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Effect of olive leaves powder supplementation on quality attributes and antioxidants of fresh milk cheese

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ABSTRACT

Background: Fortifying cheese with olive leaves powder, presents an innovative approach allowing the improvement of quality attributes of dairy products. Olive leaves, a co-product of olive oil production, are rich in bioactive compounds, which are known for their antioxidant, antimicrobial, and anti-inflammatory properties.

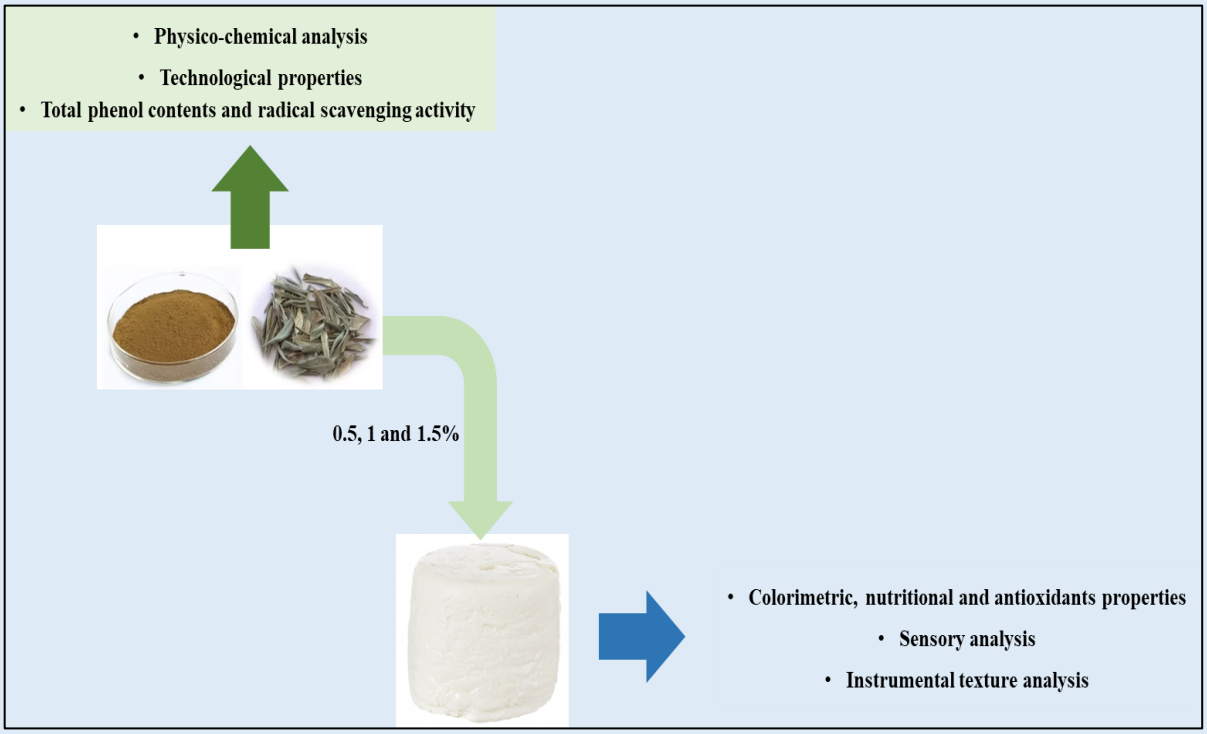
Objectives: This study aimed to evaluate the effect of olive leaf powder supplementation on fresh cheese quality attributes.

