Improvement of Physico-chemical Properties of Arbequina Extra Virgin Olive Oil Enriched with β-Carotene from Fungi

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Abstract: Nowadays the consumption of essential carotenoids is reduced due to the lower intake of fruits and vegetables, being humans not capable of synthesizing these molecules. β-carotene is one of the most important carotenoids possessing anti-oxidation, anti-inflammation and anti-cancer properties. The aim of this work consists of preparing virgin olive oils enriched in β-carotene from fungi at different concentrations (0.041 and 0.082 mg/mL) in order to obtain new functional foods. Values of quality parameters (free acidity, peroxide value, coefficients of specific extinction and p-anisidine) have been obtained, showing that quality of olive oils was improved. Furthermore, the effect of β-carotene was evaluated as possible oxidative stabilizer during microwave heating and ultra violet-light exposure of the oils. As expected, the enrichment process brought changes in olive oils color, turning them orange-reddish. The use of natural antioxidants, in particular β-carotene could be an effective way to protect virgin olive oils from degradation and is a good strategy also to enhance the consumption of bioactive compounds improving olive oils shelf-life and nutritional value.

Key words: extra virgin olive oil industry, β-carotene, quality parameters, carotenoids, functional food industry

1 Introduction

Virgin olive oil (VOO), which is one of the principal ingredients of Mediterranean diet, is a vegetable oil from the fruit of the olive tree (*Olea europaea* L.) obtained only by mechanical methods that retain its nutritional and organoleptic properties⁷. It is produced widely and has been used since ancient times in the Mediterranean region, where it is appreciated for its nutritional properties, fine taste and aroma due to its content of essential volatile and non-volatile compounds⁸. The consumption of VOO has been intensively associated with multiple healthy properties, such as prevention of coronary and degenerative diseases⁹. Furthermore, the study of the antioxidant properties of VOO have had a special attention due to their importance from health, biological and sensory points of view⁴. The different beneficial effects have been mainly attributed to polyphenols and antioxidants which present potential biologic activities such as anti-inflammatory, anti-cancer, antimicrobial and the like⁶. Unfortunately, olive oil becomes with time more prone to oxidation and change in quality attributes, being antioxidants addition an engaging strategy to prolong its shelf life, improve the acceptability and enhance nutritional value⁶.

On the other hand, carotenoids are bioactive compounds having a crucial role in a great deal of biological processes. In this sense, daily consumption of carotenoid-rich foods, such as fruits and vegetables, is considered to be beneficial for human health. Consequently, there are a lot of studies showing that carotenoids and their derivatives may contribute beneficially to fight against an important number of diseases. Therby, recent reviews involving carotenoids have shown benefits with respect to immunity and several types of cancers (prostate, breast, cervical, ovarian, colorectal)⁷,⁸. Additionally, deficiency of carotenoids result in clinical signs of conjunctiva and corneal aberration including: xerophthalmia, night blindness, keratomalacia, corneal ulceration, scarring and irreversible blindness. Carotenoids can be classified into two groups on the basis of their functional groups; xanthophylls (containing oxygen), including lutein and zeaxanthin, and carotenes (without...
One of more relevant carotenoids is βC, which is a carotenoid that has been used both as a food coloring and as a source of provitamin A and antioxidant. βC is one of the lipophilic plant pigments possessing several biological properties: anti-oxidant, anti-inflammation and anti-cancer. It can be found in vegetables as carrots, pumpkins and sweet potatoes that colors them orange, also are found together with anthocyanins in vegetables such as purple carrots and purple tomatoes10-12. Additionally, carotenoids including βC, due to their lipophilic nature, has a bioavailability relatively low13, 14, and several studies have been investigating potential dietary as well as host-related factors influencing carotenoid bioavailability aspects, including their bioaccessibility and absorptive processes. Furthermore, other studies have concluded that dietary lipids can significantly enhance carotenoid absorption fostering micellization15. Regarding safe daily intakes, the European Food Safety Authority (EFSA) establish for βC safe consumption a level below 15 mg per day8, 16. 

In light of the above, carotenoids are a group of bioactive components with antioxidant properties which play a pivotal role for reducing risk of developing several diseases. In spite of the great importance of carotenoids, the amounts of them that are found in human tissues are almost exclusively from dietary origin, mainly from fruits and vegetables, or from supplements6, 10. Unfortunately, in global terms the world intake of fruits and vegetables is not sufficient, with a particular attention to adolescents and children.

Contrasting with the reduced consumption of carotenoids in our common diet and the importance of olive oil in Mediterranean diet, olive oil enriched in carotenoids could be a good convey to improve and enhance the intake of these bioactive compounds in a daily basis. In this context, in the present work, the main goal was to study the effects of the addition of βC from Blakeslea trispora fungi to Arbequina extra-virgin olive oils. Thereby, different concentrations of βC and ripening index samples were assayed obtaining the influence of these variables in color and quality attributes in order to support βC efficiency as antioxidant while characterizing the changes induces in the olive oil legislated parameters. Furthermore, the enrichment process using βC was tested as possible stabilizer during microwave heating and ultraviolet-light(UV-light) exposure of the olive oils. On balance, a good strategy to ensure an optimal intake of carotenoids through habitual diet would be to produce enriched olive oils with βC.

