Improvement of Tunisian 'Chemlali' extra virgin olive oil stability with rosemary and laurel herbs and essential oils

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This study was carried out to investigate the effect of different flavourings (laurel and rosemary), commonly used in the Mediterranean diet, on the quality of Tunisian extra virgin olive oil derived from the variety "Chemlali". The maceration of the two herbs or the incorporation of their associated essential oils were applied.
The resistance to oxidation of flavoured and enriched olive oils was determined by measuring quality index values. During three months of storage, an increase of these indexes was recorded for all analysed olive oils. However, this increase was less pronounced in flavoured virgin olive oils when compared to control. Also, results showed more stability of total polyphenols as well as chlorophylls and carotenoids pigments essentially for macerated enriched olive oils which were characterised by a high antioxidant capacity. Finally, based on the sensory evaluation, flavoured olive oils with essential oils were more appreciated by consumers than olive oils incorporated with rosemary essential oil.
Keywords: Extra virgin olive oil, Rosemary, Laurel, Maceration, Essential oil, Stability.

1. INTRODUCTION

Since the early 1990s, the dynamics of the global olive oil market have been marked by the increase in demand and the appearance of new markets such as Canada, the United States of America, Brazil, Japan, and China. These mutations have offered Tunisian exporters opportunities to increase exports and diversify markets. However, this country has been faced with competition from European countries which are constantly increasing their market shares in these new markets. In addition, the emergence of new producer countries such as Turkey, Syria, Morocco, Jordan and recently some Latin American countries have led Tunisia to face several challenges such as the differentiation of its product to maintain its competitiveness in the world olive oil markets.
Extra virgin olive oil is a key ingredient widely produced and consumed in Mediterranean diet. It is appreciated for its nutritional properties, pleasant aroma, and delicious taste [1] and for having the most restrictive quality criteria among olive oils categories [2]. Virgin olive oil is characterised by high contents of monounsaturated fatty acids (oleic acid) and natural antioxidants known to show protective effects against many modern life-style diseases [2, 3, 4]. In recent years, the olive oil consumption is increasing due to its sensorial characteristics and health claims [4]. However, in the olive sector, face to consumer increasing demand for top quality, healthy, and innovative products, it has been shown that packaging and aromatisation of olive oils has immerging as interesting innovation practice in new olive oil markets [1, 2]. In 2010, the launch of the "Bio Tunisia" label to create AOCs in Tunisia, increased international demand for standard quality and orientation towards stabilization and prevention from oxidation of olive oil by the addition of appropriate natural antioxidants [2] could be a solution to widen the destina-

tions. In fact, Tunisian olive oil is very appreciated and 90% of its exports is in conditioned form that offer to the Tunisian olive oil its own identity [5].
Furthermore, according to Farras et al. [6], the consumption of antioxidant-rich or functional virgin olive oil promotes high-density lipoprotein (HDL) and prevent against cardiovascular diseases. They reported that bioactive compounds and essential oils can decrease low-density lipoprotein cholesterol concentrations. In this regard, several kinds of flavourings in olive oils were used particularly: essential oils, fruits (apple, orange and lemon), aromatic plants (basil, fennel, laurel, oregano, rosemary, and thyme), mushrooms, nuts, spices and vegetables (dried tomatoes, hot chili peppers, onions, pepper) [4]. Among these flavourings, the common traditional practice was the aromatization with aromatic plants and spices well known for containing essential oils with antioxidant and antimicrobial properties by using different methods [2, 7]. In fact, these flavourings could be added to the olive oil after its extraction by infusion or maceration or can be mixed directly with the olive paste fruits during the oil-productive process [1, 4, 8]. The efficiency of these bioactive flavourings, particularly rosemary and laurel, was proved in some studies on olive oils stability by protecting the oils from thermal oxidation with improvement of their sensorial properties (aroma, taste and colour) due to their health benefits and organoleptic and antioxidant characteristics [2, 7]. Also, flavouring could add further value to this precious agricultural product when increasing its use among non-traditional consumers.
In this connection, with the present study we intend to compare the influence of two enrichment methods: the maceration of two common herbal plants (rosemary and laurel) and the adjunction of their essential oils on chemical and sensory quality of flavoured olive oil as well as his stability during 84 days of storage. The effect of these aromatic plants on the quality parameters (free acidity, peroxide value and K_{232}, K_{270}), fatty acids profile, total phenols content, antiradical scavenging activity and oxidative stability were investigated.

2. MATERIALS AND METHODS

2.1. MARKET STUDY

The objective of this part is to carry out a market study for flavoured olive oil to understand the behaviour and preferences of Tunisian consumers towards this product. Studying consumer behaviour is an essential tool, especially for innovative products, because it helps guide a company's business decision and reduces uncertainty about the choice of target consumers [9].
In this study, the consumer survey was disseminated online (through a questionnaire posted on a social network) and face to face. The online questionnaire has the advantage of being able to be self-administered and does not require the presence of the interviewer. In Tunisia there are 6 million active users of the social network. This approach therefore makes it possible to reach a wide spectrum of consumers who are geographically dispersed and who use the Internet at very different frequencies.
The studied sample consists of 200 people. The mentioned data in the questionnaire such as sex, age, socio-professional category and geographical area allowed us to classify the people questioned. The processing of the survey data was carried out using two methods.

