

OLIVE OIL HUILE D'OLIVE

Genetic and environmental features for oil composition in olive varieties

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Abstract – Consumption of olive oil helps both prevent and cure heart disease. Olive oils vary in their fatty acid profiles as well as those of other secondary metabolites (phenols, sterols, and terpene compounds). We seek to distinguish the genetic bases from the environmental factors that cause these variations. The genetic base is indeed wide: varieties originate in different domestication occurrences, from different oleaster trees and in differing climatic regimes. With the aid of diagrams, we set out briefly the oil synthesis pathway for fruits in comparison with that of seeds, and the specific aspects of olive oil in particular. Varieties of olive have appeared that are adapted to regions with harsh conditions where the oleaster could not thrive. Environmental stresses have consequences on drupes and their oil profiles; these have been highlighted in European countries through the use of appellations. Whilst stresses tend to enhance the quality of the end product, they do however decrease final yields with potential negative impacts on olive growers' incomes. Irrigation experiments are underway in order to determine the optimal amount of watering. In breeding new varieties, the result sought is that of accumulating pest tolerances and fruit-quality characteristics; selection programmes are however expensive as they necessitate observations over many years. Consumers have choice across a range of appellations with different organoleptic specificities at different prices, and whatever the appellation of the oil they can expect a positive effect on their health.

Keywords: Abiotic stresses / appellations / diversity / health / oil profiles / phenolic compounds

Résumé – Les caractéristiques génétique et environnementale de la composition en huile des variétés de l'olivier. L'huile d'olive est bénéfique pour prévenir et guérir les maladies cardiovasculaires. Les profils de l'huile d'une variété sont variés tant pour les acides gras que pour les métabolites secondaires (composés phénoliques, stérols, et terpéniques). Notre but est de séparer les causes génétiques des causes environnementales qui supportent les variations des profils de l'huile. Les bases génétiques sont larges puisque les variétés sont issues d'événements de domestication distincts à partir de l'oléastre qui proliférait sous des climats différents. Nous exposons succinctement les spécificités de l'huile d'olive ainsi que les principales voies métaboliques pour les fruits par comparaison aux graines. Nous avons choisi de comparer les profils par des diagrammes. Des variétés adaptées à des stress sévères sont apparues où l'oléastre ne peut se maintenir. Les stress environnementaux ont des effets sur le fruit pour la conserve et sur le profil de l'huile, ce qui a été valorisé par les appellations dans les pays européens. Si le stress favorise la qualité des produits, il diminue en revanche le rendement et par là, le revenu des oléiculteurs. Des essais expérimentaux sont en cours pour déterminer le niveau d'irrigation sans diminuer la qualité des produits. La sélection de nouvelles variétés d'olive a pour objectif de rassembler des tolérances aux ravageurs et la production de fruits de qualité, néanmoins, les essais sont très coûteux pour des arbres observés plusieurs années. Les consommateurs ont le choix parmi de nombreuses appellations des produits, et quelle que soit l'appellation l'effet favorable de l'huile d'olive sur la santé est attendu.

1 Introduction

Olive trees have been growing throughout the Mediterranean basin for between six and seven millennia. During

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the colonization period (16–18th centuries) all the regions of the world with a similar Mediterranean-type climate experienced planting by Spanish, Italian or French settlers. It has been domesticated as the Oleaster (Besnard *et al.*, 2002; Breton, 2006; Breton *et al.*, 2006; Breton and Bervillé, 2013) and its cultivation has spread to regions where the wild olive tree (oleaster) cannot thrive. They are grown for oil and

canned fruit production; very little cultivation has a decorative purpose.

At present, most olive varieties are diffused as a clone. In a clone all the trees (each tree is a further ramet) are obtained by cuttings or by grafting from the initial tree (itself named the ortet). No genetic variation exists therefore between the ramets of each ortet. All variations in the final product are consequently attributable to environmental and physical factors – soil composition, temperature range, rain regime – and the fruit processing methods employed – mill stone, mill hammer, crushing temperature, centrifugation, filtration, and oil storage conditions. The mechanisms underlying environmentally-caused variations in oil composition are for the most part unknown.

