

Research in Olive Oil: Challenges for the Near Future

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Olive oil, a traditional food product with thousands of years of history, is continually evolving toward a more competitive global market. Being one of the most studied foods across different disciplines, olive oil still needs intensive research activity to face some vulnerabilities and challenges. This perspective describes some of them and shows a vision of research on olive oil for the near future, bringing together those aspects that are more relevant for better understanding and protection of this edible oil. To accomplish the most urgent challenges, some possible strategies are outlined, taking advantage of the latest analytical advances, considering six areas: (i) olive growing; (ii) processing, byproduct, and environmental issues; (iii) virgin olive oil sensory quality; (iv) purity, authentication, and traceability; (v) health and nutrition; (vi) consumers. The coming research, besides achieving those challenges, would increase the understanding of some aspects that are still the subject of debate and controversy among scientists focused on olive oil.

KEYWORDS: Virgin olive oil; olive processing; sensory quality; traceability; health and nutrition

INTRODUCTION

Many consumers identify olive oil as a food product that perfectly combines nutritional and sensory values. This perception has expanded olive oil consumption to figures that were unimaginable only a decade ago. Thus, whereas world production accounted for 2665.5 (1.000 tm) in the 2008/2009 season, world consumption was 2825.5 (1.000 tm), and these figures continue upward (1).

It is estimated that 7 million hectares are planted with around 600 million productive olive trees of more than 1275 autochthonous cultivars (2). Today the production and consumption of olive oil are moving slowly but inexorably beyond the Mediterranean countries, and olive trees are being planted in countries as far from the Mediterranean basin as New Zealand and Argentina. This fact is mainly due to new agricultural practices devised by traditional farmers to increase olive oil yield per hectare with no loss of sensory and nutritional properties. These practices overcome the negative benefit balance of traditional agriculture while maintaining the prestige of olive oil as a tasteful and health-promoting oil.

This revolution in agricultural techniques is not, however, exempt from challenges and even problems from the chemical viewpoint. Thus, whereas traditional orchards were planted with diverse and autochthonous cultivars and used rainfed water supply, the new orchards demand large quantities of water and the diversity of their cultivars is fewer than one dozen. Questions emerge beyond the classical issues concerning olive oil purity and nutritional benefits. How does the water demand of the new orchards fit into sustainable agriculture? How does the water quality (i.e., salinity) influence olive oil chemical composition? Are the current techniques ready to treat and make use of the increasing tons of byproducts? Is olive oil chemical composition affected by the latitude of new orchards? Are we going to lose the great diversity of olive tree germplasm with the unstoppable new monocultivar plantations? Are the numerous virgin olive oil Protected Designations of Origin (PDOs) and Protected Geographical Indications (PGIs) safeguarded from fraudulent labeling? Is the authentication of the olive oil geographical origin the great forthcoming challenge? Should the olive oil market move toward a common commercialization as daily oil instead of delicatessen marketing? These are only a few questions that highlight the current problems in olive oil field and compel food scientists to bring effort to solve them. The solutions to the current problems of olive oil may come from a high level of chemical characterization. Cultivars, pedoclimatic conditions of the orchards, and their agricultural practices, together with olive ripeness and olive oil extraction techniques, result in the great diversity of olive oil chemical profiles. In analyzing the constituents of olive fruit, water and oil account for 85-90% of the weight of the pulp, whereas the rest is mainly composed of organic matter and minerals. This fraction is composed of major compounds, mainly triacylglycerides, and a complex set of minor compounds (sterols, alcohols, hydrocarbons, pigments, volatiles, phenols, etc). All of these compounds are the basis for answering the arising questions and facing challenges that have been clustered into five main areas (Figure 1): olive growing and processing, sensory quality, authentication and traceability, health and nutrition, and consumers. They are analyzed in detail considering the recent ongoing research work and the new issues and problems that need to be investigated in the years to come.