2.1.1. The one-dimensional method (flat sorting)

It represents the distributions with a single variable giving the frequencies relating to each variable and constituting the simplest examples of statistical tables. These tables are of great importance for reading quantitative data [10].

2.1.2. The two-dimensional method (cross sorting)

It represents the two-variable distributions and consists of crossing the results of the variables two by two (cross sorting, or two-dimensional cross tables or even double entry tables) to determine whether there is a significant correlation between two well-defined variables [10].

2.2. RAW MATERIAL

Tunisian olive oil used in this study derived from the variety "Chemlali". The preliminary analysis on the obtained olive oil showed low level of oxidative degradation and then the good results of the panel test allowed classification of the oil as extra virgin. To produce flavoured and enriched olive oil, two aromatic and medicinal plants that grow in abundance in Tunisia were used. Rosemary and laurel were collected from North of Tunisia, identified and authenticated by a plant taxonomist. Essential oils were purchased from Orient Laboratory, Tunisia.

2.3. PREPARATION OF ENRICHED OLIVE OIL

Fresh aromatic plants were washed, gently dried at 40°C and then, added to olive oil at a rate of 2% (w/w) [3, 4]. Their correspondent essential oils were added at 0.2% w/w to olive oil. Before being tested, the mixtures were stored at constant temperature and humidity in hermetically sealed dark glass bottles. After that, all flavoured olive oils were sampled each 21 days during 84 days of storage at room temperature.
Three independent trials were carried out for the flavouring maceration, starting from the same olive lot. On the whole, five different flavoured and enriched olive oils were produced: Unenriched olive oil (Control), enriched olive oil using maceration of rosemary (MR), enriched olive oil using maceration of laurel (ML), enriched olive oil with rosemary essential oil (REO) and enriched olive oil with laurel essential oil (LEO).

2.4. FATTY ACIDS COMPOSITION

Fatty acids were evaluated as their methyl esters after cold alkaline transesterification with methanolic potassium hydroxide solution and extraction with n-heptane [11]. The initial fatty acid profiles of different olive oils were determined as described by Limon et al. [12].

2.5. DPPH ANTIOXIDANT ASSAY

The antioxidant activity of the phenolic extracts of olive oil with different flavourings was evaluated on the basis of the scavenging activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical. Briefly, olive oil was diluted in ethyl acetate (100 mL/mL of ethyl acetate) and mixed with a DPPH solution with a concentration of 1 10^4 mol/L in ethyl acetate. The mixture was then homogenised and kept in the dark for 30 min for reaction. After that the absorbance was registered at 515 nm against a blank solution. These assays are based on the abilities of the antioxidants present into the extracts to scavenge the radical in comparison with that of a standard antioxidant (trolox, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid). The inhibition percentage obtained for the samples was interpolated on the calibration curve to calculate the concentration in trolox equivalents (mmol/L TE) [1, 4].

2.6. QUALITY PARAMETERS DETERMINATION

Free fatty acid (FAA), peroxide value (PV), and spectrophotometric indexes (K_{232} and K_{270}) were determined following the European Union standard methods [11] and analytical methods described by Ayadi et al. [3].

2.7. TOTAL PHENOLIC CONTENT MEASUREMENTS

The total phenolic content of enriched olive oils was measured using the Folin–Ciocalteau as described by Yang et al. [13]. A 100 mg aliquot of each oil sample was mixed with the Folin–Ciocalteau reagent (0.5 mL) and methanol (2 mL). The mixture was shaken before adding 1.5 mL of 15% Na_2CO_3. After 30s of homogenization, distilled water was added to make a final volume of 7 mL. Then, the mixture was incubated at 50°C for 20 min and centrifuged (MPW Med. Instruments, MPW-350R Centrifuge, Poland) at 2000 g for 10 min. The absorbance of the obtained supernatant was measured at 750 nm. A standard curve was prepared using diluted solutions of gallic acid. The total phenolic content of the olive oil samples was expressed as milligrams of gallic acid equivalents per kg.

2.8. CHLOROPHYLLS AND CAROTENOIDS CONTENTS MEASUREMENTS

Each sample of enriched olive oil (7.5 g) was placed in a falcon tube and filled until 25 mL with cyclohexane. The chlorophyll fraction was measured in a UV spectrophotometer (Jenway 6352 spectrophotometer) at 670 nm and the carotenoids fraction at 470 nm. The concentrations of pigments were expressed following the equations described by Ayadi et al. [3].