Why is it important to determine the relative importance of genetics and environmental factors? The obtention of certain type of end-products (the fatty acid profile, for example) depends mainly on genetic factors, whereas the organoleptic quality of an oil depends mainly on environmental factors such as drought stresses.

An initial source of diversity in oil results from the diversity in domesticated oleaster trees. The genetic components of olive varieties affect the composition of the oil – alleles of the main genes that direct oil synthesis pathways, alleles that direct the synthesis of phenolic compounds synthesis and likewise that of other secondary metabolite compounds (sterols and terpenoids) found in the oil fractions. Each variety, then, displays a specific broad composition of compounds which, during the various stages of the fruit's maturity, may be more or less affected by environmental parameters. A further source of diversity lies in the unconscious selection on the part of olive growers for trees that produce the type of oil that they personally favour. Finally, the drupe is affected by the choice of canning processes, just as the oil is by the method employed to extract it from the drupes. We therefore examine here the consequences of genetic and environmental features on the current olive varieties and the trends in the breeding of new olive varieties.

2 Causes of variation from past to present times in olive oil

In the 19th century drupes were not only used as a source of food, but also as an industrial lubricant. This probably favoured led to a screening in favour of varieties with high oil content and yield, neglecting the taste aspect. The great diversity of current varieties is due to the human selection of trees (ramets) adapted to difficult environments such as the desert, mountains, northern regions, coastal areas and so on. At the present state of knowledge, we know neither the specific origin of the initial olive trees nor the approximate dates of each selective event. Nevertheless, researchers can examine molecular differences in the diverse varieties across the world and interpret what may have occurred and speculate on the more or most probable scenarios.

Olive oil is known to prevent heart diseases because of its high oleic acid content. Moreover, because of its polyphenols and sterols content, it also has many other health benefits (Gerber, 2012; Ghanbari *et al.*, 2012; Hoffman and Gerber,

2012; Visioli and Galli, 2000). The composition of olive oils varies widely depending on the varieties and the regions where they are grown. European regulations limit the use of the term “olive oil” to olive oil having an oleic acid content (OAC) greater than 55% (Regulation ALINORM 01/17 2001). Olive oil categories are consequently based upon taste and on processes employed to press the drupes (Regulation 299, 2013), but not on the fatty acid profile. The fatty acid profile determines the fluidity of the oil. However, in the oleaster the observed range in OAC is lower than in the crop (Hannachi *et al.*, 2008) – some oleaster trees may produce an olive oil with an OAC lower than 55%.

To understand the genetic trends in olive oil composition, we consider first the transition phases – probably during the period from the Mesolithic to the Neolithic – from natural populations of oleaster tree to the first land races population varieties of the olive that may have occurred. Six to seven millennia of selection have given rise to a huge variability in fruit shapes and size, oil content and oil composition. Among all the compounds of the oil, those that have direct observable consequences – the taste, for example – would be screened more rapidly than those with indirect effects – such as antioxidant activity or the prevention of ill-health. We hypothesize that during the early stages of the olive's domestication process, the oil extraction technology was poor and that fruits were probably crushed with other raw materials and released as oil into pots for cooking cereals and vegetables.

The natural selection of oleaster trees has probably favoured those that are most attractive to small mammals and birds. We consider it likely that the oil composition profile has an effect on the fruit's attractiveness to humans, and we will advocate this aspect further.

The processes of domesticating the oleaster that led to the olive trees obviously started on common oleaster trees, but humans will have unconsciously screened for trees that yielded enough fruits, with high oil content, and with stable oil composition after harvest.

Once the methods to separate the oil from the pulp became widely known (probably by the bronze age, Riley, 2002) new ortet trees with higher oil content or greater ease of oil extraction were unconsciously screened for. Subsequently, all compounds that could positively interact with the oil's long shelf storage become important in the oil composition, and again these will have been screened for unconsciously.