Methods and Results: Three formulations of fresh cheese made from cow milk and supplemented with olive leaves powder (0.5, 1, 1.5%) were prepared and compared to not-supplemented fresh cheese. The incorporation of the olive leaves powder did not affect the physicochemical characteristics of cheese, except for colorimetric parameters and fat and ash contents. However, it positively affects antioxidant content and activity. The incorporation of olive leaves powder at different levels (0.5, 1, and 1.5%) in fresh cheeses enhances the content of phenols (54.17%, 59.26%, and 64.52%, compared to control cheese). The same trend was found for the antioxidant activity. Sensory analysis showed that some sensory descriptors significantly changes, such as color, odor, taste, and after-taste. The fresh cheese supplemented with 0.5% olive leaf powder is the most appreciated

one, suggesting that olive leaves could be used in fortifying cheese and promoting consumers' antioxidant defense.

Novelty: This study presents a novel approach to fortifying fresh cheese with olive leaf powder (OLP) to enhance antioxidant properties while preserving the key quality attributes. OLP significantly increases phenolic content and antioxidant activity without compromising physicochemical attributes. Sensory analysis reveals that cheese with 0.5% OLP offers the best consumer acceptance, highlighting its potential as a functional dairy product. This innovative use of OLP supports the development of healthier dairy alternatives in response to growing consumer demand

Keywords: olive leaves, antioxidants, fresh cheese, sensory properties, chemical properties.



INTRODUCTION

Olea europaea, one of the oldest cultivated trees in the world, is primarily grown in Mediterranean countries such as Spain, Greece, Italy, Morocco, Turkey, and Tunisia. It has been considered as a key component of the Mediterranean diet. Olive cultivation represents a vital agricultural activity with significant socio-economic importance in the Mediterranean region [1]. The olive oil industry generates large amounts of byproducts, such as crude olive cake, vegetation water, twigs, and leaves [2]. Olive leaves constitute around 10% of the total weight of the harvested olive; 25 kg of olive leaves were produced per olive tree during tree pruning [3-

4-5]. Therefore, it causes not only economic and environmental problems, as the residues are disposed of either by burning or by grinding and scattering in the field, but also due to their phytotoxicity and low acidity. [6]. In the main case, olive leaves were used as animal feed. Olive leaves are considered an important low-cost source of natural antioxidants that can be used to replace synthetic additives, thus improving food safety and enhancing their potential as a functional food [2-7]. In fact, olive leaves present a natural source of bioactive compounds, especially phenols, including oleuropeosides (oleuropein and verbascoside) [8]. The most abundant phenolic compound in olive leaves is oleuropein, which represents up to 14% DW [2]. Moreover, the structure of phenolic compounds in olive leaves greatly influences their antioxidant properties.

Oleuropein and verbascoside present the o-dihydroxy (catechol) structures of their moieties [2]. Many of these compounds exhibit many biological activities and pharmacological properties: antioxidant, anti-hypertensive [7-9-10-11], anti-inflammatory, skin protective, anti-aging [12], antidiabetic, hypolipidemic, anticancer, antimicrobial, and antiviral activities, antiarrhythmic, spasmolytic, immune stimulant, cardioprotective, and antithrombotic, effects [13-14]. Therefore, olive leaves could be a source of valuable antioxidants, which can be used in functional food industries [15]. Olive leaf extract can be used as a natural ingredient to enhance the stability of edible oils, such as olive oil [16]. In fact, the olive leaf extract is rich in oleuropein and hydroxytyrosol, which are more efficient antioxidants than BHT, vitamin E, and vitamin C [17]. Moreover, olive leaf extract could be used as a functional ingredient for the stability of biscuits [18] and in the meat industry. Previous studies have demonstrated that supplementing meat with olive leaf extract enhances the inhibition of lipid and protein oxidation while also reducing microbial growth during refrigerated storage. This contributes to improved meat quality and extended shelf life, including in products such as minced beef meat [19-20]. Similarly, OLP extract has been investigated as a functional ingredient in yogurt, affecting its fermentation process, texture, sensory characteristics, and antioxidant properties [21]. Some reviews summarized the current trends in the use of plants (essential oils, plant extract, raw plant additions, etc.) and food by-products during dairy processing (milk, cheese, yogurt, and butter) [22]. This approach considers (i) a step forward toward reducing waste in the food chain, (ii) enhancement of dairy product preservation by using natural ingredients, resulting in a longer shelf life, and (iii) the development of innovative healthy dairy products.