2.9. PANEL AND CONSUMERS TESTS

Sixty trained panellists (food engineering students at the Higher Institute of Food Industries) and 8 expert panellists (National Olive Oil Center of Tunis, Tunisia) performed the sensory analysis on flavoured olive oils. The expert panellists were asked to evaluate positive sensory attributes and the defects (musty, smells of fusty, winey-vinegary, metallic, and rancid) of virgin olive oil samples, immediately after their elaboration date by using the profile sheet for virgin olive oil with a continuous unstructured line scale of 10 cm, ranging from low to high intensity [1]. The trained panellist tested by both olfactory and gustatory assessments olive oil samples for odour, taste, colour, after taste, bitterness, flavouring intensity, and overall acceptability. The various flavoured olive oils kept in the dark, at room temperature, were served to panellists in a randomised order codified by a 3-digit number and submitted to both panels. Fresh bread was used as a carrier and water as a palate cleanser between tastings [3]. The panellists were asked to rank the intensity of different attributes on a 5-point scale (1: “very weak”; 5: “very strong”). The mean sensory scores for various attributes of the flavoured oils were calculated [1].

2.10. STATISTICAL ANALYSIS

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation. An analysis of variance (ANOVA) was performed at a 5% significance level.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF THE MARKET STUDY FOR ENRICHED OLIVE OIL

3.1.1. One-dimensional method

The results showed that consumers interested in this new product are generally women aged between 20-45 years. It was shown that 97.3% of the questioned panellists consumed olive oil. Among them, 81% bought a food product, particularly olive oil, for their good quality, and taste and price were classified at the second and third place. However, 2.7% of surveyed said that they do not consume olive oil because of its strong taste. In the Tunisian market, this is an opportunity for flavoured and enriched olive oil that could be used to attract this category of consumers due to its new taste and richness in natural antioxidants. Besides, 63% of current consumers of olive oil justified their choice for this product by their nu-

tritional value and health benefits. However, 47% go towards extra virgin olive oil. Indeed, this new health concern is a favourable advantage for the consumption of flavoured olive oil.
The results showed that the attitude of consumers to try a new olive oil on the market was in progress with 67% of consumers that accepted to try new olive oil flavours against 33% who seemed attached to their habits regarding the purchased category of olive oil. This showed that the decision to bring new categories of olive oil to the market was appreciated by more than 2/3 of consumers. Moreover, 55% of respondents had a very positive attitude and 31% were in favour of the proposal to consume a new extra virgin olive oil enriched with aromatic plants or their extracts, richer in natural antioxidants and more stable against the oxidation. Also, when choosing one plant to flavour olive oil, the results of the questionnaire showed that garlic and rosemary are the two most requested plants by consumers, representing respectively 27% and 24% of choices, followed by olive leaves (16%).

3.1.2. Two-dimensional method

This method has been adapted to determine if there is a significant correlation between two variables. In this study, the calculated coefficient is the Pearson coefficient which is an index reflecting a linear relationship between two continuous variables taken in pairs. A negative value (negative correlation) means that when one of the variables increases, the other decreases. A significance level less than 0.05 reflects a significant relationship between these two variables (Data not shown).
From the results relatives to Pearson correlation coefficient, between a few variables, no statistically significant link was observed between the geographic origin of the questioned person and the plant chosen for flavouring olive oil with a significance level (Sig) (0.155) greater than 0.05. Besides, results showed a negative Pearson correlation coefficient (-0.14) between gender and attitude towards the consumption of flavoured olive oil. This result allows us to deduce that women have a positive attitude for the new offered product compared to men. On the other hand, a strong relation between the favourable attitude of consumers towards this new product and age was observed through the estimation of the correlation coefficient with a positive significant level less than 0.05 (0.046).

3.2. FATTY ACIDS PROFILES

The fatty acids profiles were assessed in the unenriched olive oil and olive oils enriched with two aromatic and medicinal herbs: rosemary and laurel. The initial composition of different olive oils samples is reported in Table I. In this study, in all analysed samples, oleic acid (C18:1) was the most abundant (57.99%) monounsaturated fatty acid (MUFA), followed by linoleic acid (C18:2) (18.18%) and palmitic acid (C16:0) which was the main (18.00%) saturated fatty acid (SFA) in olive oil. These results were partially in agreement with those reported by Limon et al. [12] and Sousa et al. [4]. In fact, they noted that C18:1 was the prominent fatty acid while, they reported higher contents of C18:1 (78.09% and 74.47%) but lower amounts of C18:2 and C16:0. However, Ollivier et al. [14] showed levels of C18:1 ranging from 59.93% to 80.97%.
Oleic acid content increased significantly ($p<0.05$) with the addition of rosemary and laurel herbs. The C18:1 content varied from 57.99% in the control to 59.15% and 59.05% respectively in olive oil added with laurel and rosemary herbs (ML and MR). Similarly, the C18:2 content (18.00%) increased in all treated olive oils. For C16:0, its content decreased for the two flavoured olive oils when compared to the

Table I - Fatty acids composition (g/100g fatty acids) of enriched and unenriched olive oils

