Effect Of Temperature And Storage Time On The Nutritional Quality Of Olive Drupes: A Case Study

E. Finotti, L. Gambelli, G. M. Mili, G. Lo Feudo, C. Benincasa, M. Pellegrino, and E. Perri

Abstract — In this study we evaluated the effect of temperature and time storage on the quality parameters of mono cultivar olive oil drupes. In particular, analyses of total free phenols, fatty acids, lipophilic and hydrophilic antioxidant capacity, sensory analysis, at different temperatures and different times of post harvest storage, were performed. All data obtained have been singularly processed by Functional Mathematical Index (FMI).

Index Terms — Functional Mathematical Index, nutritional quality, olive oil storage, sensory analysis temperature.

I. INTRODUCTION

The olive oil is an important product in the Italian traditions dishes and it is also peculiar in the Mediterranean diet. The production of this food is very ancient and the first archaeological artifacts, that testify its production, are dating back 2500 B.C. [1]. The Mediterranean area remains the most important production place, even if today other geographical area are starting to produce this food [2].

During the years 2011 and 2012, about 80% of the total olive oil has been produced in the European Union of which 77% has been produced in Spain, Italy and Greece [3]-[4].

Due to his high nutritional value, the main use of olive oil is as ingredient in different foods, and nowadays, also for pharmaceutical and cosmetological preparations [5].

During the last years, several studies underlined the healthy properties of olive oil in human diet.

The nutrient characteristics of extra virgin olive oil are well known and are due to the high content of monounsaturated fatty acids and the presence of minor compounds with antioxidant activity. The use of extra virgin olive oil is important in human diet because it seems to show a protective action on cancers, heart attack, atherosclerosis and aging processes. In 2004 the Food and Drugs Administration remarked the role played of monosaturated fatty acids against the cardiovascular disease [6].

Other studies showed that the use of olive oil in human nutrition prevents the Low Density Lipoprotein (LDL) [7] contributing to increase the High Density Lipoprotein in the human serum [8]. This is because olive oil has a perfect ratio between saturated and unsaturated fatty acids.

It is well know the high energy value of olive oil and from a nutritional point of view, present also a good ratio between saturated and unsaturated fatty acids. Moreover, the presence of minor compounds, such as phenols and tocopherols that contribute to the antioxidant capacity, olive oil increase its own healthy value [9]-[12]. Thanks to these compounds, olive oil play an important role in the human energy requirement, in particular in striated muscle, as they contribute to the transport of lipovitamin A, D, E, K [13].

Several studies have, also, showed the protective action of olive oil against cancer, cardiovascular disease and aging [14]-[16].

Pedoclimatic conditions, cultivar, harvesting methods, maturation grades of drupes, processing methods, all influence the quality of the olive oil.

During maturation, the cell wall of olive drupe, undergoes to different chemical and physic changes [17], leading to the decrease of antioxidant compounds [18]-[22] that affects the nutritional value of the final oil. The most common problem is to preserve the nutritional quality of the olive oil along its extraction processes and, in particular, the drupes storage [23]. This latter, is not just a logistic problem but, it is a very important step in the entire olive oil process, in fact a not adequate drupes storage would compromise the final product, decreasing the chemical, the nutritional and the organoleptic quality of the oil produced.

To preserve the quality of the oil, olive drupes have to be placed in suitable containers in order to reduce chemical degrades and fermentative/oxidative processes and, immediately processed after the harvest.

Long periods of storage in poorly ventilated bags or heaps piled on the ground, long times of crushing by means of mechanical blades, compromise the integrity of the fruits and then the quality of the final product. Also, the temperature plays an important role during the storage; in fact, temperatures up to 7.5 °C increase microbial growth and fermentative processes with related decrease of nutritional quality of the oil [24]-[25]. In particular, the fermentations is responsible for "heat" and in extreme cases "molds" defects.

The food organoleptic characteristics are important parameters for its overall assessment and are essential to satisfy the consumer. In fact, the volatile compounds,
present in olive oil, contributes to the taste perception, which makes it unique and also includes part of its nutraceutical characteristics.

Post-harvest step is not a well studied topic, but, it is very important because in every geographical area producers tend to collect drupes at the same time, creating what we call "funnel effect". This effect occurs in the post-harvest period when the loss of important nutritional molecules occur. So, the aim of this study is "how long can drupes be stored before processing them?"