OLIVE GROWING

In recent years, olive tree orchards have benefited from research on agricultural practices to solve current economical and environmental difficulties of olive oil production. The implementation of

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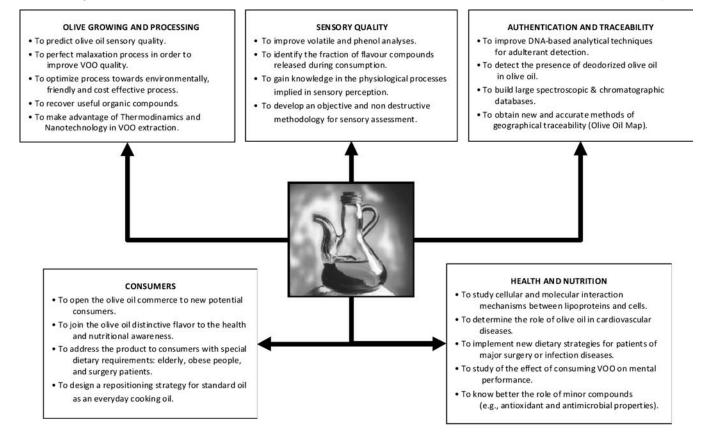


Figure 1. Main challenges for scientific research on olive oil.

modern orchards with (super-)high-density plantations has allowed producers to facilitate harvesting, reduce costs, and increase production. In this kind of agronomic practice the olive trees are planted very close, they produce faster due to automatic fertilization and irrigation systems, and specific machinery allows picking large quantity of olives rapidly with less labor costs. Only a few cultivars are, however, currently adapted to this cultivation practice. The most commonly planted cultivars are Arbequina, Manzanilla, and Picual (Spain), Barnea (Israel), and Frantoio and Leccino (Italy), Arbequina being the most universal by far (*3*).

To obtain new cultivars that are better adapted to modern agricultural practices, breeding programs are being carried out, mainly in Mediterranean countries. The breeding programs, which use a rapid genetic selection technique and variability identification (4), have as their main objective to produce cultivars that combine an optimal adaption to high-density plantations and irrigation regimes with traits of agronomic interest such as resistance to illnesses and a juvenile behavior (5). Little is known, however, about the heritability behavior of the new cultivars from their progenitors. The best progenies are being selected according to three parameters: modification of olive tree architecture to facilitate harvesting, increased oil content when possible, and modification of chemical compounds that improve olive oil quality (6). Furthermore, the crossbreeding programs are expanding knowledge in the genetic regulation of the enzymes involved in fatty acid and antioxidant biosynthesis. One of the main challenges, for example, is to improve the resistance of olive tree cultivars to Verticillium dahlia, which causes verticillium wilt, an olive tree illness that has abruptly increased with irrigation (7) and might lower the expectations of the new orchards.

It is well-known that the use of irrigation in traditional orchards increases vegetative growth and oil yield, and it explains why modern agricultural practices suggest the use of irrigation where water is available. However, the worldwide consciousness of good water management is leading researchers to pay attention to deficit irrigation regimes as well as the use of wastewater and saline water and the influence of different irrigation regimes on virgin olive oil (VOO) quality.

Ongoing research is focused on the impact of water stress on plant physiological processes, yield, and oil sensory quality. The concentrations of phenols and, to a lesser extent, volatiles are affected by water stress, whereas the effect is minimal, or nonexistent, on free acidity, peroxide value, and fatty acid composition. Early studies, for example, pointed out a decrease in the volatile compounds C6 from the lipoxygenase pathway, such as 2-hexenal (cis and trans), *cis*-3-hexenal, and *cis*-2-pentene-1-ol, when olive oils from irrigated and rainfed orchards were compared (ϑ), whereas it is well established that the total phenol content decreases with the amount of water applied during irrigation; as a consequence VOOs obtained under irrigation usually are characterized with lower bitterness (ϑ).

One of the positive aspects of the use of wastewater is the availability of nutrients (i.e., N and P) that also allows savings in fertilizers. Thus, current research is focused on the understanding of possible relationships between olive tree nutritional status and olive oil quality parameters such as the concentration of phenols that are responsible for VOO taste. Furthermore, the lower availability of fresh water, and even wastewater, has increased research on the use of saline water not only to understand the agricultural behavior of the tree but also to know its repercussion on VOO chemical composition. Thus, it seems that the concentration of NaCl affects the concentration of phenols and secoiridoid derivatives and, therefore, VOO flavor (10).

