



Article

Study of the Impact of Different Irrigation Regimes on the Quality Attributes and Phenolic Compounds Profile of Selected Goji Berry Varieties (*Lycium barbarum* L.) Cultivated Under an Organic Cultivation System in Southwestern Spain

María Elena García-Garrido ^{1,2,†} , Mónica Sánchez-Parra ^{2,3,†} , José Manuel Moreno-Rojas ^{3,*} and José Luis Ordóñez-Díaz ^{3,*}

¹ Investigación y Proyectos en la Cadena Agroalimentaria S.L., Paraje La Feria 5 Parcela 84, 10616 Cáceres, Spain

² Programa de Doctorado en Ingeniería Agraria, Alimentaria, Forestal y de Desarrollo Rural Sostenible, Universidad de Córdoba, 14071 Córdoba, Spain

³ Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) Alameda del Obispo, Avda. Menéndez-Pidal S/N., 14004 Córdoba, Spain

* Correspondence: josem.moreno.rojas@juntadeandalucia.es (J.M.M.-R.);

jusel.ordonez@juntadeandalucia.es (J.L.O.-D.)

† These authors contributed equally to this work.

Abstract

In a context of increasing water scarcity in southern Spain, improving water-use efficiency has become essential for sustainable crop production. This study evaluated the effects of two irrigation regimes (100% and 75% of crop evapotranspiration, ET_c) on the quality attributes and phenolic compound profile of three goji berry varieties (NQ1, Sweet Lifeberry, and Turgidus) cultivated under organic conditions in southwestern Spain. Key quality parameters including color, sugars, acidity, firmness, moisture, size, and weight were evaluated together with phenolic compounds profiling using UHPLC-MS/MS and antioxidant activity assays. The goji berry varieties studied showed significant differences ($p < 0.05$) in most of the variables analyzed, confirming the dominant influence of genotype. In contrast, no significant differences were detected in most quality attributes between berries cultivated under full irrigation (100% ET_c) and deficit irrigation (75% ET_c). However, deficit irrigation promoted the accumulation of hydroxycinnamic and hydroxybenzoic acids, while flavonols and flavan-3-ols showed a decreasing tendency. Despite these compositional shifts, antioxidant capacity was generally higher under full irrigation. Among the varieties, NQ1 berries exhibited the highest total phenolic content and antioxidant activity. Harvesting time further influenced phenolic distribution and antioxidant behavior. These findings suggest that moderate deficit irrigation can be applied as a sustainable water-saving strategy for goji berry cultivation under Mediterranean conditions without compromising fruit quality.

Keywords: water-use efficiency; deficit irrigation; goji berry; organic agriculture; food quality



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1. Introduction

Goji berry (*Lycium barbarum* L.) is a perennial deciduous shrub native to East Asia that belongs to the Solanaceae family. In recent years, this fruit has gained increasing attention due to its bioactive compounds and antioxidant properties, which are associated with potential health benefits for consumers. Goji berries are currently commercialized

in different forms, including fresh or dried fruits, as well as ingredients in various food products such as beverages, bakery products, purées, yogurts, and jams [1].

During the last decades, goji berry cultivation has expanded to several European countries, including Italy, Greece, Portugal, and Spain, mainly due to the increasing market demand for functional foods [2]. In Spain, berry production is well established, particularly in the southwestern regions such as Extremadura and Andalusia, where crops like strawberries, raspberries, blueberries, blackberries, and cherries are traditionally cultivated. The quality of goji berry fruits depends on several factors, such as the cultivation system, climate and weather conditions, soil composition and structure, plant nutrition, water availability, and agricultural practices. Water scarcity is an important environmental stress factor limiting agriculture worldwide, hence irrigation strategies are highly important to know the water needs of the crop as well as establishing the correct use of water. Water shortages are forcing growers to consider adopting water-saving strategies. Thus, the use of deficit irrigation (<100% of crop evapotranspiration, ETc), may be a suitable strategy to reduce the water demand for cultivation in a territory where the impact of climate change is significant.

While water availability is a critical driver of crop productivity, regulated deficit irrigation (RDI) has emerged as a strategic tool to enhance fruit quality through the deliberate induction of controlled eustress [3]. At a mechanistic level, water deficits trigger a cascade of physiological responses, including stomatal closure and increased production of reactive oxygen species (ROS). To counteract oxidative stress, plants upregulate secondary metabolic pathways, particularly the phenylpropanoid pathway, by stimulating key enzymes such as phenylalanine ammonia-lyase (PAL). This metabolic shift leads to the accumulation and partitioning of specific phenolic compounds (e.g., flavonols and hydroxycinnamic acids), which enhance the fruit's antioxidant defense system and overall functional quality [4].

However, the downstream accumulation of these bioactives is not governed by moisture stress alone. It depends on a highly complex and poorly understood interaction between genetic regulation, environmental triggers, and physiological maturity. The current literature often examines these factors in isolation, leaving a significant scientific gap regarding the simultaneous interactions of Genotype $\times$ Irrigation regimes $\times$ Harvesting times ($G \times I \times H$) on phenolic partitioning and texturometric responses in goji berries (*Lycium barbarum* L.).

To address this gap, this study evaluates how targeted water restrictions modulate the qualitative and bioactive profile of different goji cultivars across distinct commercial harvests [5,6]. By applying a comprehensive chemometric approach (PLS-DA) combined with the Index of Quality for Agricultural Foodstuffs (IQAF), we aim to decode the integrated effects of these interactions, providing a metabolic framework to optimize organic goji cultivation under climate-induced water scarcity.

Our study was conducted in the Jerte Valley (Cáceres province, Spain), a mountainous agricultural region located in southwestern Spain characterized by warm summers, cold winters, and regular rainfall throughout the year, although precipitation is scarce during summer months. These climatic conditions motivated the present study. In this context, the objective of this study was to evaluate the effect of controlled deficit irrigation on the quality attributes and phenolic compound profile of goji berry varieties cultivated under an organic agricultural system. Three varieties were grown under two irrigation regimes: full irrigation (100% ETc) and deficit irrigation (75% ETc).

2. Results and Discussion

2.1. Morphological Parameters

The data indicated significant differences between varieties for several quality parameters (Table 1). Regarding the weight and length, samples from the Turgidus variety had higher values (7.1 and 13 respectively) than the NQ1 (5.6 and 12) which showed the lowest value for width (7.1). On the other hand, no statistically significant differences were found among the values of weight, width, and length for the three varieties under study depending on the two irrigation regimes applied (100% or 75% ETc).

Table 1. Quality parameters of goji berries (morphological, physicochemical, and color analysis) and the impact of different irrigation regimes (mean).

	Varieties				Irrigation Regimes		
	NQ1	Sweet Lifeberry	Turgidus	<i>p</i> -Value	100% ETc	75% ETc	<i>p</i> -Value
Weight ¹	5.6 b	6.6 ab	7.1 a	**	6.5	6.3	ns
Width (mm)	7.1 b	7.8 a	7.9 a	***	7.5	7.6	ns
Length (mm)	12 b	13 a	13 a	***	13	13	ns
Moisture	81 a	79 ab	78 b	*	79	79	ns
TTA ²	1.19	1.20	1.20	ns	1.20	1.20	ns
pH	5.2 b	5.1 b	5.4 a	**	5.2	5.2	ns
TSS ³	15 b	14 b	18 a	***	16	16	ns
L	42 b	42 b	44 a	**	43	43	ns
a*	37	37	38	ns	38	38	ns
b*	27 b	26 b	30 a	***	28	28	ns

Different letters in the same row indicate significant differences according to Tukey's test. *, **, ***, ns: significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, and not significant, respectively. ¹ Weight expressed as 20 berries/g. ² Total titratable acidity (% citric acid). ³ Total soluble solids (°Brix).

The evolution of the quality parameters of the three varieties (NQ1, Sweet Lifeberry, and Turgidus; Figure 1) was evaluated throughout four harvesting periods (T1 to T4). In this sense, we can observe that samples from the NQ1 variety had the highest value for weight in the first sampling point (T1, Figure 1A) decreasing in T2 and then, increasing in T3 and T4 to not significantly different values. A similar behavior was observed for the length values with a lower value in T2, gradually increasing until T4 while no differences were found for width values among the different harvesting points (Figure 1). Concerning the evolution of the samples of Turgidus varieties along the harvesting time, we could conclude that the weight of the samples was statistically the same during the first three sampling times (T1, T2, T3) with a significant decrease in the data value in the T4 point (Figure 1A). A similar trend was observed for the width with statistically similar values for T1, T2, and T3 and a decrease in T4 (Figure 1B), while no differences were found for length values among the different harvesting points (Figure 1C). Finally, focusing on the third variety, the Sweet Lifeberry of the shorter period of production, we only harvested at T2 and T3 and we could highlight that the berries sampled showed statistically similar results for the three parameters studied, which were, weight, width, and length for both sampling times.