2 Materials and Methods
2.1 Chemicals
All chemicals were analytical reagent or high purity grade. Thymolphthalein, phenolphthalein, potassium hydroxide, benzoic acid, starch, sodium thiosulfate, sodium sulfate anhydrous, acetic acid glacial, chloroform, cyclohexane, ethanol 96%, diethyl ether and isooctane were acquired from Panreac (Barcelona, Spain). p-Anisidine was supplied by Sigma (Sigma-Aldrich, St. Louis, MO).

2.2 Olive oils sampling and β-carotene addition
Extra-virgin olive oils (EVOO’s) from Arbequina cultivar variety (cv. Arbequina) representative from Jaén region were selected for the present study. The olive oil samples were obtained from the same crop season (2018/2019), having different ripening index (October, November and December). Three bottles of 1 L of each olive oil (n=3) were provided by “Castillo Canera Olive Juice” company. From each bottle, three samples of 50 mL were constituted: control (control-olive oil without β-carotene); olive oil with 0.041 mg of β-carotene per mL of oil (enriched I); and olive oil with 0.082 mg of β-carotene per mL of oil (enriched II). All olive oils were filtrated in the presence of anhydrous Na2SO4 before use. After filtration, samples were homogenized and stored at 4°C in darkness using amber glass bottles without head space until analysis. For preparing enriched oils, a carotenoid solution with a concentration of 4 mg of βC/mL of oil (βC concentrated solution) was utilized. This solution was prepared using βC from Blakeslea trispora fungi, obtained following the corresponding methodology patented by DSM company (DSM, León, Spain). This βC has successfully passed the corresponding controls required by European Union legislation for food additives. Regarding βC stability, DSM company certifies that this sample is quite stable in oil under controlled storage conditions.

2.3 Physical and quality parameters determination
2.3.1 Color measurement
Color of olive oils with and without β-carotene addition was measured with a Konica Minolta model CM-5 spectrophotometer. Thereby, the different CIELAB space parameters (L*, a* and b*) were obtained from CIELAB method by the spectrophotometer after its calibration. L* is a measure of luminance or lightness component, which ranges from 100 to 0 (black and white). Parameters a* and b* are termed opponent color axes; a* ranges from negative to positive, it represents green versus red, b* also ranges from negative, blue, to positive, yellow. Color features were obtained as the average of three measurements performed on each sample. The color change was quantified using ΔE* values, which is the difference in the color from a reference point in the three-dimensional CIELAB color space, calculated with the formula given as:
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2.3.2 Quality parameters determination
The different samples were determined according to European Union standards (Annexes II, III and IX in Commission Regulation EEC/2568/91 from 11th July and amendments [17]). The quality parameters assessed were free acidity (FA, expressed as percentage of oleic acid), peroxide value (PV, expressed as mEq O₂/kg of oil) as well as the specific coefficients of extinction at 232 nm, 270 nm \(K_{232}\) and \(K_{270}\). The quality parameters assessed were free acidity (FA, expressed as percentage of oleic acid), peroxide value (PV, expressed as mEq O₂/kg of oil) as well as the specific coefficients of extinction at 232 nm, 270 nm \(K_{232}\) and \(K_{270}\) and the respective ΔK values.

3.1 Physical and chemical characterization of the olive oil samples
3.1.1 Color parameters
It is well known that consumers judge foods and drinks according to their external appearance like color or texture. For this reason, the visual aspect of olive oil could play an important role for consumers’ evaluation and preference [19, 20].

For βC enrichment appearance assessment of the EVOO’s, the color of them was evaluated. In Table 1, are shown the CIELAB main axes, \(a^*\), \(b^*\) and \(L^*\) values obtained from Arbequina EVOO’s with different concentration of BC from the fungi *Blakeslea trispora*. Firstly, it is important to highlight that βC concentrated solution, used for enriching experiments, had an intense orange color. Consequently, the visual impact of the enrichment in the different oils was quite clear as expected.

Values of \(a^*\) increase (Table 1) with the concentration of βC added similarly in the three olive oils tested. This means that green-yellow coloration initial, typical of virgin olive oils, pass to a more yellow-orange coloration. Concerning \(b^*\) parameter value, an increase was observed mainly with the 0.041 mg of βC per mL of olive oil, since oils samples become more orange with the enrichment process. Luminosity (\(L^*\)) decreases significantly in all samples being observed proportionality degree between the oil βC concentration and the luminosity, which transform olive oils darker than control olive oils.

\Delta E' = \left( \Delta L'^2 + \Delta a'^2 + \Delta b'^2 \right)^{1/2}

3 Results and Discussion
Before starting with this section it is necessary to clarify some aspects related to oils designation. According to the definition of the European Union Commission an oil designated as “extra virgin olive oil (EVOO)” must be extracted only from olives with a superior quality following a specific processing methodology. On the other hand, when an EVOO has been processed with vegetables, herbs, spices, or other compounds to improve its nutritional value, changing the sensory characteristics and increasing its stability, this oil cannot be called “extra virgin olive oil” [19, 20].

