

Appendix A

From USDA Standards for Grades of Olive Oil and Olive-Pomace Oil

§52.1539 Ascertaining the grade.

The U.S. grades of olive oil or olive-pomace oil must meet the following minimum requirements, of the respective grades listed in Table I, as appropriate.

TABLE I

Quality Criteria	US Extra Virgin Olive Oil	US Virgin Olive Oil	Lampante Virgin Olive Oil ^{1/}	US Refined Olive Oil	US Olive Oil	US Olive-pomace Oil	US Refined Olive-pomace Oil	US Crude Olive-pomace Oil
(a) Organoleptic Characteristics								
- Odor And Flavor	Excellent	Good	Poor	Acceptable	Good	Good	Acceptable	N/A
- Odor And Flavor (On A Continuous Scale):								
• Median Of Defect (M_d)	$M_d = 0$	$0 < M_d \leq 2.5$	$M_d > 2.5$ ^{2/}	N/A	N/A	N/A	N/A	N/A
• Median Of The Fruity (M_f)	$M_f > 0$	$M_f > 0$	N/A	N/A	N/A	N/A	N/A	N/A
• Color	Yellow To Green	Yellow To Green	Yellow To Green	Light Yellow	Light Yellow To Green	Light Yellow To Green	Light Yellow To Brownish Yellow	Dark Green, Brown, Or Black
(b) Free Fatty Acid Content, % m/m Expressed As Oleic Acid	≤ 0.8	≤ 2.0	> 2.0	≤ 0.3	≤ 1.0	≤ 1.0	≤ 0.3	No limit
(c) Peroxide Value, In Milleq. Peroxide Oxygen Per kg/oil	≤ 20	≤ 20	No Limit	≤ 5	≤ 15	≤ 15	≤ 5	No limit

1/ The criteria in (a), (b), and (c) is not required to be concurrent; one is sufficient (for lampante oil only).

2/ Or when the median of the defect attribute is less than or equal to 2.5 and the median of the fruity attribute is equal to 0.

TABLE I continued

	US Extra Virgin Olive Oil	US Virgin Olive Oil	Lampante Virgin Olive Oil	US Refined Olive Oil	US Olive Oil	US Olive-pomace Oil	US Refined Olive-pomace Oil	US Crude Olive-pomace Oil
Quality Criteria								
(d) Absorbency In Ultraviolet (UV) (K1% 1cm)								
- 270 nm	≤ 0.22	≤ 0.25	N/A	≤ 1.10	≤ 0.90	≤ 1.70	≤ 2.00	N/A
- Δ K	≤ 0.01	≤ 0.01	N/A	≤ 0.16	≤ 0.15	≤ 0.18	≤ 0.20	N/A
- 232 nm	≤ 2.50 ^{6/}	≤ 2.60 ^{6/}	N/A	N/A	N/A	N/A	N/A	N/A
Purity Criteria								
(e) Fatty Acid Composition As Determined By Gas Chromatography (% m/m Methyl Esters)	-Arachidic Acid (C20:0) ≤ 0.6 -Behenic Acid (C22:0) ≤ 0.2 ^{3/} -Gadoleic Acid (Eicosenoic) (C20:1) ≤ 0.4 -Heptadecanoic Acid (C17:0) ≤ 0.3 -Heptadecenoic Acid(C17:1) ≤ 0.3 -Lignoceric Acid (C24:0) ≤ 0.2 -Linoleic Acid (C18:2) 3.5 – 21.0					-Linolenic Acid (C18:3) ≤ 1.5 ^{4/} -Myristic Acid (C14:0) ≤ 0.05 -Oleic Acid (C18:1) 55.0 – 83.0 -Palmitoleic Acid (C16:1) 0.3 – 3.5 -Palmitic Acid (C16:0) 7.5 – 20.0 -Stearic Acid (C18:0) 0.5 – 5.0		
(f) Trans Fatty Acid (T) Content (%) C18:1T ^{5/}	≤ 0.05	≤ 0.05	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.40	≤ 0.40	≤ 0.20
(g) Trans Fatty Acid Content (%) C18:2T+C18:3T	≤ 0.05	≤ 0.05	≤ 0.10	≤ 0.30	≤ 0.30	≤ 0.35	≤ 0.35	≤ 0.10

^{3/} Limit raised to ≤ 0.3 for olive-pomace oils.