The interaction between variety and irrigation regime showed a limited influence on most quality parameters of goji berries, particularly fruit width, length, and moisture content (Table S2). In general, no significant differences were observed between irrigation treatments within the same variety, suggesting a minor effect of irrigation and a stronger influence of genotype, especially on fruit morphology. The Sweet Lifeberry and Turgidus varieties produced the largest fruits, showing the highest width and length values, whereas NQ1 presented the smallest fruits.

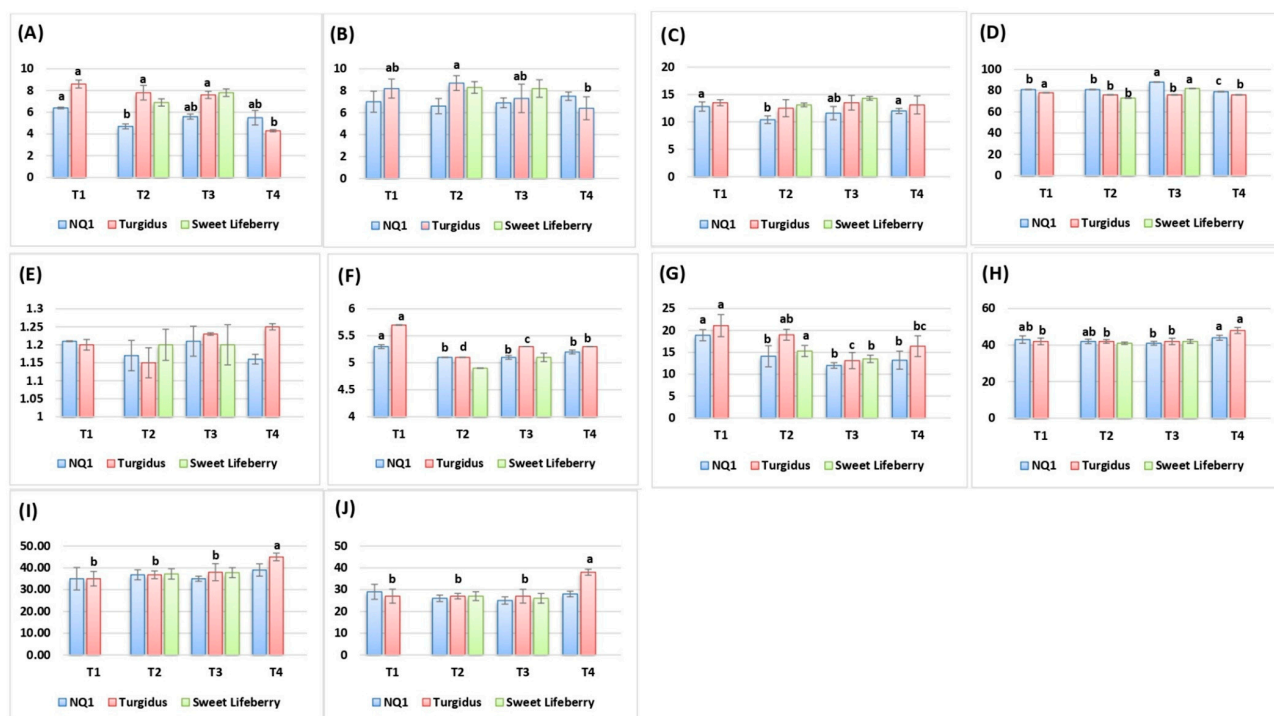


Figure 1. Evolution of quality parameters as affected by harvesting time (T1–T4): (A) weight; (B) width; (C) length; (D) moisture; (E) titratable acidity (TA); (F) pH; (G) total soluble solids (TSSs); (H) L*; (I) a*; and (J) b*. Values are expressed as mean ± standard deviation. Different letters indicate significant differences among harvesting times ($p < 0.05$).

2.2. Moisture Content, Titratable Acidity (TA), pH, and Total Soluble Solids (TSSs)

In contrast, goji berry samples from the NQ1 variety showed (Table 1) higher values for moisture (81) than samples from the Turgidus variety (78, Table 1). In relation to acidity, no significant differences were found among the samples from different varieties while significant differences were observed on the pH values, with the samples from Sweet Lifeberry being the ones with the lowest values (5.1). Samples from the Turgidus variety stood out for having the highest total soluble solids content (18.9). On the other hand, no statistically significant differences were found among these parameters for the three varieties under study depending on the two irrigation regimes applied (100% or 75% ETc).

Regarding the evolution along the harvesting times, we observed that goji berry samples from the NQ1 variety increase the moisture value at T3 (Figure 1F) with a significant decrease at T4. Samples from the Turgidus variety had a different behavior for the moisture parameter with a significantly higher value at T1, decreasing after T2, and remaining the same for T3 and T4. Finally, the samples from the Sweet Lifeberry variety showed a higher value of moisture at T3 compared to T2. We concluded that the Turgidus variety could have a better osmoregulation capacity. Regarding titratable acidity (Figure 1E), no significant differences were found during the harvesting time for any variety maintaining similar values for this parameter during the entire crop season, showing the desirable stability of this parameter and its link to sensory properties. No significant differences regarding pH values (Figure 1F) were found for the samples from the Sweet Lifeberry variety but slight differences were observed for the samples from the other two varieties along the harvesting period; both showed significantly higher values at the beginning of the season (T1), which decreased at T2 and, then, the NQ1 samples maintained constant values, while samples from the Turgidus variety showed a slight increase at T3 and T4.

Finally, after analyzing the data for TSS and the evolution with time (Figure 1G) we observed that the higher values for this parameter for the samples from the NQ1 and

Turgidus varieties were observed in the first sampling time (T1) with an important decrease for both varieties at the sampling time T3, following similar values at T4. Concerning the samples from Sweet Lifeberry we observed a significant decrease between T2 and T3. In summary, we could see that the higher values for TTS are observed at the beginning of the harvesting period suggesting that the plant reduces its reserves to the end of the harvesting season.

2.3. Color

Finally, concerning the color-related parameters, no significant differences were found for a^* values (Table 1) among the samples from the different goji berry varieties. These data indicated that all varieties maintained a similar red color base, suggesting that the content of red pigments is uniform. On the other hand, samples from the Turgidus variety showed the most intense color with higher values for L^* (44) and b^* (30) parameters than the samples from NQ1 and Sweet Lifeberry. The data obtained for NQ1 were consistent with those previously reported in the literature [2]. These results are important since the color of berries is often a key quality attribute influencing consumer perception as Kader et al. [7] and Česonienė [8] demonstrated.

In the present study, the analysis of the colorimetric parameters of the CIELAB space (L^* , a^* , b^*) reveals how genetic factors influence the visual appearance and pigment composition of goji berries, with water management having no significant impact *per se* on the three varieties of goji berries studied. In summary, the impact of irrigation on the color of the three varieties studied showed that there are no significant differences in color, meaning that pigment content is not affected by moderate water stress. This conclusion is consistent with previous studies, such as that conducted by Yiyuan et al. [9], which established that the coloration of goji berries is mainly determined by genetics.

The evolution across harvesting times showed that samples from the NQ1 and Turgidus varieties had the highest values for L (Figure 1H) at the end of the season (T4), while no significant differences were observed for the samples from Sweet Lifeberry. With respect to a^* values, we could conclude a more stable behavior for the samples from NQ1 and Sweet Lifeberry with harvesting time (no significant differences, Figure 1I) and we observed a slightly different for the samples from the Turgidus variety which presented a higher value at the end of the harvesting period, T4. Similar conclusions in regards to the variations associated with harvesting time were obtained for the b^* parameter (Figure 1J).

2.4. Texture Analyses

The texture properties of the three goji berries varieties (NQ1, Sweet, and Turgidus) under two different irrigation regimes are shown in Table 2. Texture profile analysis (TPA) enabled the determination of key mechanical parameters including hardness, springiness, cohesiveness, and chewiness, which describe the resistance, elasticity, and structural integrity of berry tissues [2]. Springiness, defined as the extent and rate at which a deformed sample recovers its original shape, is likewise regarded as a critical textural attribute [10].

The hardness values showed highly significant differences among varieties. The NQ1 and Sweet berries showed higher hardness values (4.8 and 5.4 N, respectively) compared with Turgidus (2.4 N), indicating greater resistance to deformation. Regarding springiness, significant differences were observed, with the Turgidus berries presenting the highest value (0.78 N/mm), exceeding those of the NQ1 and Sweet berries (0.66 N/mm and 0.65 N/mm, respectively). This behavior indicates a greater ability to recover shape after compression. Similarly, cohesiveness values were significantly higher in the Turgidus berries (0.37 N/mm), suggesting enhanced internal structural integrity. These trends indicate that the NQ1 and Sweet berries were characterized by firmer textures, whereas the

Turgidus berries displayed more elastic and cohesive mechanical behavior. Chewiness did not show statistically significant differences among the varieties, suggesting comparable mastication energy requirements. Regarding irrigation regimes, no significant differences were observed for any textural parameters. This suggested that moderate deficit irrigation did not substantially alter berry mechanical properties. Similar responses have been reported in fruit crops where mild water restriction did not significantly affect key quality attributes [11].

