % m / m absorbed iodine.

8.6 Determination of Unsaponifiable Matter

According to IUPAC 2.401 (part 1-5). Results are expressed as g unsaponifiable matter/kg oil.

8.7 Determination of Bellier Index (CAC/RM 20-1970)

Definition

The Bellier Index of an oil is the temperature at which precipitation of salts of the fatty acids of this oil commences, when the oil has been saponified and made into solution as described under Procedure.

Reagents

The reagents used shall be of recognized analytical reagent quality.

– Aqueous ethanolic potassium hydroxide solution.

– 42.5 g of pure KOH is dissolved in 72 ml of distilled water and adjusted to 500 ml with 95% v/v ethanol.

– 70% v/v ethanol solution (use pure ethanol or rectified spirit).

Aqueous acetic acid solution 1+2 (by volume) so adjusted that 1.5 ml exactly neutralizes (phenolphthalein indicator) 5 ml of the aqueous ethanolic potassium hydroxide solution (8.7.2.1).

Apparatus

– 220 mm x 26-27 mm test tubes.

– Condenser consisting of a glass tube with stopper.

– Thermometer graduated in 1/4° from 8 to 25°C, fixed in a stopper.

Preparation of Sample

To remove water, the oil is decanted and filtered through paper at a temperature slightly above the melting point of certain solid constituents which could separate from the fluid fatty matter.

Procedure

Place 1 ml of oil and 5 ml of the aqueous ethanolic KOH solution into a test tube. Connect to condenser and heat moderately, agitating by rotation from time to time until saponification is completed, i.e. until a perfectly clear solution in obtained. Allow to cool, disconnect condenser and add 1.5 ml of the aqueous acetic acid solution and 50 ml of the ethanol solution. Attach thermometer and homogenize. Place test tube in a beaker of water at 23-25°C. If a flocculent precipitate forms, leave standing for an hour at the same temperature and filter into a test tube. Attach thermometer to the test tube containing the clear solution. Place for a moment in a beaker of water at about 10°C less than the estimated Bellier index. Withdraw and ensure even temperature by inverting a number of times (cooling should be at the rate of about 1°C per minute). Repeat this operation until cloudiness appears. Note temperature. Allow the temperature to increase a few degrees to dissolve the precipitate. Homogenize by inverting test tube over and cool. The cooling should be slow and shaking more frequent as the temperature approaches that noted the first time.

Expression of Results

The Bellier index is the temperature °C at which the cloudiness reappears.

Repeatability

Two parallel determinations may not differ by more than 0.25°C.

8.8 Semi-siccative Oil Tests (CAC/RM 21-1970)

Principle of Method

Based on the reaction between semi-siccative (unsaturated) oils and bromine yielding substances which form an insoluble precipitate at 0°C.

Reagents

The reagents shall be of recognized analytical reagent quality.

– Hexane, or, if not available, light petroleum with distillation point between 40° and 60°C and bromine value less than 1, free of residues.

– Bromine reagent obtained by adding drop by drop while shaking 4 ml of chemically pure bromine (the presence of chlorine prevents the reaction) into 100 ml of hexane or light petroleum, chilled to 0°C and kept in the melting ice bath until required.

Apparatus

– Stoppered 50-ml Erlenmeyer flask.

– Bath of melting ice.

Procedure

The oil to be tested is filtered and dried. Place 1 ml of the oil in the previously dried Erlenmeyer flask and dissolve in 10 ml of hexane. Place the stoppered Erlenmeyer flask in the melting ice bath. After 5 min add 10 ml of bromine reagent in small quantities at a time, while shaking and maintaining the temperature at 0°C. The colour of the solution must clearly indicate excess bromine. Leave the Erlenmeyer flask in the melting ice bath for one hour, after which note appearance of solution. If semi-siccative oil is present, a flocculent precipitate will form varying in quantity according to the percentage of adulteration and the nature of the adulterating oil. The solution remains clear and transparent in the case of genuine olive oils.

Expression of Results

The result is expressed as positive or negative.

8.9 Olive-Pomace Oil Test (CAC/RM 22-1970)

Principle of Method

Based on the temperature of precipitation of salts of the fatty acids after saponification.

Reagents

The reagents used shall be of recognized analytical reagent quality.

– Aqueous ethanolic potassium hydroxide solution. 42.5 g of pure KOH is dissolved in 72 ml of distilled water and adjusted to 500 ml with 95% v/v ethanol.

– 70% v/v ethanol solution (use pure ethanol or rectified spirit).

