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FULL ARTICLE

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Variation of almond yield, biometry, α-tocopherol levels, and antioxidant properties with nitrogen fertilization

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Abstract

A two years' experiment (2015–2016) was set in a factorial design in which the effect of two application forms of nitrogen (N) (soil and soil + foliar spray) in different doses (0, 25, 50, and 100 kg N ha^{-1}) on almond (*Prunus dulcis* Mill. cv. "Masbovera") was evaluated. Kernel yield, biometric properties, phytochemicals, and antioxidant activities were assessed. The results showed that almond kernel yield increases with an increment in N rate doses ($p < 0.001$) and was positively correlated with kernel weight and thickness, and negatively with fruit weight. The levels of α-tocopherol and total polyphenol content were higher with lower N doses (25 and 50 kg N ha^{-1}). The antioxidant activities were positively correlated with polyphenol content. Based on our results, excessive N rates over 50 kg/ha depreciate the levels of α-tocopherol, total polyphenols, and antioxidant bioactivities of kernels.

Practical applications

Almond is an important nut (dry) fruit that contains high levels of α-tocopherol, moderate levels of polyphenols, and high antioxidant activities, all responsible for their claimed health-promoting properties. Almond trees are known by their alternate behavior in which kernel yield and levels of phytochemicals and other compounds are highly variable between years. This article studies the usage of a sustainable nitrogen fertilization program toward a reduction of their alternate behavior, preserving and promoting their antioxidant properties and their levels of phytochemicals, particularly α-tocopherol and polyphenols. Our findings may provide a useful guide for adequate nitrogen fertilization program toward a better almond kernel quality.

KEYWORDS

almonds, antioxidants, fertilizers, nitrogen, phytonutrients

1 | INTRODUCTION

Almond (*Prunus dulcis* Miller) is one of the most important fruit crops from Mediterranean climate areas specially in different world regions like North America, Southern and Eastern Europe, North of Africa, Middle East, Asia, and Australia (Gradziel, 2011). In the last decades, the yield of Portuguese almond orchards have been very irregular due to many factors such as irregular climatic conditions, high orchards age, low plant densities, and old pruning systems and lower knowledge about adequate crop management of the existing or new varieties onto new rootstock. Furthermore, a lack of adequate fertilization application forms has contributed to the irregular and lower yields in

the 20 last years. Recently, a new generation of farmers is increasingly appearing who are open to following new approaches of productions and innovative techniques. Nonetheless, the majority of these innovations are related with irrigation (Cornacchia, Amodio, Colelli, & Tortosa, 2010; García-Tejero et al., 2011; Phogat, Skewes, Mahadevan, & Cox, 2013; Zhu, Taylor, Sommer, Wilkinson, & Wirthensohn, 2014, 2015) and not with fertilization. Moreover, the research on fertilization effects on almond yield components is very limited to few recent studies (Muhammad et al., 2018; Saa et al., 2016).

Nitrogen (N) fertilizers are widely used in crop production as a part of balanced fertilization plan, and it is commonly accepted that N fertilization results in increased crop growth and higher biomass yield, influencing the compositional quality of fruits (Ashraf et al., 2013; Muhammad et al., 2018; Saadati, Moallemi, Mortazavi, & Seyyednejad, 2013), vegetables (Rajasree & Pillai, 2012), and cereals (Ercoli, Masoni, Pampana, Mariotti, & Arduini, 2013; Zhang et al., 2016 and Zhang et al., 2017). Nonetheless, their effects are often contradictory and excessive N rates might decrease N crop use efficiency (Rotundo & Cipriotti, 2017) thus, decreasing their quality in several ways. Higher amount of N can result in reduced ascorbic acid (vitamin C) content, lower sugar content, lower acidity, and altered ratios of essential amino acids (Rajasree & Pillai, 2012). In addition, an excessive use of N may increase crop production costs (Hirel, Tétu, Lea, & Dubois, 2011) and may deteriorate soils and groundwater quality (including drinking water) by contaminating with nitrates (Gao, Howarth, Swaney, Honq, & Guo, 2015). Therefore, in this context, we present this study, in which we evaluate the effect of different N rates and different N application forms in almond yield, biometric properties, α-tocopherol levels, and antioxidant properties, in order to determine if it is possible to modify the quality in almonds with a feasible and a sustainable N fertilization regime.

2 | MATERIAL AND METHODS

2.1 | Experimental site

The experiment was carried out at Alfândega da Fé region, Northeast of Portugal (41° 21′ N 6° 58′ O), 550 m above sea level, from 2015 to 2016 in a 13-year-old almond (*Prunus dulcis* Mill. cv "Masbovera") orchard,

TABLE 1 Meteorological data in the almonds orchards during the years 2015 and 2016, in the region of Alfândega da Fé, Northeast of Portugal[a]

Year	Month	Mean temperature (°C)	Relative Humidity (%)	Total precipitation (mm)
015	January	6.7	79.4	35.3
	February	6.0	71.7	16.8
	March	10.6	59.0	12.6
	April	13.3	63.4	43.8
	May	17.4	54.9	49.8
	June	21.9	51.2	72.4
	July	24.6	46.4	11.6
	August	22.1	50.8	8.0
	September	18.2	55.9	48.8
	October	14.1	74.9	122.6
	November	9.8	87.6	77.6
	December	7.9	86.5	50.4
2016	January	8.0	85.1	175.0
	February	7.4	75.9	83.0
	March	8.5	68.1	47.2
	April	10.7	70.1	156.0
	May	14.3	68.3	138.2
	June	20.1	57.2	15.6
	July	25.2	44.9	3.2
	August	24.9	44.3	22.0
	September	21.4	53.9	31.4
	October	16.3	70.8	41.6
	November	9.5	78.1	71.8
	December	6.5	86.7	35.6

[a]Data were recorded in the experimental filed through meteorological station.

planted in an area of 9.5 ha with a plant density of 417 trees per ha. The climate data from 2015 to 2016 are recorded and summarized in Table 1.

