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Factors Influencing the Contents of Coenzyme Q10 and Q9 in Olive Oils

(Alternative title: Investigation of Some Factors Influencing the Contents of Coenzyme Q10 and Q9 in Olive Oils)

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Abstract. The health effects of olive oil are attributed to its high content of oleic acid and other constituents, particularly its phenolic fraction. Olive oil also contains other substances with potential health effects such as coenzyme Q10 (CoQ10) and coenzyme Q9 (CoQ9). The objective of our study was to investigate some factors that could influence the quantity of coenzyme Q (CoQ) in olive oils. We analyzed almost 100 samples of commercial oil blends and fresh extra virgin olive oils of various cultivars using High-Performance Liquid Chromatography. With the investigation of various monocultivar samples we determined that genetic parameters (cultivars) have an important influence on the composition of olive oils, particularly the content of CoQ10. Possible effects of the degree of ripeness were also studied for the cultivars Istrska belica and Leccino. We determined that the highest levels of both CoQ10 and CoQ9 can be found in early maturation stages.

Keywords: *CoQ10, CoQ9, ubiquinone, harvest, maturity, health properties*

Introduction

Olive oil is a basic component of the Mediterranean diet.[1] Many studies have linked olive oil to a reduced risk of overall mortality, cardiovascular mortality, cancer incidence and mortality, as well as the incidence of neurodegenerative diseases.[2-6] Traditionally, the health effects of olive oil were attributed to its high content of oleic acid. However, more recent scientific knowledge demonstrates that these effects must also be attributed to other constituents of olive oil, particularly the phenolic fraction.[7-9] In addition to phenolic compounds, olive oil contains other substances with a potential beneficial effect for human health, such as coenzyme Q10.[10]

CoQ10 is an endogenous lipophylic compound containing 10 isoprenoid units attached to a substituted benzoquinone moiety. CoQ10 is an essential component of the mitochondrial energy metabolism[11] and widely investigated for a range of possible benefits for human health,[12] including for cardiovascular[13] and neurodegenerative conditions.[14] The human body biosynthesizes CoQ10, but its tissue levels drop progressively with increasing age.[15, 16] On the contrary, CoQ9 which can also be found in olive oil[10] is not endogenous in the human body and its role in human health is less studied.

Meat and fish are the richest dietary sources of CoQ10 and lower levels can be found in most foods of a non-animal origin.[10] Among those, nuts and vegetable oils, particularly soybean and olive oil, have been shown as possible very rich sources[10, 17] and could contribute to total CoQ10 intake significantly. However, there are significant differences in CoQ10 content in olive oils among different literature reports – ranging from 4.1 to 160 mg/kg (Table 1).[18-21] A daily intake of 20 g of olive oil, as established in a large Spanish cohort,[22] could therefore deliver from as little as 0.08 mg to up to 3.2 mg CoQ10 daily – close to the average daily intake of 3-5 mg of CoQ10 in northern European countries.[10] Vegetable oils are clearly recognized as a possible food matrix to increase CoQ10 intake and modified production processes are being developed not only to preserve CoQ10,[23] but also to enrich them. CoQ10-enriched olive oil has been developed recently and its protective effect on plasma lipoproteins has been confirmed in a small-scale intervention trial on humans.[24] Because CoQ10 is better absorbed from food than from supplements containing crystalline compound,[25] novel delivery systems have also been developed to enable the production of CoQ10-enriched functional foods.[10, 26]

It is well established that the levels and composition of the phenolic fraction of olive oils are influenced by various pre- and post-harvest factors, including the cultivar, degree of ripeness and processing[27], and it was hypothesized that these factors might also influence the content of CoQ10.[10] However, so far the CoQ10 content of olive oil has only been studied to a very limited extent, chiefly focusing on comparisons with other vegetable oils. Moreover, to our knowledge no data have been published on the factors influencing the CoQ10 content of olive oil.