The purpose of this experimental trial was, therefore, to verify, through chemical and sensory analysis, the maximum time that olives can be stored before being processed in the mill to avoid the appearing of sensory defects. In particular, analyses of fatty acids, phenols, lipophilic/hydrophilic antioxidant capacities and sensory analysis were carried out on oil samples from “Carolea” cultivar, a typical variety of Calabria [26-29]. This cultivar is present throughout the territory, in particular, it is very present in the province of Catanzaro, partly present in Cosenza and less present in Reggio Calabria. The Carolea drupe is rather large and has a dual attitude although it is mainly intended for the production of high quality oil. The results obtained in this case study may be useful for small olive-oil-producing enterprises as well as increase the knowledge of this important olive cultivar [27-29]. The drupes have been stored at different temperature and at different storage times and then processes by using a laboratory scale mill equipment. All data have been processed by a Functional Mathematic Index in order to identify the best temperature and storage time.

II. METHODOLOGY

A. Samples Collection

Olives (64 kg) from Carolea cultivar were collected and placed in open boxes in October, during the crop year 2016 from plants belonging to the olive grove of Roberti Company located in Conflenti (CZ) a city of the southern Italian region, Calabria.

B. Phytosanitary conditions of the drupes

In order to verify the phytosanitary conditions of the drupes and to check a possible Bactrocera Oleae (olive fly) infestation, one hundred drupes were monitored. The score has been 4 larvae, 8 galleries/flickering/re-infest/re-flickering, 6 pupae, 40 sterile stings and 42 flickered galleries.

C. Samples and labeling partition

All the harvested drupes were sampled and labeled as following:

Sample Control. Consisted of 14 kg of olives harvested and milled traigh after their harvest. On 100 drupes, the colour of 10 olives was green, the colour of 80 olives was yellow green and the colour of 10 olives was green with red spots.

Sample T. 25°C, 4 d. The olives (13 kg) were stored at room temperature for 4 d and then milled. On 100 drupes, the colour of 10 olives was green, the colour of 80 olives was yellow green and the colour of 10 olives was green with red spots.

Sample T. 9°C, 4 days (d). The olives (13 kg) were stored at 9°C for 4 d and then milled. On 100 drupes, the colour of 10 olives was green, the colour of 80 olives was yellow green and the colour of 10 olives was green with red spots.

Sample T. 25°C, 9 d. The olives (12 kg) were stored at room temperature for 9 d and then milled. On 100 drupes, the colour of 10 olives was green, the colour of 80 olives was yellow green and the colour of 10 olives was green with red spots.

Sample T. 9°C, 9 d. The olives (12 kg) were stored at 9°C for 9 d. On 100 drupes, the colour of 10 olives was green, the colour of 80 olives was yellow green and the colour of 10 olives was green with red spots.

D. Total free phenols

Each sample has been extracted three times with a water-methanol solution (50:50 v/v). The solution has been dried by vacuum evaporation at 40°C and stored under vacuum at 4°C. The dried sample has been dissolved with 0.5 mL of water-methanol solution and analysed by means of a spectrophotometer at 756 nm according to Folin-Ciocalteau method [30]. The amount of total phenols has been detected, comparing, for each sample, the results found with a standard solution of caffeic acid.

E. Hydrophilic and lipophilic antioxidant capacity

The antioxidant capacity of lipophilic extracts was estimated by crocin bleaching inhibition method [31-33]. This method is based on the crocin bleaching as a result of its oxidation by a source of radicals, [2,20-Azobis (2,4-dimethyl)valeronitrile] (AMVN). The reaction is monitored by recording, for ten minutes, the corresponding decrease of absorbance at 443 nm. The reaction with the crocin alone gives us the bleaching rate V0 and when an antioxidant or pseudoantioxidant is added it reacts with the free radicals and, as a consequence, the crocin bleaching rate (Va) is reduced, according to the competitive reaction equation:

\[ \frac{V_0}{V_a} = 1 + \frac{K_a}{K_c} \times \frac{[\text{Pseudo - antioxidant}]}{[\text{Crocin}]} \]