2.2 | Fertilizer treatments

The experiment was arranged as a factorial design with eight N fertilizer treatments and three replicates in two consecutive years. The experiment unit consisted of three homogeneous trees. Nitrogen was applied in two different application forms, soil and soil plus foliar sprays (Table 2). In the first regime, N was applied to the soil once a year (late of March of 2015 and 2016) at the rates 0 (N0), 25 (N25), 50 (N50), and 100 (N100) kg ha^{-1} as ammonium nitrate (27% N). The second fertilization regime consisted of supplementing similar soil N treatments (0, 25, 50, and 100 kg N ha^{-1}) with foliar sprays (0.5% N as urea) applied three times per year during the growing season (May, July, and August of 2015 and 2016). These second groups of N fertilizer treatments were, respectively, named as N0FN3, N25FN3, N50FN3, and N100FN3. Throughout the experiments, standard cultural practices (pruning and disease control) were implemented regularly.

2.3 | Data collection

2.3.1 | Yield, number of fruits per tree, and fruit biometric parameters

Trees were shaken individually using a mechanical harvester and nuts from each tree were collected and stored separately. Thirty fruits per sample were randomly selected and allowed to dry for one month. After that period, biometric parameters of fruits with shell (weight, length, width, thickness) were determined and they were hulled, cracked, and the same biometric parameters of kernels and kernel yield were determined. The kernel yield was calculated using the equation: kernel yield (%) = kernel mass (g)/nut weight (g) × 100. The samples were collected at commercial maturation in September 2015 and September 2016.

TABLE 2 Fertilizer treatments performed

Treatment designation	Nitrogen rates	Application
N0	0 kg/ha	soil
N25	25 kg/ha	soil
N50	50 kg/ha	soil
N100	100 kg/ha	soil
N0FN3	0 kg/ha + 0.5% nitrogen as urea	soil + foliar sprayer
N25FN3	25 kg/ha + 0.5% nitrogen as urea	soil + foliar sprayer
N50FN3	50 kg/ha + 0.5% nitrogen as urea	soil + foliar sprayer
N100FN3	100 kg/ha + 0.5% nitrogen as urea	soil + foliar sprayer

2.3.2 | Total protein

The total protein of kernels was determined by classical method of Lowry, Rosebrough, Farr, and Randall (1951), with small modifications. Briefly, 4 ml of phosphate buffer with pH 7.0 was added to 50 mg of dry kernels from each sample, mixed thoroughly in a vortex, and kept at 4°C for 1 hr with agitation every 20 min. The mixtures were then centrifuged at 4,000 rpm for 15 min and the supernatants were collected and stored at −20°C until further determinations. Simultaneously, a work solution (2 ml of 1.56% copper sulfate solution in 2.37% of sodium potassium tartarate with 100 ml of 2% sodium carbonate in 0.1 N NaOH solution) needed to quantify the total protein content was prepared. Then, 0.4 ml of each extract was added to 4 ml of work solution, mixed thoroughly in a vortex, and kept at room temperature for 10 min. Next, 0.4 ml of Folin–Ciocalteu's phenol reagent (1 N) was added and the mixtures incubated at room temperature in the dark for 30 min. The absorbance values at 660-nm wavelength were then recorded in a spectrophotometer against blank (solvent used in sample extraction instead of sample) and a calibration curve with the BSA standard (bovine serum albumin-protein standard) was performed to quantify the total protein content as mg g^{-1} dry weight (dw).

2.3.3 | Total polyphenol content

Total polyphenol content (TPC) of kernels was determined based on the classical colorimetric method (Singleton & Rossi, 1965), but conducted with small modifications in a 96-well microplate (Costar 3590, Corning, NY, USA). First, it was necessary to extract polyphenols and for that 40-mg dw kernels were weighed to 2-ml centrifuge vials and 1 ml of methanol 70% (methanol:water) was added. Each mixture was agitated thoroughly in a vortex and then heated at 70°C for 30 min. Afterward, the mixtures were centrifuged at 11,000 rpm at 1°C for 15 min (Eppendorf Centrifuge 5,804 R, Hamburg, Germany), the supernatants were collected and filtered with Spartan filters (0.2 µm) to HPLC amber vials (Chromabond 2-SVW(A) ST-CPK, Sigma-Aldrich, Tauferkichen, Germany) and the precipitate was rejected. These extracts were used to quantify TPC and in the antioxidant activity bioassays. Then, 20 µl of each extract was added to each microplate well followed by the addition of 100 µl of Folin–Ciocalteu's phenol reagent (1:10 in dd H_2O) and 80 µl of 7.5% Na_2CO_3. Then, the microplates were heated at 45°C for 15 min and the absorbance values were measured at 765-nm wavelength in a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland), against blank (all reagents except sample). A calibration curve with a commercial standard of gallic acid at different concentrations (mg ml^{-1}) was performed and the results were expressed as mg gallic acid equivalent (GAE) g^{-1} dw.