The objective of this study was to investigate some factors that could influence the quantity of CoQ10 and CoQ9 in olive oils. We particularly concentrated on the influence of genetic factors (the cultivar) and the degree of ripeness. We also compared the composition of commercial oil blends and fresh extra virgin olive oil samples.

Materials and Methods

Olive oil samples

The commercial olive oil and olive-pomace oil samples were purchased in stores in Slovenia, Spain, the USA and Japan (seven samples of extra virgin olive oil blends, two samples of refined olive oil, two samples of olive-pomace oil) (Table 2).

The monocultivar oil samples were provided by local producers or from local laboratories in Slovenia, Spain, Italy, Greece, France, Italy, Morocco, South Africa, Chile and New Zealand (Table 3, Table 4). Monocultivar samples (n=79) were stored in amber-colored glass at 4 °C in a nitrogen atmosphere until analyses. Thirteen of those samples were frozen when fresh for one season (-70 °C) and considered as fresh in the further analyses. Olive oils of the following cultivars (number of samples) were also analyzed: Picual (7 samples), Istrska belica (7), Leccino (7), Arbequina (4), Hojiblanca (4), Coratina (4), Frantoio (4), Maurino (4), Blanqueta (3), Koroneiki (3), Aglandau (2), Alfafara (2), Buga (2), Farga (2), Manzanilla (2), Olivière (2), Picholine (2), Tanche (2), Villalonga (2) and one sample per cultivar for Arbusana, Ascolano, Athinolia, Barnea, Barouni, Cacereña, Carolea, Itrana, Kalamata, Kolovi, Ladolia, Manaki, Mission and Nocellara.

The monocultivar fresh samples of extra virgin olive oils for studying the CoQ10 and Q9 content as a function of the harvest time within a season were provided by the Experimental Centre for Olive Growing (COG) at Agricultural and Forestry Institute in Nova Gorica, Slovenia. 17 samples of the cultivars Istrska Belica and Leccino (9 and 8 samples, respectively) were included for the analyses. Samples were collected from an experimental grove at Beneša (Slovenia) as part of a national program to monitor the maturity of olives. Trees with a similar fruit load are selected for the monitoring program. The earliest harvest date was set for mid-October, and subsequent harvest dates were approximately one week apart. COG also provided an olive maturity index (MI) for these samples that was determined[28] on a sample of 100 randomly sampled fruits.

Quantification of CoQ10 and CoQ9 levels in the olive oil samples

CoQ10 and CoQ9 levels were determined based on ESA App Note[29] (ESA, Dionex Corp.) modified as follows: Olive oil samples were diluted 1:100 shortly before the analyses using 2-propanol. After 30 s of vortexing at 1,500 rpm at room temperature in polypropylene tubes, the solution was centrifuged for 5 min at room temperature prior to High-Performance Liquid Chromatography (HPLC) injection. Determinations of CoQ9 and CoQ10 were performed using HPLC equipment with a diode array UV-Vis detector (HPLC System Gold, Beckman Coulter Ltd.) and electrochemical coulometric detector (ESA Coulochem III, Dionex Corporation). Separation was carried out at a 40 ºC flow rate 0.8 mL/min using an Ultrabase C18 column (5 µm, 4.6 x 150 mm) (Análisis Vínicos, Spain). The mobile phase was composed of solvent A (Methanol:Propanol:0.33M Ammonium acetate pH 4.4, 84:10:6) and solvent B (Propanol:0.33M Ammonium acetate pH 4.4, 98:2). Initial conditions were 95% solvent A 5% solvent B, at min 12 the mobile phase turned to 65% solvent A 45% solvent B in 4 min. At min 33 the mobile phase composition returned to the initial conditions in 3 min. The typical sample injection volume was 50 µL. Electrochemical detection was used for the CoQ determination. The ESA Coulochem III electrochemical detector was programmed with the following settings: conditioning guard cell +400 mV, reducing cell at -500 mV followed by the analytical cell at +500 mV, full scale was 100 nA. HPLC-grade solvents were purchased from Scharlab (Spain). The CoQ9 and CoQ10 standards were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and available from commercial suppliers. CoQ9 and CoQ10 concentrations were calculated using calibration curves. Stock solutions of CoQ10 and CoQ9 standards were prepared by reading their absorbance at 275 nm in ethanol. They were then used to prepare five calibration standards for each compound studied by dilution with an extraction solvent (25 pmol, 50 pmol, 75 pmol, 100 pmol, 125 pmol). Values of the slope (b), intercept (a), correlation coefficient (R) and standard deviation of the slope V(b) were calculated using the 32Karat™ software (Beckman Coulter Ltd.).