Contò et al. [23] reported that the supplementation of sheep cheese with olive leaves affected the fatty acid profile, allowing a higher content of C18:1 cis-9 and C18:3 n-3. Moreover, the combination of olive and walnut leaves has been recognized for its potential to lower blood glucose levels [24]. Furthermore, Innosa et al. [25] reported that the supplementation of goat diet by olive leaves improved the oxidative stability of ricotta cheese during storage time due to higher content of phenolic compounds/antioxidant activity and also provided an increase in unsaturated fatty acid (vaccenic acid, linolenic acid…) and a decrease of ω-6/ω-3ratio. It has been reported that supplementing cheese and yogurt with olive leaves and their extract can promote public health as functional foods. However, adding them at high concentrations negatively may affect the sensory properties of the final product [26]. The advantage of using olive leaf powder instead of its extract is that it requires less processing, consumes less energy, and does not generate secondary byproducts, making it a

more sustainable approach while also preserving the viability of probiotic *Lactobacillus* strains, which are beneficial for health [27].

The objective of this work is to fortify a fresh cheese made from cow milk by OLP and (ii) to determine the effect of OLP amount (0.5, 1, 1.5%) on the main cheese properties (antioxidant, sensory, and physicochemical properties).

MATERIAL AND METHODS

Plant material: Olive leaves (OL) of the Chetoui variety were provided by the Olive Institute farm in Tunis, Tunisia. OL was oven-dried at 60°C until a constant weight for 13h and then ground to a fine powder using a coffee grinder (Moulinex®, France). The OLP was then vacuum packaged and stored at 4°C until use.

Physicochemical analysis of OLP: Proximate chemical composition: Moisture, protein, and fat contents were determined using the gravimetric, Kjeldahl, and Soxhlet methods [28], respectively. The ash content was assessed using a muffle furnace. Carbohydrate content was estimated as 100 - (sum of percentages of moisture, ash, protein, and fat).

Technological properties: Water retention capacity (WRC), milk retention capacity (MRC), and oil retention capacity (ORC) of OLP were evaluated as reported by Mkadem et al. [29]. For water and milk retention capacity, 30 ml of distilled water or milk was added to 1 g of OLP and incubated for 1 h at 25°C. The mixture was centrifuged at 3000 × g for 25 min, and the excess supernatant was decanted. Water and milk retention capacities were expressed as g water/g dry matter and g milk/g dry matter, respectively. To determine the oil retention capacity, 6 ml of sunflower oil were added to 0.5 g of OLP and were incubated for 1 h at 25°C. The mixture was then centrifuged, and the excess supernatant was decanted. ORC was expressed as g oil/g dry matter.

Total phenol contents and radical scavenging activity: Five grams of OLP were extracted with 50 ml of ethanol (96%). The mixture was then shaken in the dark using a magnetic stirrer for 30 min at 55°C. The crude extract was centrifuged at 3000g for 10 min, and the supernatant was filtered through Whatman paper n°4. The residue was extracted twice with 50 ml of the same solvent under the same extraction conditions. A combination of the three extracts was collected and stored at 4 °C [30].

Total phenol contents (TPC) and Radical Scavenging Activity (RSA) were determined at 520 nm using the DPPH assay according to Ben Abdallah et al. [30]. TPC was expressed as g of gallic acid equivalent (GAE) per 100 of dry matter (DW). The percentage of inhibition of DPPH radical was calculated between A_0 and A_t, according to the following equation (Eq.1), with A_0 as the initial optical density and A_t as the final optical density. Appropriate solvent blanks were run in each assay [30].

$$Percentage of inhibition = \frac{(A_0 - A_t)}{A_0} 100 \quad \text{(Eq.1)}$$

Cheese manufacture: Fresh cheese is an unripened cheese that is ready for consumption shortly after manufacture and is characterized by moisture on a fat-free basis of about 54-69. Four fresh cheeses were prepared at the pilot scale from cow milk supplemented with or without OLP (control: cheese not supplemented with OLP; C_1: cheese supplemented with 0.5% OLP; C_2: cheese supplemented with 1.0% OLP; and C_3: cheese supplemented with 1.5% OLP). A simplified flowchart of the fresh cheese-making process is shown in Figure. 1.