where Kc and Ka are the respective absolute second order constants. The slope Ka/Kc has been calculated by means of the [Pseudo-antioxidant]/[crocin] versus V0/Va linear regression plot. Its value indicates the relative capacity (antioxidant capacity) of different molecules to interact with the ROO• radicals. The AMVN (40mM) and crocin (0.24 mM) were added to the toluene extract and bleaching rate of crocin was determined after 10 min from the beginning of the reaction. The reaction was carried out at 40°C. Blank without sample was run to rule out spectral interferences between compounds and crocin. All lipophilic extracts corresponding to each millstream under investigation were tested. Each kinetic analysis was compared with kinetic crocin bleach containing only AMVN (with bleaching rate V0) and used for the calculations according to the competitive reaction equation. The same method was used for the measurement of the hydrophilic antioxidant capacity, using the APAB [2,20-Azobis(2-methylpropionamidine) dihydrochloride] as free radical source [31-34]. Solvents were from Sigma Aldrich (Mi, Italy). AMVN and APAB were from Waco Chem.
**Fatty Acid Composition**

Fatty acid composition was analyzed by gas chromatography [32]-[35]. Briefly, 2 g of oil were extracted with 10 mL chloroform/methanol (2:1 v/v) and 100 µl were evaporated to dryness by nitrogen, transmethylated by boron trifluoride (BF3) and heated under reflux with methanol at 72 °C for 30 min. The obtained methyl esters were extracted with n-hexane and evaporated by nitrogen to dryness. Transmethylated samples were performed in duplicate and, 1 µl of each sample solute in n-hexane, was injected.

**G. Organoleptic Analysis**

The olive oils produced in the experimental trial were evaluated by sensory analyses in order to estimate their organoleptic characteristics according to the Regulation No. 2568/91 and subsequent amendments and additions [36].

**H. FMI (Functional Mathematical Index)**

This index introduces the concept of ‘optimal product’ by applying the Euclidean standard (modified) to a vector of a dimensional N-parameters in space. Where N is the number of selected parameters, which are normalized and then added together [37]-[42]. Since this index is able to processes not only continuous numeric value, but also discontinuous numerical value, such as the organoleptic results, and in order to use this results in the FMI score, we decided to consider the bitter, spicy and fruity as positive characteristics, on the contrary heat and mold as negative characteristics, assigning at each of them the value of one (i.e. if we have 3 positive characteristics such as bitter, spicy and fruity, and each of them value is one, the final score will be 3).

**I. Statistics**

The data reported represent the mean value obtained by performing the experiment in triplicate. The statistical variations were evaluated by Student’s t-distribution (p>0.05).

### III. RESULT AND DISCUSSION

**A. Total Phenols**

Table I list the amount of total phenols contained in the samples under investigations. It is possible to observe that the concentration values of phenols decrease. However, there is no statistically significant differences (p<0.05, P=0.3294). From these results we can say that, in the sample studied, the temperature and storage do not influence the phenols production.

**B. Lipophilic and hydrophilic antioxidant capacity**

Table II a shows the lipophilic antioxidant capacity. From the result listed in the Table, it can be observed that the lipophilic antioxidant capacity does not decrease at 25 °C after 4 d of storage (P=0.9378). Instead, it can be noted a remarkable reduction of it when the sample is stored at 9 °C (P=0.0151). After 9 d of storage, the sample kept at 25 °C showed the same antioxidant capacity when the sample kept at 9 °C for 4 d (P=0.0237) and, the sample kept at 9 °C for 9 d (P=0.0004) does not showed any antioxidant capacity. The hydrophilic antioxidant capacity is reported in Table II b. Again, the samples stored at 25 °C for 4 d show a slight difference respect to the control (P=0.4135), but at 9 °C the value reaches about half of sample control (P=0.0131). After 9 d of storage, a decrease of hydrophilic antioxidant capacity value in both samples kept at 25 °C (P=0.0151) and at 9 °C has been recorded but, in this last sample the decrease is not statistically significant (P=0.0770) and its value is similar to the sample kept at 9 °C for 4 d.

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| TABLE I: TOTAL POLYPHENOLS | Samples | mg kg⁻¹ | ± |
|----------------------------|---------|---------|---|
| Control                    | 218.26  | 20.00   |
| T. 25°C, 4 d              | 273.06  | 19.30   |
| T. 9°C, 4 d               | 253.35  | 21.30   |
| T. 25°C, 9 d              | 190.49  | 17.20   |
| T. 9°C, 9 d               | 228.20  | 20.40   |

All values are the mean of three replicates, n.s.= not significative, * = p<0.05.