Table 2. Textural parameters of goji berries.

	Varieties				Irrigation Regimes		
	NQ1	Sweet Lifeberry	Turgidus	<i>p</i> -Value	100% ETc	75% ETc	<i>p</i> -Value
Texture analysis profile (TPA)							
Hardness (N)	4.8 a	5.4 a	2.4 b	***	3.6	4.3	ns
Springiness (N/mm)	0.65 b	0.66 ab	0.78 a	*	0.72	0.69	ns
Cohesiveness (N/mm)	0.26 b	0.25 b	0.37 a	**	0.30	0.30	ns
Chewiness (N)	0.79	0.91	0.64	ns	0.75	0.76	ns
Mini Kramer/Ottawa sensor							
Maximum force (N)	21	23	15	ns	18	19	ns
Shear force (N/g of sample)	170 a	165 a	98 b	***	136	143	ns

Data are expressed as mean values. Different letters in the same row indicate significant differences by Tukey's test. *, **, ***, ns: significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, and not significant, respectively.

Additionally, the Miniature Kramer–Ottawa sensor provided complementary information regarding overall tissue resistance. Although the maximum force values did not differ significantly among the varieties, the shear force values reinforced the TPA findings. The NQ1 and Sweet berries (170 and 165 N/g of sample, respectively) required greater cutting energy than the Turgidus berries (98 N/g of sample), consistent with their higher hardness values. Likewise, the values obtained under both irrigation regimes were not significantly different.

The interaction between variety and irrigation significantly influenced some textural attributes, particularly fruit hardness and shear force (Table S3). NQ1 and Sweet Lifeberry exhibited higher hardness values under deficit irrigation (75% ETc), suggesting that moderate water stress may enhance tissue firmness in these varieties. Similarly, differences in shear force were observed in Sweet Lifeberry, where slight deficit irrigation increased fruit resistance in this variety. Other parameters, such as springiness, cohesiveness, chewiness, and maximum force, were not significantly affected by the interaction, indicating that these mechanical attributes are relatively stable across irrigation regimes.

2.5. Phenolic Compound Profile

A total of 31 phenolic compounds were tentatively identified and quantified in the goji berry samples (Table 3), comprising hydroxycinnamic acids ($n = 16$), hydroxybenzoic acids ($n = 6$), flavonols ($n = 8$), and flavan-3-ol ($n = 1$). Total phenolic compounds showed significant varietal differences, with the NQ1 berries showing the highest values (31 mg/100 g DW), followed by the Sweet Lifeberry (29 mg/100 g DW) and Turgidus (26 mg/100 g DW) samples. This variability indicated that phenolic accumulation capacity differs among genotypes, likely reflecting differences in phenylpropanoid pathway regulation and carbon allocation. Comparable genotype-dependent variability in goji berry phenolic composition has been widely documented in previous studies [2,12].

Table 3. Concentration of phenolic compounds identified in goji berry samples of different varieties and irrigation regimes.

	Varieties				Irrigation Regimes		
	NQ1	Sweet Lifeberry	Turgidus	<i>p</i> -Value	100% ETc	75% ETc	<i>p</i> -Value
Total hydroxycinnamic acid	7.5 b	9.0 a	7.7 b	***	7.9 b	8.3 a	**
Caffeoyl dihexoside	0.21 b	0.22 a	0.12 c	***	0.16 b	0.21 a	***
Caffeoyl hexoside I	0.18 b	0.21 a	0.16 c	***	0.15 b	0.21 a	***
2'-Hydroxycinnamic acid (o-coumaric acid)	0.025 c	0.114 a	0.060 b	***	0.037 b	0.096 a	***
4'-Hydroxycinnamic acid (p-coumaric acid)	0.042 c	0.098 a	0.052 b	***	0.040 b	0.088 a	***
Caffeoyl hexoside II	0.49 b	0.53 a	0.41 c	***	0.45 b	0.50 a	***
Coumaric acid glucoside	0.18 c	0.58 a	0.27 b	***	0.20 b	0.48 a	***
Chlorogenic acid	0.65 b	1.12 a	0.57 c	***	0.73 b	0.83 a	**
Ferulic acid hexoside	2.9 a	2.7 b	2.9 a	***	2.7 b	3.0 a	***
Caffeoyl hexoside III	0.14 b	0.16 a	0.13 b	***	0.15	0.14	ns
Sinapic acid-O-hexoside	0.86 b	0.98 a	0.59 c	***	0.81	0.80	ns
Caffeoylquinic acid	0.26 b	0.62 a	0.22 c	***	0.45 a	0.28 b	***
Caffeic acid	0.065 a	0.068 a	0.051 b	***	0.058 b	0.064 a	***
(3',4'-Dihydroxycinnamic acid)	0.089 a	0.095 a	0.060 b	***	0.084 a	0.079 b	*
Sinapic acid	0.17 c	0.26 b	0.36 a	***	0.27	0.26	ns
3'-Hydroxycinnamic acid (m-coumaric acid)	1.02 b	1.02 b	1.25 a	***	1.22 a	0.98 b	***
Ferulic acid	0.29 b	0.26 c	0.50 a	***	0.40 a	0.30 b	***
Isoferulic acid	8.5 a	8.7 a	5.1 b	***	6.9 b	7.9 a	***
Total hydroxybenzoic acid	2.05 b	2.61 a	0.57 c	***	1.45 b	2.04 a	***
Hydroxybenzoic acid hexoside	0.48 b	0.51 a	0.37 c	***	0.46 a	0.44 b	*
4-Hydroxybenzoic acid	3.9 a	3.6 b	2.4 c	***	3.1 b	3.4 a	***
3-galloyl-gallic acid	0.56 a	0.39 b	0.29 b	***	0.33 b	0.50 a	***
3-Hydroxybenzoic acid	0.90 ab	1.08 a	0.72 b	**	0.88	0.92	ns
Mono-galloyl-glucose	0.62 b	0.56 b	0.79 a	***	0.71 a	0.60 b	***
Vanillic acid	14 a	10 c	11 b	***	13 a	11 b	***
(4-hydroxy-3-methoxybenzoic acid)	0.60 a	0.47 b	0.29 c	***	0.46	0.45	ns
Total flavonols	0.066 b	0.095 b	0.225 a	***	0.157 a	0.102 b	***
Quercetin trihexoside	10.4 a	7.0 b	5.3 c	***	7.8 a	7.3 b	**
Quercetin-3-O-rutinoside	0.115 a	0.110 a	0.081 b	***	0.096 b	0.108 a	**
Quercetin dihexoside	1.56 b	1.62 b	4.53 a	***	3.25 a	1.89 b	***
Rutin isomer	0.75 a	0.66 b	0.24 c	***	0.51 b	0.59 a	***
Rutin	0.16 c	0.28 a	0.22 b	***	0.20 b	0.24 a	**
Laricitrin 3-rutinoside	0.151 b	0.119 c	0.253 a	***	0.178	0.170	ns
Kaempferol 3-rutinoside	1.14 b	0.61 c	2.37 a	***	1.85 a	0.89 b	***
Quercetin	1.14 b	0.61 c	2.37 a	***	1.85 a	0.89 b	***
Total flavan-3-ols	31 a	29 b	26 c	***	29 a	28 b	**
(+)-Catechin							
Total phenolic compounds							

Data are expressed as mean values (mg/100 g DW). Different letters in the same row indicate significant differences by Tukey's test. *, **, ***, ns: significant at t $p < 0.05$, $p < 0.01$, and $p < 0.001$, and not significant, respectively.

Concerning the total hydroxycinnamic acids content, significant differences were observed among the varieties. The Sweet Lifeberry variety showed the highest concentration (9.0 mg/100 g DW), while NQ1 and Turgidus showed lower values (7.5 and 7.7 mg/100 g DW, respectively). This pattern was largely driven by chlorogenic acid (5-O-caffeoylquinic acid) and an isomer of caffeoylquinic acid, which were significantly

enriched in the Sweet Lifeberry berries. Chlorogenic acid was consistently reported as the dominant hydroxycinnamic acid in goji berries [13,14], confirming in our study its quantitative importance within this family. A similar trend was observed for caffeoylquinic acid, which reached its maximum concentration in Sweet Lifeberry (0.62 mg/100 g DW). These results were consistent with previous studies highlighting the strong genotype- and maturity-dependent variability of chlorogenic acid [13,14]. The elevated abundance of caffeoyl and coumaric acid derivatives in the Sweet Lifeberry berries suggested enhanced conjugation and glycosylation processes, which contribute to phenolic stabilization and storage [14,15]. In contrast, ferulic and isoferulic acids were significantly higher in the Turgidus berries, indicating genotype-specific metabolic allocation toward ferulic acid derivatives, compounds frequently associated with structural and protective functions. Glycosylated derivatives, particularly ferulic acid hexoside, showed higher concentrations in NQ1 and Turgidus compared to the Sweet Lifeberry samples, further supporting the importance of conjugation reactions in modulating hydroxycinnamic acid stability and distribution [16]. Additionally, Zhao & Shi [14] suggested that glycosides compounds played an important role in plant growth, while aglycones served as precursors for further metabolic transformations.