Cow milk was pasteurized at 70-72°C for 30s and cooled to 35± 0.5°C. Calcium chloride (0.3 to 0.4 ml/L), microbial rennet (0.1 ml/L, M. Miehei, Strength 1:10.000 UI, Sectorial Center in Agrifood d'El Khadra., Tunisia), and starter of mesophilic lactic cultures Frozen Direct Vat Set (F-DVS) R-704 (Chr. Hansen A/S), primarily composed of *Lactococcus lactis subsp. cremoris* and *Lactococcus lactis subsp. lactis* were added to the pasteurized cow milk. The preparation was homogenized and incubated at 22°C for 18 h. The obtained gel was allowed to drain for 3 h and then salted (1% of NaCl). OLP was then added at three concentrations: 0.5, 1, and 1.5% (w/w), and the formulations were homogenized by mixing. The fresh cheeses were stored at 4°C for three weeks and sampled for analysis after processing.

Colorimetric parameters: The colorimetric parameters L*, a*, and b* of OLP and of different cheeses were evaluated using a colorimeter (CPCE, TCR200, Spain). The total color difference (ΔE) was determined for the different cheeses by using the following equation (Eq. 2):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

(Eq. 2)

Where L* is the value of the brightness, and it varies from 0 (black) to 100 (white); a* value varies from -100 (green) to 100 (redness), and b* ranges from -100 (blue) to +100 (yellow). The subscript "0" in the equation refers to the control sample (not supplemented cheese).

Nutritional and antioxidant properties of fresh milk cheese: The cheeses were analyzed for protein, fat, dry matter, and ash using the Kjeldahl, Gerber, and oven drying methods, respectively [31].