^{4/} Linolenic acid values between 1.0 and 1.5 percent would be subject to further testing listed in Table II.

^{5/} Fatty acid with 18 Carbon atoms (C) and one *trans* isomer (T)

^{6/} Commercial partners in the country of retail sale may require compliance.

TABLE I continued.

Purity Criteria	US Extra Virgin Olive Oil	US Virgin Olive Oil	Lampante Virgin Olive Oil	US Refined Olive Oil	US Olive Oil	US Olive-pomace Oil	US Refined Olive-pomace Oil	US Crude Olive-pomace Oil
(h) Desmethylsterol Composition (% Total Sterol)								
(i) Total Sterol Content (mg/kg)	≥1000	≥1000	≥1000	≥1000	≥1000	≥1600	≥1800	≥2500

^{z/} Limit raised to ≤ 0.2 for olive-pomace oils.

^{8/} Campesterol values between 4.0 and 4.5 would be subject to further testing listed in Table II.

Appendix B

Description of Olive Oil Analyses Tests

Paul Vossen

- **Color:** A subjective visual rating is made of an oil to make sure it does not have unusual color that is not common to olive oil. It can also be evaluated using spectrometric instruments according to the American Oil Chemists Society (AOCS) and International Union of Pure Applied Chemistry (IUPAC) methods for specific hue, purity, and brightness. Colors other than green to golden might indicate a problem.
- **Aspect:** A subjective visual rating is made to determine if the oil is clear (limpid) or cloudy after it has been maintained at a temperature of 68°F (20°C) for 24 hours. Aspect can indicate the abnormal presence of saturated fatty acids or moisture in olive oil.
- **Free Fatty Acids:** This is a crude indicator of oil quality based on fruit handling procedures prior to milling. It is a measurement of hydrolytic breakdown of the fatty acid chains from triglycerides into diglycerides and monoglycerides, liberating free fatty acids. It is determined easily with a titration of potassium hydroxide that neutralizes the acidity. It is usually expressed as percent (%) free fatty acids on the basis of the oleic acid, because that is the predominant fatty acid in olive oil. It is commonly called the percent acidity or free acidity percent. Free acidity in an oil is not the same as sourness or acidity in other foods. Free fatty acids (acidity) cannot be tasted in olive oil, at least not at the levels normally present. (*ISO 660 & AOCS Cd 3d-63*)
- **Peroxide Value:** This is a crude indicator of the amount of primary oxidation that has occurred, forming peroxide compounds within the oil. A high value indicates that the olives or paste was likely handled improperly, the oil could be defective, and the oil might not keep well. The Peroxide Value is a measure of the active oxygen content. It is done through a titration that liberates iodine from potassium iodide and is expressed as a value in milliequivalents of free oxygen per kilo of oil (meq O₂/kg). (*ISO 3960 & AOCS Cd 8b-90*)
- **UV Light Absorbency:** This is a more delicate indicator of oxidation, especially in oils that have been heated in the refining process. It measures the quantity of certain oxidized compounds that resonate at wavelengths of 232 and 268 nanometers (nm) in the ultraviolet spectrum in a spectrophotometer. Delta (Δ) K detects oil treatments with color removing substances and the presence of refined or pomace oil by measuring the difference between absorbance at 270 nm and 266 nm – 274 nm. (*ISO 3656*)
- **Moisture and Volatiles:** This is a measure of the water and volatile material content that may be present by placing a measured weight of oil in a drying stove for 30 minutes and measuring the weight difference until a difference is no longer detected. It is expressed as a percent (%) of the total weight. (*ISO 662*)
- **Insoluble Impurities:** A measure of the presence of dirt, minerals, resins, oxidized fatty acids, alkaline soaps of palmitic and stearic acids, and proteins that are suspended in the oil. It is determined by dissolving some oil in petroleum ether and filtering out the impurities and is expressed as a percentage (%) of the total. (*ISO 663*)