Fatty acids (%)	*Control*	*REO*	*LEO*	*MR*	*ML*
Palmitic acid: $C_{16:0}$	18.18	17.27	17.88	17.23	17.02
Palmitoleic acid: $C_{16:1}$	2.47	2.45	2.40	2.33	2.33
Margaric acid: $C_{17:0}$	0.03	0.03	0.06	0.05	0.05
Heptadecenoic acid: $C_{17:1}$	0.08	0.07	0.10	0.08	0.09
Stearic acid: $C_{18:0}$	2.25	2.20	2.20	2.19	2.23
Oleic acid: $C_{18:1}$	57.99	59.08	58.71	59.05	59.15
Linoleic acid: $C_{18:2}$	18.00	18.01	17.95	18.20	18.12
Linolenic acid: $C_{18:3}$	0.59	0.54	0.49	0.53	0.64
Arachidic acid: $C_{20:0}$	0.27	0.24	0.13	0.23	0.24
Eicosenoic acid: $C_{20:1}$	0.13	0.10	0.07	0.10	0.12
SAFA	20.73	19.74	20.27	19.70	19.54
MUFA	60.67	61.7	61.28	61.59	61.69
PUFA	18.59	18.55	18.44	18.73	18.76

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
Values are means of three replicates, standard deviation are in the range [±0.00 to ±2.06]. Means with different superscripts are significantly different ($p < 0.05$).

Table II - Radical scavenging activity (DPPH) of enriched and unenriched olive oils at the beginning of storage

Day	*Control*	*REO*	*LEO*	*MR*	*ML*
0	70.285±0.00^{aA}	67.082±0.00^{aA}	67.438±0.00^{aA}	70.641±0.00^{aA}	70.818±0.00^{aA}
4	62.149±1.7$^{b\,A}$	61.74±0.00^{bB}	59.11±0.04^{aB}	69.039±0.00^{cA}	69.395±0.10^{cB}
8	57.167±1.89^{aB}	60.158±0.00^{aB}	58.756±0.30^{aB}	67.182±0.00^{bB}	67.616±0.01^{bC}
12	54.156±0.04^{aB}	57.957±0.00^{cB}	56.534±0.28^{bC}	67.013±0.01^{dB}	67.275±0.00^{dC}
16	52.775±0.01^{aC}	57.438±0.02^{cC}	55.174±0.15^{bC}	65.836±0.28^{dB}	65.48±0.09^{dD}

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
Data are mean ± standard deviation, n=6. Means with different superscripts are significantly different ($p < 0.05$).

control (18.18%) with significantly higher values when olive oils were incorporated with EOs. These findings were in line with the standard set by the European Community Regulation [11] which requires C16:0 and C16:1 levels ranged respectively from 7.5% to 20% and from 0.3 to 3.5% for an extra virgin olive oil. In fact, it has been proved that C16:1 is a minor fatty acid since its content is generally low in a good quality olive oil [14]. On the other hand, all enriched olive oils presented equal or inferior values to the control for different fatty acids fractions that compose the studied olive oils (Tab. I) which mean that herbal incorporation didn't influence significantly ($p>0.05$) the contents of C17:0, C17:1, C20:0 and C20:1 as described before by Sousa et al. [4].

Furthermore, the obtained results revealed that MUFA were the most abundant with values ranging from 60.67% (Control) to 61.7% (REO) and 61.69% (ML), followed by SFA and polyunsaturated fatty acids (PUFA). In this connection, the addition of both rosemary and laurel herbs decreased significantly SFA contents and increased MUFA contents. Otherwise, olive oils incorporated with EOs contained higher SFA amounts than oils added with respective herbs. Also, MUFA amounts in flavoured olive oils were always higher than the untreated control. Concerning PUFA, the addition of rosemary and laurel herbs significantly influenced their contents (18.73% and 18.76%, respectively) which were higher than control. These findings were in accordance with the maximum levels to be considered as extra-virgin olive oils as recommended by the European Community Regulation [11] and the results reported by Sousa et al. [4].

3.3. ANTIOXIDANT ACTIVITY

The results of the evolution of the antioxidant activity of control and treated samples during the first two week of storage are illustrated in Table II. A significant decrease ($p>0.05$) of this parameter was observed, during storage.

At production, the results did not show significant difference ($p>0.05$) among control and tested enriched olive oils. The highest antioxidant activity was reported for ML (70.82%) and MR (70.64%) oils when compared to oils incorporated with their respective EOs and the control.

During storage, the antioxidant capacity of control and enriched olive oils decreased significantly ($p<0.05$). These findings were in line with those of Taoudiat et al. [2] reporting that antiradical activity decreased in virgin olive oils with laurel EO. However, Sousa et al. [4] showed that enrichment of olive oil by dried laurel, oregano and pepper did not protect olive oil against oxidation. Moreover, the results partially agreed with those of Baiano et al. [8] who found that the antioxidant potential of olive oils enriched with herbs like oregano and rosemary decreased significantly during 9 months of storage but more slowly in control olive oil.

After 16 days of storage, results showed that the antiradical activity of virgin olive oils enriched with herbs was greater with no significant difference ($p<0.05$) observed between incorporation of rosemary and laurel. This finding was in accordance with that of Yang et al. [13] reporting that the major active compound in rosemary extract known as carnosic acid had an important antioxidant activity.

