Evaluation of the oxidative stability of blends of 'Arbequina' olive oils with other monovarietal olive oils

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Abstract

Like elsewhere in the Mediterranean, the olive oil sector is one of the strategic branches of the Moroccan economy owing to its social and economic significance. This research entailed evaluation of the oxidative stability of olive oils made up of a blend of 'Arbequina' olive oil with 'Arbosana' and 'Koroneiki' monovarietal oils known for their high content of natural antioxidants (phenols and tocopherols) and their superior oxidative stability compared with 'Arbequina' oil. The monovarietal oils produced from the 'Arbequina', 'Arbosana' and 'Koroneiki' varieties, which have recently been introduced under intensive cultivation in the eastern region of Morocco, underwent physico–chemical characterisation to determine quality criteria, natural antioxidant content, fatty acid composition and triacylglycerol profile. The Rancimat test* was performed to assess the oxidative stability of these monovarietal oils and their three-variety blends. The oxidation tests were performed on five freshly prepared blends **(A)** of the three varieties – 'Arbequina'/'Arbosana'/'Koroneiki' – according to the following volume ratios: $\mathbf{A}_1$: 60/30/10; $\mathbf{A}_2$: 60/20/20; $\mathbf{A}_3$: 60:10:30; $\mathbf{A}_4$: 50:25:25 and $\mathbf{A}_5$: 40:30:30. The test results show that blends $\mathbf{A}_4$ and $\mathbf{A}_5$ displayed the best oxidative stability, recording respective values of 72.67 h and 75.42 h. These results are comparable to those obtained for 'Arbosana' monovarietal oil (75.42 h), which is considered to be relatively stable. Hence, blending is an excellent tool for enhancing oils produced from varieties which, despite their excellent initial quality and their organoleptic attributes, are handicapped by their poor stability, as is the case of 'Arbequina' oil.

Key words

Eastern region of Morocco, 'Arbequina', 'Arbosana', 'Koroneiki', monovarietal olive oil, oil blend, natural antioxidants, oxidative stability.

*The Racimat test is not an official IOC method

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Introduction

World consumption of olive oil continues to climb as more and more new consumers across the globe incorporate it into their diet. Until now Spain and Italy have been the world's top two olive oil producers but this panorama has started to change since countries in the Maghreb like Morocco and Tunisia embarked on olive growing expansion programmes. This orchard expansion has been accompanied in recent years by big changes in olive cultivation, olive harvesting and olive oil processing prompted by the introduction of new varieties, olive harvest mechanisation and increasing mill automation. In Morocco, new varieties of olive such as 'Arbequina', 'Arbosana' and 'Koroneiki' are being introduced specifically for olive oil production. Originating in Catalonia, 'Arbequina' is one of the chief varieties of olive cultivated in Spain (Tous and Romero, 1992; Tous *et al.*, 1997; Tous *et al.*, 2001; Rallo, 2002). It is also being planted increasingly more in olive orchards on the southern shores of the Mediterranean, mainly in Tunisia and Morocco (Ait-Hmida, 2010; Mahhou *et al.*, 2011; Rkhis *et al.*, 2010; El Mouhtadi *et al.*, 2014). Olive farmers choose 'Arbequina' for several reasons: it is suited to the soil and climatic conditions in high density orchards in the southern Mediterranean region (Boulouha, 2006), its entry into production is early and it has a high oil content compared with native varieties (Rkhis *et al.*, 2010; Mahhou *et al.*, 2011; El Mouhtadi *et al.*, 2014). The oil produced from 'Arbequina' olives is very fruity, smooth and aromatic; it is not bitter and has virtually no pungency. On the downside, its oxidative stability is poor because of its low content of natural antioxidants and its high level of polyunsaturated fatty acids (Terouzi *et al.*, 2010; Gharby *et al.*, 2012; Mansouri *et al.*, 2013).