Statistics

The results are the average of at least two replicates analyses; the standard deviation of the *de novo* analyses was determined to be below 10%. Average ± SD was calculated for various groups of samples (commercial samples: extra virgin olive oils, refined olive oils and olive-pomace oils; monocultivar oil samples: a group of all mono-cultivar samples, a group of samples of the same cultivar). Group comparisons were performed using an unpaired t-test and differences of $P<0.05$ were considered significant. Between group comparisons for olive oils from various cultivars are presented in Table 3.

Results and Discussion

CoQ10 and CoQ9 levels in the commercial olive oil blends

We analyzed extra virgin olive oils, refined olive oils and olive-pomace oils purchased in different markets (Table 2). While the average levels of CoQ10 and CoQ9 in the extra virgin olive oils (56±4 and 23±12 mg/L, respectively) were a little higher than in the refined olive oils (54±10 and 14±9 mg/L, respectively), the differences were not significant. However, higher levels of both CoQ10 and CoQ9 were observed in the olive-pomace oil (83±12 and 61±19 mg/L, respectively) although we note that only two samples of olive-pomace oils were investigated. Olive-pomace oil is obtained by treating olive pomace with solvents or other physical treatments[30] and this might explain the better extraction of CoQ from the fruit. Due to its undesirable organoleptic properties olive-pomace oil is considered a low-quality oil and is very rarely found in markets; it was therefore not studied further. Nevertheless, if an opportunity were to emerge for the sustainable extraction of valuable biological compounds from vegetable oils, olive pomace oil might be a valuable raw material in such processes. Such an extraction procedure was studied by Laplante et al. using mackerel and herring oils as starting material.[31]

CoQ10 and CoQ9 levels in the fresh monocultivar extra virgin olive oil samples

Commercial olive oil samples are commonly blends produced from the fruits of various cultivars. To gain an insight into pre-harvest factors that could affect the composition of olive oils monocultivar samples are more appropriate. A series of 79 monocultivar olive oil samples was therefore selected; samples were provided by local producers or local laboratories from different geographical regions (Chile, Spain, Italy, Slovenia, South Africa, the USA, Greece and France). To limit the influence of post-harvest processing, only extra virgin olive oil samples were included. Although CoQ10 is relatively stable in food matrixes,[32] the included samples were either fresh or fresh-frozen.

In case of the cultivars Arbequina, Coratina, Frantoio, Hojiblanca, Istrska belica, Leccino, Maurino, Picual four to seven samples of olive oil per cultivar were available. Results of the determination of CoQ in those samples are presented in Table 3 together with between group statistical comparisons. For the cultivars Aglandau, Alfafara, Buga, Farga, Koroneiki, Manzanilla, Olivière, Picholine, Blanqueta, Tanche and Villalonga where less than four samples were available, we did not perform between group statistical comparisons (Table 4). Results for cultivars where only one sample per cultivar was available (Arbusana, Ascolano, Athinolia, Barnea, Barouni, Cacereña, Carolea, Itrana, Kalamata, Kolovi, Ladolia, Manaki, Mission and Nocellara) are presented in aggregate form in Table 4 (Other cultivars).

