Influence of regulated deficit irrigation strategies applied to olive trees (*Arbequina* cultivar) on oil yield and oil composition during the fruit ripening period†

M José Motilva,[1]* M Jesús Tovar,[1] M Paz Romero,[1] Simón Alegre[2] and Joan Girona[2]

[1]Food Technology Department, Escuela Técnica Superior de Ingeniería Agraria, Universidad de Lleida, Av/Alcalde Rovira Roure 177, E-25198 Lleida, Spain

[2]Institut de Recerca i Tecnología Alimentáries (IRTA), Àrea de Tecnologia Frutícola, Centre UdL-IRTA, Av/Alcalde Rovira Roure 177, E-25198 Lleida, Spain

Abstract: This study evaluated the effect of regulated deficit irrigation (RDI) strategies applied to olive trees (*Arbequina* cv) during the fruit ripening and harvest periods on oil yield and oil composition. Fatty acid composition, pigments, colour, polyphenol content and stability of oils were evaluated. The results indicate that regulated deficit irrigation induces fruit ripening; at harvest, oil yield increased when water supply was decreased, probably as a consequence of lower water content in the olive. Acidic composition was not affected by irrigation treatments. Irrigation affected pigment content and oil colour primarily during the early stages of olive ripening. RDI increased polyphenol concentration and stability of oils at all picking dates, especially during the first stages of the ripening period, probably owing to water stress.

Keywords: olive oil; oil composition; *Arbequina* cultivar; regulated deficit irrigation

INTRODUCTION

The olive (*Olea europea*) is drought-resistant[1–3] and is usually grown in areas with limited water resources. However, olive trees respond positively to irrigation,[4] and regulated deficit irrigation is reportedly useful for olive oil production.[5] In mediterranean areas, where summer rainfall is scarce, olive and olive oil production depend on climatic conditions. Thus irrigation could influence oil production.

Most olive oil produced in the southern part of Lleida (Catalonian, Spain) is included in the protected territorial quality label 'Les Garrigues'. The oils correspond to extra virgin classification and have a distinctive sensorial quality as a consequence of the specific cultivar (*Arbequina* cv) and the careful elaboration of oil.[6–8] Since the availability of irrigation water for olive is very limited (approximately 100 mm year^{-1}), the available water should be utilised efficiently to regulate oil production and oil quality.

Certain developmental periods in olive are especially sensitive to low soil moisture. During its bloom period, olive is very sensitive to dry soil conditions, particularly in warm, dry weather. These conditions also cause excessive fruit thinning, fruit drop and alternate bearing. Insufficient soil moisture during summer reduces shoot growth and carbohydrate production.[9] The regulated deficit irrigation applied to olive tree is based in its seasonal sensitivity to water stress as an indicator of total and seasonal water requirements.[10]

Many trials have been carried out on different olive cultivars to determine the effect of auxiliary drip irrigation with low quantities of water[2,11–14] and single-season drought irrigation strategies.[4] In all cases, irrigation improved vegetative growth and olive productivity.

However, there is very little information on the effect of regulated deficit irrigation on oil quality. Preliminary studies have shown that regulated deficit irrigation strategies applied to *Arbequina* olive trees negatively affected leaf water potential, stomatal conductance and fruit fresh weight.[15] Oil composition was also affected by irrigation: polyphenol content and oil stability increased with decreased water supply.[13]

* Correspondence to: M José Motilva, Food Technology Department, Escuela Técnica Superior de Ingeniería Agraria, Universidad de Lleida, Av/Alcalde Rovira Roure 177, E-25198 Lleida, Spain

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However, the classical oil quality parameters (titratable acidity and peroxide value) were not affected.[8]

The objectives of this study are to evaluate the effects of regulated deficit irrigation on oil yield and oil composition during the olive ripening period and at harvest time.