This research focused on the characterisation of the oils obtained from three varieties – 'Arbequina', 'Arbosana' and 'Koroneiki' – recently introduced in the eastern region of Morocco under super-intensive, irrigated olive cultivation. After undergoing physico–chemical characterisation, the 'Arbequina' oils were blended with 'Arbosana' and 'Koroneiki' oils, which are known for their high natural antioxidant content, and the different blends were tested for their oxidative stability using the Rancimat test. The objective was to achieve a blend combining good organoleptic attributes and better oxidative stability. If this methodology were to be mastered by oil mills, it would no doubt help them to expand their range of commercial products by creating new brands of monovarietal blends to satisfy the growing diversity of consumer tastes.

Materials and methods

Plant material

The samples of olive oil studied were monovarietal oils produced from 'Arbequina', 'Arbosana' and 'Koroneiki' olives in the 2013/14 crop year. The olives were grown in irrigated, super-intensive orchards planted at a density of 1 300 trees/ha. The trees were 6–7 years old and were cultivated in the same soil and climatic conditions and received the same cultural care. The olives were crushed and the oil was stored and bottled at the on-farm mill.

Quality criteria

Free acidity (% C18 :1), peroxide value (PV, meq/kg) and the specific coefficients of extinction in ultraviolet at 232 nm (K_{232}) 270 nm (K_{270}) and ΔK measured by spectrophotometry were determined according to the methods recommended by the International Olive Council and specified in the EU regulation on the characterisation of olive oils (EEC, 2003).

Total phenols

The phenolic compounds were extracted according to the method described by Ollivier *et al.* (2004). Total phenol content was determined by the Folin-Ciocalteu method using caffeic acid as the standard. The results were expressed in milligrams of caffeic acid/kg of olive oil.

Tocopherols

Alpha-tocopherol (the majority compound) was determined by HPLC chromatography using a diode array detector according to the amended AOCS method (1989). Separation was performed on a silica column using hexane/isopropanol (99/1, v/v) as the mobile phase at a flow rate of 1 mL/min. An alpha-tocopherol standard was used for identification purposes and a calibration curve for quantification.

Fatty acid composition

The methyl esters of the fatty acids were analysed by gas chromatography using a flame ionisation detector (FID). Separation was performed on an HP-5880A capillary column (25 m × 0.25 mm, 0.25 µm). The temperature of the FID detector was 250 °C. Optimal separation was obtained under the following conditions: initial oven temperature of 50 °C, increased to 150 °C at a rate of 30 °C/min, and then to 240°C at a rate of 4 °C/min and maintenance at this temperature for 10 minutes. An injection volume of 1µL was used (splitless mode).

Triacylglycerol (TAG) composition

TAG composition was determined by HPLC according to the amended method (Abaza *et al.*, 2002):

10 µL of the oil in acetone (10%, P/V) were fractionated in a Shimadzu CBM 20A HPLC chromatograph (equipped with a refractive index detector, RID, 10A). Isocratic separation was performed with the aid of an ODS C18 apolar reverse-phase column (250 mm × 5 mm, 5 µm). The mobile phase was a mix of two solvents, acetone and acetonitrile (63.6/36.4 V/V), at a flow rate of 1 mL/min.

Oil oxidative stability

Oxidative stability was evaluated by the Rancimat method (Gutiérrez Rosales, 1989). The Rancimat induction time (expressed in hours) was determined using a Metrohm Rancimat 743 with a 3 g test sample of oil at an air flow of 15 l/h and a temperature of 101 °C.

Blending

The three varietals ('Arbequina', 'Arbosana' and 'Koroneiki') were blended in varying volume/volume percentages as shown in Table 1. The 'Arbequina' oil was always predominant in the blends (at least 40% V/V).

Table 1: Identification of the different volume/volume blends (A1, A2, A3, A4, A5) of 'Arbequina', 'Arbosana' and 'Koroneiki' oils produced in the eastern region of Morocco in the 2013/14 crop year.

Blend code	% monovarietal oil		
	% Arbequina	% Arbosana	% Koroneiki
A_1	60	30	10
A_2	60	20	20
A_3	60	10	30
A_4	50	25	25
A_5	40	30	30

Statistical analysis

The results reported are the means of triplicate analyses and are given as means ± standard deviation. Significant differences between the means were determined by analysis of variance using SPSS statistics software (SPSS 20, USA).