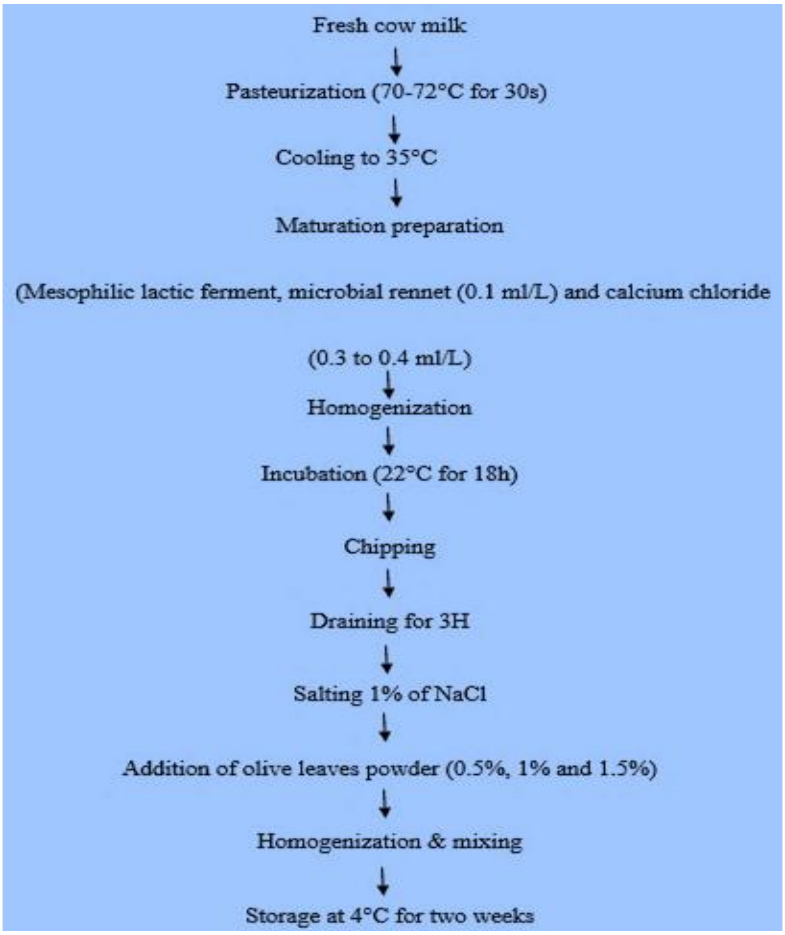


Figure 1. Flowchart of the making process of the fresh milk cheese fortified with OLP.

To extract phenols from cheeses, 10 g of cheese was added to 15 ml of a solution of ethanol (70%) HCl (0.1%) (v/v) and incubated for 24h at 4°C. The obtained solutions were filtered through the Whatman No.1 paper. Total phenol content and radical scavenging activity were determined on the extracts as described by Ben Abdallah et al. [30]. TPC was expressed as g gallic acid equivalent (EAG) per 100 g of cheese. Antioxidant capacity was compared to that of Trolox, which is commonly used as a standard antioxidant molecule to measure the antioxidant capacity of foods and beverages. The results are expressed as g Trolox Equivalent/100g of cheese.

Sensory analysis of fresh milk cheese: Fresh cheese samples were sensory evaluated by a panel of 12 trained panelists at the Olive Institute of Tunis (Tunisia). Each member of the panel served 20 g of the cheese sample placed on small white plates coded with three-digit random numbers. The overall acceptability of the cheeses was scored on a scale of 1-10. The hedonic and the descriptive tests were carried out, and the panelists assessed a total of 18 parameters, divided into five main groups: color (white, green, and homogeneity), odor (lactic, vegetable, and olive odor), texture (granular, viscous, melting, smooth, pasty, sticky), taste (acidity, saltiness, bitterness, sweetness, olive, after taste). Each of these descriptors was scored on a scale of 1-10, where 1 indicated “non-existent,” and 10 was “extremely strong.”

Instrumental texture analysis of fresh milk cheese: The firmness of the cheeses was evaluated with a texture analyzer (TAXT2, Surrey, UK) equipped with a 25 mm diameter acrylic cylinder probe. The penetration speed of the probe was 5 mm/s to a distance of 1 to 50 mm from the surface.

Statistical analysis: All experiments and analyses were triplicated; average and standard deviations were calculated. Statistical analysis was performed by using the software package IBM. SPSS 20.0 (Statistical Package for Social Science, 2011). The comparison of averages of each treatment was based on the analysis of variance (ANOVA) at a significance level of 5%.

RESULTS AND DISCUSSION

Characterization of olive leaf powder: Table 1 illustrates the physicochemical characteristics of OLP. The dried OL presented a low moisture content (0.10 ± 0.01 g/g dry matter. The results of the nutrient profile showed that OLP is rich in essential nutrients, including soluble sugars (27.99 ± 0.41 g/100 g dry matter), protein (9.20 ± 0.82 g/100 g dry matter), and minerals (7.59 ± 0.57 g/100 g dry matter). However, this byproduct contains a low-fat content (3.70 ± 0.53 g/100 g dry matter). These findings align with previous studies highlighting the nutritional richness of olive leaves. Studies have shown that olive by-products hold increasing potential in various industries, including nutraceuticals, pharmaceuticals, and cosmetics [9].

Additionally, the obtained results showed that OLP possesses interesting water and oil retention capacities (1.96± 0.73 g water/g DM; 2.06 ± 0.16 g oil/g DM, respectively) and a noticeably high milk retention capacity (2.48 ± 0.29 g milk/g DM).