Also, after two weeks of storage, a significant difference ($p>0.05$) was observed between the control, the macerated virgin olive oils with herbs and aromatized virgin olive oils with essential oils. Contrastingly, Taoudiat et al. [2] and Ben Rached et al. [15] suggested an improvement of antioxidant activity with EOs addition and thus, their efficiency in virgin olive oils compared to fresh and dried herbs.

3.4. QUALITY PARAMETERS EVOLUTION DURING STORAGE

An extra-virgin olive oil is a liquid fat free of defects and compliant with a serial of chemical parameters with maximum levels permitted by the International Olive Council IOC [16] (Free fatty acid percentage ≤0.8 g oleic acid/kg oil, peroxide value ≤20 meq O_2/kg, K_{232}≤2.50, K_{270}≤0.22, median of fruity >0) [3].

3.4.1. Free Acidity

Free acidity changes of unenriched control and enriched olive oils during storage are presented in Table III. Values of free acidity expressed in oleic acid showed that enrichment increased slightly this acidity. Thus, initial free acidity increased significantly ($p<0.05$) in unenriched and enriched olive oils with rosemary and laurel plants to reach about 0.26±0.0%, 0.32±0.07%

Table III - Evolution of quality parameters and pigments of enriched and unenriched olive oils during storage

Day	Analyses	*Control*	*REO*	*LEO*	*MR*	*ML*
0						
	FA(%)	0.23±0.00^{aA}	0.24±0.00^{aA}	0.25±0.00^{aA}	0.25±0.00^{aA}	0.24±0.00^{aA}
	PV (mg.O_2)	10.02±0,00^{aA}	10.02±0,00^{aA}	10,02±0,00^{aA}	10,02±0,00^{aA}	10,02±0,00^{aA}
	K_{232}	2.001±0,00^{aA}	2.001±0,00^{aA}	2,001±0,00^{aA}	2,001±0,00^{aA}	2,001±0,00^{aA}
	K_{270}	0.117±0,00^{aA}	0.117±0,00^{aA}	0,117±0,00^{aA}	0,117±0,00^{aA}	0,117±0,00^{aA}
	Carotenoids (ppm)	1.03±0,00^{aA}	1.03±0,00^{aA}	1,03±0,00^{aA}	1,03±0,00^{aA}	1,03±0,00^{aA}
	Chlorophylls (ppm)	1.21±0.00^{aA}	1.21±0.00^{aA}	1,21±0.00^{aA}	1,21±0.00^{aA}	1,21±0.00^{aA}
	Total phenols (mg GAE/kg)	1510±56,57^{aA}	1510±56,57^{aA}	1510±56,57^{aA}	1510±56,57^{aA}	1510±56,57^{aA}
21						
	FA(%)	0,25±0,07^{aA}	0,27±0,14^{aB}	0,28±0,007^{bB}	0,28±0,14^{bB}	0,27±0,07^{bB}
	PV (mg.O_2)	13,90±0,04^{cB}	11,74±0,08^{bAB}	11,12±0,05^{Ab}	11,78±0,08^{bB}	12,58±0,14^{cB}
	K_{232}	2,448±0,03^{dB}	2,141±0,01^{bB}	2,174±0,02^{bB}	2,031±0,04^{aA}	2,316±0,05^{aB}
	K_{270}	0,127±0,001^{Ab}	0,125±0,004^{aA}	0,126±0,004^{aB}	0,121±0,007^{aB}	0,122±0,005^{aB}
	Carotenoids (ppm)	0,98±0,01^{aA}	0,99±0,04^{aA}	1,01±0,028^{aA}	1,07±0,02^{aA}	1,05±0,05^{aA}
	Chlorophylls (ppm)	1,16±0,02^{aA}	1,17±0,02^{aA}	1,19±0,04^{aA}	1,27±0,04^{bA}	1,29±0,02^{bA}
	Total phenols (mg GAE/kg)	1400±28,28^{aA}	1430±25,45^{aB}	1421±12,71^{aB}	1550±7,07^{bA}	1590±28,07^{bA}
42						
	FA(%)	0,25±0^{aA}	0,27±0,14^{aB}	0,28±0,14^{bB}	0,29±0,14^{bB}	0,27±0^{aB}
	PV (mg.O_2)	17,22±0,02^{eC}	16,17±0,14^{cC}	16,75±0,08^{dC}	15.36±0,02^{aC}	15,90±0,04^{bC}
	K_{232}	2,503±0,02^{dC}	2,257±0,008^{aC}	2,396±0,01^{cC}	2,239±0,01^{aB}	2,319±0,009^{bB}
	K_{270}	0,131±0,004^{aB}	0,129±0,004^{aB}	0,128±0,005^{aB}	0,123±0,007^{aB}	0,124±0,007^{aB}
	Carotenoids (ppm)	0,97±0,02^{aA}	0,98±0,05^{aA}	0,99±0,02^{aA}	1,06±0,04^{aA}	1,01±0,02^{aA}
	Chlorophylls (ppm)	1,04±0,01^{aB}	1,09±0.04^{aB}	1,12±0,02^{bA}	1,11±0,02^{aB}	1,21±0,02^{cA}
	Total phenols (mg GAE/kg)	1318±29,02^{aA}	1377±29,69^{bC}	1377±12,66^{bC}	1452±9,89^{cA}	1483±28,80^{cA}
63						
	FA(%)	0,26±0,14^{aA}	0,28±0.00^{aB}	0,28±0,07^{aB}	0,31±0.00^{bC}	0,28±0,01^{aB}
	PV (mg.O_2)	18,21±0,02^{Dd}	17,19±0,04^{aD}	17,61±0,02^{bD}	17,93±0,08^{cD}	17,71±0,12^{bD}
	K_{232}	2,511±0,00^{eC}	2,348±0,00^{aD}	2,486±0,01^{dD}	2,386±0.00^{Bc}	2,408±0,009^{cC}
	K_{270}	0,134±0,005^{aB}	0,132±0,00^{aC}	0,131±0,00^{aB}	0,126±0,00^{aB}	0,127±0,00^{aB}
	Carotenoids (ppm)	0,93±0,02^{aA}	0,95±0,04^{aA}	0,94±0,02^{aA}	0,98±0,04^{aA}	0,97±0,01^{aA}
	Chlorophylls (ppm)	1,02±0,01^{aB}	1,04±0,02^{aB}	1,06±0,04^{aB}	1,07±0,00^{aB}	1,19±0,04^{bA}
	Total phenols (mg GAE/kg)	1273±28,12^{aA}	1359±14,14^{bD}	1341±14,01^{bC}	1395±16,97^{cA}	1410±12,73^{cA}
84						
	FA(%)	0,26±0^{aA}	0,28±0,01^{bB}	0,29±0,00^{bB}	0,32±0,07^{cC}	0,3±0.00^{dC}
	PV (mg.O_2)	20,55±0,01^{cE}	19,39±0,04^{aE}	19,46±0,05^{aE}	19,90±0,07^{bE}	19,82±0,04^{bE}
	K_{232}	2,823±0,00^{eD}	2,512±0,01^{cE}	2,613±0,009^{dE}	2,446±0,01^{aD}	2,486±0,007^{aC}
	K_{270}	0,15±0,004^{aC}	0,149±0,008^{aD}	0,145±0,005^{aC}	0,141±0,004^{aC}	0,142±0,004^{aC}
	Carotenoids (ppm)	0,91±0,01^{aA}	0,92±0,01^{aA}	0,92±0,01^{aA}	0,96±0,02^{aA}	0,94±0,02^{aA}
	Chlorophylls (ppm)	1,01±0,04^{aB}	1,03±0,00^{aB}	1,04±0,02^{aB}	1,06±0,05^{aB}	1,12±0,01^{bB}
	Total phenols (mg GAE/kg)	1218±43,84^{aB}	1333±14,04^{bD}	1327±16,17^{bD}	1363±11,13^{cB}	1374±16,01^{cB}