In this context, in the present work we have utilized as starting samples extra virgin olive oils being these samples EVOO’s. Further, we have generated enriched oils adding carotenoids extract to the starting EVOO’s and these enriched samples cannot be designated in the same way.

2.4 Degradation study
2.4.1 Heating procedure using microwave
Concerning heating study 1, 3, 5, 10 and 15 min, were the different times selected for microwave treatment to simulate conventional times used in home cooking. For each olive oil and heating time, three sub-samples of 50 mL were individually heated in bottles of 100 mL in a domestic microwave oven (LG) at maximum potency (700 Watt). As control unheated olive oil (0 min) was used. Afterwards, the samples were kept in Falcon tubes and refrigerated until analysis. The heating procedure only was applied to oils from cv. Arbequina harvested in November.

2.4.2 Light degradation using UV-radiation exposure
Regarding the photostability study, 1, 2, 4, 6 and 8 weeks, were the different times selected for the exposure to UV-light. For each olive oil and weeks of light exposure, sub-samples of 50 mL were individually placed in bottles of 100 mL in a control cabin and color comparison CAC 60 (VeriVide), with a light source UV lamp (Black Light-Blue, 18 Watt). As control, unexposed olive oil (0 week) was used. Afterwards, the samples were kept in Falcon tubes and refrigerated until analysis. For this experiment oils from cv. Arbequina harvested in October were selected.

2.5 Statistical analysis
Regarding olive oils parameters analysis, \(a^*\), \(b^*\), \(L^*\), FA, PV, \(K_{232}\), \(K_{270}\), ΔK, \(p^*\)-AV and TOTOX were done in triplicate on three levels (without, enriched with 0.041 mg/mL and enriched with 0.082 mg/mL). Data were expressed as means ± SD and analyzed using a one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. The significance level was set at a 5%. In addition, data obtained during microwave heating and light exposure treatments, were analyzed by two-way ANOVA followed by Bonferroni’s multiple comparison post-test. The statistical analysis was performed using different packages of the open source statistical program R (version 3.6.0).
These results showed that oils enrichment process brought significant changes in their color (Figs. 1 and 2). The color difference value ($\Delta E$) is a color parameter that represents the color difference contrasting enriched and control oils. So, when the amount of $\beta$C increase in oil by continuous addition of carotenoid concentrated solution, color difference values ($\Delta E$) increased as expected. Figure 1 shows three different coloration curves obtained during the enrichment process. Regarding oils without $\beta$C, those corresponding to October and November have a similar behavior because they have a strong green color due to a high chlorophyll concentration in comparison with oils from December. In this sense, the curves describing these months in Fig. 1 reach a lower $\Delta E$ value that the corresponding to December as expected.

In addition, Fig. 2 describes $\Delta E$ values which represent color differences that are higher with the amount of $\beta$C added to olive oils, which means that color differences increase as expected.

### 3.1.2 Quality parameters

The quality of the EVOO’s was evaluated to verify if the $\beta$C enrichment process promotes quality changes. Regarding the different values obtained and shown in Table 2, it is possible make certain that no quality degradation is undertaken by $\beta$C addition.

With reference to FA values obtained in all samples from different ripening index (October, November and December) and with different $\beta$C concentration (enriched I and II) no significant changes were observed. These outcomes are positive since the $\beta$C enrichment process does not trigger hydrolytic reactions in the olive oils samples, maintaining the levels of free fatty acids within the “extra virgin” category, as stated by Regulation EEC 2568/1991 and modifications (17).

### Table 1: CIELAB space color parameters ($a^*$, $b^*$ and $L^*$) of Arbequina EVOO’s without (control) and enriched with $\beta$C from *Blakeslea trispora* dissolution (enriched I: 0.041 mg/mL; enriched II: 0.082 mg/mL). Results are expressed as mean ± SD of three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey’s Test $p<0.05$) among different olive oils.

| Olive Oil         | Color parameters           |   |   |   |
|-------------------|---------------------------|---|---|---|
|                   | $a^*$                     | $b^*$ | $L^*$ |
| control           | $-2.91 \pm 0.00$ a        | $111.92 \pm 0.00$ a | $87.06 \pm 0.01$ a |
| Arbequina October | enriched I: $24.48 \pm 0.00$ b | $130.10 \pm 0.06$ b | $77.28 \pm 0.00$ b |
|                   | enriched II: $34.23 \pm 0.00$ c | $123.35 \pm 0.06$ c | $74.21 \pm 0.00$ c |
| control           | $-3.12 \pm 0.00$ a        | $115.16 \pm 0.01$ a | $86.41 \pm 0.01$ a |
| Arbequina November| enriched I: $24.71 \pm 0.01$ b | $128.99 \pm 0.07$ b | $76.54 \pm 0.00$ b |
|                   | enriched II: $35.53 \pm 0.01$ c | $120.70 \pm 0.01$ c | $70.71 \pm 0.00$ c |
| control           | $-4.68 \pm 0.01$ a        | $94.87 \pm 0.01$ a  | $90.79 \pm 0.01$ a  |
| Arbequina December| enriched I: $26.52 \pm 0.01$ b | $133.56 \pm 0.10$ b | $79.48 \pm 0.00$ b  |
|                   | enriched II: $35.53 \pm 0.01$ c | $127.72 \pm 0.01$ c | $75.08 \pm 0.00$ c  |
Regarding the rest of parameters (PV, K_{232}, K_{270}, ΔK and $p$-AV), they were calculated in order to elucidate the possible appearance of oxidative processes in the EVOO’s studied (Table 2). The peroxidation of the samples is reduced by βC addition, since in all oils a decrease in PV was observed. This fact could be explained as a consequence of the antioxidant effect produced by βC enrichment, being inhibited the formation of primary oxidation products (mainly hydroperoxides)\(^{26}\).