Results and discussion

Quality criteria

Table 2 reports the results of the basic physico–chemical analyses performed on the monovarietal olive oils. These show that the quality criteria, namely free acidity, peroxide value and specific extinctions (K_{232}, K_{270} and ΔK) were distinctly lower than the limits recommended for extra virgin olive oils (EVOO) in the IOC trade standard applying to olive oils and olive pomace oils (IOC, 2013).

Table 2: Quality criteria of monovarietal 'Arbequina' 'Arbosana' and 'Koroneiki' oils produced in the eastern region of Morocco in the 2013/14 crop year

Quality criteria	Variety			EVOO*
	Arbequina	Arbosana	Koroneiki	
Acidity (% C18:1)	0.24 ± 0.02^{ab}	0.21 ± 0.04^{a}	0.29 ± 0.04^{b}	≤ 0.8
Peroxide value	6.78 ± 1.16^{a}	9.08 ± 0.69^{ab}	10.89 ± 1.13^{a}	≤ 20
K_{232}	0.10 ± 0.00^{a}	0.12 ± 0.00^{b}	0.15 ± 0.01^{c}	≤ 0,22
K_{270}	1.66 ± 0.06^{b}	1.73 ± 0.03^{b}	1.50 ± 0.03^{a}	≤ 2.5
ΔK	0.002 ± 0.0001^{a}	$0.006 \pm 0.0003b$	$0.007 \pm 0.0004b$	≤ 0.01

Different letters (a-c) on the same line indicate significant differences.
*Extra virgin olive oil (IOC, 2013).

Fatty acid profile of the monovarietal oils

Fatty acid composition is central to the nutritional and organoleptic quality of olive oil. Olive oil is made so original and healthy by its high content of monounsaturated fatty acids, amongst which oleic acid can account for as much as 83%. Several factors such as degree of fruit ripeness, climate or variety affect the fatty acid composition of olive oil (Garcia *et al.*, 1996; Judde, 2004, Pardo *et al.*, 2007). The data given in Table 3 show that the fatty acid composition of the oils tested complied with the requirements of the IOC trade standard (IOC, 2013).

Table 3: Fatty acid composition of the 'Arbequina', 'Arbosana' and 'Koroneiki' olive oils produced in the eastern region of Morocco in the 2013/14 crop year

Fatty acid (%)	Monovarietal olive oil			EVOO*
	Arbequina	Arbosana	Koroneiki	
Myristic acid	0.02 ± 0.00[b]	ND[a]	ND[a]	<0.03
Palmitic acid	16.42 ± 0.01[c]	14.84 ± 0.01[b]	12.08 ± 0.39[a]	7.5 - 20.0
Palmitoleic acid	1.71 ± 0.01[c]	1.35 ± 0.01[b]	0.66 ± 0.09[a]	0.3 - 3.5
Margaric acid	0.10 ± 0.00[b]	0.17 ± 0.00[c]	0,04 ± 0,01[a]	≤ 0.3
Margaroleic acid	0.22 ± 0.00[b]	0.36 ± 0.00[c]	0.08 ± 0.01[a]	≤ 0.3
Stearic acid	1.88 ± 0.01[a]	2.21 ± 0.01[ab]	2.27 ± 0.32[b]	0.5 - 5.0
Oleic acid	65.67 ± 0.02[a]	73.10 ± 0.01[b]	77.15 ± 0.2[c]	55.0 - 83.0
Linoleic acid	12.54 ± 0.02[c]	6.40 ± 0.00[b]	6.26 ± 0.11[a]	55.0 - 83.0
Alpha-linolenic acid	0.56 ± 0.00[a]	0.65 ± 0.00[b]	0.63 ± 0.05[b]	3.5 - 21.0
Arachidic acid	0.40 ± 0.01[a]	0.44 ± 0.00[a]	0.40 ± 0.07[a]	≤ 1.0
Gadoleic acid	0.29 ± 0.01[a]	0.30 ± 0.00[a]	0.26 ± 0.05[a]	≤ 0.6
Behenic acid	0.13 ± 0.01[a]	0.17 ± 0.00[b]	0.13 ± 0.02[a]	≤ 0.4
ΣSFA	19.02 ± 0.02[c]	17.83 ± 0.01[b]	14.93 ± 0.04[a]	≤ 0.2
ΣMUFA	67.89 ± 0.02[a]	75.11 ± 0.01[b]	78.15 ± 0.12[c]	
ΣPUFA	13.09 ± 0.02[c]	7.05 ± 0.00[b]	6.89 ± 0.08[a]	
O/L ratio	5.24 ± 0.01[a]	11.41 ± 0.00[b]	12.33 ± 0.21[c]	