3 Methods and data representation

The profile of a particular olive oil is dependent on the method used to analyse each compound. Consequently, the regulations are based upon the methods and the IOOC has published a list of protocols for each of them (Regulation 299, 2013). Obviously, comparative studies between varieties must be based on identical methods otherwise biases may occur. These aspects are not addressed here and but are examined elsewhere in this OCL dossier. However, as explained in (Pouyet and Ollivier, 2014, in this OCL issue) fatty acids are not free in the oil, but each esterifies one of the three alcohol radicals of a glycerol molecule. Each position is not esterified at random, but there is some specificity in the esterification,

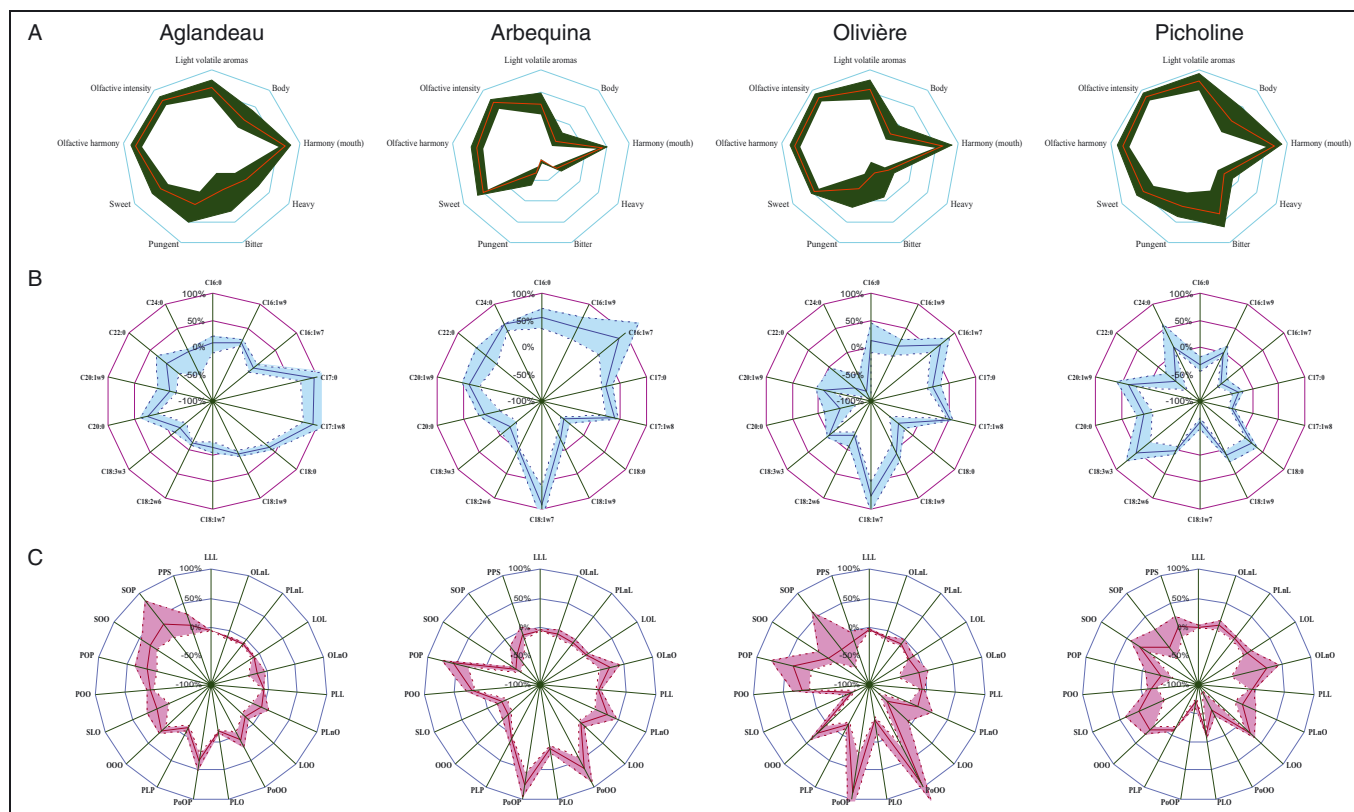


Fig. 1. Morphogram to describe the oil profiles of the varieties *Aglandau*, *Arbequina*, *Olivière* and *Picholine* based on several tens of oil samples (from Pinatel *et al.* leaflets, www.afidol.fr). Ligne A : organoleptic description. Ligne B : fatty acid composition. Ligne C : triglycerid composition.