2.3.4 | α-Tocopherol

The levels of α-tocopherol in the kernel samples were determined by high-performance liquid chromatography (HPLC), according to

the adaptation of the method used by Gueguen, Herbeth, Siest, and Leroy (2002) with several modifications. Ten milliliters of a solvent mixture, methanol:dichcorlometane (1:1), were added to 1 g of dw of kernels, mixed thoroughly in a vortex, and sonicated for 20 min. Then, the mixtures were centrifuged at 4,000 rpm and room temperature for 10 min. The supernatants were collected, filtered (PTFE 0.2 µm, Ø 13 mm (Teknokroma, Spain) and stored in HPLC amber vials (Chromabond 2-SVW(A) ST-CPK, Sigma-Aldrich, Tauferkichen, Germany) and immediately injected in HPLC–DAD–UV/Vis system to avoid any substantial losses of vitamin E by degradation. The HPLC system was set with the following parameters: a C18 column (150 × 46 × 5 µm) (ACE® HPLC columns, Advanced Chromatography Technologies, Ltd., Aberdeen, Scotland); an eluent of absolute methanol with 0.1% of trifluoroacetic acid (TFA); and an isocratic gradient with a flow of 1 ml min^{-1} in a run length of 15 min. The detection was performed at 296-nm wavelength. The identification and quantification of α-tocopherol was made using the retention time (7.06 min.) of the peaks obtained in the chromatograms, UV spectra of detected compound compared with external commercial standard of α-tocopherol (Sigma-Aldrich, Tauferkichen, Germany), and standard curve for external standardization. The results are expressed as mg g^{-1} of α-tocopherol.

2.3.5 | Antioxidant activity

Anti-lipid peroxidation bioassay

The modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media, described previously by Adithya, Lakshmi, Christabel, and Sasikumar (2013) in a 96-well microplate (Costar 3590, Corning, NY, USA). Briefly, 20 µl of substrate (homogenate of egg yolk at 10% in 0.1 M phosphate buffer, pH 7.4) and 5 µl of $FeSO_4$ (1 mM in water) were added, followed by addition of 20 µl of extract (same extracts used to quantify TPC) and 65 µl of ddH_2O. Then, the microplates were incubated for 15 min at 37°C. After, 50 µl of trichloroacetic acid (50% in water) and 100 µl of tiobarbituric acid (TBA) (0.8% in phosphate buffer) were added sequentially, and the microplates were incubated again at 95°C for 15 min until a pink color appears. Then, the absorbance values (Abs) were recorded at 532 nm in a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland). Simultaneously, a complete oxidized extract (egg yolk + $FeSO_4$, without extract) was used as control. The results were expressed as % lipid peroxidation inhibition using the following formula: (%) lipid peroxidation inhibition = $(Abs_{control} - Abs_{sample}/Abs_{control}) \times 100$.

Ferric reduction capacity

The ferric-reducing capacity of almond extracts was determined by the ferric reduction capacity (FRAP) method described by Stratil, Klejdus, and Kubán (2006) with several modifications. In the dark, FRAP reagent was made with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol 2,4, 6-tripyridyl-S-triazine 10 (TPTZ) (Sigma-Aldrich, Tauferkichen, Germany) in a 40 mmol L^{-1} HCl (Sigma-Aldrich, Tauferkichen, Germany) solution and 20 mmol L^{-1} $FeCl_3.6H_2O$ (Sigma-Aldrich, Tauferkichen, Germany). Then, 25 µL of each extract (same extracts used to quantify TPC) or standard solutions was combined with 275 µL of FRAP reagent. After incubating for 5 min in the dark and room temperature, the absorbance values were recorded at 593 nm in microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland) against blank. Simultaneously, a calibration curve with $FeSO_4$ was produced in order to express the results as equivalent to µM FeSO4.

DPPH free radical scavenging activity

The free radical scavenging activity of almond extracts were determined by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay (Siddhraju & Becker, 2003) conducted in a 96-well microplate (Costar 3590, Corning, NY, USA). Briefly, 285 µl of freshly DPPH (Sigma-Aldrich, Tauferkichen, Germany) solution (4 mg of DPPH in 100 ml of 95% of ethanol) was added to each microplate well, followed by the addition of 15 µl of extract (same extracts used to quantify TPC). A blank sample (all reagents and extraction solvent instead of sample) added to one well. Then, the microplates were incubated in the dark, at room temperature for 30 min. After this period, the absorbance values (Abs) were recorded at 517-nm wavelength in a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland). The results were expressed as % free DPPH radical scavenging capacity using the following formula: $\%\ DPPH_{scavenging\ capacity} = (Abs_{blank} - Abs_{sample}/Abs_{blank}) \times 100$.

2.4 | Statistical analysis

All determinations were done in triplicate and the results were analyzed using a two-way ANOVA in factorial arrangement followed by Tukey test, based on confidence level equal to or higher than 95% ($p < 0.05$). The results were expressed as mean values ± standard deviation (*SD*) of three replicates. The software SPSS V.17 (SPSS-IBM, Orchard Road-Armonk, New York, NY, USA) was used to carry out these analyses. A linear regression was determined between kernel yield and all biometric properties of almond shells and kernels. In addition, a Pearson's correlation was performed between the chemical parameters and antioxidant activity results in order to establish the influence between them.

3 | RESULTS AND DISCUSSION

Table 1 summarizes the parameters of temperature, precipitation, and humidity for 2015 and 2016. The average temperatures were very similar between both years but in the second year the precipitation and humidity was higher particularly in the first 5 months of the year.