Different letters (a-c) on the same line indicate significant differences.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; O/L: oleic/linoleic ratio. *Extra virgin olive oil, IOC, 2013.

Significant differences were observed between all the varieties studied ($p<0.05$) for all the parameters. Several authors have documented the heavy effect of variety on the fatty acid profile of olive oils. The oils produced from the 'Arbosana' and 'Koroneiki' varieties had comparable fatty acid profiles characterised by high levels of oleic acid (77.15 and 73.10%, respectively) compared with the 'Arbequina' oil (65.67%) and relatively low levels of palmitic acid (12.08 and 14.84% respectively) and linoleic acid (6.26 and 6.40%, respectively). The fatty acid profile of the 'Arbequina' oil differed from that of the other two varieties in that it recorded high levels of palmitic acid (16.42%) and saturated fatty acids (SFA: 19.02%), a low level of monounsaturated fatty acids (MUFA: 67.89%) and the lowest oleic/linoleic acid ratio (O/L) owing to its low content of oleic acid (65.67%) and its high content of linoleic acid (12.54%), which are major fatty acids.

Composition of triacylglycerol molecular species in olive oils

Nine different molecular species of triacylglycerols (TAGs) were distinguished in the olive oils tested: OOO, POO, LOO, LPO, SOO, POP, LOL, LPL and POLn (Figure 1). Listed by descending order of quantity, they are triolein (OOO), accounting for almost half of the TAGs in the 'Koroneiki' oil (48.02%) but only one-third in the 'Arbequina' oil (31.51%); dioleopalmitin (POO) with levels of 20.45 to 29.80%; dioleolinolein (LOO) with rates from 10.32 to 16.97% and palmitooleolinolein (LPO) with levels between 4.20 and 11.10%. These four major species of TAG account on their own for more than 91% of the total TAGs; the rest represent small quantities ranging from 0.5 to 4%. These results concur with those reported for olive oils produced from these varieties in Tunisia (Abaza *et al.*, 2002). The predominance of four molecular types of TAGs with at least one oleate (OOO, POO, LOO, POL) is linked to the fatty acid composition of the oil, characterised by its high oleic acid content.

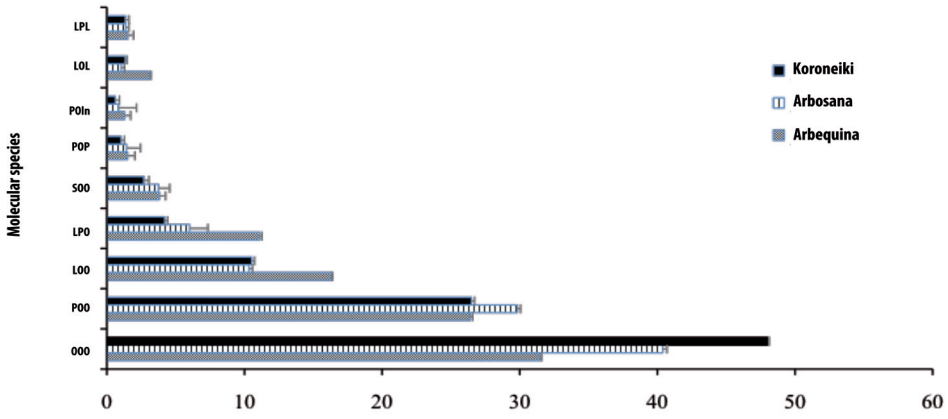

Figure 1: Composition of molecular species of TAGs of monovarietial 'Arbequina', 'Arbosana' and 'Koroneiki' olive oils produced in the eastern region of Morocco in the 2013/14 crop year (P: palmitate; S: stearate; O: oleate; L: linoleate; Ln: linolenate)