EXPERIMENTAL

Samples

The study was carried out in a commercial olive orchard (*Olea europea* L cv *Arbequina*) located in the olive-growing region of Les Garrigues (Lleida, Spain) in 1996. Annual rainfall for 1996 was 511 mm, with almost no precipitation during the summer period. Average air temperatures were 20.8, 23.0, 23.0 and 16.4 °C for June, July, August and September respectively. Annual reference crop water use Eto was 968–518 mm for the June–October period. These weather conditions are close to what is a typical year for the area.

Eighty 100-year-old trees, spaced 10 m × 10 m, were used in a randomised complete block design with five replications and three to four trees per plot.

Four irrigation treatments were applied: control and three regulated deficit irrigation (RDI) treatments. Control trees were fully irrigated during the whole season, using crop evapotranspiration (ET_c) calculated from modified Penman-determined reference crop water use (ET_o) (from a weather station close to the experimental plot),[16] with estimated crop coefficient $K_c = 0.7$ adapted from Ref 4. A reduction of 60% was imposed ($K_r = 0.4$) to account for the area shaded by the canopy,[17] and doses were modified *in situ* based on plant water status.[15] Additionally, three RDI treatments were imposed which were irrigated like the control for the whole season, but applying only 75% (T-75), 50% (T-50) and 25% (T-25) of the dose applied to the control from the beginning of massive pit hardening (5 July for the 1996 season) to the third week of September (2 weeks before the beginning of ripening).

Olive trees were irrigated daily with eight compensating droppers ($6 l h^{-1}$) placed around the tree. Irrigation requirements for each treatment were determined using the water budget approach.[18]

Olive fruits from each irrigation treatment (control, T-75, T-50 and T-25) were selected randomly for sampling throughout ripening, from immature stage to normal harvest date.

Three representative subsamples from each treatment (3 subsamples × 4 treatments) were picked and brought to the laboratory for oil extraction and physical and chemical analyses. Sampling corresponding to the ripening period of olive was done on three dates: 18 October, 30 October and 6 November. At harvest time (27–30 November), two representative subsamples from each subplot were picked (2 subsamples × 4 treatments × 5 subplots).

Olive analyses

Ripeness index

The olive ripeness index (RI) was determined according to the method proposed by the National Institute of Agronomical Research of Spain, based on a subjective evaluation of the olive skin and pulp colours.[19] The procedure consists of distributing a randomly taken sample of 100 fruits in eight groups: intense green (group $N = 0$), yellowish green (group $N = 1$), green with reddish spots (group $N = 2$), reddish brown (group $N = 3$), black with white flesh (group $N = 4$), black with $< 50\%$ purple flesh (group $N = 5$), black with $\geq 50\%$ purple flesh (group $N = 6$) and black with 100% purple flesh (group $N = 7$). The index is expressed as $\Sigma\ (N_i n_i)/100$, where N is the group number and n is the fruit number in that group. Ripeness index values range from 0 to 7.

Water and oil content determination

The water content of olive was determined by desiccation of the milled fruit according to the UNE standard Spanish method.[20] The fat content was determined by Soxhlet extraction[21] and is expressed on a dry weight basis (% fruit dw).

Oil yield

An Abencor analyser (MC2 Ingenierias y Sistemas, Sevilla, Spain) was used to process the olives in a pilot extraction plant and to determine oil yield. This unit consists of three basic elements: a mill, a thermobeater and a pulp centrifuge.[22] The oil was separated by decanting and the amount obtained was evaluated. Oil samples were transferred into dark glass bottles and stored in the dark at 4 °C.

The oil yield of olives is expressed on both a fresh and dry weight basis (% fruit fw and % fruit dw respectively).

Olive oil analyses

Fatty acid composition

The fatty acid composition of oils was determined by gas chromatography (GC) as fatty acid methyl esters (FAMEs). FAMEs were prepared by saponification/methylation with sodium methylate according to CEE 2568/91 as modified by (León Camacho and Cert.[23] A chromatographic analysis was performed in a Hewlett Packard 5890 Series II gas chromatograph using a capillary column (SP 2330, Supelco). Column temperature was isothermal at 190 °C and injector and detector temperature was 220 °C. Fatty acids were identified by comparing retention times with standard compounds. Five fatty acids, palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2), expressed as percentages of fatty acid methyl esters, were used in this study. Percentages of minor fatty acids are not shown.