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
FA: Free Acidity; PV: Peroxyde value; Specific extinction (K_{232} and K_{270}).
Data are mean ± standard deviation, n=6. Means with different superscripts are significantly different ($p < 0.05$).

and 0.29±0.0%, respectively for the control, MR and ML, at the end of storage. Indeed, the enrichment with natural antioxidants using maceration of plants recorded the highest free acidity content particularly when rosemary aromatic plant was used.
In all tested samples, all values of acidity were lower than the limits set by EEC [11] for extra virgin olive oil. Also, acidity values in this study were in line with those found by Limon et al. [12] and lower than those reported in previous studies [1, 2, 3].

3.4.2. Peroxide value

The results related to the evolution of the peroxide value (PV) of the control and enriched olive oils during about three months of storage at room temperature are illustrated in the Table III. The PV indicates the formation of primary compounds of oxidation [4]. In this study, initial PV was about 10.02±0.0 meq O_2/kg showing a low rate of oxidation as described by other authors [3, 12]. This result disagreed with the results found by Sousa et al. [4] and Taoudiat et al. [2] re-

porting lower PV (4.8 meq O_2/kg and 3 meq O_2/kg, respectively) after addition of laurel herb and EO in virgin olive oil.
During storage at room temperature, the initial peroxide values of all analysed samples increased significantly ($p>0.05$) to reach, after 84 days of storage, values of about 20.55±0.01 meq O_2/kg; 19.9±0.07 meq O_2/kg; 19.82±0.04 meq O_2/kg; 19.39±0.04 meq O_2/kg and 19.46±0.05 meq O_2/kg, respectively, for the control, MR, ML, REO and LEO. It should be noted that the difference was significant ($p>0.05$) between the control and the flavoured samples and that the enrichment using incorporation of essential oils was remarkably more accentuated than that by maceration in term of peroxide value. Nevertheless, the difference was not significant ($p<0.05$) between both MR and ML as well as between REO and LEO. As a result, all the samples analysed underwent a slight increase in peroxide value without exceeding 20 meq O_2/kg, value overcoming the maximum permitted limit except in the untreated control that consequently lost the classification of virgin olive oil category after 84 days of storage [8].