The average content of CoQ10 of all the monocultivar olive oil samples was 52 ± 17 mg/L and is comparable with our results for the commercial extra virgin olive oil samples. However, the averages for different cultivars of which at least four samples of olive oil were available (Arbequina, Coratina, Frantoio, Hojiblanca, Istrska belica, Leccino, Maurino and Picual) ranged from 24 ± 4 to 98 ± 20 mg/L and several statistically important differences in CoQ10 levels (Table 3) among them were observed. The highest content of CoQ10 was observed in the cultivar Hojiblanca [98

± 22 mg/L; a statistically significant difference when compared to the Istrska belica (30 ± 4 mg/L; P=0.01), Leccino (41 ± 11 mg/L; P=0.02), Frantoio (36 ± 7 mg/L; P=0.02), Coratina (48 ± 5 mg/L; P=0.03) sets], followed by Picual [63 ± 6 mg/L; a statistically significant difference when compared to Istrska belica (P>0.01), Leccino (P>0.01), Frantoio (P>0.01), Coratina (P=0.01)] and Maurino [63 ± 11 mg/L; a statistically significant difference when compared to Istrska belica (P=0.01), Leccino (P=0.02), and Frantoio (P=0.01)]. This confirms our hypothesis that the expression of CoQ10 is also driven by genetic factors. For the Picual cultivar, our data on CoQ10 levels (63 ± 6 mg/L) are comparable with the report by Venegas et al. (77 ± 12 mg/L),[21] whereas the other cultivars were not investigated in previous studies.

The average content of CoQ9 of all the monocultivar olive oil samples was 21 ± 8 mg/L (23 ± 12 mg/L in the commercial extra virgin olive oil samples). A comparison of the CoQ9 contents between the cultivars shows less significant differences as with CoQ10. The CoQ9 levels in the studied cultivars ranged from 10 ± 1 to 30 ± 5 mg/L. A statistical comparison of the CoQ9 content between sets of olive oils of different cultivars for which at least four samples were available showed the highest CoQ9 content in the Coratina series (30 ± 5 mg/L) with statistically significantly lower levels in Leccino (22 ± 7 mg/L; P=0.05) and Istrska belica (19 ± 3 mg/L; P=0.01). The observed CoQ9 levels are a little higher than reports in the literature where levels of up to 18 ± 2 mg/L were observed.[20] However, we also observed lower CoQ9 levels in some cultivars (i.e. 10±1 mg/L in Koroneiki).

Foods containing over 50 mg CoQ10/kg are considered to be *very rich sources* of CoQ10.[10] We established that extra virgin olive oils generally meet this criterion and can be considered among the best natural sources of dietary CoQ10. In contrast, our results confirm that olive oils are not a very important source of CoQ9, especially when compared to other vegetable oils (i.e. corn oil with 93 to 373 mg CoQ9/L).[17, 20]

Levels of CoQ10 and CoQ9 content in fresh olive oil with different harvest times

During the maturation process the color, oil content and composition change dramatically in olive fruit. The magnitude of these changes depends on the cultivar and parameters such as geoclimatic and growing conditions with processing also having an important effect.[27, 33] Due to variability in cultivar response between growing seasons and the influence of varying crop loads on maturation rates, the determination of the optimal harvest time of olives is difficult.

In our study we also observed relatively high average standard deviations in CoQ10 and CoQ9 contents within oil samples of the same cultivar (on average 20% and 28%, respectively). To gain further insights into the impact of pre-harvest conditions on the composition of olive oil, we investigated whether CoQ levels in olive oil are also affected by the degree of ripeness of olive fruit at the time of pressing.