Although there is consensus as to the effect of irrigation on some chemical and sensory characteristics, the current irrigation strategies that are proposed today are diverse and differ greatly in Table 1. Objectives and Drawbacks of the Steps in the Olive Oil Supply Chain: Chemical and Sensory Variables Affected by the Processes

process	objective	drawbacks	parameters to be controlled
hedgerow orchards	high yields kg per ha automatic fertirrigation	low diversity of cultivars high water consumption high risk of the olive oil tree illnesses (e.g., verticillium wilt)	harvesting time by chemical/sensory parameters effect of wastewater on oil production phenol content to avoid excessive reduction
traditional orchards	sustainable production high diversity of cultivars low pesticide and water consumption	variability of chemical composition between seasons difficulty in automation more affected by pest outbreak	chemical composition related to cultivars
harvesting (by shakers)	lower cost harvesting better harvesting for olives	in case of small olive mill capacity, olives might be stored for days before processing	sensory defects due to long olive storage
washing	cleaning olives removing earth, mud, leaves, twigs, etc. protecting machinery from mineral materials avoiding undesirable sensory attributes	loss of olive oil with over-ripen olives need of a water recycling system	<i>E</i> -2-hexenal and bitterness perception if leaves are added moldy and earthy sensory perceptions and related volatiles if uncleaned olives are used
crushing by metallic crushers	lowering cost increasing hourly working load	emulsions temperature of the paste increases up to 6-10 °C	metallic sensory perception
	several kinds of crushers at various speeds	lesser amount of volatiles than old granite crushers	bitterness, if there is violent crushing
nixing	automating control of time and temperature applying inert atmosphere (N ₂) while mixing	occasional addition of coadjuvants (micronized mineral talc)	sensory perception and volatiles if temperature is too high amount of phenols with N ₂ atmosphere
pressure	lowering investment and energy/water consumption	hygienic working conditions lower scores in sensory assessment high labor costs	chlorophylls, peroxide value, alcohols, ketones, and acetic acid
	minimizing OMW ^a production	low working load discontinuous process	
percolation	working at room temperature with no water addition higher olive oil quality	semiautomated process low yield not applicable to all olives low working load	yield
three-phase decanters	lowering labor cost with fully automated process increasing oil yield, <3-5% oil in olive pomace	high investment energy and water consumptions high volume of OMW need for OMW treatment plants	compounds related to taste
two-phase decanters	reducing low OMW effluents and high amount of phenols	lower yield highly wet olive pomace need for a second decanting of "alperujo"	compounds related to flavor and taste, induction time
modern storing	enhanced storing procedure (stainless steel containers under inert atmosphere)	high cost room temperature control	rancid (hexanal and nonanal) and <i>o</i> -diphenols
bottling	avoid oxidation with brown glass and use of N ₂ headspace	selling in low quality plastic containers	rancid and cucumber defects (2,6-nonadienal), pyropheophytins, shelf life

^aOMW, olive mill wastewater.

water amount and irrigation period. Because fresh water availability is a worldwide problem, research on the best irrigation strategy is mainly focused on the use of wastewater or saline water that should be optimized with two parameters: water-use efficiency and improvement of chemical composition related to VOO flavor.

PROCESSING, BYPRODUCT, AND ENVIRONMENTAL ISSUES

Once the olives are collected from the tree, oil is extracted. Today modern olive mills extract VOO by means of centrifugation systems because they allow obtaining high-quality oils with less production cost. Today, however, some bottles of VOO are still labeled with "cold pressed", which is an anachronistic and largely unregulated label description for olive oil.

Although centrifugation is a procedure that was set up in the 1960s, still there are studies on how some parameters should be optimized for a better VOO quality. All of the factors during the extraction procedure affect the content of minor compounds and, hence, VOO sensory characteristics (**Table 1**). Thus, there is high

interest in chemical changes of the oil occurring as consequences of crushing mechanism, malaxation time and temperature, kind of decanter centrifuge, and type of coadjuvant, among many others (11-13). The resulting information from these studies is assisting in the improvement of the manuals of good manufacturing practices as well as modifying the machinery in conformity with a higher efficiency, a better VOO sensory quality and an optimum management of the olive byproducts.

The whole process of olive oil production produces a series of byproducts that basically are olive mill wastewater (OMW), olive pomace, and, of lesser importance, twigs and leaves. However, new centrifugation machinery (the so-called two-phase system) separates VOO from a watery product made of OMW and olive pomace that is named "alperujo". Whatever the machinery used to extract VOO, three phases or two phases, the resulting byproducts as well as some of their constituents have an increasing economic interest. OMW, either alone or as part of alperujo, is a dark red acidic (pH 4.6-5.3) liquid of high conductivity with high biological oxygen demand (20-55 g/L) that has to be treated to reduce its pollutant effect prior to being discharged (14). It is the combination of environmental problems and economical interest, mostly on the phenols, that has raised the research activity on byproduct management. Thus, the first studies were centered on its application as water for clay brick manufacturing, growth media for microbial production of enzymes, material for fertilization of poor soils, and also its transformation into products of high added value (15) among other applications. Alperujo and, to a lesser extent, OMW contain mineral elements (N, Na, P, Ca, He, K), ash, sugars, organic acids (e.g., acetic, glyceric), phenols, vitamins, fermentable proteins, waxy and resinous materials, and other organic substances. Thus, more recent studies are devoted to perfecting the extraction of OMW natural antioxidants, to apply olive mill wastewater as biofungicides, to study the enrichment of edible oils with OMW extracted phenols, to produce algal biomass rich in polyunsaturated fatty acids, and to other applications related to health products (16-18). Furthermore, other current studies have focused on the design of industrial systems that allow extracting squalene and tocopherols, among other compounds, from olive oil deodorizer distillates with the highest purity (19).