Hydroxybenzoic acids also exhibited significant varietal differences (Table 3). In this sense, the NQ1 and Sweet Lifeberry samples showed similar total concentrations of this chemical family (8.5 and 8.7 mg/100 g DW, respectively), while Turgidus displayed significantly lower values (5.1 mg/100 g DW). The predominant compound was 3-galloyl-gallic acid in the NQ1 and Sweet Lifeberry samples, which highlighted the importance of gallic acid derivatives, compounds recognized for their antioxidant potential [17]. Previous studies have reported the presence of galloyl derivatives in goji berries, although their concentrations vary considerably among cultivars [13,15]. The Sweet Lifeberry berries exhibited higher concentrations of hydroxybenzoic acid hexoside (2.61 mg/100 g DW), suggesting enhanced glycosylation processes associated with phenolic stabilization [14].

Flavonols represented a main phenolic family and exhibited pronounced varietal differences. The NQ1 berries showed the highest total flavonol concentration (14 mg/100 g DW), followed by the Turgidus (11 mg/100 g DW) and Sweet Lifeberry berries (10 mg/100 g DW). This difference was mainly driven by the content of quercetin dihexoside and rutin. Quercetin glycosides were widely reported as predominant flavonols in goji berries [13,16] and they were known to contribute significantly to antioxidant activity. On the other hand, rutin was markedly higher in the Turgidus samples (4.53 mg/100 g DW), reinforcing genotype-dependent differences in flavonol profiles. In the literature, it was observed that the MYB1 transcription factor was shown to positively regulate the biosynthesis of quercetin glycosides in goji berries, supporting the genetic basis of flavonoid variability [18]. Flavan-3-ols were represented by (+) catechin, which the Turgidus variety showed the highest concentration. This compound has been associated with antioxidant activity and sensory attributes such as bitterness and astringency [17].

Concerning the effect of irrigation regimes, significant differences between the 100% and 75% irrigation treatments were observed (Table 3), indicating that water availability had markedly influenced phenolic metabolism. Total phenolic compound values were significantly affected by irrigation regime. Goji berries cultivated under the 75% irrigation treatment exhibited lower total values compared to 100% irrigation (28 mg/100 g DW vs. 29 mg/100 g DW). This trend suggests that moderate drought stress induced a selective modulation of phenolic biosynthesis rather than a uniform enhancement, as different phenolic families responded distinctly under varying irrigation conditions. Although water deficit is often associated with increased phenolic accumulation in plants, the absence of a consistent increase in total phenolic content observed in this study contrasts with some

previous reports [3,19]. This discrepancy may be related to the moderate intensity of the water deficit applied (75% ETc), which may not have been severe enough to strongly stimulate secondary metabolism. In addition, genotype-dependent responses and environmental factors such as soil characteristics and climatic conditions may also influence phenolic biosynthesis and explain the differences observed among studies [12–14].

Hydroxycinnamic acids increased under the 75% ETc irrigation treatment, primarily driven by most caffeoyl derivatives, including chlorogenic acid, and caffeoyl hexosides isomers I and II, among others. Comparable responses have also been described in other species subjected to drought stress [19], while water deficit irrigation has been shown to enhance the accumulation of multiple phenolic classes in grape berries [20]. In this context, drought-induced stimulation of phenolic acids via the activation of phenylpropanoid metabolism has been widely reported in several cultivars, such as lettuce or pomegranate [21,22]. In addition, the increased accumulation of glycosylated hydroxycinnamic acids may reflect enhanced phenolic stabilization and storage mechanisms, as glycosylation improves compound solubility and reduces chemical reactivity [23]. Similarly, coumaric acid derivatives also increased, suggesting drought-induced modulation of upstream phenylpropanoid intermediates [24]. In contrast, ferulic acid content was higher under 100% ETc. This compound has been associated with structural roles and cell wall reinforcement, and its relative decrease under deficit irrigation may indicate metabolic redistribution toward other stress-responsive phenolic compounds.

Hydroxybenzoic acid content exhibited a similar response, with higher concentrations under 75% ETc (8.3 and 7.9 mg/100 g DW). This increase was mainly driven by 3-hydroxybenzoic acid, 3-galloyl-gallic acid, and hydroxybenzoic acid hexoside (0.50 mg/100 g DW, 3.4 mg/100 g DW and 2.04 mg/100 g DW, respectively). The enhanced accumulation of gallic acid derivatives under moderate drought stress has been previously linked to increased antioxidant protection and stress adaptation [25]. Conversely, the flavonol and flavan-3-ol groups decreased under 75% ETc, suggesting that moderate drought stress had an impact on flavonoid biosynthesis. Similar responses have been described where stress intensity determined the balance between the activation and constraint of flavonoid pathways [18,26]. For all above, a moderate water deficit induced a metabolic reallocation within the phenylpropanoid pathway, favoring phenolic acids (hydroxycinnamic and hydroxybenzoic acids) accumulation at the expense of downstream flavonoid biosynthesis [27].

Harvesting time significantly influenced phenolic accumulation patterns in the three varieties of goji berry cultivated with 100% ETc (Figure 2). Total phenolic compounds varied across harvesting times, indicating that harvest stage played a central role in modulating the overall phenolic content in mature fruits. Specifically, Turgidus and Sweet Lifeberry exhibited higher total phenolic contents at later harvests, whereas the NQ1 berries showed higher values in the intermediate harvesting samples (Figure 2E).

As shown in Figure 2, distinct trends were observed among the different phenolic families. Hydroxycinnamic acids displayed variety-specific temporal trends: the NQ1 berries increased in intermediate harvest, Turgidus showed the highest content in T1, whilst Sweet Lifeberry's highest was at T3 (Figure 2A). Hydroxybenzoic acids increased toward later harvests, particularly in Turgidus and Sweet Lifeberry, suggesting ripening-associated phenolic remodeling [16]. Conversely, this pattern differed in the NQ1 samples, where the highest content of hydroxybenzoic acid was detected in T1 (Figure 2B).

Flavonols exhibited the strongest genotype dependence that generally increased at later harvesting times, mainly for Sweet Lifeberry in T3 and Turgidus in T4, which is consistent with their maturation-dependent biosynthesis [28] (Figure 2C). Flavan-3-ols showed a similar trend in the three varieties evaluated, reaching their highest content at

T3 (Figure 2D). The increase of flavan-3-ols observed at T3 may be associated with the progression of fruit maturation and the activation of phenylpropanoid metabolism during the intermediate ripening stages [2,12].

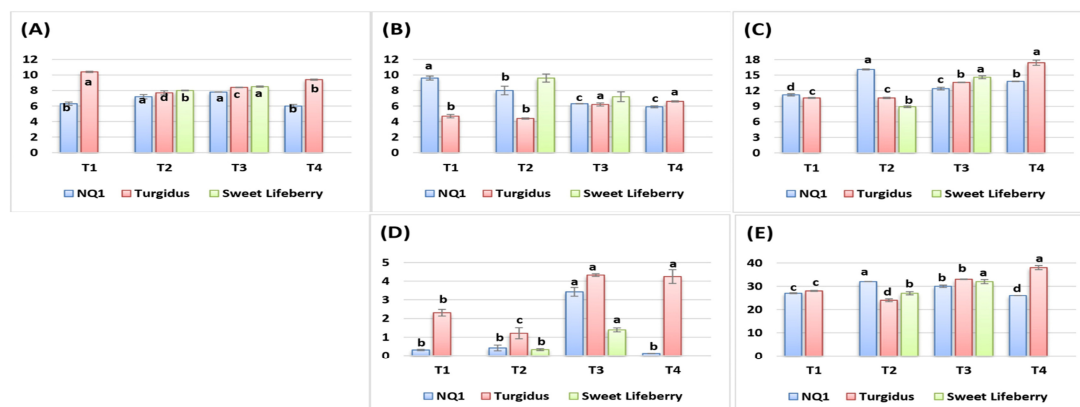


Figure 2. Evolution of polyphenol families as affected by harvesting time (T1–T4): (A) total hydroxycinnamic acids; (B) total hydroxybenzoic acids; (C) total flavonols; (D) total flavan-3-ols; and (E) total phenolic compounds. Values are expressed as mean $\pm$ standard deviation. Different letters indicate significant differences among harvesting times ($p < 0.05$).