Table 2 presents the treatments performed and Table 3 presents the average levels for each biometric property (weight, length, width, and thickness) of almond shells and kernels. Figure1 presents the variation of kernel yield between years and treatments.

TABLE 3 Average value of biometric properties of almond nuts, submitted to different N treatments[1,2,3]

Year	Treatment	Production (kg per tree)	Shell + kernel				Kernel			
			Weight (g)	Length (mm)	Width (mm)	Thickness (mm)	Weight (g)	Length (mm)	Width (mm)	Thickness (mm)
2015	N0	6.25 ± 2.57a	5.27 ± 0.81b	40.35 ± 2.62b	24.32 ± 1.60a	17.13 ± 0.90ab	1.14 ± 0.18b	26.90 ± 1.94a	13.86 ± 0.97b	6.52 ± 0.35a
	N25	8.96 ± 3.76a	4.43 ± 0.48a	38.25 ± 1.80a	23.57 ± 1.21a	16.80 ± 0.79a	1.04 ± 0.13a	26.24 ± 1.36a	13.16 ± 0.74a	6.51 ± 0.32a
	N50	13.49 ± 2.68b	4.66 ± 0.54a	38.95 ± 1.95a	23.58 ± 1.38a	17.27 ± 0.81b	1.02 ± 0.15a	26.56 ± 1.37a	13.15 ± 0.81a	6.39 ± 0.32a
	N100	13.18 ± 3.30b	4.65 ± 0.75a	41.68 ± 2.24c	24.33 ± 1.42a	17.41 ± 0.96b	1.18 ± 0.20b	27.83 ± 1.43b	13.69 ± 0.78b	6.58 ± 0.47a
	p value	*0.000***	*0.000***	*0.000***	*0.041**	*0.048**	*0.001***	*0.001***	*0.001***	*0.244 n.s.*
	N0FN3	7.06 ± 2.52a	5.42 ± 0.53c	39.89 ± 1.69b	24.26 ± 1.22b	17.64 ± 0.84b	1.09 ± 0.07a	27.33 ± 1.07ab	13.74 ± 0.59b	6.23 ± 0.37a
	N25FN3	9.09 ± 1.80a	5.75 ± 0.90d	40.06 ± 2.71b	24.99 ± 1.60c	18.08 ± 0.91c	1.24 ± 0.18b	26.78 ± 2.02a	14.35 ± 0.84c	6.86 ± 0.40c
	N50FN3	8.72 ± 3.56a	4.82 ± 0.65b	39.98 ± 2.69b	24.03 ± 1.48b	17.46 ± 0.74b	1.15 ± 0.15a	28.11 ± 1.76b	13.27 ± 0.82a	6.50 ± 0.43b
	N100FN3	14.19 ± 6.79b	4.28 ± 0.46a	37.59 ± 1.93a	23.17 ± 1.08a	16.94 ± 0.74a	1.15 ± 0.12a	26.81 ± 1.47a	13.51 ± 0.59ab	6.79 ± 0.50c
	p value	*0.006***	*0.000***	*0.000***	*0.000***	*0.000***	*0.001***	*0.005***	*0.000***	*0.000***
2016	N0	0.10 ± 0.11a	5.30 ± 0.94b	39.34 ± 2.56a	25.51 ± 1.82b	18.45 ± 1.40b	1.36 ± 0.26a	25.97 ± 1.75a	14.73 ± 0.83b	7.57 ± 0.65a
	N25	0.23 ± 0.12a	4.73 ± 0.89a	39.02 ± 2.54a	24.59 ± 1.40ab	17.99 ± 1.30ab	1.43 ± 0.29a	26.57 ± 1.64a	14.52 ± 0.88ab	7.77 ± 1.03a
	N50	0.21 ± 0.24a	5.34 ± 0.77b	39.78 ± 2.56a	25.26 ± 1.25b	17.98 ± 0.95ab	1.44 ± 0.24a	26.86 ± 1.93a	14.51 ± 0.69ab	7.85 ± 0.99a
	N100	0.10 ± 0.06a	4.54 ± 1.08a	38.96 ± 3.70a	24.26 ± 2.41a	17.59 ± 1.58a	1.40 ± 0.33a	26.81 ± 2.45a	14.15 ± 1.37a	7.86 ± 1.22a
	p value	*0.144 n.s*	*0.001***	*0.682 n.s.*	*0.028**	*0.104 n.s*	*0.713 n.s.*	*0.287 n.s.*	*0.153 n.s.*	*0.657 n.s.*
	N0FN3	0.21 ± 0.21a	5.20 ± 0.90b	38.62 ± 3.44a	25.24 ± 1.85a	18.33 ± 1.27ab	1.34 ± 0.27a	25.66 ± 2.33a	14.65 ± 1.08a	7.72 ± 1.12a
	N25FN3	0.18 ± 0.14a	5.76 ± 0.98c	41.90 ± 3.18b	25.69 ± 1.67a	18.82 ± 2.06b	1.57 ± 0.25b	27.88 ± 1.85b	14.95 ± 0.89a	7.81 ± 0.85a
	N50FN3	0.23 ± 0.17a	4.80 ± 1.09ab	39.85 ± 2.91a	25.46 ± 1.74a	17.86 ± 1.49a	1.41 ± 0.28a	26.59 ± 1.82a	14.81 ± 1.02a	7.61 ± 1.20a
	N100FN3	0.11 ± 0.11a	4.60 ± 0.92a	39.98 ± 2.91a	25.17 ± 1.71a	17.66 ± 1.05a	1.40 ± 0.19a	26.66 ± 1.81a	14.47 ± 0.94a	7.67 ± 0.84a
	p value	0.390 n.s.	0.000***	0.001**	0.645 n.s	0.017*	0.006**	0.000***	0.263 n.s.	0.889 n.s.
Source of Variance		*p* values								
Treatments (T)		0.000***	0.099. n.s.	0.710 n.s.	0.000**	0.000**	0.000**	0.006 **	0.000***	0.000***
Year (Y)		0.000***	0.000***	0.000***	0.000***	0.000***	0.000**	0.001**	0.000***	0.256 n.s.
T × Y		0.000***	0.013*	0.000***	0.007**	0.076 n.s.	0.095 n.s.	0.000***	0.022*	0.206 n.s.