The production of high-quality VOO with optimum yield and minimum cost is not the only demanded objective of olive oil sector. An environmentally friendly olive oil production, combined with the individual availability of olive byproducts, is more and more demanded by farmers and a society that is conscious of the need for environmental protection. Today the machinery of olive mills is not designed from a global but a partial vision of olive oil production, and it is designed to extract VOO exclusively. Thus, its scientific background is on the basis of mechanical laws when the above demands fit better to systems regulated by thermodynamic laws. The extraction with supercritical fluids, the use of ceramic materials in the milling step, the implementation of nanotechnology in the malaxation process that allows modifying the oil/water interface, or the use of nanomaterials for a better selective extraction would contribute to a revolution of the industrial process similar to that of centrifugation with respect to pressure in the past.

SENSORY QUALITY

The cherished VOO aroma is due to the presence of numerous volatile compounds, whereas the concentration of phenols is responsible for attributes related to taste (e.g., bitterness and pungency). Therefore, an objective measurement of VOO sensory quality should be based on the quantification of these compounds.

The official method, however, is based on sensory assessment by trained assessors, the so-called panel test (henceforth, sensory assessment management or SAM). Although the scientific basis and methodology (training of assessors and evaluation process) of the sensory assessment are well-established (20), the use of SAMs to classify VOOs into categories is not exempt from risks up to the point that errors in the classification may cause a serious economical loss for producers and retailers; a fairly high percentage of approved SAMs may classify a virgin olive oil as lampante, an oil that is not fit for consumption and must be refined (21).

The proposal of replacing SAM with an analytical procedure has two challenges at present. The first is the determination of the chemical compounds (volatiles and phenols) responsible for VOO flavor from the large set of currently identified compounds. The second challenge is how to explain those important VOO sensory attributes that are described with considerable vagueness as fruitiness and green. These challenges have to be addressed with a combined action of new analytical techniques and statistical procedures.

Many volatile compounds, with diverse molecular weights and chemical natures, have been described in VOOs (22). The qualitative and quantitative profiles of the volatile fraction depend on different factors such as cultivar, geographical origin, pedoclimatic conditions, ripeness and health conditions of olives at harvesting time, processing conditions, and olive oil storage (15). Thus, the challenge of analytical procedures is the design of methodologies with adequate values of selectivity that ultimately affect the two extreme precision measures, repeatability and reproducibility.

The preconcentration step has traditionally been the main technological bottleneck as static and dynamic headspace techniques failed in the precision measures; the former due to repeatability (within-laboratory precision) and the latter due to reproducibility (between-laboratory precision) (23, 24). New instrumentations combine the advantages of those techniques including automation and the use of a nonselective trap with a large absorbent surface. Thus, volatiles are trapped in a tube containing a polymer (e.g., Tenax) after the headspace of an agitated vial has been swept by an inert gas.

With regard to the quantification of volatiles, the main challenges are in perfecting the chromatographic resolution and in computing the recovery percentage of the peaks as well as in determining their odor thresholds (25). Tens of recent papers have documented the concentrations of VOO volatiles, but only a few take into account their recovery percentage prior to comparing their concentrations in VOOs with their odor threshold. The main problem is still that the chromatographic method is often applied without a proper calculation of the recovery factors. Thus, the values of recovery factors for many volatiles (e.g., those identified by GC-MS) are supposed to be 1, whereas the actual value might be much lower. As a consequence, the potential contribution of these volatiles to VOO aroma may be underestimated or overestimated because a volatile contributes to VOO aroma if, and only if, its odor activity value (OAV) (26) (the ratio between its concentration in VOO and its odor threshold) is > 1.