To evaluate the possible influence of harvesting time on CoQ content in olive oil two cultivars prevalent in Slovenia were selected. The cultivar Istrska belica (Istrian white olive) is a domestic variety grown in Slovenian Istra and the neighboring countries, while the cultivar Leccino originates in Tuscany (Italy).[34] CoQ levels were monitored in oil samples taken from an experimental grove at Beneša (Slovenia) during the approximately three-month harvesting season. We determined that the CoQ10 and CoQ9 levels in both cultivars were strongly influenced by the harvest time (Figure 1, Figure 2). We observed a lowering of total CoQ levels with maturation, an almost linear lowering was observed for the first 2–3 weeks of the investigation (R^2 0.98 and 0.99 for Istrska belica and Leccino, respectively) and after that the CoQ levels continued to be stable. In the case of Istrska belica, the total CoQ dropped by 53% in the first three weeks of the investigation, while in Leccino a 56% reduction was observed. The changes in the CoQ9 levels (75% and 65% for Istrska belica and Leccino, respectively) were higher than in the CoQ10 levels (29% and 47%). Although these measurements were performed on samples collected within a single season and focused only on investigation of the maturation phase, the results reveal that olive oils that are harvested early might be richer in CoQ10 and CoQ9. Further studies are needed to examine this phenomenon in greater detail and should include sampling of the same cultivars in different locations and in different growing seasons. We should note that these results cannot be simply generalized to other cultivars as it has already been established that the influence of harvest and maturity index on olive oil yield and quality can fundamentally differ between various cultivars.[33]

In conclusion, this study shows for the first time the important influence of some pre- and post-harvest factors on the content of CoQ10 and CoQ9 in olive oil. By investigating the effects of the degree of ripeness on the Istrska belica and Leccino cultivars we established that the maturity of fruits significantly affects the content of CoQ in olive oil; the highest levels of both CoQ10 and CoQ9 can be found in the early maturation stages. Our investigation of CoQ10 and CoQ9 levels in numerous monocultivar samples allowed us to determine that genetic parameters (cultivars) also have an important influence on the composition of olive oils, particularly the content of CoQ10. However, we observed relatively high variations in CoQ levels also within cultivars. In addition to the maturity effects, there could be several other reasons for this, including agricultural and geo-climatic effects. Therefore, in further studies, comparisons within cultivars should be made also using samples with comparable maturity and growing conditions. While such studies would enable a comparison between only a small number of cultivars, they would provide an insight into several other possible factors influencing the composition of olive oils. Interestingly, the results of our study indicate the limited influence of post-harvest processing, except when the processing technique is changed drastically, i.e. by introducing organic solvents which not only improve the extraction of oil from olive fruits but also the extraction of CoQ. However, to explore this in detail more studies are needed, covering a higher number of processed oils, preferably involving the use of the same sample of olive fruit as the starting material. These results are an important contribution to knowledge on the composition and quality of olive oils and will provide a basis for additional studies to further explore the factors influencing the contents of Coenzyme Q in olive oils.

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Figure captions

Figure 1*: Coenzyme Q9 and Q10 Content in Olive Oil as a Function of Harvest Time for the Cultivar Istrska Belica (Experimental Grove, Beneša, Slovenia)*

Figure 2*: Coenzyme Q9 and Q10 Content in Olive Oil as a Function of Harvest Time for the Cultivar Leccino (Experimental Grove, Beneša, Slovenia)*

***Table 1**: Literature Data on Coenzyme Q9 and Q10 Content in Olive Oils*

		Content (mg/L)		
Description	***Source (year)***	***CoQ9***	***CoQ10***	***CoQ***
Olive oil[a]	Kamei et al.[18] (1986)	6.0	3.8	9.8
Olive oil	Pregnolato et al.[19] (1994)	12.3	100.6	112.9
Extra virgin olive oil	Cabrini et al.[20] (2001)	18 ± 2	94 ± 4	109 ± 4
Extra virgin olive oil[a,b]	Venegas et al.[21] (2011)	5.9 ± 2.3	77.5 ± 11.5	83.4

[a]Recalculated to mg/kg with an approximation of oil density: 0.92 g/cm^3
[b]Picual cultivar, Spain

***Table 2**: Coenzyme Q9 and Q10 Content in Olive Oils Purchased in Different Markets*

Description	***No. of samples***	***Content (mg/L)***		
		CoQ9	***CoQ10***	***CoQ***
Olive oil (market)				
- extra virgin	7	23 ± 12	56 ± 4	79 ± 12
- refined	2	14 ± 9	54 ± 10	68 ± 2
- olive-pomace oil	*2*	61 ± 19	83 ± 12	144 ± 31
Fresh olive oil (extra virgin, monocultivar)	***79***	**21±8**	**52±17**	**73±20**