Pigment quantification

The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption

spectrum of each virgin olive oil sample (7.5 g dissolved in cyclohexane (25 ml).[24] The chlorophyll and carotenoid contents are expressedin mg kg^{-1} of oil.

Colour

A colorimeter (chromameter type Color-Eye 3000, Macbeth) with a computer program Optiview 1.1 was used to assess the oil colour, and the Hunter colorimetric system was applied (L^*, lightness; a^*, redness; b^*, yellowness).[25] Oil samples were examined without dilution to avoid colour variation.

Polyphenol content

The polar fraction of oil was obtained using the modified method described by Vázquez-Roncero *et al.*[26] The oil sample (10 g), dissolved in hexane (50 ml), was extracted with methanol/water (60:40 v/v, 3×20 ml). The aqueous fractions were collected in a volumetric flask (100 ml) to obtain the total polyphenol extract. The total polyphenol content was measured colorimetrically at 725 nm using Folin–Ciocalteau reagent. The results are expressedin mg kg^{-1} of caffeic acid.

Stability test

A stability test was carried out using a 679 Rancimat apparatus (Metrohm Co, Basel, Switzerland) at 120 °C and 20 $l\,h^{-1}$ air flow.[27,28] The oil stability is expressed as the induction time (h) of hydroperoxide decomposition.

Statistical analysis

Two replicates were tested for each parameter. Results were analysed using an analysis of variance with version 6.12 of the SAS package (SAS Institute Inc, Cary, NC, USA). Differences and confidence levels were determined by calculating the least significant difference (LSD), and significant difference was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Irrigation treatments clearly affected tree water status.[15] Although yield (kg of olives per tree) was lower for T-25 (mainly owing to smaller fruits), % of oil yield was higher for this treatment (Table 1), resulting in no statistically significant differences between irrigation treatments in oil yield per tree (data not presented).[15]

Fruit ripening was slightly advanced by RDI (Table 1). The highest values of ripeness index (RI) were recorded from trees experiencing the greatest water deficit (T-25) throughout the ripening period (October–November). At harvest the RI value was 2.1 (green with reddish spots epicarp) and 3.0 (reddish brown epicarp) for control and T-25 respectively.

The water content of olive fruit decreased with fruit ripening. The lowest fruit water content corresponded to the most severe treatment (T-25) at all picking dates, although differences may not always have been statistically significant.

The oil content (expressed as% dry weight) increased with fruit ripening in all treatments and was not significantly influenced by irrigation (Table 1). The rate of oil accumulation and dry weight relative content (% oil content) increased with fruit ripening under all irrigation treatments and was more advanced for control, T-75 and T-50 treatments than for T-25 at the begining of the ripening period (October). At harvest (27–30 November) there were no differences between treatments. The delay in oil accumulation for T-25 may be a consequence of hydric stress of trees at the end of the summer period; similar observations

Table 1. Ripeness index, water and oil content of olive and oil yield in relation to picking date and irrigation treatment of *Arbequina* cultivar