– Aqueous acetic acid solution 1+2 (by volume) so adjusted that 1.5 ml exactly neutralizes (phenolphthalein indicator) 5 ml of the aqueous ethanolic potassium hydroxide solution.

Apparatus

– 100 m1 balloon-flask equipped with reflux condenser.

– 50 ml test tubes.

– Heating arrangement to keep balloon-flask at about 80°C.

– Thermometer graduated from 15° to 60°C.

Preparation of Sample

To remove water, the oil is decanted and filtered through paper at a temperature slightly above the melting point of certain solid constituents which could separate from the fluid fatty matter.

Procedure

Place about 1 g of the oil, prepared as above, into the balloon-flask. Add 5 ml of aqueous ethanol potassium hydroxide solution. Attach condenser and bring to boil holding at this temperature for 10 minutes, shaking from time to time. Allow to cool at ambient temperature. Add 1.5 ml of acetic acid solution and 50 ml of ethanol solution previously heated to 50°C. Mix by shaking, introduce thermometer and allow to cool, noting the appearance of the solution once 45°C in reached. If a flocculent precipitate forms at a temperature above 40°C, the test is positive. Allow to cool to ambient temperature (not lower than 18°C) over at least 12 hours. Observe solution again; the formation of a flocculent precipitate, floating in the middle of the liquid also indicates that the test is positive. A cloudiness not forming into flakes does not indicate the presence of olive-pomace oil.

Expression of Results

The result is expressed as positive or negative.

NOTE : On rare occasions some virgin olive oils, obtained by second pressing, yield a positive result.

8.10 Cottonseed Oil Test (CAC/RM 23-1970)

Principle of Method

Based on red colour developed by cyclo-propenoic acids under the operating conditions in the presence of sulphur.

Reagents

The reagents used shall be of recognized analytical quality.

Sulphur reagents : Mix equal volumes of amyl alcohol and a solution of 1 g of sulphur in 100 ml of carbon disulphide.

Apparatus

- 250 mm x 25 mm test tubes.
- Water bath with constant temperature control.
- Heating apparatus to keep the test tubes at 110^{o}-120^{o} C.

Procedure

Place approximately 10 ml of the oil under examination into a test tubes add the same volume of sulphur reagent; shake and keep in water bath at 70°- 80°C, shaking until the carbon disulphide has completely evaporated (generally 5 minutes are enough), which is confirmed by the appearance of slight fuming above the liquid. Transfer the test tube to the heating apparatus and keep at 110°- 120°C for 2.hours. A red, or pink colour indicates the presence of cottonseed oil. However, the appearance of an orange colour must not be interpreted as being proof of the presence of cottonseed oil.

Expression of Results

The result in expressed as positive or negative.

NOTE: The heating of the cottonseed oil to a temperature above 170°C brings about a progressive destruction of the cyclo-propenoic acid responsible for the coloration. This destruction in practically complete at 200°C.

8.11 Teaseed Oil Test (CAC/RM 24-1970)

Principle of Method

Based on Fitelson (modified Lieberman-Burchard) tests i.e. red colour developed by acetic anhydride in the presence of sulphuric acid in chloroform solution of the oil.

Reagents

The reagents used shall be of recognised analytical quality.

- Chloroform
- Concentrated sulphuric acid (d = 1.84)
- Acetic anhydride
- Diethyl oxide

Apparatus

– 150 mm x 15 mm test tubes.

– 2 ml pipette, graduated in tenths.

– Dropper so calibrated that *7* drops of oil weigh approximately 0.22 g.

– Water bath at 5°C.

Procedure

Using the graduated pipette, place 0.8 ml of acetic anhydride, 1.5 ml of chloroform and 0.2 ml of sulphuric acid in a test tube. Cool to 5°C, then add approximately 0.22 g of oil. If cloudiness appears add acetic anhydride drop by drop with shaking until the solution becomes clear. Keep at 5°C for *5* minutes. Add 10 ml of diethyl oxide previously cooled to 5°C. Stopper the test tube and mix immediately by inverting it twice. Return the test tube to the bath at 5°C and observe the colour. After about one minute a red colour will appear if tea oil is present.

Expression of Results

The result is expressed as positive or negative.

NOTE: A pink colour shall be regarded as negative, since some olive oils yield this colour.

8.12 Sesameseed Oil Tests (CAC/RM 25-1970)

Principle of Method

Based on the detection of sesamoline, a glycoside, and sesamine, a complex cyclic ether, which are present in small amounts in sesameseed oil.