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| TABLE II A: LIPOPHILIC ANTIOXIDANT CAPACITY | Samples | Ka/Kc | ± |
|--------------------------------------------|---------|-------|---|
| Control                                    | 0.34    | 0.05  |
| T. 25°C, 4 d                              | 0.35 n.s.| 0.13 |
| T. 9°C, 4 d                               | 0.15 *  | 0.06 |
| T. 25°C, 9 d                              | 0.16 *  | 0.07 |
| T. 9°C, 9 d                               | 0 *     | 0.1  |

All values are the mean of three replicates, n.s.= not significative, * = p<0.05.

| TABLE II B: HYDROPHILIC ANTIOXIDANT CAPACITY | Sample | Ka/Kc | ± |
|---------------------------------------------|--------|-------|---|
| T. 25°C, 0 d                               | 0.68   | 0.15  |
| T. 25°C, 4 d                               | 0.58 n.s.| 0.11 |
| T. 9°C, 4 d                                | 0.35 * | 0.16  |
| T. 25°C, 9 d                               | 0.44 * | 0.18  |
| T. 9°C, 9 d                                | 0.39 n.s.| 0.15 |

All values are the mean of three replicates, n.s.= not significative, * = p<0.05.

**C. Fatty Acids**

Table III shows the most representative fatty acids present in the oil, in which oleic acid, palmitic acid and stearic acid, are the most representative fatty acids. Palmitic acid values do not present differences vary in each sample tested. Oleic acid decreases during the time between the forth and the ninth day, and, only in the last period, its value decreases significantly (25 °C 9 d P=0.063, and 9 °C 9 d P=0.0002). Searic acid increases its concentration from the fourth day to the ninth day (25 °C 4 d P=0.0001, 9C° 4 d P=0.0004, 25°C 9 d P=0.0001, 9°C 9 d P=0.0001), miristic acid (25 °C 4 d P=0.0001, 9 °C 4 d P=0.0001, 25 °C 9 d P=0.0002, 9 °C 9 d P=0.0001) and linoleic acid (25 °C 4 d P=0.0001, 9°C 4 d P=0.0001, 25 °C 9 d P=0.0001, 9 °C 9 d P=0.0001) are present at low concentrations and their trend is very similar along all the storage time.

In the fatty acid profile (Table III) we can observe a common fatty acids pattern of the olive oil, but the most important differences are due to oleic acid, that decreases.
during the time between the forth and the ninth day and to Linoleic acid, that in the last period, its value decreases significantly. In our opinion this parameter does not seems related to the temperature, but to the storage time. On the contrary Stearic acid increases its concentration from the fourth day to the ninth day, also in this case it own trend does not seem related to the temperature.

| Samples | C14:0 | ±SD | C16:0 | ±SD | C18:0 | ±SD | C18:1 | ±SD | C18:2 | ±SD |
|---------|-------|-----|-------|-----|-------|-----|-------|-----|-------|-----|
| Control | 6.0   | 0.4 | 31.8  | 2.9 | 11.5  | 0.9 | 50.7  | 3.5 | 4.33  | 0.25|
| 25°C, 4 d | 1.9 * | 0.15 | 30.5 n.s. | 2.5 | 27.2 * | 1.1 | 40.3 n.s. | 3.7 | 3.0 * | 0.0 |
| 9°C, 4 d | 2.0 * | 0.18 | 26.6 n.s. | 2.4 | 22.9 * | 1.5 | 48.3 n.s. | 3.2 | 1.14 * | 0.09|
| 25°C, 9 d | 2.6 * | 0.15 | 28.4 n.s. | 2.5 | 32.3 * | 1.3 | 36.7 * | 3.0 | 0.0 * | 0.0 |
| 9°C, 9 d | 2.4 * | 0.16 | 36.7 n.s. | 2.2 | 37.2 * | 1.8 | 22.7 * | 1.4 | 0.0 * | 0.0 |

All values are the mean of three replicates, n.s.= not significative, * = p<0.05.

### D. Organoleptic analysis

Sensory analysis is reported in Table IV. Sample Control: the organoleptic examination of the oil highlighted a light green fruity; a slight hint of bitter and spicy with a persistence of the bitter and a light aftertaste of sweet almonds.

Sample kept at 25 °C for 4 d: the organoleptic examination of the oil highlighted a slight fruity. The bitter and spicy were light and the persistence of the spicy exceeded one of the bitter. Sample kept at 9 °C for 4 d: the organoleptic examination showed a medium fruity of green olives with persistent spicy and bitterness. A definite aftertaste of almond and artichoke was perceived. Sample kept at 25 °C for 9 d: the organoleptic examination showed a complete absence of fruity with defects of mold and heat that were perceptible to the nose and markedly to the taste.