Picking date	*Irrigation treatment*	*Ripeness index*	*Water content (% dw)*	*Oil content (% dw)*	*Oil yield (% fw)*	*Oil yield (% dw)*
18 October[a] (ripening)	Control	1.4	52.8±0.4	43.8±1.3	14.6±0.3	31.0±0.7
	T-75	1.5	49.8±1.5	41.3±1.6	15.8±0.5	31.4±0.8
	T-50	1.8	49.3±1.1	44.1±0.8	16.5±0.9	32.6±1.3
	T-25	2.1	48.0±1.3	45.9±0.8	18.3±0.9	35.8±1.2
30 October[a] (ripening)	Control	1.9	52.2±0.3	50.0±1.9	14.7±0.7	30.7±0.8
	T-75	1.9	48.0±1.2	48.1±1.4	16.2±0.6	31.2±0.8
	T-50	2.3	49.0±0.9	47.9±0.6	15.1±1.0	29.5±1.7
	T-25	2.5	47.2±1.7	45.4±1.1	17.7±0.8	33.6±1.1
6 November[a] (ripening)	Control	2.2	49.0±0.9	49.3±1.1	16.0±1.0	31.4±1.3
	T-75	2.3	47.1±0.3	51.7±2.0	16.8±0.9	31.8±1.9
	T-50	2.5	48.0±0.6	52.9±1.3	17.4±0.6	33.6±1.5
	T-25	2.7	47.0±0.5	54.7±1.1	17.7±0.5	33.5±1.0
27–30 November[b] (harvest)	Control	2.1	47.4±1.5	53.4±2.5	18.2±1.9	35.1±3.2
	T-75	2.5	44.4±2.2	51.1±3.5	17.8±1.8	33.2±2.1
	T-50	2.6	45.2±1.2	52.9±3.4	19.0±1.8	34.7±3.3
	T-25	3.0	44.6±1.2	54.5±1.7	21.6±1.5	39.2±2.9

[a] Mean ± SD, $n = 3$.
[b] Mean ± SD, $n = 10$.

were reported by Lavee and Wodner[29] under no-irrigation conditions.

There were no substantial differences between irrigation treatments in oil yield, expressed as fresh and dry weight of fruit, during the olive ripening period. However, at harvest time the highest oil yield corresponded to the most limiting irrigation regime, probably as a consequence of fruit water content (47.44 and 44.63% for control and T-25 respectively). Parallel studies have shown that the number of fruits per tree was lower when irrigation was most restricted, 36 000 under T-25 and 42 000 under control treatment.[15] Therefore oil yield per tree may not be affected by regulated deficit irrigation. This observation agrees with the results published by d'Andria *et al*[13] for different Italian cultivars. The higher oil yield (expressed as fresh weight of fruit) for T-25 could be the result of ripening advance with decreased water supply, or the result of higher water content in the control treatment fruit, which may affect oil extraction.

In relation to oil quality, fatty acid composition was similar in all irrigation treatments at different picking dates (ripening and harvest) (Table 2). Oleic acid content decreased slightly throughout ripening, while the amount of saturated fatty acids, palmitic and stearic and linoleic, increased slightly, but no differences in oil fatty acid composition were observed among irrigation treatments. This observation agrees with the results published by d'Andria *et al*,[13] who found that oil yield and acidic composition for *Ascolana tenera* and *Kalamata* cultivars were affected only by varietal factors and not by water regime. Salas *et al*[30] showed differences in acidic composition between dry-farming and irrigated olive orchards, but differences between irrigation treatments were insignificant.

The main differences in pigment content and colour of oils between irrigation treatments (Table 3) were observed at the first stages of ripening (18 October picking date): the chlorophyll and carotenoid concentrations in oils of the driest treatment (T-25) were 17.63 and 13.37 mg kg^{-1} respectively as compared with 8.54 and 8.38 mg kg^{-1} for the control treatment. However, oil pigment content for the control treatment remained relatively constant over time, while a gradual decrease, mainly at the first stages of ripening, could be observed in oils when irrigation was withheld. At harvest (27–30 November) the highest pigment content corresponded to oils extracted from fruit on fully irrigated trees. Manzi *et al*[31] reported a significant linear correlation ($R^2 = 0.95$) between the olive ripeness index and the olive carotene content, but they reported no correlation between the oil carotene content and the corresponding olive ripeness index in different cultivars. Other authors have observed a rapid decrease in chlorophyllic and carotenoid pigments in fruit as well as in the extracted oil during the period of olive harvesting.[32]

The values of the chromatic ordinates L^*, a^* and b^* obtained from the absorption spectra of the oils were used to evaluate the effect of irrigation treatment and picking date on colour (Table 3). The L^* values were similar at different ripening stages for all irrigation treatments. The values of a^* correspond to the green zone and the values of b^* are located in the yellow zone. As fruits ripened, the a^* values in oils increased slightly and the b^* values decreased in all irrigation treatments. The main variations were observed in chromatic ordinate b^*, which decreased similarly to oil pigment content (chlorophyll and carotenoid): at the first stages of fruit maturity (18 October picking date) the b^* value for control treatment was lower than for regulated deficit irrigation treatments (T-75, T-50 and T-25), which is