- **Flash Point:** This is a measure of the temperature at which the sample spontaneously begins to burn. Refined olive oil, pomace oil, and seed oils have a lower flash point temperature than virgin olive oil. Virgin olive oils have a flash point around 410°-428°F (210°-220°C), while most seed oils begin to burn at 374°-392°F (190°-200°C). (*FOSFA International Method*)
- **Metal Traces:** This is a measure of the amount of Iron and Copper in a sample taken by burning the oil in a special high temperature graphite oven and analyzing the ash with atomic absorption. (*ISO 8294*)
- **Halogenated Solvents:** This is a measure of the quantity of chloroform, trichloroethylene, and tetrachloroethylene that may be present as residuals in solvent extracted oils. Headspace volatile gases are measured in a gas chromatograph and expressed in mg/kg. (*COI/T.20/Doc. No. 8*)
- **Additives:** For virgin olive oils none are permitted. For refined olive oil, olive oil, refined pomace oil and pomace oil a maximum of 200 mg/kg of alpha-tocopherol is permitted. (*ISO 9936*)
- **Heavy Metals & Pesticide Residues:** All oils must comply with Codex Alimentarius maximum limits. Different and very complex tests are conducted for each suspected pesticide.
- **Sterol composition and content:** Sterols are important components of the non-glycerin fraction of olive oil. Their presence is determined by gas chromatography. Seed and nut oils have types of sterols and different levels of specific sterols that olive oil. Pomace oil has a higher total sterol levels because it is solvent extracted. Total sterol levels and and specific sterol types must be within certain maximum levels. (*ISO 12228 & AOCS Ch 6-91*)
- **Fatty acid composition:** is measured by gas chromatography and can help distinguish between varieties and growing region, but also between some seed oils and olive oil. The basic percentages of fatty acid types are well documented for each oil type within a certain range. (*ISO 5508 & AOCS Ch 2-91*)
- **Saturated fatty acid content in position 2 of the triglyceride:** The middle carbon of the triglyceride molecule (2 or beta position) in natural virgin olive oil always contains the non-saturated fatty acids such as oleic or linoleic. Reesterified oils that are processed artificially do not conform to this same fatty acid distribution and can be detected with gas chromatography. (*ISO 6800*)
- **Unsaponifiable material:** is the content of components that would not turn to soap under the process of saponification (addition of lye). Limits must be within those specified. (*ISO 3596 & AOCS Ch 3-91*)
- **Wax content:** is used to identify the presence of pomace oil and is determined by gas chromatography. Wax content of oils is higher in pomace oil, because pomace contains a greater proportion of fruit skin where most of the waxes originate. It is also solvent extracted, which dissolves waxes that otherwise would not be extracted. Waxes are not easily removed in the refining processes. (*AOCS Ch 8-02*)