8.12.1 Detection of Sesamoline

Reagents

The reagents used shall be of recognized analytical quality.

– Concentrated hydrochloric acid (d = 1.18).

– Solution of 2% v/v freshly distilled furfural in 95% v/v ethanol.

Apparatus

Graduated 50-m1 stoppered test tube.

Procedure

Place 10 ml of the oil and 10 ml of conc. hydrochloric acid in the graduated test tube. Stopper and shake vigorously for 30 seconds. Allow to stand. Add 0.5 ml of the solution of furfural. Stopper and shake again. Allow to stand until decantation. If the lower layer does not turn red, the test is negative. If a red coloration appears, add 10 ml of water and shake gently and allow the liquid to settle. If the coloration disappears, the test is negative. If the coloration remains, the test is positive. Refined sesame oils do not always give a positive reaction by this method.

Expression of Results

The result is expressed as positive or negative.

8.12.2 Detection of Sesamine

Reagents

The reagents used shall be of recognized analytical quality.

– Concentrated sulphuric acid (d = 1.84).

– Solution of freshly distilled furfural in acetic anhydride, 0.35/ml v/v.

Apparatus

– 25-ml, stoppered graduated test tube.

– Decanting beaker approximately 50-ml.

– Flat-bottomed porcelain dish approximately 60 mm in diameter.

Procedure

Place 10 ml of the oil and 5 ml of the solution of furfural in the test tube. Stopper and shake vigorously for approximately one minute. Pour the mixture into the decanting beaker and allow to settle. Transfer a portion of the deposit into the dish and add 6 or 7 drops of sulphuric acid. Mix by shaking the dish gently.

The test is positive if a greenish-blue colour appears. Sesame oils, even when refined, give a positive reaction.

Expression of Results

The result is expressed as positive or negative.

8.13 Determination of the Sterol Content

According to IUPAC 2.403.

Results expressed as % of the sum of beta-sitosterol, campesterol and stigmasterol.

8.14 Determination of the Fatty Acids in the 2 Position

According to the IUPAC method (1979, 6th edition) no. 2.210. "Determination of the Fatty Acids in the 2-position in the Triglycerides of Oils and Fats".

The saturated fatty acids at position 2 means the sum of palmitic (16 :0) and stearic (18 :0) acids expressed as a percentage m/m of the total fatty acids at position 2.

8.15 Determination of Free Acidity

According to IUPAC 2.201.

Results are expressed as % m/m oleic acid and/or as the number of mg KOH required to neutralize 1 g oil.

8.16 Determination of Peroxide Value

According to IUPAC 2.501 or ISO 3960 :1998

Results expressed as milliequivalents active oxygen/kg.

8.17 Determination of Specific Extinction in Ultra-Violet (CAC/RM 26-1970)

Principle of Method

The degree of oxidation of olive oil is reflected by its specific extinction at 232 nm and 270 nm. In fact, virgin olive oils, of good quality and correctly stored, contain very few products of oxidation; these mainly peroxidic in nature, have a maximum absorption at approximately 232 nm. The values of E ?, at 232 and 270 nm in such olive oils are below the maximum provided for in the standard. On the other hand, when the oil is treated with a decolourising agent (i.e. an absorbent earth) during the refining process, conjugated trienoic compounds are formed. These compounds have a maximum absorption situated at approximately 270 nm; this means that refined oils have higher values of E ? at 270 nm.

NOTE: Measurement of specific extinction in ultra-violet is essentially a measurement of the state of alteration of the oil. It is not specifically a measurement of the refining. In some particular cases, abnormally altered virgin oils can show spectral characteristics close to those of refined oils.

Reagents

– Spectrophotometrically pure cyclohexane: Minimum transmittance at 220 nm: 40% and minimum transmittance at 250 nm: 95% by comparison with distilled water.

– Basic alumina of known grade

Basic alumina of Brockmann grade 1 (0% water) is obtained by heating for 3 hours at 380-400ºC basic alumina (chromatographic quality) of particle size 30µ to 130 µ (mean 80 µ). To 100g of this product add 5 ml of distilled water to produce basic alumina of Brockmann grade close to IV.

NOTE: Method used to check the activity index of the alumina.