Some VOO sensory descriptors are not satisfactorily explained yet due to the difficulty in interpreting the impact of volatile compounds on VOO aroma. The intensive research on volatile analysis in past decades has led to the identification of a great number of chemical compounds (22, 27), and some of them have been identified as mainly responsible for VOO sensory defects (28) as defined by the International Olive Council (IOC) (29). However, the explanation of most of the positive sensory descriptors is still tentative. Although many papers have been published relating volatile compounds with sensory attributes by means of

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regression algorithms or principal component analysis or other statistical procedures, almost none of them have been validated with external samples or have checked OAVs of selected compounds. The projection of volatiles onto attributes (30) is still a good approach to determine the contribution of each volatile to basic sensory descriptors because this approach is not based in casual relationships expressed with mathematical equations.

In addition to the attempts to explain sensory descriptors by means of equations, the vagueness in some definitions contributes to the difficulty. The explanation of some sensory attributes by chemical compounds is not an easy matter because the fuzziness in the semantics of some descriptors does not allow the chemical compounds perceived by the assessors to be identified, resulting in a vicious circle.

The difficulties in explaining sensory descriptors of taste are not quite different from volatiles. There is still controversy about which individual phenols are the main contributors to taste attributes. Despite bitterness and pungency having been associated with phenol content for a long time, little is known about other factors influencing bitterness such as the lipid matrix and the volatile composition. A complete mapping of sensory characteristics of individual phenols would require emulating the wellknown OAV using the same methodology as used in volatile compounds and aroma (31). Furthermore it would require studying the evolution of phenols during storage (32) and in culinary practices (33) as well as varietal characterization (34, 35).

With regard to the analytical aspects, the highly complex composition and lability of phenols result in the great variety of analytical methods used for their quantification (36). The result is the current confusion not only in their identification (authors have quantified compounds in VOO that are not even identified by others) but also in the range of their concentration values, even though some authors have used internal standards for identification and for calculation of response factors.

Despite extensive knowledge of the volatile and phenol composition of olive oil, VOO sensory quality is determined by an evaluation of positive and negative sensory descriptors. This method of sensory assessment for classifying VOOs, which is mainly based on determining if they have no defects (29), has opened the way to the application of chromatographic techniques to detect off-flavors. The critical decision of whether sensory defects are present in a sample of VOO has, as it has been said before, an enormous influence in commercial and economical terms, and there is a general demand from participants in VOO business for objective methods of sensory evaluation. Chromatographic techniques are offering fully objective results (28) in a cheaper and more rapid way than the official VOO sensory assessment does, thus avoiding the endless litigation caused by opposing evaluations from different SAMs.

Notwithstanding the cited recent developments in chromatography, research is going to be focused on the application of new sensors for the development of rapid and nondestructive techniques. Acoustic sensors, and other kinds of gas sensors, for example, seem to have enormous possibilities as nondestructive chromatographic detectors (37). Further works should also contribute with more information on chemical compounds, which, being present at very low concentration, influence VOO acceptability by consumers. In addition, the prediction of the potential quality of olive oil prior to the olives being milled (21) is a new niche of research for improving VOO quality as it allows the blending of olives that will produce olive oils with similar sensory quality characteristics.

However, the analytical methods sometimes fail in reproducing the results of the panelists and consumers. The problem comes from the misinterpretation of the chemical results from a sensory standpoint or vice versa. This problem is not solved only with improvements in instrumentation and chemical analysis. Satisfactory solution may come only from a better knowledge of the physiological implications of the compounds responsible for sensory attributes. Thus, information from panelists, consumers, and chemical analysis could be considered the three vertices of a hypothetical "sensory triangle" that is based on the interrelationships between these data interpreted from a chemical/physiological perspective.

The physiological processes implied in the olfaction and taste perceptions, as a result of the chemical stimuli, have not been explored yet and they are part of the black box associated with sensory assessment and consumers' decision. New approaches are being developed to measure the hemodynamic response of the brain during the smelling and tasting process by functional magnetic resonance imaging (fMRI) (*38*). The application of this technique could open a new area of research for a full understanding of sensory perceptions and consumers' decisions.

In the attainment of knowledge of the sensory processes, another challenge is the elucidation of how the flavor compounds of VOO are distributed in the mucosa/saliva and in the air phase during consumption. The impact of the protein composition of saliva on flavor release is also scarcely known (39), and it may have a significant effect on the kind and intensity of perception. Research in this regard would allow us to understand what fraction of volatile compounds is available in the air phase and can reach the olfactory receptors by means of retronasal delivery. Likewise, only the fraction of compounds (volatile and phenols) that remain in the proteic/aqueous phase of saliva could be responsible for taste and post-taste perceptions (e.g., bitterness, pungency, astringency). New researches based on chemical, sensory, and physiological approaches would make sensory assessment dispensable to some extent, and it would give coherence and unity to the sensory information, thus avoiding the current confusion and fuzziness that affect the sensory assessment of VOOs.