In addition, with the evaluation of the specific extinction coefficients (K_{232} and K_{270}) it is also possible to verify the degree of olive oil oxidation, complementing the PV determinations. It is well known that these specific coefficients are indicative of the conjugation of trienes (K_{232}) and the presence of carbonyl compounds (K_{270}), respectively\(^{27}\). The addition of βC to the olive oil did not bring significant changes to K_{232} and ΔK. On the other hand, it is curious that βC enrichment process increase moderately K_{270} values. In a first approximation, this fact could represent formation of secondary oxidation products but evaluating the values obtained in $p$-AV, this increase could be related with the color conferred to olive samples by the βC solution added. Focusing the attention in $p$-AV, this parameter is an empirical determination utilized to assess advanced oxidative rancidity of vegetable oils, being a methodology based on the aldehyde carbonyl bond on the p-anisidine amine groups, leading to the formation of Schiff base that absorbs at 350 nm. Consequently, it allow the estimation of secondary products of oxidation of unsaturated fatty acids, principally dienals and 2-alkenals\(^{28,27}\). Subsequently, studying the data shown in Table 2, is so clear that enrichment process causes neither primary (PV and K_{232}) nor secondary ($p$-AV) oxidative reactions.

Finally, TOTOX value is a complementary parameter when assessing oxidative deterioration of oils, being also reported as the total oxidation value according to ISO 6885 (2006). In all enriched samples a 1997 decrease in TOTOX value was obtained in comparison with the control oil value. Whereby, these oils reported lower oxidative deterioration with βC addition.

In light of the above, the results obtained involving quality parameters of the olive oils with and without βC, all oils could be classified as EVOO’s according to Commission Regulation EEC/2568/91 from 11\(^{th}\) July and amendments\(^{19,20}\). Nevertheless, even if the limit established for quality parameters are accomplished, enriched oils cannot be named EVOO following the definition of the European Union Commission\(^{19,20}\).

Regarding color and quality parameters obtained (Tables 1 and 2), it seems clear that a dependency may exist with the olive oil production date (different months), because olive oils composition depends on maturity index. In this context, new experiments must be conducted in future works in order to determine other bioactive compounds such as phenolic compounds, fatty acids and tocopherols. The determinations of these parameters from olive oils will allow us understand the influence of production date on samples characterization.

### 3.2 Stability of oils during microwave heating

Currently, microwave heating studies are being developed in order to know the effect of this process on olive oil chemical, nutritional, biological and sensory properties\(^{28-30}\).

In this context, the effect of microwave heating in the stability of samples has been evaluated using Arbequina EVOO’s from November and a βC concentration of 0.08 mg/mL, obtaining color and quality parameters for a correct understanding.

For the analyzed samples the free acidity values were similar at all the studied times with the exception of 15 min...
during microwave heating treatment FA values with the heating time. On the other hand, others changes were determined between the olive oil samples in the most usual behaviour values increased after microwave heating treatment as the temperature increases, after that an important increase showed little variations, after that an important increase was observed for all the coefficients. At the end of the heating process was observed for all the coefficients. Additionally, studies developed with olive oils added with tea extract for testing oxidation during microwave heating, showed that the presence of this extract apparently acts as pro-oxidant. In all cases the values obtained were similar to the obtained previously. As observed, in both samples the increasing microwave heating exposure did not cause severe changes in FA values. This behavior could be explained by the inexistence of water in the olive oils, as observed by previous studies. Our results are in agreement with those described in previous works where authors reported that no statistical changes were determined between the olive oil samples in FA values with the heating time. On the other hand, others researchers reported significant differences in FA values during microwave heating treatment.