3.4.3. Specific extinctions

The values of the PV<20 meq O_2/kg of olive oil does not always mean the absence of the oxidation phenomenon. The use of ultraviolet absorbance coefficients (K_{232}, K_{270}) provides information on the presence or absence of secondary oxidation products in the oil. The hydro-peroxides of the early stages of oxidation absorb at 232 nm, while the secondary oxidation products such as ketones absorb near 270 nm [17] and their presence is indicative of an extensive oxidation [4].
The evolution of specific extinction parameters for the different samples are reported in Table III. Initial values were lower than registered values in other studies [8]. During the storage period, all analysed olive oils underwent a significant increase ($p<0.05$) of these parameters until the 84th day. Referring to the table, this increase was significantly different ($p<0.05$) over time for all analysed samples with maximum attributed to unflavoured control confirming the protective effect of natural herbs and their essential oils. As expected, this increase was attributed to the primary oxidation product's evolution into secondary oxidation products such as hydroperoxides as described by Taoudiat et al. [2].
Regarding the obtained results in the present study, unflavoured olive oils reported the highest K232 values and could not be considered as extra virgin olive oil, after 63 days of storage, according to the European legislation [11]. In contrast, flavoured olive oils with EOs exceeded the recommended limit (2.5), after 84 days. It was observed that maceration using natural herbs gave more stability to the olive oil than enrichment with essential oils.
Indeed, the secondary oxidation content of control and those relative to enriched olive oils with EOs appeared slightly higher than those of enriched olive oils using herbal maceration. By comparing the obtained pairs of values (MR and ML or REO and LEO), no significant difference ($p<0.05$) was observed when adding herbs contrary to flavouring with EOs. All tested oils did not exceed the maximum legal value for K_{270} values (0.22). Therefore, particular attention must be given to these two quality parameters to avoid the declassification of the olive oil from the extra virgin or virgin categories.

3.5. PIGMENTS AND POLYPHENOLS CONTENTS EVOLUTION DURING STORAGE

Olive oil contains minor compounds that give it its organoleptic and nutritional quality. Among these compounds are pigments known for their antioxidant nature in the dark and pro-oxidising in the light. They play an important role in the oxidative stability of the oil during its storage [2, 18] and in the preservation of its quality [18, 19].

3.5.1. Beta carotene content

The main carotenoids present in olive oil are lutein and β-carotene. The presence of carotenoids in olive oil is closely related to that of green pigments and influenced by the same factors. Numerous studies have shown the anti-carcinogenic activity of β-carotene and other carotenoids and their role in the prevention of cardiovascular diseases and eye diseases [20]. The results related to the evolution of the β-carotene level during 84 days of storage at room temperature are registered in the Table III.
During storage at room temperature, the initial value (1.03±0.00 ppm) of β-carotene content of all analysed samples decreased to reach, after 21 days of storage, values of the order of 1.07 ppm and 1.05 ppm, respectively for MR and ML. This result was explained by the richness of plants with β-carotene and the instantaneous migration of pigments from plants to olive oil. Then, β-carotene content decreased and reached, after 84 days, about 0.91 ppm; 0.92 ppm; 0.92 ppm; 0.96 ppm and 0.94 ppm, respectively for control, REO, LEO, MR and ML without any significant differences ($p>0.05$).
This could be due to β-carotene degradation following the presence of oxygen, as described by Criado et al. [21]. They reported that a significant loss of β-carotene could occur even at a low oxygen concentration and that the existence of free radicals can also accelerate the rate of degradation of carotenoids. In this connection, rosemary oil proved to be very rich in β-carotene with a maximum content compared to other oils.

3.5.2. Chlorophyll content

The colour of olive oil is the result of green and yellow hues due to the presence, respectively, of chlorophylls and carotenoids [2]. During the first 3 weeks,

the analysis of chlorophyll content showed that the enrichment of olive oils with rosemary and laurel maceration increased this content slightly but significantly of about 0.06 ppm and 0.08 ppm, respectively (Tab. III). This can be explained by the instantaneous migration of pigments from plants to olive oil during maceration.
After 84 days of storage, the behaviour of these pigments showed a slight decrease for the control and the other enriched olive oils. It should be noted that the difference was not significant ($p>0.05$) between all samples and macerated olive oil with laurel.
Thus, olive oil enrichment using rosemary or laurel maceration ensured more pigment stability for olive oil than other studied oils. In fact, the addition of aromatic plants helped strengthen the antioxidant activity of olive oil by increasing its levels of β-carotene and chlorophyll contributing to olive oil stability.