PURITY, AUTHENTICATION, AND TRACEABILITY

Olive oil is a strictly regulated food product because its high price and reputation make it a preferred target for defrauders. Thus, a series of limits established for analytical parameters determine the purity of the oil and the absence of adulterants. The limits and the methods to determine these parameters are approved by the IOC (www.internationaloliveoil.org) through the proposals of its Chemists' Working Group. In general terms, these purity criteria are adopted by the European Union (EU) regulatory body and the Codex Alimentarius. The objective is to ensure safety and consumer protection and to avoid the image of a hypothetical uncontrolled distribution of adulterated olive oil into the market.

Instrumental advances have led to a greater success in the fight against adulteration, although, at the same time, the perpetrators also use this advanced analytical information to invalidate the usefulness of some standard methods. Hence, the pace of research activity on authentication should be rapid enough to counteract fraudulent practices. A considerable investment in perfecting techniques and developing new ones is still required to solve new authenticity issues (40).

In the current context, the purpose of olive oil authenticity is not only to detect possible adulterations but to determine that an olive oil is genuine with regard to authenticity issues, such as geographical origin and botanical variety. There is no rapid and universal method that can be used for all authentication purposes. Instead, each authenticity issue can be solved by determining the selected chemical parameters by different techniques, either chromatographic or spectroscopic. Despite the arsenal of analytical techniques available at the moment, there are still some adulterations that are difficult to detect. Today, there is available industrial technology that allows VOO deodorization at a moderate temperature (≤ 100 °C) to remove volatiles that are responsible for undesirable attributes. The resulting oil (so-called "deodorato") might be added to VOO with irreproachable sensory perception, which is banned because VOO cannot undergo any thermal process (29). Researchers have tried the ratio between pheophytin A (a natural chlorophyll) and pyropheophytin A (a thermal degradation compound) to detect this kind of adulteration. However, pyropheophytin A also increases along VOO shelf life, which invalidates this ratio for detecting the presence of deodoratos, although it is still adequate to apprise consumers of VOO freshness (41).

Attempts have been made to combine the information of chlorophylls and diacylglycerols with uneven success. The determination of alkyl esters (methyl and ethyl esters) of fatty acids has been proposed (42) because these compounds are highly correlated with low sensory quality VOOs (43). Thus, a high value of the ratio between ethyl and methyl esters would be an indicator of the presence of deodorato as an irreproachable VOO has low quantities of ethyl esters. However, the oils that are set apart to be deodorized presumably have mild defects, and the question is whether these oils have sufficient amounts of ethyl esters to be detected in the blend.

Another authenticity issue is the geographical identification of oils. Consumers' awareness of the importance of geographical traceability in food safety and quality assurance has increased the interest for new methods that can assess VOO geographical origin. Several methods have been proposed (44); some of them are based on the study of the chemical fingerprint of the oils by spectroscopic methods, but they need of nonexistent large databases to build a consistent classification model (40).

The development of new systems of geographical identification could benefited from the recent advances in "omics" technologies (45). DNA extracted from olive oil is being used in traceability (46), although intensive research is required to avoid DNA contamination from other sources and to overcome the difficulties in extracting good-quality DNA (47).

The traceability, or geographical characterization, cannot be based whatsoever on chemical compounds for which concentration changes during VOO shelf life, such as phenols, chlorophylls, and carotenoids, or physical-chemical parameters related to VOO purity/quality, such as free acidity, peroxide index, K_{232} , and diacylglycerides among others, which have been used in many papers. The results of geographical traceability are corrupted if variables explaining quality, adulteration control, purity, or shelf life are used.

The alternative is the identification and quantification of major and minor compounds (e.g., fatty acids, hydrocarbons, alcohols, sterols) of which concentrations do not change over time but depend on the cultivar and, to a lesser extent, the soils and climate of the orchards. The information should be compiled to build a large database that allows determining the geographical origin of the most representative European VOOs with fine certainty factors (48). Through this approach, the geographical identification of samples can take advantage of multidisciplinary approaches such as those that use data of very different natures managed with chemometric procedures (49).