The peroxide value is a widely used indicator of fat oxidation, measuring lipid peroxides and hydroperoxides formed during the initial stages of oxidation. The PVs prior heating (time zero) were 7.4 and 5.2 meq O₂/kg of olive oil for control and enriched samples respectively. PVs increase until 3 min and decrease afterwards but in all cases the values corresponding to the enriched oil were lower than the control oil. This observation indicates that, when heating time increases, the presence of added βC apparently acts as anti-oxidant. In all cases the values obtained were under the maximum permitted limit for the classification of virgin olive oil category (20 meq O₂/kg of olive oil) according to Commission Regulation EEC/2568/91. Previous works using different olive oils have reported that PV values increased after microwave heating treatment as most usual behaviour. Nevertheless, other authors using two Portuguese olive oils, reported that PV values showed a decline when treated with microwave heating for up 10 min, being in agreement with results obtained in this work. Additionally, studies developed with olive oils added with tea extract for testing oxidation during microwave heating, showed that the presence of this extract apparently acts as pro-oxidant. The peroxide value may not be a good index for measuring oxidation because hydroperoxides are unstable when heated at high temperatures, being necessary to use other additional parameters such as specific extinction coefficients.

Table 3 shows changes in K₃₅₂ and K₃₇₀ specific coefficients and ΔK under different exposure times at microwave heating. In the initial heating periods (under 5 min) the specific coefficients values showed little variations, after that an important increase was observed for all the coefficients. At the end of the heating process (15 min) oils presented a K₃₅₂ higher than 3.0 that indicates a rapid degradation process. Regarding K₃₇₀ values of both oils presented a positive correlation with the exposition time, being exceeded the maximum value permitted for EVOO (0.22, from IOC 2018) for times values greater than 3 minutes. These results indicate that after intense microwave heating (more than 3 minutes) an acceleration must occur in the oxidation process. These results are similar to the obtained previously demonstrating the worsening effect of microwave heating in olive oil. Table 3 also shows significant differences in the analy-

### Table 3

Comparison of microwave heating effect in the quality parameters of olive oils without (control) and with βC from *Blakeslea trispora* dissolution (enriched II: 0.082 mg/mL). FA: free acidity; PV: peroxide value, specific extinction coefficients (K₃₅₂, K₃₇₀, ΔK), p-AV: p-Anisidine value and TOTOX. Results are expressed as mean ± SD of three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey’s Test p < 0.05) among different olive oils. Results from two-way ANOVA followed by Bonferroni’s multiple comparison test, were shown as interaction "concentration versus time".

| Olive Oil: Arbequina November | Quality parameters | 0 min | 1 min | 3 min | 5 min | 10 min | 15 min |
|------------------------------|-------------------|------|------|------|------|-------|-------|
|                              | FA (%)            | PV (meq O₂/kg) | K₃₅₂ | K₃₇₀ | ΔK   | p-AV  | TOTOX |
| control                      | 0.13 ± 0.01 ns    | 7.4 ± 0.3 a    | 1.82 ± 0.02 ns | 0.11 ± 0.00 a | 0.00 ± 0.00 ns | 7.8 ± 0.1 a | 22.6 ± 0.6 a |
| enriched II                  | 0.13 ± 0.01      | 5.2 ± 0.2 b    | 1.82 ± 0.03   | 0.15 ± 0.01 b | 0.00 ± 0.00   | 8.7 ± 0.3 b  | 19.1 ± 0.5 b  |
| control                      | 0.12 ± 0.00      | 9.7 ± 0.1 b    | 1.65 ± 0.23 b | 0.14 ± 0.01 b | 0.00 ± 0.00   | 8.0 ± 0.6   | 27.4 ± 0.6 b  |
| enriched II                  | 0.12 ± 0.01      | 14.9 ± 0.8 a   | 1.50 ± 0.12 a | 0.16 ± 0.00 a | 0.00 ± 0.00   | 9.5 ± 0.1 ns | 39.3 ± 1.6 b  |
| control                      | 0.11 ± 0.00      | 11.1 ± 0.3 b   | 2.08 ± 0.23 b | 0.24 ± 0.01 b | 0.01 ± 0.01   | 9.7 ± 0.3   | 31.9 ± 0.7 b  |
| enriched II                  | 0.10 ± 0.01 a    | 8.2 ± 0.2 a    | 2.25 ± 0.15 a | 0.40 ± 0.00 ns | 0.02 ± 0.00   | 22.3 ± 0.0 a | 38.7 ± 0.4 b  |
| control                      | 0.12 ± 0.00 b    | 7.2 ± 0.5 b    | 2.11 ± 0.10   | 0.41 ± 0.01   | 0.02 ± 0.00   | 20.4 ± 0.3 b | 34.8 ± 1.0 b  |
| enriched II                  | 0.14 ± 0.00      | 6.0 ± 0.5      | 2.81 ± 0.05 b | 0.56 ± 0.01   | 0.03 ± 0.00   | 27.2 ± 0.4   | 39.2 ± 1.1   |
| control                      | 0.13 ± 0.01 ns   | 6.7 ± 0.4 a    | 3.17 ± 0.15 a | 0.52 ± 0.03 ns | 0.03 ± 0.00   | 26.6 ± 0.1 ns | 40.0 ± 0.8 ns |
| enriched II                  | 0.14 ± 0.00      | 6.0 ± 0.5      | 2.81 ± 0.05 b | 0.56 ± 0.01   | 0.03 ± 0.00   | 27.2 ± 0.4   | 39.2 ± 1.1   |
| control                      | 0.23 ± 0.01 a    | 7.2 ± 0.5 a    | 3.66 ± 0.26 a | 0.58 ± 0.00 a | 0.03 ± 0.00 ns | 28.3 ± 0.4 ns | 42.7 ± 1.1 ns |
| enriched II                  | 0.28 ± 0.00 b    | 6.7 ± 0.3      | 4.57 ± 0.31 b | 0.64 ± 0.01 b | 0.03 ± 0.00   | 29.2 ± 0.9   | 40.0 ± 1.1   |