Place 30 g of the basic alumina (as obtained above) in a chromatographic column, 450 mm long with a diameter of 35mm; through this column pass, under the conditions laid down in the method, a mixture of 95% virgin olive oil, having a specific extinction coefficient below 0.18 at 270 nm, and of 5% arachis oil previously treated, during the refining process, with decolourising agent (absorbent earth) and having a specific extinction coefficient equal to or above 4 at 270 nm. If this mixture shows a specific extinction coefficient greater than 0.11, the activity of the alumina is acceptable. Should the elution of conjugated trienes not have taken place using this alumina, an alumina at a higher hydration should be used after verifying that it agrees with the preceding test.

Apparatus

– Ultra-violet spectrophotometer for measurements between 210 and 300 nm.

– Quartz cells of 1cm thickness.

– 50-ml and 500-ml volumetric flasks.

– Chromatographic column, 450 mm long with a diameter of 35 mm.

Adjustment of Spectrophotometer: dissolve 0.2 g of dry potassium chromate in exactly 1 litre of a 0.05 N solution of potassium hydroxide. Place exactly 25 ml of this solution in a 500-ml flask and bring up to the 500-ml mark with the 0.05 N solution of potassium hydroxide. Determine the optical density of this latter solution by comparison with the 0.05 N solution of potassium hydroxide as a reference solution, in a 1 cm cell. This, at 275 nm should be 0.200 ± 0.005.

Procedure

If the oil is not completely clear at ambient temperature, filter before attempting measurements. Place approximately 0.5 g, weighed accurately, of the oil in the 50-ml flask. Add the cyclohexane up to the mark and shake. Fill a cell with this solution and measure the optical density using the cyclohexane as a reference solution. Make determinations at 232 and 270 nm. Determine, in the region of 270 nm, the wavelength of the maximum absorption λ_m and determine the optical density at λ_m nm, λ_{m-4} nm and λ_{m+4} nm.

Calculation and Expression of Results

Calculation of Specific Extinction at 232 and 270 nm

$$E\lambda = \frac{A\lambda}{c\,l}$$

where:

E ? = specific extinction at wavelength ? nm

A ? = optical density at wavelength ? nm

c = concentration of the test solution in g/100 ml

l = thickness of the cell in cm

NOTE: If the optical density is less than 0.2, re-measure with a more concentrated solution. If it is more than 0.8, re-measure with a weaker solution.

Calculation of the variation of the specific extinction at the wavelength of maximum absorption near 270nm

$$\Delta E = E\lambda_m - \frac{(E\lambda_{m-4}) + (E\lambda_{m+4})}{2}$$

Where:

? E = variation of specific extinction at ? $_m$

E ?$_m$ = specific extinction at the wavelength of maximum adsorption near 270 nm.

E ?$_{m-4}$ and E ?$_{m+4}$ = specific extinctions at wavelengths plus ? $_m$ plus or minus 4 nm.

Additional procedure for determination of the specific extinction after passage through alumina

Place 30 g of basic alumina (as in the reagents section earlier) in a chromatography column approximately 450 mm long and 35 mm in diameter, furnished with a draining tube of about 10 mm diameter. Pack the alumina mechanically by repeatedly tapping the column, held vertically, on a wooden surface. Place in the column thus prepared 100 ml of a solution of 10 % oil in hexane. Collect the drainings and evaporate the solvent in a vacuum at less than 25°C. Using the oil so obtained, immediately determine the specific extinction at 270 nm, as previously described.

8.18 Determination of Alpha-Tocopherol

According to IUPAC 2.404 "Identification and determination of tocopherols", method A.

Results are expressed as mg tocopherol/kg oil.

8.19 Determination of Matter Volatile at 105°C

According to IUPAC 2.601 or ISO 662 :1998. Results are expressed as % m/m.

8.20 Determination of Insoluble Impurities

According to the IUPAC 2.604. Results are expressed as % m/m.

8.21 Soap Test (CAC/RM 27-1970)

Principle of Method

Detection of alkalinity using bromophenol blue as indicator.

Reagents

- Solution of 0.10 of bromophenol blue in 96º v/v ethanol.
- Freshly distilled acetone, 20 v/v water content.
- A few drops of the solution of bromophenol blue should give a yellow to yellow-green colour to the acetone with 2% water.

Apparatus

150 mm x 15 mm test tube.

Procedure

Place 10 ml of the acetone and 1 drop of the bromophenol blue solution in a test tube. The solution should have a yellow colour. If not, rinse the test tube with acetone until the blue colour disappears. Place 10 g of the oil in the test tube, stopper with a clean stopper, shake and allow to settle. The presence of blue colour in the upper acetonic layer indicates the presence of soap.

Expression of Results

The result is expressed as positive or negative.