Techno-functional properties are critical for various industrial applications. OLP's capacity to

retain both water and oil suggests that this powder is suitable for formulations where moisture and fat stability are to be optimized. OLP showed multifunctional properties combining nutritional and health properties [32].

As shown in Table 1, the TPC of OLP was 3.23 ± 0.24 g GAE/100 g of OLP, and it contains 0.763 ± 0.05 g oleuropein/100 g dry basis, which provides significant antioxidant activity (RSA ~ 82.91 ± 0.79%). These results are consistent with the literature. Indeed, olive leaves are a rich source of nutrients and antioxidants, and their phenolic compounds protect biological systems from oxidative stress [15].

The antioxidant properties of OLP justified its uses in traditional medicine, particularly for managing hyperglycemia and diabetes. Moreover, the high nutrient content, including soluble sugars, proteins, and minerals, further enhances the potential health benefits of olive leaves, making them an attractive source of functional food ingredients.

Table 1. Physicochemical techno-functional properties and antioxidants of olive leaves powder

Parameter	Average value
Moisture (g/g dry matter)	0.10± 0.01
Ash (g/100 g dry matter)	7.59± 0.57
Fat (g/100 g dry matter)	3.70± 0.53
Proteins (g/100 g dry matter)	9.20± 0.82
Soluble sugars (g/100 g dry matter)	27.99± 0.41
TPC (g GAE/100 g d.b)	3.23± 0.24
Oleuropein (g/100 g d.b)	0.763± 0.05
RSA (% percentage of inhibition)	82.91±0.79
Techno-functional properties	
WRC (g/g)	1.96 ± 0.73
ORC (g/g)	2.06 ± 0.16
MRC (g/g)	2.48 ± 0.29
L* (-)	3.10 ± 0.11
a* (-)	0.82 ± 0.27
b*(-)	2.50 ± 0.45

WRC: water retention capacity; ORC: oil retention capacity; MRC: milk retention capacity.

TPC: total phenols content; RSA: DPPH- radical scavenging activity

Physicochemical profile of the fresh milk cheese: The physicochemical characteristics of cheeses supplemented with or without OLP are illustrated in Table 2. There was no significant difference in pH values (4.95 to 5.00), moisture (65.11-66.71%), dry matter (33.29-34.89%), and protein content (24.40-25.83%) between the different cheese formulations. Ash contents were slightly affected by OLP supplementation, with a maximum for C3 (1.61 ± 0.01%), compared to the other formulations, which was higher in OLP. However, fat content shows a significant decrease with OLP addition (from 23.5± 0.05 for not-supplemented cheese to 17.30± 0.09 g /100 g dry matter in cheese supplemented with 1.5% OLP).

Table 2. Physicochemical parameters and antioxidants of the cheeses supplemented or not with olive leaves powder.