*p*Interaction (concentration-time) df F 13.019 6.775 16.961 8.207 3.089 10.468 5.611

\[ p \leq 0.001 \]
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The color of the EVOO’s was evaluated during the heating process. In Table 4 are presented the CIELAB main axes L*, a* and b* values obtained from oils. In general terms values of a* decrease with time. Regarding b* value in the control oil is observed a decrease in this parameter. On the contrary, the enriched oil displayed an increase in this value. The luminosity (L*) of olive oils increase slightly in both samples during the heating process being observed a proportion between exposition time and the luminosity observed which turn olive oils paler. In this sense, comparing the unheated and heated samples at 1, 3 and 5 min, using visual assessment only slight changes were observed. However, comparing the unheated samples with the samples heated at 10 and 15 min, the latest show discoloration and less viscosity. This effect could be related also with the destruction and constant decrease in the content of chlorophylls and other pigments including added βC.

### 3.3 Photostability of oils during UV-light exposure

It is well known that oils storage conditions (such as light exposure and temperature) and duration of storage and exposures affect olive oil quality, changing its final nutritional, health and sensory properties, leading to the appearance of sensory defects like rancidity. In this context there are previous works which study the effect of storage parameters using different approaches. On the other hand, other researchers have studied how adding natural additives, like carotenoids and similar, stability of olive oils can be improved during storage.

In light of the above, a pivotal degradation variable that must be taken into account in foods during storage is light exposure. Consequently, a photostability study under UV-light exposure was developed using Arbequina EVOO’s from October and using a βC concentration of 0.08 mg/mL.

Regarding free acidity, the obtained values during the eight weeks under UV-light exposure were similar for oils without βC and enriched (0.082 mg/mL) (Table 5). In certain way these results were expected and are in accordance with the results obtained by others authors studying light exposure, using different light sources and periods of time. It is well known that hydrolysis in fatty acids promote changes in FA being involved hydrolytic enzymes. In general terms these enzymes are presents in the olive fruit or surrounding microorganisms. Once in the present work has utilized high quality olive oils that are filtered and dehydrated, the probability of enzymes occurrence is low or inexistnt.

Peroxide amounts were used for an estimation of oxidative degradation. Before UV-light exposure the control olive oil had a PV of 6.6 (meq. O₂/kg of olive oil), until the enriched oil had 5.9. When the olive oils were exposed to UV-light the PV increased continuously in both kind of oils but a higher formation of peroxides was observed in the control samples rather than in the olive oil enriched. In this sense, enriched olive oil showed values within the range of EVOO category (≤ 20 meq·Kg⁻¹) during the initial two weeks, exceeding this maximum value from here until the

### Table 4

Colorimetric values of Arbequina EVOO’s without (control) and with βC from Blakeslea trispora dissolution (enriched II: 0.082 mg/mL), as a function of time under microwave heating. Results are expressed as mean ± SD of three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey’s Test p < 0.05) among different olive oils.

| Olive Oil: Arbequina November | Color parameters |   |
|------------------------------|-------------------|---|
|                              | a*                | b*| L*     |
| 0 min control                | -3.12 ± 0.00 a    | 115.16 ± 0.01 a | 86.41 ± 0.01 a |
|                              | 35.53 ± 0.01 b    | 120.70 ± 0.01 b | 70.71 ± 0.00 b |
| 1 min control                | -1.12 ± 0.01 a    | 113.39 ± 0.01 a | 86.67 ± 0.00 a |
|                              | 32.01 ± 0.01 b    | 125.62 ± 0.03 b | 73.99 ± 0.02 b |
| 3 min control                | -1.64 ± 0.01 a    | 109.30 ± 0.01 a | 87.31 ± 0.00 a |
|                              | 30.03 ± 0.00 b    | 127.83 ± 0.08 b | 75.58 ± 0.01 b |
| 5 min control                | -5.28 ± 0.01 a    | 67.50 ± 0.01 a  | 90.72 ± 0.00 a |
|                              | 24.50 ± 0.01 b    | 132.43 ± 0.10 b | 79.21 ± 0.01 b |
| 10 min control               | -4.92 ± 0.01 a    | 21.70 ± 0.01 a  | 95.50 ± 0.01 a |
|                              | 29.56 ± 0.00 b    | 94.75 ± 0.00 b  |  |
| 15 min control               | -4.69 ± 0.00 a    | 22.97 ± 0.01 a  | 95.34 ± 0.00 a |
|                              | 26.89 ± 0.00 b    | 94.97 ± 0.00 b  |  |
Table 5  Comparison of UV-light exposure effect in the quality parameters of olive oils without (control) and with βC from Blakeslea trispora dissolution (enriched II: 0.082 mg/mL). FA: free acidity; PV: peroxide value, specific extinction coefficients (K₂₃₂, K₂₇₀), ΔK, p-AV: p-Anisidine value and TOTOX. Results are expressed as mean ± SD of three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey’s Test p < 0.05) among different olive oils. Results from two-way ANOVA followed by Bonferroni’s multiple comparison post-test, were shown as interaction “concentration versus time”.