Natural antioxidant content and oxidative stability of monovarietal oils and monovarietal blends

Virgin olive oil is virtually the only oil to contain notable amounts of natural antioxidants (phenols and tocopherols). Besides lending olive oil its distinctive taste – both fruity and bitter – phenols are believed to be largely responsible for its oxidative stability (Boskou *et al.*, 1996; Mansouri *et al.*, 2013). The phenol and tocopherol content of olive oil, and hence its oxidative stability, is variety-dependent (Table 4). All the oils tested contained considerable amounts of phenolic compounds. The 'Koroneiki' olive oil had the highest phenol content (566.30 mg kg^{-1}) and the best oxidative stability (102.44 h). In contrast, the 'Arbequina' oil recorded the lowest content (286.51 mg kg^{-1}), which translates into poor oxidative stability (53.78 h). Hence, a clear correlation was observed between natural antioxidant content (chiefly phenols) and Rancimat oxidative stability; this finding concurs with results published in the literature (Tanouti *et al.*, 2011; Grati Kammoun and Laroussi, 2013).

Table 4: Natural antioxidant content and evaluation of the oxidative stability of monovarietal olive oils produced from 'Arbequina', 'Arbosana' and 'Koroneiki' olives in the eastern region of Morocco in the 2013/14 crop year

	Monovarietal olive oils		
	Arbequina	Arbosana	Koroneiki
Total phenols (mg kg^{-1})*	286.51 ± 5.63[a]	454.8 ± 11.87[b]	566.30 ± 8.87[c]
Alpha-tocopherol (mg kg-1)	322.36 ± 13.54[b]	460.07 ± 15.16c	344.58 ± 11.54b
Oxidative stability (hr)	53.78 ± 1.81[a]	78.81 ± 0.90[b]	102.44 ± 0.19[c]

Different letters (a-c) on the same line indicate significant differences.
*Polyphenol content is expressed in milligrams of caffeic acid per kilogram of oil.

Rancimat evaluation of the oxidative stability of the monovarietal oils (Table 4) revealed significant between-variety differences ($p<0.05$). Variety is therefore a factor that clearly influences oil stability. Concomitant comparison of the oxidative stability, phenol content and fatty acid composition of the three varieties shows that the phenol-rich oils ('Koroneiki' and 'Arbosana') were more stable in oxidative terms. Besides having a very high content of these antioxidant compounds, they had low levels of polyunsaturated fatty acids and a high MUFA/PUFA ratio. The higher the PUFA content of the oil, the more susceptible it is to attack from oxidation reactions. Conversely, phenolic compounds limit such reactions through their antioxidant effect (by free radical scavenging). The combined effect of low PUFA content and high phenol content is believed to be the reason for the great stability of olive oil and its low peroxide value (Allalout *et al.*, 2009; Aparicio *et al.*, 1999; Baccouri *et al.*, 2008; Bendini *et al.*, 2007; Boselli *et al.*, 2009, Gómez-Alonso *et al.*, 2002; Gutierrez *et al.*, 2001; Mansouri *et al.*, 2013).

High phenol content is definitely involved in oil resistance to oxidation but it also plays a part in its or-

ganoleptic properties. Several studies (Ollivier et *al.*, 2004) have reported a close relationship between high polyphenol and ortho-diphenol content and the bitter, astringent and pungent tastes in olive oil.