[a]Values expressed as mean ± standard deviation (*SD*) of three replicates.
[b]Probability test values obtained by ANOVA variance analysis; number with different letters in same column are significantly different from another at $p < 0.05$ by Duncan test.
[c]The symbols means: not significant (n.s.); significant (*), very significant (**); highly significant (***).

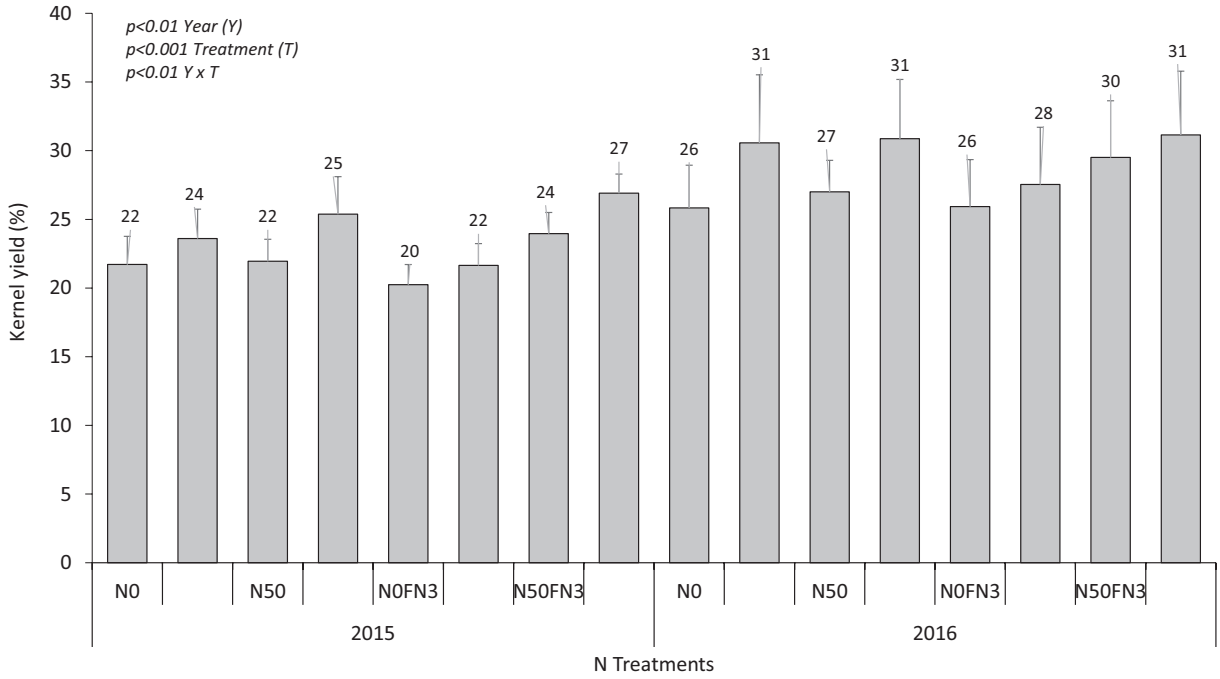

FIGURE 1 Kernel yield expressed as percentage ± standard deviation (*SD*), throughout 2015–2016

Figure 2wpresents the influence of each almond biometric property on kernel yield by a linear regression calculation with the respective equations. In general, treatments and year influenced significantly the shells + kernels width and thickness and kernels' weight, and width ($p < 0.001$). The length of kernels was less influenced by both factors ($p < 0.01$) and thickness of kernels was only significantly affected by treatments ($p < 0.001$). Comparatively, the kernel yield was higher in 2016 than in 2015, particularly when higher N rates were applied either to the soil (N100, 100 kg ha^{-1}) or in combination with N foliar sprayers ($N100FN_3$, 100 kg/ha) (Figure 1). The increments in the kernel yields were positively correlated with kernel weight and thickness, and negatively with fruit weight (shell + kernel) (Figure 2).

The average levels of α-tocopherol, total soluble proteins, and TPC, and the antioxidant activity of kernels are presented in Table 4 and Figure 3, respectively. In general, the average values of tocopherols, total soluble proteins, and TPC were similar in both years of experiment, while the antioxidant activities followed an opposite trend depending on the method used in their determination (Figure 3). However, the antioxidant activity seems to be related to the variation of TPC content as shown by the Pearson correlation results presented in Table 5. The levels of α-tocopherol and TPC were higher when lower N doses (N25, N50, N25FN3) were applied, particularly in the second year of experiment, which was followed by the antioxidant activity results, except for FRAP method (Figure 3). While, the amount of total soluble proteins were higher when the highest N doses (N100 and N100FN3) were applied in both years of experiment.