The high number of VOO PDOs has raised even more the concern of producers and consumers about the particular characteristics of the oils produced from PDOs. Consumer demand has resulted in a European Community regulation (50) that establishes a controlled labeling of food products based on geographical indications such as PDO. The implementation of PDOs

intends to ensure consumers' expectations and a better protection of VOOs from diverse areas of olive growing with specific sensory and chemical characteristics against falsification or mislabeling. Olive oils within the regime of a PDO usually have a higher market price and are, therefore, vulnerable to frauds (51). However, these regulations do not suggest any analytical procedure to verify the information provided on the label. As a consequence, the geographical declaration of VOO is never controlled by physical--chemical parameters and, in most of cases, it is exclusively supervised by administrative controls. Therefore, future work on geographical traceability will be focused on building an olive oil map, whereby the most productive cultivars and all of the approved PDOs are characterized by chromatographic, spectroscopic, and isotopic techniques. The resulting databases, in conjunction with new procedures of classification and visualization techniques, would allow evaluation of the best combination of these techniques in VOO traceability.

HEALTH AND NUTRITION

The chemical characteristics of olive oil also support the unquestionable healthy food claim as a consequence of the balance between mono- and polyunsaturated fatty acids and the bioactivity of minor compounds.

Olive oil, being one of the main ingredients of the Mediterranean diet, has been pointed to as mainly responsible for the health benefits of the nutritional pattern of the Mediterranean countries in numerous studies. In 2004 the U.S. Food and Drug Administration (FDA) evaluated the studies carried out on the healthy properties of olive oil up until that time. The FDA concluded that there is enough evidence to include a health claim in the labels of olive oil bottles to inform consumers about its health benefits (52). Today, the increment of worldwide preference for olive oil as a daily cooking oil is explained by a combination of health and sensory concerns of consumers. In addition to their known properties as nutraceuticals, cardioprotective agents, and topically applied skin care, new healthy properties are being extensively studied once the bioavailability and antioxidant properties of their microconstituents are known.

Researchers have pointed out facts and perspectives regarding the protective effect of olive oil intake on cardiovascular diseases. The effects have been ascribed to the presence of monounsaturated fatty acids (oleic acid) in serum as result of olive oil intake, which has proven to diminish the concentration of total cholesterol and low-density lipoprotein (LDL)-cholesterol. Several mechanisms have been proposed for the protective effect of oleic acid (15). Thus, it has been proposed that LDL rich in oleate is less susceptible to oxidation in comparison with linoleate-rich LDL (53), and it has been observed that oleic acid prevents the activation of endothelium by avoiding the expression of adhesion molecules or by improving NO production (54), which affects the inflammatory processes implied in atherosclerosis. The monounsaturated fatty acid (MUFA) content is not, however, enough to predict the lowering effect on cholesterol concentration (55). There are minor compounds with biological activity that could explain some of the observed protective effects of olive oil consumption (56). Special attention is being paid to compounds that have shown antioxidant properties such as phenols, squalene, and triterpenic alcohols.

The phenols have shown antioxidant activity preventing the oxidation of LDL isolated from blood (57). Individual phenols such as oleuropein and hydroxytyrosol are scavengers of reactive oxygen species near or within membranes (58). However, it is quite unlikely that the observed anti-inflammatory effect of olive oil can be due to a single compound. It is more reasonable to

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formulate the hypothesis that it is due to the combined action of several compounds (52). They can be tocopherols, sterols, terpenic compounds, and squalene, which have shown antioxidant and anti-inflammatory properties (59). Thus, for example, a lower systolic blood pressure has been associated with higher intake of phenols and triterpenoids (60).

The effect of olive oil consumption on the prevention of some types of cancers is receiving attention as well. The consensus report of the international conference on the healthy effect of virgin olive oil (61) specifies in one of the conclusions that the incidence of some types of cancer is lower in Mediterranean countries where olive oil is the principal source of fat, such as Spain, Italy, and Greece. Although MUFA could be involved in the cancer protective effect, due to their antioxidant and antiaging properties, the prevention effect of olive oil can be explained by the action of bioactive compounds altering tumor eicosanoid biosynthesis and cell signaling pathways modulating gene expression and preventing DNA damage induced by reactive oxygen metabolites (62).

The epidemiological studies carried out to date show major evidence in the case of breast cancer, which has been verified in animal research as well. Olive oil consumption seems to reduce cancer incidence, and research in this field is demonstrating that the efficiency of this edible oil against tumor diseases may justify its use in nutritional—pharmacological combinations to reduce some types of cancers (62).