The impact of the interaction between variety and irrigation regime on the total phenolic content of goji berries showed some differences (Table S4). Thus, NQ1 and Sweet Lifeberry did not show significant differences between the irrigation regimes evaluated. Conversely, Turgidus exhibited lower total phenolic content under deficit irrigation (75% ETc), decreasing from 28 to 25 mg/100 g DW.

Regarding phenolic families, hydroxycinnamic acids were significantly influenced by the interaction between variety and irrigation, showing different behaviors among the varieties. Although the total hydroxycinnamic acid content in NQ1 did not differ between irrigation regimes, differences were observed at the individual compound level. In particular, caffeic and ferulic acid hexosides were higher in the samples under deficit irrigation (75% ETc). Sweet Lifeberry showed an increase in this phenolic family under deficit irrigation (75% ETc), from 8.3 to 9.8 mg/100 g DW, suggesting stimulation of this pathway under moderate water stress in this variety. This increase was largely associated with the accumulation of major compounds such as chlorogenic acid, coumaric acid glucoside, and coumaric acids. In contrast, Turgidus showed a different trend, with a decrease in total hydroxycinnamic acids under deficit irrigation (75% ETc), mainly associated with lower levels of ferulic and isoferulic acids.

On the other hand, differences were also observed in the interaction between variety and irrigation regimes for the total content of hydroxybenzoic acids. Sweet Lifeberry and Turgidus did not show significant differences between the irrigation regimes evaluated. In contrast, the NQ1 berries under deficit irrigation (75% ETc) showed an increase in this phenolic family, mainly driven by the accumulation of hydroxybenzoic acid hexoside and 3-galloyl-gallic acid, two of the most abundant compounds within this group.

Regarding flavonoids, including flavonols and flavan-3-ols, a similar trend was observed. These phenolic families were affected by deficit irrigation (75% ETc), showing lower contents compared to the 100% ETc treatment. These differences were mainly associated with decreases in rutin in NQ1 and quercetin dihexoside in Sweet Lifeberry and Turgidus. In addition, (+)-catechin levels decreased under deficit irrigation (75% ETc) in all varieties.

2.6. Antioxidant Activity

Total antioxidant activity of the samples was evaluated following three scavenging methods: ABTS, DPPH, and ORAC assays. The results are displayed in Table 4 for the varieties and irrigation regimes. Regarding varieties, the NQ1 berries exhibited the highest antioxidant capacity values across the three assays whereas the Sweet Lifeberry and Turgidus berries showed significantly lower and comparable activities. These results were strongly associated with total phenolic content, a main chemical group contributing to antioxidant capacity. This pattern confirms a strong genotype-dependent effect, consistent with previous studies reporting marked varietal differences in goji berry antioxidant potential [2,13,14].

Table 4. Influence of variety and irrigation regimen on antioxidant capacity measured by ATBS, DPPH, and ORAC (mmol/100 g) of goji berries.

	Varieties			<i>p</i> -Value	Irrigation Regimes		
	NQ1	Sweet Lifeberry	Turgidus		100% ETc	75% ETc	<i>p</i> -Value
ABTS	1.05 a	0.90 b	0.85 b	***	1.01 a	0.86 b	***
DPPH	1.05 a	0.91 b	0.90 b	***	1.00 a	0.90 b	***
ORAC	35 a	28 b	27 b	***	32 a	28 b	**

Data are expressed as mean values. Different letters in the same row indicate significant differences by Tukey's test. **, ***: significant at $p < 0.01$, and $p < 0.001$, respectively.

Regarding irrigation regimes, antioxidant capacity was significantly higher under 100% ETc compared with deficit irrigation (75% ETc) for the three antioxidant assays. These results indicate that moderate water restriction did not enhance antioxidant activity, suggesting that drought stress may limit the accumulation or effectiveness of radical scavenging compounds. Although water deficit is often associated with enhanced phenolic biosynthesis, several studies have reported that non-severe drought conditions can instead reduce antioxidant capacity, depending on stress intensity, duration, and genotype [1,19,22]. Moreover, it was observed that the content of flavonoids (flavonols and flavan-3-ols) was highest in the 100% ETc samples (Table 3). The stronger association between antioxidant activity and flavonoid content may be attributed to the structural characteristics of flavonoids, including multiple hydroxyl groups and extended conjugation, which enhance their radical scavenging efficiency [29].

On the other hand, harvesting time significantly influenced antioxidant capacity across the three varieties (Figure 3). The NQ1 samples showed a similar trend in the ABTS and DPPH assays, exhibiting stable activity from T1 to T3 followed by a significant decline at T4, suggesting reduced antioxidant potential at a delayed harvest (Figure 3A,B). A significant decrease at the last harvesting time (T4) was also observed in the ORAC. These results were highly correlated with total phenolic compound in this variety, which decreased significantly at T4 (Figure 3C). In contrast, the antioxidant activity of the Turgidus berries increased from the first sampling stages, keeping the highest values in T3 and T4. These results were related to the increase of hydroxybenzoic acids and flavan-3-ols in the last harvesting time (Figure 2). The Sweet Lifeberry samples did not display significant variation among the sampling stages. In general, these temporal shifts are consistent with maturation-dependent modulation of phenolic composition and antioxidant efficiency [13,30].

The interaction between variety and irrigation regime significantly influenced the antioxidant capacity of goji berries (Table S5), with different responses observed depending on the genotype. In NQ1, antioxidant activity remained relatively stable across irrigation treatments, did not show significant differences in ABTS or DPPH values between 100% and 75% ETc, but ORAC values decreased under deficit irrigation, suggesting a moderate

reduction in peroxy radical scavenging capacity under water stress. In contrast, Sweet Lifeberry exhibited a clear decline in antioxidant activity under deficit irrigation, with significantly lower ABTS and DPPH values compared to the fully irrigated samples. Similarly, Turgidus showed reduced antioxidant capacity under deficit irrigation in all assays.

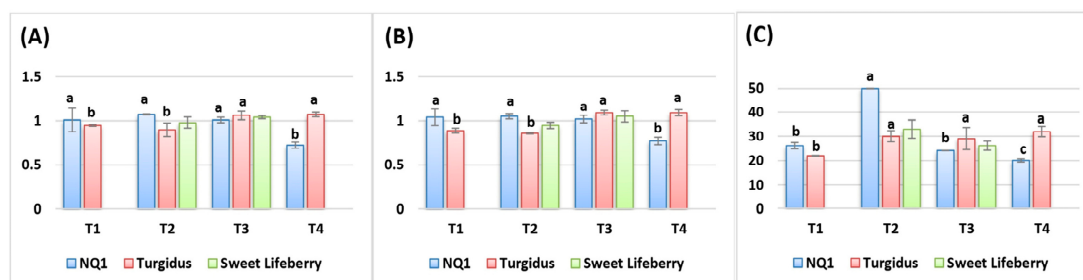


Figure 3. Evolution of antioxidant activity as affected by harvesting time (T1–T4): (A) ABTS; (B) DPPH; and (C) ORAC. Values are expressed as mean $\pm$ standard deviation. Different letters indicate significant differences among harvesting times ($p < 0.05$).

2.7. Pearson Correlation Analysis

Pearson correlation analysis was performed among the quality parameters, including color parameters (L , a^* , b^*), total soluble solids (TSSs), pH, titratable acidity (TTA), and moisture content, in order to explore the relationships between these physicochemical quality traits (Figure 4). As expected, the color coordinates were strongly and significantly intercorrelated (L – b^* : $r = 0.96$; L – a^* : $r = 0.81$; a^* – b^* : $r = 0.76$; $p < 0.001$ in all cases), reflecting that lightness, redness, and yellowness co-vary jointly as pigment content and fruit ripening progress, rather than representing independent sources of variation.