3.5.3. Polyphenol content

The initial polyphenols level was about 1510±56.57 mg GAE/Kg (Tab. III). This content was higher than that (1036.72 mg GAE/Kg) found by Taoudiat et al. [2] confirming that this content is influenced by several factors like the extraction system and olive variety.
Total polyphenols in MR and ML registered an increase, during the first three weeks. However, the other analysed enriched samples with EOs showed a slight decrease ($p>0.05$) during the same period. Thus, virgin olive oils with rosemary and laurel herbs exhibited significantly ($p<0.05$) higher total phenolic content than control and treated oils with EOs. This rise in polyphenols is probably due to the migration of phenolic compounds, which are very abundant in laurel and rosemary plants, to olive oil during its storage.
After that and as expected, phenols content decreased significantly ($p>0.05$), during storage period, for all analysed oils with a significant difference ($p>0.05$) between the control and treated oils. In fact, the phenols contents of enriched and unenriched olive oils decreased with increasing storage time. This decrease in total phenols content may be caused by the decomposition and oxidation of phenolic compounds in oils which undergo qualitative and quantitative modifications during storage [22]. In addition, the total antioxidant activity agreed with the phenolic levels, with higher values in the enriched oils obtained by herbal maceration confirming their effective and protective role in virgin olive oil [2]. In fact, the phenolic compounds in oils may act as an antioxidant by donating H-atom(s) to free radicals which contributes to its decrease [13]. Nevertheless, oils added with rosemary and laurel herbs still showed the highest total phenolic content until the end of storage. These findings disagreed with those of Sousa et al. [4] and Taoudiat et al. [2] suggesting that flavouring olive oil with essential oils was more efficient compared to dried herbs.

3.6. SENSORY ANALYSIS

A descriptive sensory study was carried out on olive oils flavoured by the maceration or addition of essential oils of rosemary and laurel to determine which of these flavoured oils was most appreciated by consumers. The results of a panel test carried out on the studied oils showed, from the sensory profiles of control and flavoured olive oils obtained by using the two different flavouring methods, that all of them were devoid of defects.
From Table IV, it was concluded that the addition of flavourings to olive oil influenced several properties and improved their sensorial characteristics. As reported by Sousa et al. [4], Consumer's acceptability of olive oil-aromatic plants is very important for the introduction of these products to the market. Thus, several descriptors evaluated this acceptability. Regarding colour, MR and ML oils presented a dark yellowish green colour while the colour of REO and LEO oils was light green. The panellists preferred the colour of oils flavoured with rosemary and laurel essential oils and appreciated the taste of olive oils flavoured with rosemary essential oil more. In fact, olive oils incorporated with essential oils were less bitter than macerated ones.
It was also noticed that panellists preferred olive oil flavoured with rosemary essential oil considering the colour, smell, taste, texture, and overall acceptability. Moreover, macerated oils with tested herbs proved to be the less appreciated. Thus, the flavoured olive oils can be classified by ascending order based on the consumer's preference as follow; REO>LEO>MR>

Table IV - Sensory evaluation of enriched and unenriched olive oils

Descriptor	Odor	Color	Taste	Aftertaste	Bitterness	Flavoring intensity	Global appreciation
Control	3.4±0.15a	1.8±0.04a	3.67±0.18b	2.9±0.1b	2.2±0.2a	3±0.14a,b	3.6±0.2a
REO	3.8±0.25a	2±0.02a,b	4.62±0.32b	2.9±0.18b	2.7±0.2b	3.6±0.22a,b	3.95±0.1b
LEO	3.7±0.16a	2.24±0.03b	4.61±0.28b	2.6±0.16a	2.5±0.1a,b	3.8±0.25b	3.7±0.2a,b
MR	3.52±0.26a	2.25±0.02b	3.85±0.35a	2.5±0.2a	2.48±0.15a,b	3.2±0.18a,b	3.55±0.25a
ML	3.5 ±0.2a	2.26±0.02b	3.9±0.3a	2.45±0.22a	2.3±0.09a,b	2.8±0.24a	3.5±0.2a

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
Means with different superscripts are significantly different ($p < 0.05$).

ML>C. These findings were partially in agreement with other studies reporting that the inclusion of essential oils at low or moderate concentrations avoided over-aromatisation, improved their sensorial characteristics and acceptability by consumers [4]. Also, according to Ayadi et al. [3], flavoured olive oils prepared with the maceration of aromatic plants should not only satisfy the sensory requirements of consumers, but also other qualities needed in the food market when compared to standard olive oils.

4. CONCLUSION

In this study, the enrichment of olive oil using maceration or EOs incorporation improved the chemical and sensory qualities as well as oxidative stability of analysed olive oils. This treatment added further value to this precious product due to the abundance of natural antioxidants which were transferred into olive oils following the maceration of rosemary and laurel herbs or the addition of their EOs. The results showed that rosemary and laurel herbs and EOs exhibited good antioxidant properties that control lipid oxidation during storage. Besides, flavoured olive oils had desired aromatic characteristics when compared to the control. These results may be an opportunity for Tunisia to improve its competitiveness in the world's olive sector, precisely on the import markets where conditioned Tunisian olive oil is highly appreciated.

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