The quality attributes in almonds (shell + kernels) like in other fruit species are highly dependent on cultural practices and environmental conditions. According to the climate data recorded in the almond orchard (Table 1), the precipitation was very limited in the first 5 months of 2015, while in 2016, excess of precipitation and humidity was observed in a similar period when blooming, flowering, and pollination occurred, which might have a negative impact on fruit setting and therefore less yield occurred (Table 3). Although the constrain effect of precipitation and humidity it was possible to observe that biometric properties of almonds shells + kernels and kernels increased in the second year of production, maybe due to the positive action of nitrogen fertilizations (Table 3). Similar trend was observed for compositional attributes (Table 4). In fact, after N fertilizations, particularly in the second year of experiment, kernel yields were higher when high N rates were applied either to the soil (N100, 100 kg ha^{-1}) or in combination with N foliar sprayers ($N100FN_3$, 100 kg/ha to the soil plus three doses of 0.5% N foliar) (Table 3). This increment was positively correlated with kernel weight, width, and thickness, and negatively with fruit weight (shell + kernel) (Figure 2). These results are in agreement with previous studies of Raese (1997), Saenz, DeJong, and Weinbaum (1997), Gray and Garrett (1998) and Gunes, Okay, Koksal, and Koroglu (2010) which recorded high yields with high rates of N, in different types of tree crops.

A different trend was observed for α-tocopherol, total proteins, and TPC of kernels (Table 3). The levels of α-tocopherol, total proteins, and TPC were higher when moderate N rates (N25, N50, N25FN3) were applied. In addition, the increment was higher in the second year of experiment. This year-to-year variation could be due to the cumulative effect of N fertilization. Pérez-Zamora and Cigales-Rivero (2001) in cantaloupe melon found that increasing N fertilization produces an increase in the fruit quality attributes up to a maximum value, due to a cumulative effect of N. However, the same author reported that if the supply continues increasing,

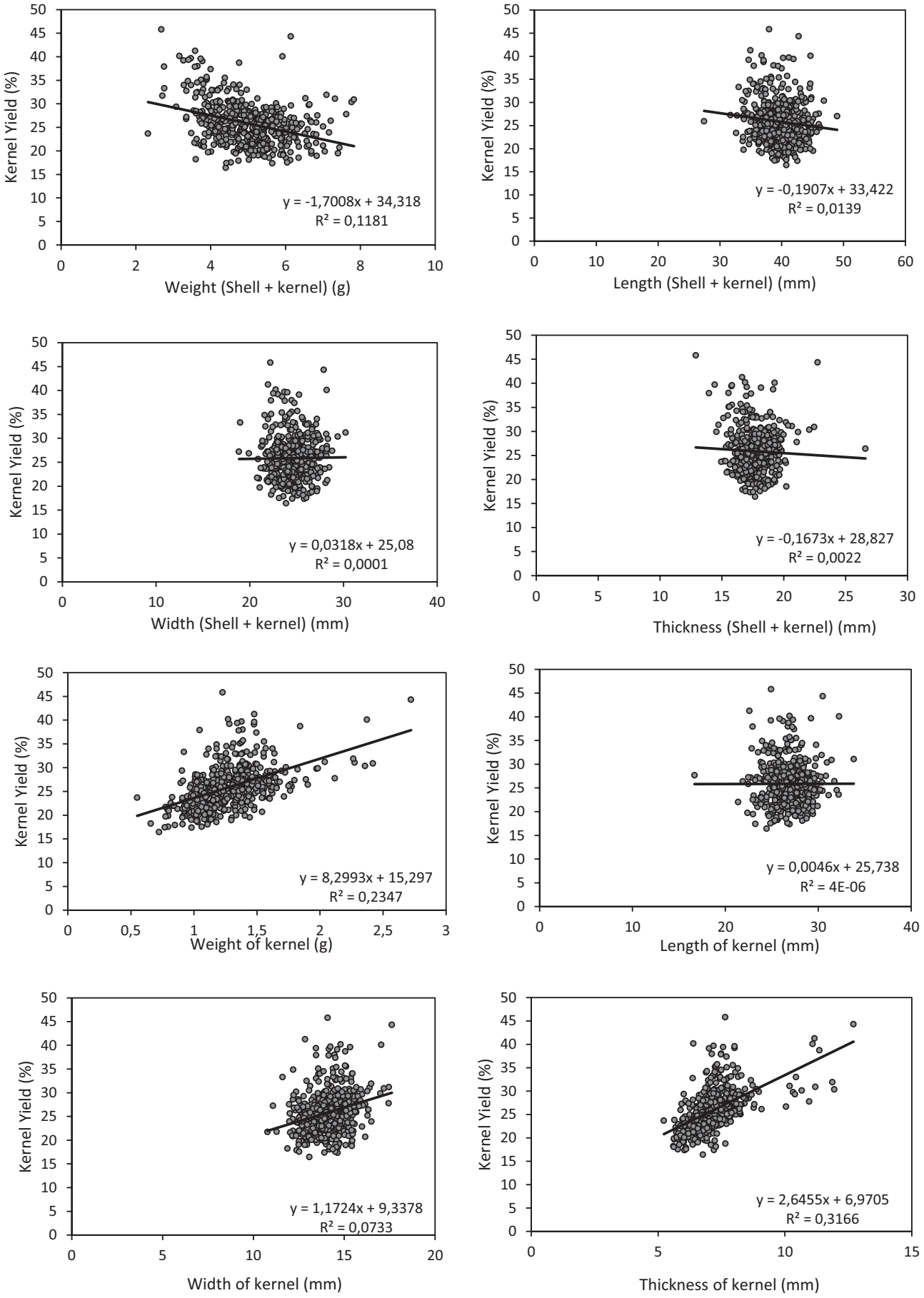

FIGURE 2 Relation between kernel yield and the different biometric parameters measured