Table 2. Fatty acid composition of virgin olive oil in relation to picking date and irrigation treatment of *Arbequina* cultivar

Picking date	Irrigation treatment	Fatty acid (%)				
		Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)
18 October[a] (ripening)	Control	14.44±0.68	1.40±0.08	1.85±0.06	73.48±0.50	8.84±0.30
	T-75	14.13±0.27	1.31±0.06	1.81±0.14	73.76±0.36	8.99±0.27
	T-50	14.15±0.20	1.28±0.03	1.88±0.05	73.63±0.86	9.07±0.25
	T-25	14.05±0.59	1.21±0.03	2.02±0.11	73.65±0.93	9.09±0.16
30 October[a] (ripening)	Control	13.05±0.42	1.31±0.16	1.72±0.14	74.52±0.59	9.58±0.21
	T-75	13.10±0.78	1.12±0.18	1.68±0.17	74.04±0.78	10.06±0.18
	T-50	14.29±0.85	1.15±0.11	1.71±0.12	73.10±0.86	9.75±0.25
	T-25	12.48±0.71	1.80±0.18	1.80±0.18	74.31±0.68	9.62±0.19
6 November[a] (ripening)	Control	14.62±0.73	1.76±0.15	1.43±0.17	72.68±0.99	9.52±0.31
	T-75	14.72±0.47	1.61±0.10	1.49±0.12	72.60±0.87	9.59±0.16
	T-50	14.47±0.45	1.70±0.14	1.56±0.16	72.00±1.06	10.28±0.29
	T-25	14.71±0.32	1.51±0.08	1.65±0.11	73.65±0.95	9.99±0.20
27–30 November[b] (harvest)	Control	15.19±0.53	1.58±0.17	1.56±0.12	72.52±0.71	9.14±0.63
	T-75	15.53±0.60	1.71±0.15	1.37±0.14	71.71±0.75	9.68±0.56
	T-50	15.20±0.41	1.57±0.16	1.65±0.15	72.07±0.98	9.50±0.54
	T-25	15.24±0.65	1.52±0.15	1.55±0.13	72.25±0.84	9.44±0.30

[a] Mean ± SD, $n = 3$.
[b] Mean ± SD, $n = 10$.

Table 3. Chlorophyll and carotenoid concentration and colour (expressed as chromatic ordinates *L**, *a** and *b**) of virgin olive oil in relation to picking date and irrigation treatment of *Arbequina* cultivar

Picking date	Irrigation treatment	Chlorophyll (mg kg^{-1})	Carotenoid (mg kg^{-1})	Chromatic ordinate		
				L*	a*	b*
18 October[a] (ripening)	Control	8.54±1.07	8.38±0.60	87.78±2.81	−3.32±0.28	102.80±3.32
	T-75	11.82±0.97	10.36±0.80	84.91±2.70	−2.36±0.26	109.73±5.54
	T-50	15.95±0.86	12.38±0.88	87.90±2.34	−2.34±0.20	111.39±6.00
	T-25	17.63±1.18	13.37±0.95	85.88±3.84	−2.69±0.19	114.22±5.95
30 October[a] (ripening)	Control	8.48±0.44	7.93±0.62	90.43±3.75	−5.31±0.28	90.07±4.12
	T-75	7.61±0.42	6.97±0.49	88.78±2.39	−4.95±0.36	88.64±3.76
	T-50	7.90±0.54	6.95±0.75	87.67±3.31	−4.19±0.19	88.06±5.14
	T-25	7.34±0.61	7.04±0.70	86.97±2.98	−4.37±0.35	86.73±3.13
6 November[a] (ripening)	Control	8.56±0.63	7.19±0.84	85.85±3.10	−8.84±0.20	87.72±3.85
	T-75	7.57±0.55	6.18±0.58	91.40±2.62	−6.66±0.34	86.53±2.78
	T-50	7.84±0.62	6.44±0.67	92.83±3.27	−5.63±0.16	88.52±4.62
	T-25	7.17±0.59	6.40±0.70	90.66±3.12	−4.86±0.13	84.70±3.54
27–30 November[b] (harvest)	Control	8.79±0.89	8.23±0.81	86.15±3.74	−6.48±0.39	92.30±6.65
	T-75	7.25±0.78	7.14±0.59	85.89±5.07	−5.14±0.45	91.89±6.71
	T-50	6.67±0.56	7.07±0.77	88.02±4.54	−5.09±0.41	89.46±6.94
	T-25	6.44±0.64	6.04±0.89	85.20±3.48	−4.74±0.51	91.32±7.76