and thus analysis of the TAG spectrum may assist not only in differentiating between oil samples drawn from trees grown in different regions, but also identifying the specific geographic origin (Ollivier *et al.*, 2006).

4 Specificities of olive oil

Seed oils are synthesized by the embryo tissues and are accumulated in the cotyledons. By contrast, olive oil is a fruit oil synthesized by and accumulated in the drupe mother tissues. For any particular clone variety, all the ramets have the same alleles. All the fruits of the same tree carry the same alleles directing oil composition. Thus, all variation in oil composition from a given variety is due to environmental factors only; these include the time of harvesting and the process used to press the drupes.

In the olive drupe the embryo synthesizes its own oil. This is stored in the cotyledons with the purpose of providing the energy to enable the embryo's germination, before the young tree is able to produce its own through photosynthesis. The composition of embryonic oil is quite different from drupe oil, being richer in linoleic A. (C18:2). However, the proportion of embryonic oil in the drupe as a whole is very small. Making oil with de-stoned drupes, a process that some mills offer, therefore has no discernable impact on the composition of the resultant oil.

Olive oil is a cold-pressed oil extracted at low temperature; the regulation (virgin oil) specifies less than 30 °C. Seed oil

is extracted at high temperature (above 100 °C, up to 240 °C with solvents) to facilitate draining the oil from the seed coat. For fruit oils maturity stages and fruit conservation techniques prior to oil extraction influence the oil profile.

5 Short survey on the fatty acid synthesis pathway

Fatty acids are built from acetate (2 C for carbon residues) by a metabolic network with some steps in the mitochondria, in the chloroplast, and in the cytosol (Browse, 1997). This explains why most fatty acids carry an even number of C atoms – 16 (Palmitic A) 18 Oleic A, 20 (Arachidonic A), these are saturated; further desaturase (D) enzymes introduce a double bond in the molecules at a specific position counted from the acid radical of the fatty acid (D6 leads to Linolenic A; D9 leads to Oleic A) and further enzyme activities may introduce hydroxyl groups such as exists in Palmitoleic A. Thus, the fatty acid synthesis pathway is sequential. It is stage-dependent since each enzyme's activity is variable along stages, and the qualitative profile of an oil therefore depends upon the enzyme activities expressed in the drupe at the stage at which they are pressed.

However, along the synthesis chain fatty acids are not free, rather combined with a carrier, and finally with glycerol. The place of the fatty A. that esterifies one of the three alcohol functions is not attributed at random, but is defined by an enzyme. The mechanism is not well-known.

6 The mean to expose olive oil composition

The olive oil literature is rich in descriptions of methods for highlighting oil profile variations. However, data set out in tables and graphs are not easy to read as they lack a clear visual display. By contrast, the diagrams deployed by Pinatel *et al.* (2004) from the CTO-AFIDOL are in our opinion much more clear. For each French variety they gave the centred mean, the minimal value, the maximal value and the median for “fruits composition and yields”; “organoleptic analysis”; “specific aromas”, “description of the oil”; they also indicated the presence of 15 fatty acids and 20 TAGs that may be found in olive oil samples. Moreover, and interestingly, they set out a morphogramme to display the profile of each variety. The thickness of the line for each compound gives information on the whole variability of the compounds. If the variation is narrow then the characteristic is determined mainly by genetic factors; conversely if the variation has wide range, the characteristic is predominantly affected by environmental factors. Consequently, the composition in triacyl-glycerol (TAG) of the oil is due partly to the variety and partly to environmental factors.