TABLE 4 Average value of chemical properties of almond nuts, submitted to different N treatments[a,b,c]

Year	Treatment	α-Tocopherol (mg g^{-1})	Total soluble protein (mg g^{-1})	Total polyphenols (mg g^{-1})
2015	N0	0.155 ± 0.01b	239,61 ± 29,64a	1,31 ± 0,20b
	N25	0.155 ± 0.01b	258,88 ± 5,23ab	1,18 ± 0,09ab
	N50	0.125 ± 0.02a	254,67 ± 10,54ab	0,96 ± 0,27ab
	N100	0.130 ± 0.00a	273,76 ± 6,14b	0,87 ± 0,22a
	p value	0.02*	0.160 n.s.	0.106 n.s.
	N0FN3	0.123 ± 0.00ab	223,95 ± 4,08a	1,21 ± 0,09a
	N25FN3	0.133 ± 0.02b	232,87 ± 11,50a	1,25 ± 0,27a
	N50FN3	0.129 ± 0.01ab	232,25 ± 6,88a	1,27 ± 0,44a
	N100FN3	0.109 ± 0.01a	242,30 ± 25,28a	0,93 ± 0,02a
	p value	0.078 n.s.	0.524 n.s.	0.398 n.s.
2016	N0	0.150 ± 0.01a	281,53 ± 27,66a	1,10 ± 0,14a
	N25	0.156 ± 0.05a	318,98 ± 28,43a	1,28 ± 0,06a
	N50	0.154 ± 0.02a	286,71 ± 59,31a	1,09 ± 0,33a
	N100	0.130 ± 0.02a	237,48 ± 86,41a	1,45 ± 0,65a
	p value	0.698 n.s.	0.413 n.s.	0.628 n.s.
	N0FN3	0.144 ± 0.01a	203,24 ± 44,26a	1,39 ± 0,50a
	N25FN3	0.143 ± 0.02a	268,29 ± 20,38a	1,17 ± 0,76a
	N50FN3	0.142 ± 0.01a	274,81 ± 50,63a	0,88 ± 0,17a
	N100FN3	0.148 ± 0.00a	249,55 ± 64,86a	1,23 ± 0,17a
	p value	0.559 n.s.	0.375 n.s.	0.633 n.s.
Source of Variance		*p* values		
Treatments (T)		0.095 n.s.	0.124 n.s.	0.628 n.s.
Year (Y)		0.352 n.s.	0.510 n.s.	0.830 n.s.
T × Y		0.925 n.s.	0.276 n.s.	0.555 n.s.

[a]Values expressed as mean ± standard deviation (*SD*) of three replicates.
[b]Probability test values obtained by ANOVA variance analysis; number with different letters in same column are significantly different from another at $p < 0.05$ by Duncan test.
[c]The symbols means: not significant (n.s.); significant (*), very significant (**); highly significant (***).

the amount and quality of production might be affected negatively, which is in agreement with our findings, because at higher N doses (N50, N100, and N50FN3, N100FN3), the levels of tocopherol, total proteins, and TPC decreased. Therefore, we can state that extreme N levels (to low or to high) had a depressive effect on biometric and compositional attributes of almonds. These results have also consequences in the antioxidant properties of kernels (Figure 3). The variation found for antioxidant properties of kernels (Figure 3) measured by lipid peroxidation, FRAP, and DPPH bioassays, was only affected significantly affected by TPC (Table 5). The difference in trends observed for the twee methods in particularly for FRAP compared to DPPH and Lipid peroxidation assays could reside in the presence of other compounds not determined in current work. Surprisingly, the levels of α-tocopherol in samples had no significant effect ($p > 0.05$) on their antioxidant activity. α-Tocopherol has been reported as having important antioxidant activity (Ryo, 1997); however, in this work that seems not the case, maybe because is in lower levels or their influence might be supplanted by TPC.

Another important finding of this study resides in the fact that N applied only to the soil seems to be enough to preserve and promote the quality of almond kernels. In both years of experiment, the average content of α-tocopherol, total proteins and TPC were always higher in trees submitted to a unique N dose (Table 4). This result suggests that single application of N into the soil at lower N rates is enough to promote, improve, or preserve the almond compositional quality, which is in agreement with the previous cited authors who found that excessive N has a depreciative effect in produces quality.

Based on the results of this study, we can state that extreme N levels (to low or to high) might have a positive effect on almond kernel yield but have a depressive effect on their compositional quality and antioxidant properties. The lack of significant tree response to high N rate applications may reflect the use of stored nutrients in perennial tree parts but not for the fruit composition as was observed by Ding et al. (2017) for dwarf apple trees. Liu, Sung, Chen, and Lai (2014), Miceli and Miceli (2014) and Saa et al. (2016), found