With the assistance of an olive oil mill in the region, we prepared blends of the three monovarietal oils (Table 1). 'Arbequina' oil accounted for at least 40% (V/V) of the blends. Blending 'Arbequina' with 'Arbosana' and 'Koroneiki' gives oils that are highly rated for their sweetness and rich fruity aromas and which have a high content of phenols and tocopherols that makes them more resistant to oxidative instability. Comparison of the Rancimat induction times of the blends (Table 5) and the 'Arbequina' monovarietal oil (Figure 2) clearly shows the effect of blending in correcting the poor oxidative stability reported for oils produced from the 'Arbequina' variety.

Table 5: Oxidative stability of three-variety blends of monovarietal 'Arbequina', 'Arbosana' and 'Koroneiki' olive oils produced in the eastern region of Morocco in the 2013/14 crop year

	Three-variety blends of monovarietal olive oils				
	A_1	A_2	A_3	A_4	A_5
Oxidative stability (h)	63.22 ± 1.09[a]	67.19 ± 1.14[b]	69.28 ± 1.02[b]	72.67 ± 2.2[c]	75.42 ± 0.67[d]

Different letters (a-c) on the same line indicate significant differences.

The better oxidative stability of the blends of 'Arbequina' with 'Arbosana' and 'Koroneiki' is certainly due to the increase in their phenol content and decrease in their PUFA content. It is common knowledge that the last two oils contain a large quantity of natural antioxidants and small amounts of PUFAs (see Table 3). Figure 2 shows that the oxidative stability of the blends (going from A1 to A5) was distinctly better, owing to the reduction in the percentage share of 'Arbequina' oil. Significant differences were detected between the different blends (p<0.05): the most stable blend proved to be A5 (75.42 h), which contained the least amount of 'Arbequina' oil. Although blends A1, A2 and A3 contained the same percentage of 'Arbequina' (60%), they varied in oxidative stability. Stability appears to depend above all on the proportion of 'Koroneiki' oil. Hence, blends A2 and A3 containing 20 and 30% 'Koroneiki' oil respectively were more stable (with Rancimat induction times of 67.19 h and 69.28 h, respectively) than blend A1, which contained only 10% 'Koroneiki' oil and recorded an induction time of 63.22 h.

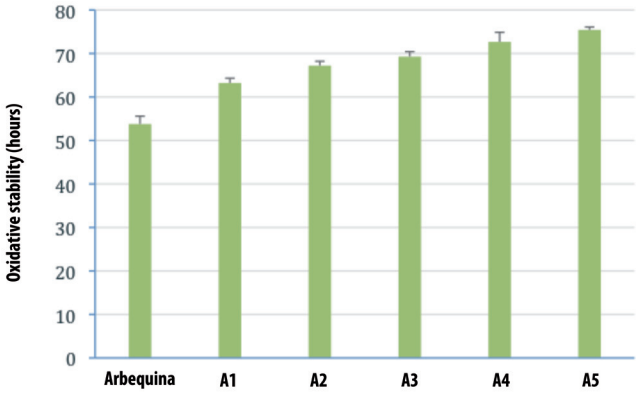

Figure 2: Changes in the oxidative stability of 'Arbequina' olive oil and of blends of such oil with 'Arbosana' and 'Koroneiki' monovarietal olive oils.

Conclusions

Olive oil oxidative stability is basically variety-dependent. It is closely linked to the natural antioxidant content (phenols and tocopherols) and MUFA/PUFA ratio of the oil. These parameters have to be taken into account to produce quality blends. The recommendation is to limit the amount of poor stability oil (the case of 'Arbequina') to less than 50%; the rest should be made up of oils that help to lower the PUFA content and raise the natural antioxidant content of the resultant blend. This would help to ensure better control of autoxidation and to preserve the organoleptic attributes of the blends. In this research, the improved oxidative stability achieved on blending 'Arbequina' with other oils helped to preserve and enhance its sensory properties, at least partially. Blending 'Arbequina' oil with two or three other monovarietal oils – 'Arbosana', 'Koroneiki' and 'Picholine' – is one solution for counteracting its poor resistance to oxidation. It is also a way of responding to market needs by creating new brands of olive oils.

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