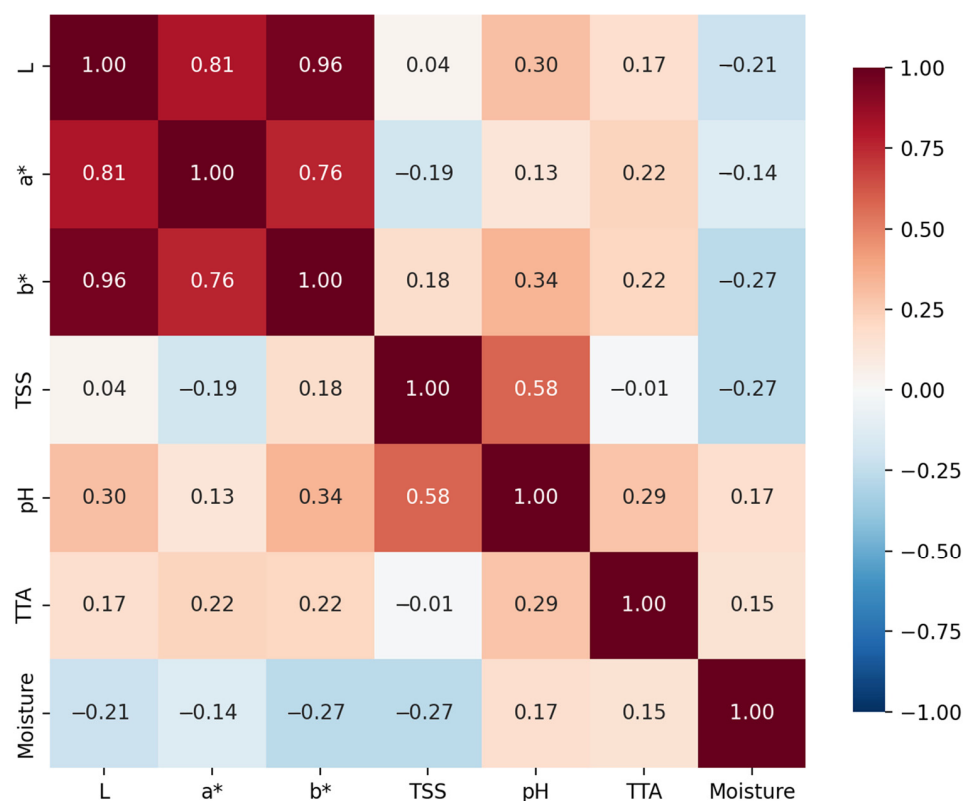


Figure 4. Pearson correlation heatmap of the physicochemical quality parameters of goji berries. The color scale represents Pearson's correlation coefficients (r), with red indicating positive correlations and blue indicating negative correlations.

Among the physicochemical maturity indices, TSS and pH showed the strongest association outside the color block ($r = 0.58$, $p < 0.001$), consistent with the well-established physiological link between sugar accumulation and a decline in organic acid concentration during fruit ripening, which jointly drive an increase in both soluble solids content and pH. A weaker but still significant positive correlation was found between pH and TTA ($r = 0.29$, $p < 0.05$); although pH and titratable acidity are commonly expected to be inversely related, this positive, modest association suggests that buffering capacity and the specific organic acid profile of goji berries modulate this relationship, so that pH in this matrix should not be regarded as a direct proxy for TTA.

Interestingly, moisture content showed significant negative correlations with TSS ($r = -0.27$, $p < 0.05$) and with the color parameter b^* ($r = -0.27$, $p < 0.05$), indicating that fruits with higher water content tended to present lower soluble solids and a less intense yellow coloration, a pattern compatible with the dilution effect of sugars and pigments at higher moisture levels. The remaining associations involving TTA and moisture with the color parameters (L , a^* , b^*) and with TSS were weak and not statistically significant ($p > 0.05$), suggesting that, within this dataset, titratable acidity and moisture content behave largely independently of fruit color, while TSS and pH remain the physicochemical parameters most closely linked to color development.

2.8. Partial Least Squares Discriminant Analysis (PLS-DA)

A PLS-DA model was built to explore sample discrimination according to variety (NQ1, Sweet Lifeberry and Turgidus) and irrigation regime (100% and 75% ETc). The scores plot (Figure 5A) showed that Component 1 (18.3%) mainly separated Turgidus, particularly under 100% ETc, from NQ1, with Sweet Lifeberry in an intermediate position, while Component 2 (11.9%) further distinguished a subset of NQ1 under 75% ETc and Turgidus 75% under the ETc samples. The substantial overlap between irrigation levels within each variety indicated that genotype was a stronger source of variability than irrigation regime, although partial separation between irrigation levels within Turgidus suggested an additional, secondary irrigation effect. VIP scores (Figure 5B,C) identified moisture content as the most influential variable ($VIP > 1.7$), followed by total hydroxybenzoic acids, flavan-3-ols, and hydroxycinnamic acids ($VIP 1.4$ – 1.8), which were all above the relevance threshold ($VIP > 1$). TTA also showed consistently high VIP values, while textural and morphometric traits, such as weight, maximum force, length, shear force or TSS, and the color parameters including L and b^* , contributed to a lesser extent. These results highlight moisture and phenolic composition as the main drivers of variety and irrigation-related discrimination, with the directionality of these effects (e.g., higher moisture in NQ1 or higher hydroxybenzoic acids and flavan-3-ols in Turgidus) consistent with the genotype-dependent phenolic patterns discussed above.

Model robustness was confirmed by cross-validation, with R^2 and Q^2 reaching optimal values at five components ($R^2 = 0.90$, $Q^2 = 0.80$) (Figure S1), and by a permutation test (1000 permutations, $p < 0.001$) (Figure S2), confirming that the observed class separation was not due to chance.

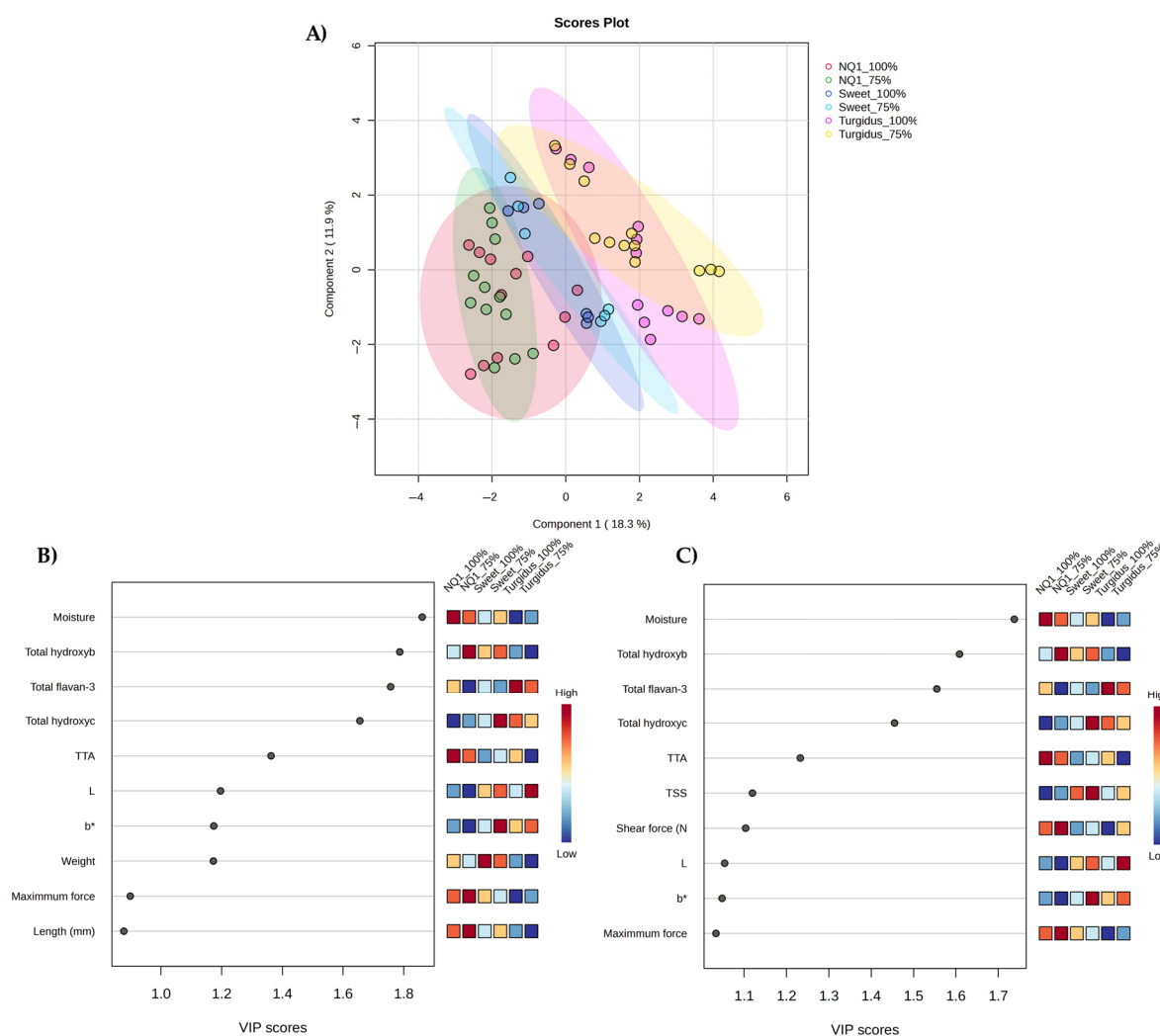


Figure 5. Partial least squares discriminant analysis (PLS-DA) of goji berry samples according to variety (NQ1, Sweet Lifeberry, and Turgidus) and irrigation regime (100% and 75% ETC). **(A)** Scores plot showing sample distribution based on the first two latent components (Component 1: 18.3%; Component 2: 11.9%). **(B)** Variable Importance in Projection (VIP) scores for Component 1. **(C)** Variable Importance in Projection (VIP) scores for Component 2. Variables with VIP > 1 were considered the main contributors to sample discrimination.