- ***Erythrodiol and uvaol:*** are two terpenic alcohols that can be detected with gas chromatography. Some secondary extracted oils, refined pomace oil, and crude pomace oil can exceed the legal limit. Most of these compounds are found in the skin of the fruit. (*IUPAC no. 2.431*)
- ***ECN 42 content:*** Seed oils can also be detected by the difference of Equivalent Carbon Number (ECN) of the oil molecules determined by High Performance Liquid Chromatography (HPLC) and the theoretical ECN calculated from fatty acid content. Trilinolein is a triglyceride molecule that is not naturally present in olive oil, but appears in seed oils especially in sunflower oil. HPLC is used to detect this molecule that has the configuration of 3 Linoleic fatty acids attached to glycerin (more double bonds); levels cannot exceed the percentages in each category. (*AOCS 5b-89*)
- ***Hydrocarbons:*** Certain hydrocarbons such as stigmastadienes and the relationship of stigmasta-3, 5 diene, and campesta-3, 5 diene (R_1) can be identified in refined olive oils that have been heated and de-colored. They are not present in virgin olive oil. (*ISO 15788-1 & AOCS Cd 26-96*)
- ***Trans fatty acid isomer:*** When oils are exposed to high heat or pressure they can change from the “cis” (natural) form to the “trans” form. Detection is with gas chromatography. This method can also detect if oil has been exposed to color removal substances. Trans fat has been shown to raise “bad” LDL cholesterol, lower “good” HDL cholesterol, have adverse effects on the inner lining of blood vessels, and raises the risk of diabetes. Normal cis olive oil has fatty acid molecules that have a curved shape, which allows them to be metabolized properly. Heat and hydrogenation twists the shape (trans) so it does not “fit” correctly with enzymes. New labeling laws in the US will require products to be labeled with the content of trans fatty acids. (*ISO 15304 & AOCS Ce 1f-96*)
- ***Total aliphatic alcohols:*** are low in virgin olive oil, but much higher in solvent extracted pomace oil, because their levels are higher in fruit skin. Aliphatic alcohol content is measured with gas chromatography. Extra virgin – virgin – ordinary olive oils cannot exceed 250 mg/kg, lampante oil should be < 400, and refined olive oil must be less than 350 mg/kg. (*COI/T.20/Doc. No. 26 – June 03*)
- ***DNA characterization:*** is the recovery of intact or large fragments of DNA from the protein residue in olive oil by using electrophoresis and SSR analysis to develop a fingerprint. This is very new technology and a standard for it has not yet been developed.
- ***Total Polyphenols:*** are the complex compounds in olive oil that inhibit oxidation and make the oils bitter and pungent. Levels are determined by variety, fruit maturity, moisture content, tree irrigation level, and oil age. Polyphenol levels are an indicator of shelf life and freshness. (Gutfinger, T. 1981. J. Am. Oil Chem. Soc. 62: 895-898).
- ***Triacylglycerols:*** (TGA’s) are the lipid backbone of olive oil consisting of an ester of three fatty acids and glycerol. Seed, nut, and olive oils have different specific TGA arrangement profiles. Determined with gas chromatography and used to identify olive oil adulteration with seed and nut oils. Percent of total triacylglycerols limit and the TGA profile methodology has not yet been officially established in any standard (DGF standard method).

- **1,2 and 1,3-Diacylglycerol (DAG's):** are lipid compounds that are found at different levels in fresh and refined olive oil. Fresh olive oil contains a high proportion of 1,2-DAG's and refined olive oil contains a higher proportion of 1,3-DAG's. During breakdown a greater proportion of 1,3-DAG's are formed. According to the Australian Olive Oil Association, the percentage of 1,2-DAG's should be greater than 40% and a higher percentage indicates fresher oil. Determined with gas chromatography (DGF method C-VI 16(06) – ISO 29822:2009)
- **Pyropheophytins (PPP):** are chlorophyll pigment breakdown compounds from the thermal degradation of olive oil. Heat and long storage time break down chlorophyll into pheophytins then into PPP. Used to indicate the presence of adulteration with refined oil. Percent pyropheophytin content is determined with high performance liquid chromatography (HPLC) (DGF method C-VI-15(06) – ISO 29841:2009).
- **Sensory characteristics:** The sensory characteristics of odor and taste, also called organoleptic characteristics, of an oil must be evaluated by a minimum of 8 official taste panel members according to specific guidelines set forth by the IOC. According to the results of the tasting, the oil would be placed in one of the standard grade categories. (COI/T.20.Doc no. 15)

ISO – International Organization for Standardization. <http://www.iso.com>

AOCS – American Oil Chemists Society <http://www.aocs.org>

IUPAC – International Union of Pure and Applied Chemistry <http://www.iupac.org>

IOC – International Olive Council <http://www.internationaloliveoil.org>

FOSFA – Federation of Oils Seeds and Fats Association <http://www.fosfa.org>

AOA – Australian Olive Association <http://www.australianolives.com.au>

DGF - German network for science and technology of fats, oils and lipids <http://dgfett.de>