[a] Mean±SD, $n=3$.
[b] Mean±SD, $n=10$.

consistent with the loss of chlorophyll and carotenoid contents in the oils. These differences declined as the season advanced, and at harvest time there were no differences between irrigation treatments. Therefore the colour of oils was not affected by water reduction and there was a relationship between *b** values and pigment concentration as shown by Mínguez-Mosquera *et al*[24] for different olive varieties and stages of ripeness.

Under the most severe irrigation treatment (T-25), oils showed a polyphenol concentration and stability significantly higher than under control, T-75 and T-50 treatments throughout the ripening period, but especially in the early stages (18 October picking date) (Table 4). No differences were observed between oils of control, T-75 and T-50 treatments, nor were there important variations in polyphenol concentration and stability of oils throughout the ripening period under all irrigation treatments, with the exception of oils corresponding to the 6 November picking date, coinciding with a rainfall period, where a reduction in polyphenol concentration was observed (data not presented); however, oil stability was not affected, especially for the T-25 treatment. Other authors have also shown that irrigation reduces the polyphenol concentration and stability of olive oil, probably as a consequence of water stress produced by water reduction.[13,14,30]

At harvest, polyphenol concentration and stability of the oils produced by T-25 trees reached the highest values. This fact could be attributed to the more

Picking date	Irrigation treatment	Polyphenol (mg kg^{-1})	Stability (h)
18 October[a] (ripening)	Control	357±12	17.9±0.7
	T-75	376±12	18.7±0.6
	T-50	428±9	18.9±0.5
	T-25	555±22	21.6±1.1
30 October[a] (ripening)	Control	397±13	17.7±0.9
	T-75	382±18	20.1±0.6
	T-50	415±18	21.9±0.7
	T-25	469±19	22.1±0.9
6 November[a] (ripening)	Control	299±29	18.8±0.8
	T-75	330±11	19.2±0.4
	T-50	343±25	19.4±0.5
	T-25	368±14	21.3±0.6
27–30 November[b] (harvest)	Control	384±29	20.7±0.9
	T-75	412±26	21.1±1.0
	T-50	408±22	21.7±1.2
	T-25	488±35	23.7±1.8

Table 4. Polyphenol concentration and stability of virgin olive oil in relation to picking date and irrigation treatment of *Arbequina* cultivar

[a] Mean±SD, $n=3$.
[b] Mean±SD, $n=10$.

advanced ripening stage of the fruit under this irrigation treatment. Considering each irrigation treatment, the polyphenol concentration of oils increased slightly as fruits ripened (Table 4), with the exception of oils corresponding to the 6 November picking date. The oils obtained from T-25 olives represented an exception to this general rule, showing a higher polyphenol concentration at the first stages of ripening, coinciding with the end of the water reduction period. The differences in polyphenol concentration in the oils seem to be more a consequence of the advance in fruit ripening than of the irrigation treatment.

CONCLUSIONS

In the mediterranean area, where summer rainfall is scarce, a regulated deficit irrigation applied to *Arbequina* olive accelerated fruit ripening and affected fruit and oil composition during the early stages of ripening. However, at harvest, differences in oil content and yield due to irrigation treatment were minimal, with the exceptions of polyphenol concentration and oil stability, which were marginally affected. Long-term effects of regulated deficit irrigation at harvest time need to be evaluated.

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