2.9. Integrated Quality Assessment Framework for Goji Irrigation Management (IQAF)

Although the present study was conducted under Mediterranean conditions, the results support the proposal of an Integrated Quality Assessment Framework for Goji Irrigation Management (IQAF), which may provide a standardized approach for evaluating irrigation strategies in goji berry cultivation (Figure 6). Rather than relying on individual quality traits, this framework integrates multiple complementary indicators to assess fruit responses to water management.

The IQAF comprises three main dimensions: (i) commercial quality, including fruit size, weight, texture, color, and total soluble solids; (ii) physiological stability, represented by moisture content, pH, and titratable acidity; and (iii) functional quality, including total phenolic content, the main phenolic families, and antioxidant activity (ABTS, DPPH, and ORAC). Together, these indicators provide a comprehensive evaluation of irrigation performance by considering not only fruit marketability but also nutritional quality and physiological response.

Application of the IQAF to the present study indicates that genotype was the primary factor determining fruit quality, whereas moderate deficit irrigation (75% ETc) preserved most commercial and physicochemical attributes while inducing selective changes in phenolic composition. Therefore, under the conditions evaluated, the 75% ETc treatment can be considered a suitable water-saving strategy without compromising overall fruit quality.

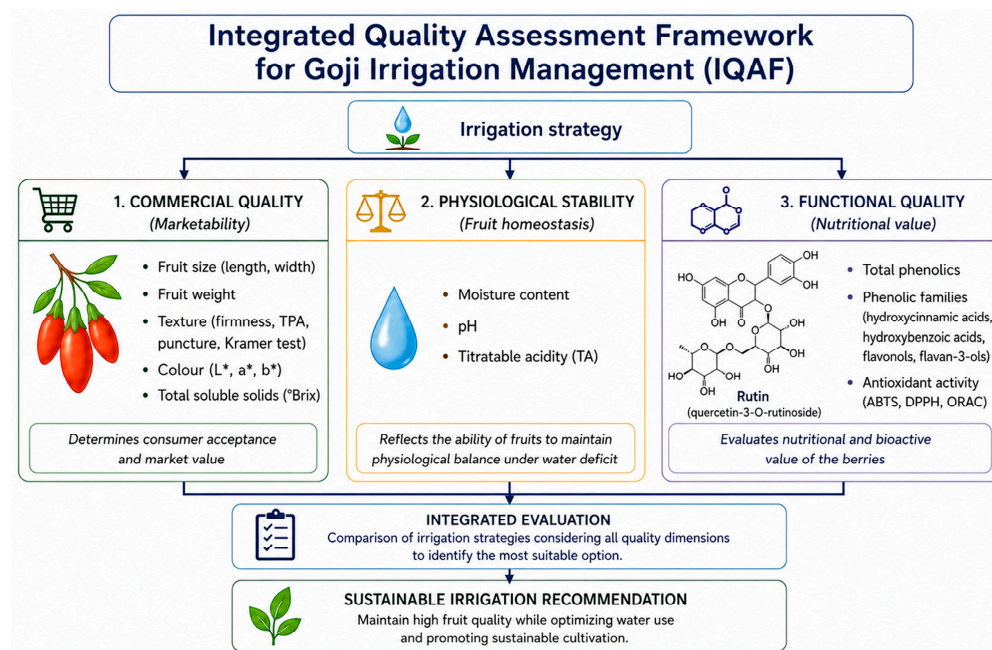


Figure 6. Integrated Quality Assessment Framework for Goji Irrigation Management (IQAF).

Although additional agronomic variables, such as yield, water-use efficiency, and postharvest performance, should be incorporated in future studies, the proposed IQAF provides a practical and transferable framework for comparing irrigation strategies across genotypes and growing environments.

3. Materials and Methods

3.1. Chemicals

HPLC-grade reagents, including methanol, acetonitrile, and deionized water, as well as gallic acid (3,4,5-trihydroxybenzoic acid) and potassium hydroxide, were sourced from Panreac Applichem ITW Reagents (Darmstadt, Germany). Sodium hydrogen carbonate was procured from VWR International Eurolab (Barcelona, Spain) and HPLC-grade formic acid from Fisher Scientific (Madrid, Spain). The radical scavenging reagents ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), DPPH (2,2-diphenyl-1-picrylhydrazyl), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), together with the phenolic compound reference standards, were obtained from Sigma-Aldrich (Steinheim, Germany).

3.2. Material and Sample Collection

This study was conducted in 2024 in Jerte Valley (Cáceres, Spain; 40°06'54.4" N, 5°56'08.8" W), a mountainous agricultural region in southwestern Spain. During the study period, the mean annual temperature was 16.58 °C, with winter minima averaging 10.80 °C and extremes down to 0.29 °C (January), while the highest temperature (40.76 °C) was recorded in July. Annual rainfall totaled 1283 mm, and was distributed unevenly across the year, with the driest month (August) recording no precipitation and seven months accumulating less than 100 mm. The mean relative humidity was 59.62%, with

seasonal peaks in the winter and troughs in July–August. Reference evapotranspiration (ET_o) averaged 1120.59 mm/year, with maximum values in the summer (174–185 mm) and minimum values in the winter (29–31 mm). Solar radiation averaged 15.53 MJ/m², with the maximum values in April–August (up to 30 MJ/m²) and minimum values in November–January (0.7–2.88 MJ/m²). According to the Iberian Wind Map, the prevailing wind directions were NE and SW, which also corresponded to the highest wind speeds (>18 m/s). Light winds (0–3 m/s) were mainly from the SW. The mean wind speed was 1.24 m/s, with average maximum and minimum velocities of 2.30 and 0.73 m/s, respectively.

Three *L. barbarum* varieties, namely NQ1, Sweet Lifeberry, and Turgidus, were grown under certified organic management at the same private plantation in the Jerte Valley. The plants were four years old at the time of this study, established in open field in 2021 in a north–south-oriented row configuration with an inter-row and intra-row spacing of 2 × 1.5 m. Anti-weed fabric with ridges was used as ground cover. Water was supplied via drip irrigation lines with an emitter flow rate of 2 L/h. The experiment followed a randomized block design with five biological replicates per treatment. Each biological replicate consisted of five plants of each variety subjected to the same irrigation regime.

Fruits were harvested at four time points corresponding to commercial ripeness, assessed by external color and morphological characteristics. Berries were collected randomly from multiple plants within each plot to ensure representativeness, keeping samples from each irrigation treatment separate. The four harvest dates corresponded to early August (T1), late September (T2), early October (T3), and late October (T4). A total of 20 samples were generated across varieties, harvest times, and irrigation treatments (100% and 75% ET_c), with five samples per variety per harvest period. After collection, the fruits were stored in PET punnets with lids at 5 ± 2 °C and transported to the laboratory within 24 h. All analytical determinations were performed in triplicate.

3.3. Morphological Measurements

Three morphometric variables (fresh weight, width and length) were measured on samples of 20 berries per variety and irrigation treatment. Fresh weight was recorded using a precision analytical balance (sensitivity 0.01 g; Nimbus, Adam Equipment, Oxford, CT, USA). Berry width and length were measured with a digital caliper (precision 0.01 mm; Comecta SA, Barcelona, Spain).

3.4. Moisture Content

Moisture content was determined gravimetrically in triplicate following AOAC international methods [31]. Approximately 1 g of each sample was dried in a forced-air oven at 103 ± 2 °C until constant mass was achieved (minimum 2 h). The results are expressed as percentage of fresh weight.

3.5. Titratable Acidity (TA), pH, and Total Soluble Solids (TSSs)

Prior to physicochemical analysis, 10 g of goji berries were homogenized with 15 mL of distilled water using an Ultra-Turrax disperser for 2 min to obtain a uniform slurry. Titratable acidity (TA) was quantified by potentiometric titration following the procedure reported by Sánchez-Parra et al. [32], using a Mettler Toledo T70 automatic titrator (Mettler Toledo, Greifensee, Switzerland), and expressed as percentage of citric acid. The pH of the slurry was measured directly using a combined glass electrode (DGi111-SC, Mettler Toledo). Total soluble solids (TSSs) were determined refractometrically with an Atago PAL-1 digital handheld refractometer (Atago Co., Ltd., Tokyo, Japan), calibrated with distilled water before each series of measurements, and reported in °Brix.

3.6. Color

The surface color of berry skin was characterized using a Konica Minolta CM-700D portable spectrophotometer (Minolta Corporation Ltd., Osaka, Japan), with illuminant D65 and a 10° standard observer. Three consecutive readings were taken at equatorial positions on each berry, and the device was calibrated against a standard white tile before use. Color coordinates were expressed in the CIELAB space: L* (0 = black, 100 = white), a* (negative = green, positive = red), and b* (negative = blue, positive = yellow).