Sample kept at 9 °C for 9 d: the organoleptic examination showed a light fruity with an aftertaste of heat and mold.

In order to use this results in the FMI score, we decided to consider the bitter, spicy and fruity as positive characteristics (+), on the contrary heat and mold as negative characteristics (-), assigning at each of them the value of one (i.e. if we have 3 positive characteristics such as bitter, spicy and fruity, and each of them value is one, the final score will be 3). The increase of storage time increases the negative attributes, regardless the storage temperatures.

In accordance with the data reported in Table 4, in the organoleptic analysis the positive characteristics decrease with the storage time and increase the negative characteristics, until the complete absence of fruity with defects of mold and heat that were perceptible to the nose and markedly to the taste.

In our opinion, probably, the samples kept at 25 °C are more airy and for this reason the fermentative processes are more mitigate because this environment present a low humidity content, how also underlined by the sensory analysis and the fatty acids profile. Instead the sample kept at 9 °C, in a very closed environment, in which is more difficult remove the humidity and so to create a more suitable environment for microbial growth. However for all samples the storage time (4 d) appear a critical point for nutritional quality drupes.

### E. FMI score

Table V reports the Total and Local FMI scores obtained. The total FMI score, in all tested samples, falls in to the range between 0 and 1; where 0 indicates the best quality and 1 the worst one. In this study the best quality was found in the sample kept at 25 °C for 4 d (Total FMI score 0.45) and the worst one in the sample kept at 9 °C for 9 d (Total FMI score 0.85). Fig. 1 reports the Total FMI trend.

The FMI analysis has been performed in order to obtain an index able to integrate all the results obtained and to give a complete response about the processes in fact in Figure 1, it is possible to observe that both trends are very similar, but, the sample kept at 25 °C presents a best Total FMI score, that means a best nutritional quality. The increase of storage time increases the negative attributes and the fatty acids pattern, regardless the storage temperatures.

### TABLE III: FATTY ACIDS (%)

### TABLE IV: ORGANOLEPTIC ANALYSIS

| Samples | Positive characteristics | Negative characteristics |
|---------|--------------------------|--------------------------|
| 25°C, 0 d | 3 | 0 |
| 25°C, 4 d | 3 | 0 |
| 9°C, 4 d | 2 | 0 |
| 25°C, 9 d | 0 | 2 |
| 9°C, 9 d | 1 | 2 |

All values are the mean of three replicates, n.s.= not significative, * = p<0.05.

### TABLE V: TOTAL AND LOCAL FMI SCORES

| Parameters          | 25°C | 25°C | 9°C | 25°C | 9°C |
|---------------------|------|------|-----|------|-----|
|                    | 0 d  | 4 d  | 9 d | 4 d  | 9 d |
| Total FMI score     | 0.65 | 0.45 | 0.59 | 0.70 | 0.85|
| Lipophilic antioxidant capacity | 0 | 0 | 0.11 | 0.09 |
| Hydrophilic antioxidant Capacity | 0 | 0.01 | 1 | 0.28 | 0.60|
| Poliphenols         | 0.19 | 0 | 0 | 1 | 0.09|
| C14:0               | 1 | 1 | 0.82 | 0.19 | 0.33|
| C16:0               | 0 | 0 | 0.01 | 1 | 0.25 |
| C18:0               | 1 | 0 | 0 | 0.15 | 1|
| C18:1               | 1 | 0 | 0.47 | 0 | 1|
| C18:2               | 1 | 1 | 0.05 | 1 | 1|
| Positive characteristics | 0 | 0 | 0.01 | 1 | 0.20|
| Negative characteristics | 0 | 0 | 0 | 1 | 1|
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IV. CONCLUSION

In our opinion, probably, the samples kept at 25 °C are more airy and for this reason the fermentative processes are more mitigate because this environment present a low humidity content, how also underline by the sensory analysis and the fatty acids profile. Instead the sample kept at 9 °C, in a very closed environment, in which is more difficult remove the humidity and so to create a more suitable environment for microbial growth. However, for all samples the storage time (4 d) appear a critical point for nutritional quality drupes.

In conclusion the temperature and the storage time play important role in the nutritional quality of drupes, and by the FMI it is possible monitoring the storage processes.

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