The healthy effect of olive oil intake in other pathologies has also been studied, although there is scarce knowledge about the mechanisms that lead to these effects. Thus, a notable improvement of carbohydrate metabolism control in diabetes patients has been observed when the diet is supplemented with oleic acid (63). Reduced obesity has been also related to diets rich in olive oil (64).

Many pathologies in developed countries, with elderly populations, are related to the oxidation process and aging. Recently, the antioxidant property of some foods has gained special interest among the population, olive oil being one of these antiaging foods. Some cognitive disorders such as Alzheimer's and Parkinson's diseases could be prevented with higher intakes of olive oil, and therefore one of the recent challenges in health research is to prove the negative association between olive oil intake and cognitive disorders in elderly populations (65). Scarmeas et al. (66) have concluded the Mediterranean diet is associated with a reduction in the risk for Alzheimer's dysfunction. On the other hand, Parkinson's disease is related with a fatty diet, and some studies have related the intake of vitamin E with a lower risk of this motility pathology (67). However, the relationship between olive oil intake and protection against these diseases needs to be confirmed with further research before a general recommendation of olive oil as a protective agent (65). In the meantime, current research is setting the basis of known benefits of olive oil consumption while new healthy properties are being analyzed. The interactions between genes and the bioactive components present in olive oil studied by the emergent discipline of nutrigenomics (44) may help in the explanation of health benefits properties.

CONSUMERS

The demand for olive oil by consumers is slightly higher than the production, and the prospect seems to be favorable, particularly in nontraditional markets. In fact, the world olive oil demand has undergone a continual increment in the past decade due to the discovery of olive oil as an "everyday" cooking oil by the new consumers, who are abandoning the idea of buying olive oil as a gourmet oil to be used in only selected meals. The highest consumption rate in terms of kilograms per person and year is found in Greece (24 kg), followed by Spain (13.1 kg) and Italy (12.5 kg), with a sharp increase of 5-7% per year, whereas the United States and Japan lead the consumption increase at 12% and 30%, respectively (68). With the new intensive plantations, an optimum equilibrium will be reached between supply and demand very soon.

Besides the optimistic market prospects, standard olive oil needs to be repositioned and distanced from EVOO by giving customers enough cues to revise their opinions and consumption strategies. Furthermore, the awareness of consumers for a higher sensory quality and a clear geographical identity has encouraged producers to develop new products based on monovarietal VOOs characterized by different sensory attributes and VOOs with a declared geographical origin (PDO) that are controlled by a Regulatory Council when produced within the European Union. Today, olive oil authenticity, sold by PDOs and reputed sellers, is guaranteed, and consumer expectations of sensory quality are fulfilled when they buy a bottle of VOO. On the other hand, producers have recently released new products based on virgin olive oil flavored with spices and herbs, from garlic to rosemary, and occasionally mixed with vinegar, ready to be used as salad dressing or dipper. This application needs a more complex labeling regulation that has not launched yet.

Deep- and pan-frying is a millenarian culinary practice of the Mediterranean countries. In fact, olive oil composition, with its low content of saturated fatty acids and high content of antioxidants (e.g., phenols), makes it an excellent oil to be applied in culinary processes that require high temperatures such as frying and baking. Thus, after 10 h of heating at 180 °C the total polar compounds of VOO can be lower than 25%, whereas other vegetable oils, such as sunflower oil or commercial blends intended for frying purposes, easily reach 29% polar compounds (*33*). The use of olive oil as frying oil creates a new market niche to be promoted in the future, offering stability, healthy properties, and additional flavor.

The new challenge for researchers in consumer science is to establish all of the key factors affecting the purchases of consumer buying olive oil in nonproducer countries. Intensive research in this field may provide the right strategies to open the market for olive oil. Information on the willingness of consumers to pay a high price, consumers' knowledge and usage of the product, and their attitude toward organic oils and oils with geographical indication may adapt the market for each country. Recent studies suggest that reasons for buying olive oil are the new finding on health issues, the geographical association of the product, and the excellent flavor. Thus, results from recent consumer surveys seem to support the ongoing research in the areas of health and nutrition, oil authentication, and flavor chemistry.

Although olive oil is one of the most studied oils, many aspects are still unknown, and the rapid evolution of markets and consumer demands lead to new challenges that must be addressed. The development and improvement of instrumental analysis, the decoding of metabolome through "omics" technologies, and the integration of findings across diverse disciplines will help in successfully meeting these challenges.

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