3.7. Evaluation of Texture Properties

Texture properties were evaluated using a TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 5 kg load cell for the puncture test and texture profile analysis (TPA), and a 50 kg load cell for the miniature Kramer–Ottawa shear test. Data were acquired and processed with Exponent software v.5.1.1.1 (Stable Micro Systems Ltd.). These three complementary probes were selected for their demonstrated suitability for characterizing texture in berry fruit crops [33,34]. A total of seven textural parameters were determined as described elsewhere [35]. For the Kramer–Ottawa assay, five berries (approximately 1 g total) were arranged in a single layer at the base of a five-blade chamber, perpendicular to the blades, and compressed at a crosshead speed of 10 mm/s. All measurements were performed in triplicate at a controlled temperature (23 ± 2 °C).

3.8. Extraction of Phenolic Compounds

Phenolic compounds were extracted from lyophilized goji berry samples using an aqueous methanol solution (methanol–water, 80:20, *v/v*) acidified with 1% formic acid. A mass of 0.2 g of freeze-dried material was combined with 1 mL of extraction solvent, vortexed for 10 s, sonicated for 10 min, and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was recovered and the pellet was subjected to a second extraction under identical conditions. The two supernatants were pooled, transferred to vials, and stored at -80 °C until analysis.

3.9. Antioxidant Activity

Total antioxidant capacity was assessed by three independent methods (ABTS, DPPH, and ORAC), all measured using a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA).

3.9.1. ABTS Radical Scavenging Assay

Radical scavenging capacity against the ABTS•⁺ cation (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was determined by the decolorization method [36]. The results are expressed as mg Trolox equivalents per 100 g dry weight (mg TE/100 g DW) and represent the mean of three independent measurements.

3.9.2. DPPH Radical Scavenging Assay

The capacity to quench the DPPH• radical (1,1-diphenyl-2-picrylhydrazyl) was assessed following the method described by Gulçin & Alwasel [37]. Antioxidant activity is reported as mg Trolox equivalents per 100 g dry sample (mg TE/100 g DW), calculated as the average of three independent readings.

3.9.3. Oxygen Radical Absorbance Capacity (ORAC) Assay

ORAC values were determined according to Huang et al. [38]. Extracts were dissolved in 500 µL of a 7% randomly methylated β-cyclodextrin (RMCD) solution prepared in acetone–water (1:1, *v/v*). In each well of a 96-well microplate, 25 µL of sample extract,

Trolox standard, or blank (75 mM phosphate buffer, pH 7.4) was combined with 150 μ L of a fluorescein working solution (8.5×10^{-5} mM in 75 mM phosphate buffer, pH 7.4). Fluorescence was monitored at excitation/emission wavelengths of 485/528 nm every 2 min for 120 min. The peroxy radical generator AAPH (153 mM in 75 mM phosphate buffer, pH 7.4) was added at 30 μ L per well to initiate the reaction. The results are expressed as mmol Trolox equivalents per 100 g sample (mmol TE/100 g).

3.10. Phenolic Compounds Profile

Phenolic compounds were separated and quantified by UHPLC-MS/MS using a Vanquish Flex UHPLC system coupled to a TSQ Fortis™ Plus triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separation was carried out on a Zorbax SB-C18 RRHD column (100 $\times$ 2.1 mm, 1.8 μ m; Agilent, Santa Clara, CA, USA), fitted with an in-line guard column of the same stationary phase, held at 40 °C. Analytical conditions followed the method reported by Tuárez-García et al. [39].

Mass spectrometric detection was performed in negative electrospray ionization (ESI-) mode, using selected reaction monitoring (SRM). Source parameters were optimized as follows: vaporizer temperature 300 °C, capillary temperature 325 °C, spray voltage 3.5 kV, and sheath, auxiliary, and sweep gas flow rates of 35, 20, and 1 arbitrary units, respectively. Compound identification was based on retention time matching and precursor/product ion correspondence against authentic standards and publicly available databases (PubChem, Phenol-Explorer, METLIN). Full details of the tentatively identified compounds, including retention times, molecular formulae, precursor and product ions, and collision energies, are compiled in Table S1.

3.11. Statistical Analysis

Statistical analyses were carried out using R software (v.4.3.3; R Core Team, Vienna, Austria). Before performing the analysis of variance, data normality and variance homogeneity were checked by means of the Shapiro–Wilk and Levene’s tests, respectively. A one-way ANOVA was then applied, with mean comparisons conducted through Tukey’s post hoc test, setting the significance threshold at $p < 0.05$. In addition, Pearson correlation analysis was performed to explore the relationships between color parameters (L^* , a^* , b^*) and physicochemical quality attributes (total soluble solids, titratable acidity, pH, and moisture content). Correlation coefficients (r) were obtained, and statistical significance was denoted at three levels ($p < 0.05$, $p < 0.01$, and $p < 0.001$). The correlation matrix was built in Python (v.3.12) using the pandas, SciPy, and Matplotlib(v.3.11) libraries. Finally, a partial least squares discriminant analysis (PLS-DA) was performed using MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca>, accessed on 30 June 2026). Model performance was evaluated by 5-fold cross-validation and a permutation test, and the variables contributing most strongly to sample discrimination were identified from the VIP scores.

4. Conclusions

This study evaluated the effect of two irrigation regimes (100% and 75% ETc) on the quality attributes and phenolic profile of three goji berries varieties (*L. barbarum*) cultivated in southwestern Spain under an organic production system. Significant differences were observed among the varieties in terms of morphological and textural attributes, confirming the strong influence of the genotype. Among the evaluated cultivars, Turgidus showed the best commercial quality, characterized by higher sweetness (>18 °Brix), greater size and weight, and brighter color.

Deficit irrigation did not significantly affect most of the physicochemical and textural attributes of the berries, suggesting that goji plants are able to maintain fruit hydration

and structural properties under moderate water restriction. However, irrigation regime influenced phenolic metabolism. Deficit irrigation (75% ETC) promoted the accumulation of hydroxycinnamic and hydroxybenzoic acids, whereas flavonols and flavan-3-ols showed a decreasing tendency. Despite these compositional changes, antioxidant capacity remained higher under full irrigation conditions (100% ETC), likely due to the greater contribution of flavonoid compounds to radical scavenging activity.

Harvesting time also influenced phenolic composition and antioxidant behavior, with varietal differences in the temporal patterns observed. In particular, the NQ1 berries exhibited the highest total phenolic content and antioxidant capacity, whereas Turgidus showed increased antioxidant activity at later harvesting stages.

In addition, the proposed Integrated Quality Assessment Framework for Goji Irrigation Management (IQAF) integrates commercial, physiological, and functional quality indicators into a standardized approach that supports the evaluation of irrigation strategies. Based on this framework, moderate deficit irrigation (75% ETC) represents a suitable water-saving strategy while maintaining the overall fruit quality of the evaluated genotype.

Finally, these findings indicated that moderate deficit irrigation can be applied as a sustainable water-saving strategy for goji berry cultivation under Mediterranean conditions without compromising fruit quality.

Despite the robust chemometric and metabolomic discrimination achieved in this study, some limitations must be acknowledged. The current work lacks direct mechanistic validation at the molecular level to fully elucidate the enzymatic upregulations driving the observed phenotypic plastic responses under eustress. Future research lines should focus on integrating transcriptomic approaches to unravel the underlying gene expressions and further studies should evaluate the long-term effects of irrigation management on yield stability and postharvest shelf life.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12070852/s1>: Table S1. Tentative identification of phenolic compounds in goji berry by HPLC-MS/MS. Table S2. Interaction between variety and irrigation regimes in quality parameters of goji berries. Table S3. Interaction between variety and irrigation regimes in textural parameters of goji berries. Table S4. Interaction between variety and irrigation regimes in the concentration of phenolic compounds identified in goji berry samples. Table S5. Interaction between variety and irrigation regimen on antioxidant capacity of goji berries. Figure S1. Cross-validation performance of the PLS-DA model as a function of the number of latent components. Accuracy, R^2 and Q^2 values are shown for models with 1 to 5 components; the asterisk indicates the optimal number of components (five) selected for the final model. Figure S2. Permutation test (1000 permutations) of the PLS-DA model. The histogram shows the distribution of the separation distance statistic obtained under random class permutation, compared with the observed statistic for the true class assignment ($p < 0.001$).

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed at the corresponding authors.

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Conflicts of Interest: The authors declare no competing interests. One of the authors is enrolled as a PhD student in a Doctoral Programme of Cordoba University (Programa de Doctorado en Ingeniería Agraria, Alimentaria, Forestal y de Desarrollo Rural Sostenible) and has a second affiliation with “Investigación y Proyectos en la Cadena Agroalimentaria S.L.”. Her contribution was made exclusively as PhD student and the company had no involvement in the conception, execution, or publication of this study.

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