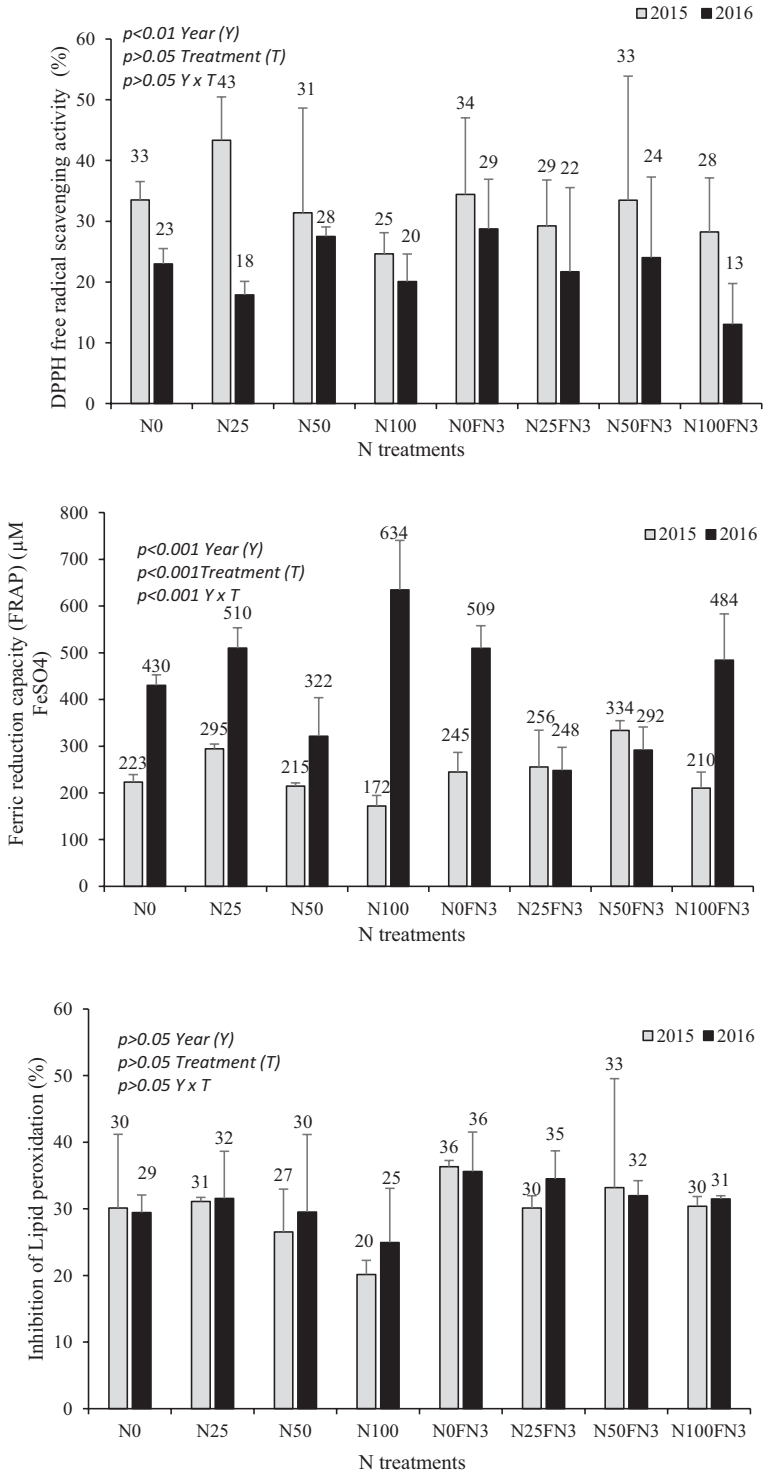

FIGURE 3 Mean ± standard deviation (*SD*) values of antioxidant activity of almond kernels, measured by three different methods

that high rates of N decrease N crop use efficiency, cause a loss of minerals and bioactive compounds of edible part of plants, and an excessive accumulation of undesirable nitrates in plants, soil, and ground water. Therefore, lower N rates of N increase and preserve the quality of almonds without affecting the environment. Our results suggest that for almond and from quality point of view, there are no significant benefit of increasing N over 50 kg/ha.

To conclude, increasing N fertilization rates increases kernel yield through an increment of kernel weight and thickness, but an excessive N application can depreciate the nutritional quality and bioactive properties of almonds. Therefore, a regime with N rates of 25 kg/ha and 50 kg/ha applied only to the soil is enough to promote and preserve the nutritional quality and bioactive properties of almonds and probably reduce their alternate behavior.

TABLE 5 Pearson's correlation coefficients[1,2,3]

	α-Tocopherol	Total soluble protein	Total polyphenols	Inhibition of Lipid peroxidation	Ferric reduction capacity	DPPH free radical scavenging activity
α-Tocopherol	1	0.170 (0.254)	0.133 (0.372)	0.119 (0.424)	0.193 (0.193)	0.119 (0.427)
Total soluble protein		1	−0.291 **(0.047)***	−0.015 (0.920)	−0.015 (0.920)	−0.063 (0.676)
Total polyphenols			1	0.352 **(0.015)***	0.406 **(0.005)****	0.181 (0.223)
Inhibition of Lipid peroxidation				1	0.080 (0.593)	0.282 (0.055)
Ferric reduction capacity					1	−0.209 (0.159)
DPPH free radical scavenging activity						1

[a]*, **, *** Correlation is significant at the $p < 0.05$, 0.01 and 0.001 level, respectively.
[b]Between the brackets the p value determined by Pearson's correlation.
[c]The values in bold reports the most significant correlations.

The next steps of research is to understand how nitrogen affects the biosynthetic mechanisms of α-tocopherol and other antioxidant compounds as well as the levels of antioxidant enzymes and pigments, which may be critical to the carbohydrates balance of plant and physiological behavior of almond trees, particularly for the formation of leafs, spurs, flowers, and fruits. This information will be important to understand how antioxidant compounds can be maximized.

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CONFLICT OF INTEREST

Authors declared that they have no conflict of interest.

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