7 Appellations – labels

Like grapes and wine, olives and their oil are the object of appellations and labels attached to the packaging that attest to the typicity of the product, and which in many cases assure its high quality. Appellations for the olive have several bases, depending on the country. Delimitations by geographical area they might include fewer or more varieties. Appellation details are provided in other sources [www.afidol.fr] and are therefore not listed here. As an example, the appellation “olive de Nyons”, it is a PAO (Protected Origin Appellation), which combines a geographic area and one admissible variety the *Tanche*. However, whilst the *Tanche* is partially self-fertile, cross pollination by suitable pollinizers is required for a successful crop. In the past *Sauzin* was used; now *Cayon* is prevalent. These pollinizers cannot exceed 5% of the trees in the orchard. This means that outside this area *Tanche* products that are not labelled, due to they may have less typicity. *Tanche* is also widely cropped in other regions however, the products have less typicity and do not harbour labels.

Because all *Tanche* ramets have the same genetic base, the typicity of the PAO “olive de Nyons” product is attributable to environmental factors. Several factors have been suggested, all linked to the specific climate of the Nyonsais, for example the effect in this area. One had speculate that among the physical factors is the north wind called the “Mistral”. There is however no direct evidence to support any such an opinion since the mechanisms by which physical factors might affect the specific alleles of the *Tanche* genes to modulate oil profile remain unknown. Indeed, the range of potential environmental factors is too wide for researchers to isolate any particular one and its influence on gene expression.

Even at the most detailed level, analyses of oil composition variation remain an ineffective tool for identifying the genes that are targeted by environmental parameters. Obviously, one

can examine the origin of the *Tanche* attempt to ascertain when its selection occurred and the nature of its genetic base.

Another example is provided by the PDO-IGP (Protected Denomination of Origin – Protected Geographical Indication) attributed to a list (potentially wide) of varieties that may be used in the production of oil in a particular region with, as a consequence, potentially wide ranges of variation in the oil’s composition from one olive grower to another. The trend, though, is towards the production of oils homogeneous in their physical-chemical properties. For the olive oil market as whole, the proportion carrying an appellation is much low than the proportion without. Behind this situation lies the fact that the cost of oil bearing an appellation is much high than for other oil, and people believe that the health benefits of olive oil are the same, regardless of where and how it has been produced (Afidol, www.afidol.fr).

Comparisons of oil composition between different olive varieties have been widely documented in Australia (Mailer, 2005) France (Pinatel *et al.*, 2004; Fiches Afidol, see www.afidol.fr), Iran (Movahed *et al.*, 2012), Italy (Muzzalupo *et al.*, 2011) Spain (Aparicio and Luna, 2002) and Tunisia (Dhifi *et al.*, 2005; Zarrrouk *et al.*, 2009). Breeding olive varieties consists in gathering and combining the most desirable characteristics. Of course, though, the characteristics segregate in the progenies (examples can be found for oil composition in several publications such as Léon *et al.*, 2004; Bellini *et al.*, 2008), variations in the progenies of crosses cannot be predicted from the oil composition of the parents since olive varieties are highly heterozygous. The permutations of alleles for oil composition are therefore very numerous.

8 Olive and watering

The effects of watering on crops have been widely studied. Water stress reduces photosynthetic activities and consequently the end-yield in fruits and oil. Data exist comparing wholly rain-fed (*pluvial* in French) with irrigated plots. However, rain-fall varies over time; such experiments are therefore poorly replicable. Experiments comparing dry plots covered by a shelter with watered plots are demanding in their design and their costs are high. They are therefore rarely used to study fruit crops. In any case, watering affects the development of plants by temporarily retarding their maturity (Hendrickson and Veihmeyer, 1949) and as a consequence comparison at days immediately after watering and at the end of the particular development stage may lead to contradictory conclusions. So far as the effects of watering on fruit yield are concerned we can expect only trend results.