| Olive Oil: Arbequina October | Quality parameters | p-AV | TOTOX |
|------------------------------|--------------------|------|-------|
|                              | FA (%)             | PV (mEq O₂/kg) | K₂₃₂ | K₂₇₀ | ΔK   |       |
| 0 weeks                      | control            | 0.13 ± 0.01 ns | 6.6 ± 0.5 ns | 1.72 ± 0.04 ns | 0.10 ± 0.01 a | 0.01 ± 0.01 ns | 7.7 ± 0.1 ns | 20.9 ± 1.0 ns |
|                              | enriched II        | 0.11 ± 0.01    | 5.9 ± 0.3  | 1.79 ± 0.07  | 0.15 ± 0.01 b | 0.00 ± 0.00  | 7.5 ± 0.3  | 19.3 ± 0.7  |
| 1 weeks                      | control            | 0.12 ± 0.01 ns | 24.8 ± 0.4 a | 2.67 ± 0.31 a | 0.42 ± 0.06 a | 0.00 ± 0.00 ns | 6.4 ± 0.5 a | 56.0 ± 0.9 a |
|                              | enriched II        | 0.12 ± 0.00    | 14.3 ± 0.1 b | 2.11 ± 0.01 b | 0.14 ± 0.01 b | 0.01 ± 0.01 | 7.7 ± 0.3 b | 36.3 ± 0.4 b |
| 2 weeks                      | control            | 0.12 ± 0.01 ns | 31.5 ± 0.5 a | 2.74 ± 0.08 ns | 0.12 ± 0.01 a | 0.00 ± 0.00 ns | 6.2 ± 0.3 a | 69.2 ± 1.0 a |
|                              | enriched II        | 0.11 ± 0.00    | 20.4 ± 0.3 b | 2.65 ± 0.07  | 0.16 ± 0.00 b | 0.00 ± 0.00  | 7.5 ± 0.4 b | 48.3 ± 0.7 b |
| 4 weeks                      | control            | 0.12 ± 0.00 ns | 42.4 ± 0.6 a | 2.58 ± 0.01 a | 0.14 ± 0.04 ns | 0.00 ± 0.00 ns | 5.7 ± 0.1 a | 90.5 ± 1.2 a |
|                              | enriched II        | 0.12 ± 0.00    | 28.1 ± 0.7 b | 2.75 ± 0.06 b | 0.13 ± 0.00   | 0.00 ± 0.00  | 5.3 ± 0.4  | 61.5 ± 1.5 b |
| 6 weeks                      | control            | 0.13 ± 0.01 a  | 39.6 ± 0.6 a | 2.64 ± 0.25 a | 0.17 ± 0.01 ns | 0.00 ± 0.00 ns | 6.2 ± 0.2 a | 85.4 ± 1.2 a |
|                              | enriched II        | 0.11 ± 0.00 b  | 33.4 ± 0.4 b | 3.26 ± 0.17 b | 0.17 ± 0.00   | 0.00 ± 0.00  | 5.8 ± 0.4  | 72.6 ± 0.9 b |
| 8 weeks                      | control            | 0.12 ± 0.01 ns | 39.4 ± 0.7 ns | 2.79 ± 0.04 a | 0.18 ± 0.01 ns | 0.00 ± 0.00 ns | 5.5 ± 0.4 ns | 84.3 ± 1.5 ns |
|                              | enriched II        | 0.12 ± 0.00    | 38.5 ± 0.2  | 3.66 ± 0.17 b | 0.17 ± 0.01   | 0.00 ± 0.00  | 5.3 ± 0.3  | 82.3 ± 0.5 |

Interaction
(concentration-time )

|                | df | 5  | 5  | 5  | 5  | 5  | 5  |
|----------------|----|----|----|----|----|----|----|
|                | F  | 2.860 | 144.602 | 21.546 | 47.455 | 2.066 | 9.437 | 138.894 |
| p              | 0.037 | ≤ 0.001 | ≤ 0.001 | ≤ 0.001 | 0.105 | ≤ 0.001 | ≤ 0.001 |

end of the studied period. This oxidative degradation observed could be explained through the combined effect of light and oxygen availability resulting in rapid oils oxidation during exposure time. Furthermore, similar results denoting growth in PV values, were reported in previous works using fluorescent light exposure (90 days) and other artificial light exposure (5 months) respectively.27, 30 However, despite this increasing in PV values, they did not exceed the maximum limit during the initial two month (8 weeks) treatment. This difference with respect to our results could be explained by the high degradation effect of the ultraviolet light used for us compared to the other light sources utilized by others authors.