***Table 3**: Coenzyme Q9 and Q10 Content in Fresh Extra Virgin Olive Oils of the Cultivars Picual, Istrska Belica, Leccino, Arbequina, Hojiblanca, Coratina, Frantoio and Maurino*

Cultivar	***No. of samples (country origin[a])***	***Content (mg/L)***		
		CoQ9	***CoQ10***	***CoQ***
Arbequina	4 (2 ES, CL, US)	21±10	58±22	79±28
Coratina	4 (ES, IT, CL, US)	30±5 **(i,l)*	48±5 **(p,i,h,f)*	79±9 **(i,f)*
Frantoio	4 (2 CL, SA, IT)	25±6	36±7 **(p,h,c,m)*	61±6 **(p,i,h,c,m)*
Hojiblanca	4 (ES)	24±16	98±22 **(i,l,c,f)*	122±38 **(i,l,f)*
Istrska belica	7 (SI)	19±3 **(c,m)*	30±4 **(p,l,h,c,m)*	48±7 **(p,l,h,c,f,m)*
Leccino	7 (4 SI, IT, ES, SA)	22±7 **(c)*	41±11 **(p,i,h,m)*	63±18 **(p,i,h,m)*
Maurino	4 (SI)	26±4 **(i)*	63±11 **(i,l,f)*	89±14 **(i,l,f)*
Picual	7 (6 ES, CL)	22±7	63±6 **(i,l,c,f)*	85±13 **(i,l,f)*

[a]*Country origin:* ES=Spain; CL=Chile; SI=Slovenia; IT=Italy; US=USA/California; SA=South Africa; *Statistical difference ($P<0.05$) in comparison with other cultivars: [c]Coratina; [f]Frantoio; [h]Hojiblanca; [i]Istrska belica; [l]Leccino; [m]Maurino; [p]Picual.

Table 4: *Coenzyme Q9 and Q10 Content in Other Fresh Monocultivar Olive Oils*

Cultivar	***No. of samples (country origin[a])***	***Content (mg/L)***		
		CoQ9	***CoQ10***	***CoQ***
Aglandau	2 (GR)	15±7	50±9	65±2
Alfafara	2 (ES)	11±3	47±23	58±27
Blanqueta	3 (ES)	12±2	56±4	68±5
Buga	2 (SI)	20±4	49±7	69±11
Farga	2 (ES)	14±4	24±4	39±8
Koroneiki	3 (GR)	10±1	62±4	71±5
Manzanilla	2 (ES, SA)	17±5	27±3	44±8
Olivière	2 (FR)	11±1	52±17	63±15
Picholine	2 (FR)	40±20	64±10	104±30
Tanche	2 (FR)	11±2	58±1	68±3
Villalonga	2 (ES)	21±4	81±18	103±14
Other cultivars	14[b]	23±7	49±19	71±23

[a]*Country origin:* ES=Spain; CL=Chile; SI=Slovenia; IT=Italy; US=USA/California; SA=South Africa; GR=Greece; FR=France;

[b]Other cultivars: Arbusana (CL), Ascolano (US), Athinolia (GR), Barnea (CL), Barouni (US), Cacereña (ES), Carolea (IL), Itrana (SL), Kalamata (SA), Kolovi (GR), Ladolia (GR), Manaki (GR), Mission (US), Nocellara (CL).