For fruit crops the main question is whether watering might enhance yield, without negative effects on quality. The traditional cultivation of olive trees without watering has for over the course of centuries given rise to a broadly consistent end-product. The watering of orchards to improve olive-growers’ incomes has given rise to questions regarding the quality of the resulting fruit. Numerous experiments have been conducted in arid, semi-arid and traditional regions, employing a variety of watering methods.

8.1 Does watering lead to sustained increases in fruit and oil yields ?

In general the response is yes, but the result is not regular each year, and watering delays the maturity stage slightly (Breton *et al.*, 2009; Xiloyannis *et al.*, 1999). Thus comparison of growth results date by date may show that watering is unfavourable, and comparison at equivalent stages may show that watering is indeed slightly favourable. It should be noted too that watering favours the production of larger fruits, a characteristic appreciated in table olives. The characteristic of large size is nevertheless complex and may depend on surprising effects of cross pollination as revealed by Farinelli *et al.* (2012) and may consequently depend on partial incompatibility with pollinizers (Breton and Bervillé, 2012).

8.2 What are the effects on the quality of the products?

The effect of watering on fatty acid composition is not significant, and thus the oil produced in drought and watered conditions will be classed the same. The stability in fatty acid composition in cultivars grown in stressed and in watered conditions has been also observed for seed oils such as sunflower oil (Bervillé, 2009).

However, in the olive water stresses increase phenol fraction accumulation in the plant and the oil. This fraction serves as an antioxidant in the leaves, and in the oil affects the taste and aroma. We may therefore observe different tastes and aromas in oil fractions from cultivars from drought and watered conditions. A wide experimental plot has been observed for ten years by AFIDOL and SERFEL on Picholine and Aglandau. The results so far (Organoleptic diagrams, oil profiles, polyphenol content) are available on the SERFEL site (www.serfel.fr). Such studies will assist olive growers in formulating strategies to cultivate olive varieties with or without watering, according to type of end-product they wish to market.

9 Breeding for olive varieties

Like other oleaginous fruits, olives have a wide range of oil profiles. Since the appellation “olive oil” is applied only to oil profiles with and OAC above 55%, olive oil so labelled prevents certain cardiovascular diseases as well as other illnesses. The role of polyphenols in health prevention is supported by epidemiological studies; their quantity and quality are therefore not parameters in such studies. At present, epidemiological studies are focussing on olive oil with high phenols content such as from Picholine (Hiroko *et al.*, 2012), though the highest concentration of polyphenols is in the leaves, which would argue for consuming olive leaf infusions.

Matches are made between olive varieties that display complementary oil profiles: breeders hope to find in the progenies a combination of the best traits of each variety. However, on field olive experiments are long require wide surfaces and thus are costly. Consequently, studies on segregating progenies for the main genetic traits in olive oil composition (QTL) have not been mapped yet. When this strategy is applied to

seed oil species, the chances of success are high because the number of screened progenies can easily number in the thousands. For the olive, plots with trees are expensive and so the number of progenies examined is much lower – a few hundred – and the chance of discovering a valuable new variety is correspondingly lower. Moreover, the hierarchy between of desirable characteristics is not well understood nor is there sufficient information from which to estimate the probability of screening an improved variety. Moreover, even if an apparently improved variety does emerge, for it to be a real improvement it would need not only to display a better oil profile, but also improved biotic stress tolerance factors for the main diseases and pests and be adapted to modern olive growing techniques.

10 Conclusion

Olive oils are diverse in their fatty acid and polyphenol profiles. Their beneficial effects in helping to prevent cardiovascular diseases are probabilistic as opposed to causally established: they depend not only on the oil profile but also, and in ways not yet fully understood, on the genetic makeup of each consumer. Consumers should make their choice according to their tastes and their budget. Based on a Afidol survey on a significant statistical population sample, the main criteria to buy olive oil is the price, neither the label nor the taste. Among abiotic stresses, drought stress tends to enhance the quality and typicity of most cultivars. However, drought stresses are complex in their effects and their impacts on quality are not guaranteed.

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