In reference with specific extinction coefficients the addition of βC to the olive oil did not bring significant changes during UV-light exposure process (Table 5). It is important to highlight that both kind of oils reported K₂₇₀ values below the legal limit established for EVOO, being in agreement with previous studies showing slight increments but not exceeding the maximum limit for EVOG.7, 30, 43 Regarding K₂₃₂ values, only during the initial two weeks both oils showed values under the maximum limit for EVOO according to legislation.27 The rest of the weeks studied promoted values that exceeded the maximum limit. Table 5 also shows significant differences in the analysis of the concentration-time interaction.

The color of the EVOO’s was also evaluated during the light exposure process. In Table 6 are presented the CIELAB main axes L*, a* and b* values obtained from oils. Values or a* remain more or less stable for both kind of samples with different time exposure. Regarding b* value in the control oil is observed a decrease and concerning enriched olive oil a smooth increase is detected. Luminosity (L*) follows a similar behavior pattern as in microwave heating study, showing a continuous increase with time exposure. In comparison with microwave heating experience, the colors observed remain more stable during the period utilized for developing this experiment. An explanation for this phenomenon could be the higher stability of EVOO’s when are irradiated with UV-light.

4 Conclusions
The addition of βC brought significant changes in olive oils quality, stability and color. The enrichment process turns olive oils more yellow-orange due to βC original color. Oxidative stability is enhanced, improving olive oils shelf-life, protecting them from oxidative processes. Olive oils peroxidation is reduced as well the formation of oxidation products, enhancing olive oils quality. The addition of 0.41 or 0.082 mg/mL not affects decisively to olive oils quality and composition, therefore the 0.082 mg/mL would be an appropriate concentration to consume a daily amount of 3

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mg of βC considering the average oil intake in Spain\(^{46}\), being this intake value under the maximum limit established (15 mg per day)\(^{8, 16}\).

The present work also proves that microwave heating and light exposure produce losses in olive oil quality. With the exception of free acidity values, the rest of parameters are affected by the time of heating and light exposure. However, enriched oils cope better the influence of this degradation processes showing a positive effect of the βC addition. The possible introduction of this kind of olive oils as new functional foods would increase the daily intake of essential bioactive molecules in human diet, enhancing health properties for consumers and conferring crucial nutritional assets. Other pivotal variable to be considered is olive oils storage time and for this reason we are evaluating the enrichment effect in oil shelf life using a long time experiment along 3 years (unpublished results). These results are similar to the obtained by others authors previously, with oil enriched with lutein-zeaxanthin\(^{44}\) or using oils enriched with lutein from *Scenedesmus almeriensis*\(^{40}\).

With the results obtained in this work, we also concluded that the fungi *Blakeslea trispora* is a good source of βC. Consequently, oils enriched in this bioactive compound could be used for different companies in order to incorporate in the market new products for fighting against certain diseases.

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### Conflict of Interest

The authors declare no conflict of interest.

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### Table 6

Colorimetric values of Arbequina EVOO’s without (control) and with βC from *Blakeslea trispora* dissolution (enriched II: 0.082 mg/mL), as a function of time under UV-light exposure. Results are expressed as mean ± SD of three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey’s Test *p* < 0.05) among different olive oils.

| Olive Oil: Arbequina October | Color parameters |
|-----------------------------|------------------|
|                             | *a*              | *b*              | *L*              |
| 0 weeks control             | −2.91 ± 0.00 a   | 111.92 ± 0.00 a  | 87.06 ± 0.01 a   |
| enriched II                 | 34.23 ± 0.00 b   | 123.35 ± 0.06 b  | 72.41 ± 0.00 b   |
| 1 week control              | 2.59 ± 0.01 a    | 106.14 ± 0.01 a  | 88.22 ± 0.00 a   |
| enriched II                 | 34.35 ± 0.00 b   | 126.78 ± 0.04 b  | 74.66 ± 0.00 b   |
| 2 weeks control             | 2.22 ± 0.01 a    | 103.90 ± 0.01 a  | 89.49 ± 0.00 a   |
| enriched II                 | 34.92 ± 0.01 b   | 128.51 ± 0.08 b  | 75.75 ± 0.01 b   |
| 4 weeks control             | 1.26 ± 0.00 a    | 101.46 ± 0.01 a  | 90.62 ± 0.00 a   |
| enriched II                 | 34.30 ± 0.00 b   | 130.36 ± 0.05 b  | 76.94 ± 0.00 b   |
| 6 weeks control             | 0.35 ± 0.00 a    | 95.09 ± 0.02 a   | 92.25 ± 0.00 a   |
| enriched II                 | 33.95 ± 0.01 b   | 131.04 ± 0.06 b  | 77.35 ± 0.00 b   |
| 8 weeks control             | 1.04 ± 0.00 a    | 91.29 ± 0.01 a   | 92.75 ± 0.00 a   |
| enriched II                 | 33.38 ± 0.01 b   | 132.20 ± 0.06 b  | 78.11 ± 0.00 b   |
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