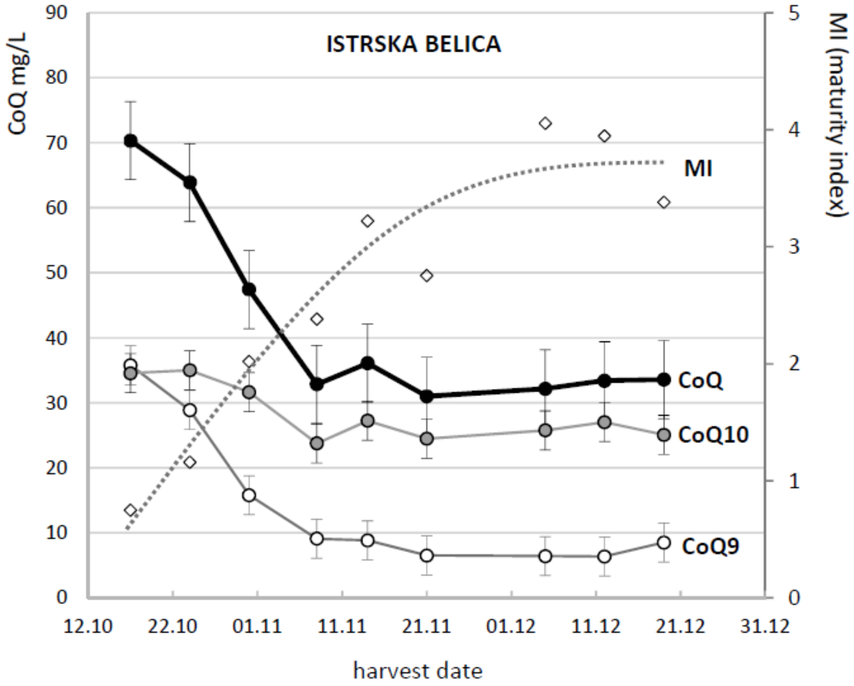
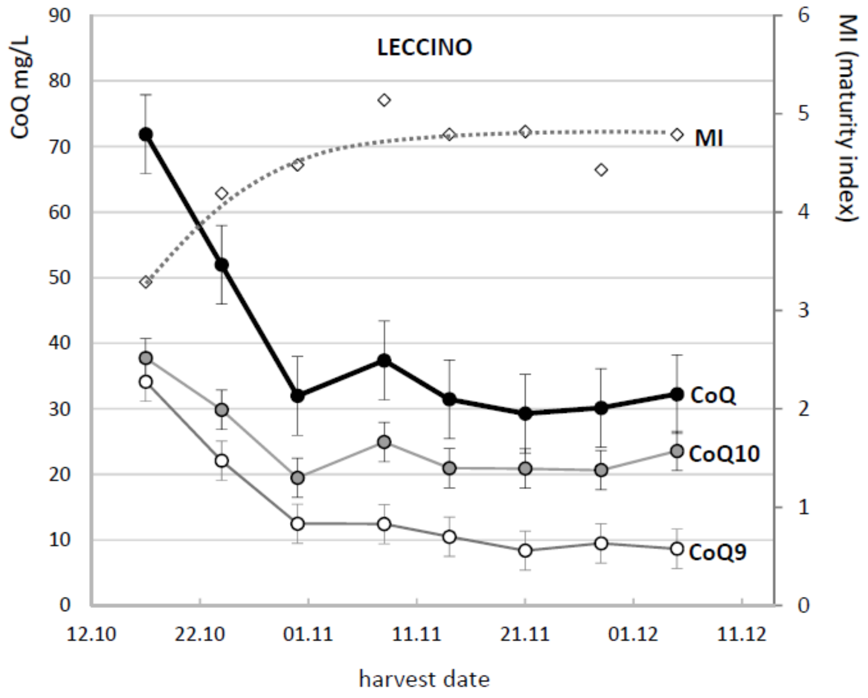

ISTRSKA BELICA
CoQ mg/L
MI (maturity index)
MI
CoQ
CoQ10
CoQ9
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
12.10
22.10
01.11
11.11
21.11
01.12
11.12
21.12
31.12
harvest date

Figure 1



LECCINO
CoQ mg/L
MI (maturity index)
MI
CoQ
CoQ10
CoQ9
harvest date
12.10
22.10
01.11
11.11
21.11
01.12
11.12
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6

Figure 2

Graphic for table of contents