	Cheese formulations			
Parameters	**Control**	**C1**	**C2**	**C3**
pH (-)	4.99 ± 0.01^{a}	5.00 ± 0.01^{a}	5.00 ± 0.01^{a}	4.95 ± 0.06^{a}
Moisture (%)	65.11 ± 0.15^{a}	65.83 ± 1.18^{a}	66.71 ± 2.42^{a}	66.71 ± 2.42^{a}
Dry matter (%)	34.89 ± 0.15^{a}	34.17 ± 1.18^{a}	33.29 ± 2.42^{a}	33.29 ± 2.42^{a}
Ash (%)	1.51 ± 0.04^{ab}	1.42 ± 0.05^{b}	1.48 ± 0.06^{b}	1.61 ± 0.01^{a}
Fat (%)	23.5± 0.05^{a}	18,78± 0.13^{b}	17.51± 0.10^{c}	17.30± 0.09^{c}
Protein (%)	24.40± 0.16^{a}	25.21± 0.5^{a}	25.72± 0.86^{a}	25.83± 0.94^{a}
L(-)	14.04 ± 0.50^{a}	8.80 ± 0.42^{c}	7.97 ± 0.74^{c}	10.68 ± 0.39^{b}
a*(-)	0.62 ± 0.72^{a}	0,47 ± 0,19^{a}	0.76 ± 0.68^{a}	0.63 ± 0.23^{a}
b*(-)	6.23 ± 0.63^{a}	4.61 ± 0,31^{ab}	3.21 ± 0.09^{b}	4.16 ± 1.02^{b}
ΔE (-)	-	5.49 ± 0.37^{a}	6.79 ± 0.70^{a}	4.02 ± 0.59^{b}
Firmness (N)	0.496 ± 0.006^{c}	0.667 ± 0.018^{b}	0.815 ± 0.024^{a}	0.868 ± 0.027^{a}
TPC (g GAE/100 g d.b)	0.011±0.003^{c}	0.024±0.002^{b}	0.027±0.001^{ab}	0.031±0.002^{a}
RSA (g equivalent Trolox/100 g of cheese)	0.080±0.031^{d}	0.162±0.010^{c}	0.340±0.018^{b}	0.679±0.021^{a}

Control: cheese not supplemented with olive leaves powder; *C1*: cheese supplemented with 0.5% of olive leaves powder; *C2*: cheese supplemented with 1.0% of olive leaves powder; *C3*: cheese supplemented with 1.5% of olive leaves powder. Means ± standard deviation (SD) of three separate determinations. %: g/100 g of cheese. Values with the same letter are not considered as significantly different at $p<0.05$.

The highest luminance value, L*, was obtained for the control cheese (14.04 ± 0.50) compared to the three cheeses supplemented with OLP. However, the addition of OLP leads to the decrease of the L* value from 8.80 ± 0.42 for C1 to 7.97 ± 0.74 for C2, whereas it is 10.68 ± 0.39 for C3. Carotenoids present in OLP are responsible for the b* value (yellowness/blueness); the b* value decreased by about 48.48% with OLP supplementation when compared to control cheese. This result could be explained by the richness of OLP in carotenoids. However, there was no difference between the three supplemented cheeses, whatever the added OLP, due to the lowest level of OLP added to the cheese. These results can be explained by the addition of OLP, characterized by a greenish and matte color leading to the diminution of the luminance (L*) and b* value of formulated cheese. On the other hand, the value of a* (green/redness) remained constant with the addition of different levels of OLP compared to the control cheese. Considering the values of the total difference of color ΔE of the cheese samples and according to Barba et al. [43], it can be noticed the presence of a slight difference (1.3) of color between C1 and C2, whereas this difference is noticeable between C1 and C3 (2.77). Therefore, we can conclude that the addition of different levels of OLP leads to a moderate change in cheese color.

Table 2 also shows that the control cheese presented a low TPC (0.011±0.003 g GAE/100 g d.b). However, the addition of OLP leads to an increase in the TPC, in order of 54.17%, 59.26%, and 64.52% for C1, C2, and C3, compared to the control cheese. The increase in the levels of OLP for 0.5% and 1.5% was proportional to the TPC and varied from 11.00% for C2 to 22.58% for C3, compared to C1. The same trend

was observed for the RSA of the four cheese samples, to reach the maximum 0.679±0.021 g Trolox Equivalent/100 g of cheese, for C3, compared to control cheese (0.080±0.031 g Trolox Equivalent/100 g). The increase in the percentage of OLP from 0.5 to 1.5% led to an increase in RSA (50.62 %, 76.47 %, and 88.21%, respectively, compared to control cheese). Therefore, OLP incorporation improved the antioxidant potential of supplemented cheeses. The textural results showed that the firmness of the control cheese was the lowest (0.496 N) compared to that of the other three supplemented cheeses. Moreover, the increase in the percentage of OLP led to a slight increase in the firmness of the cheese from 0.667 ± 0.018 N for C1 to 0.868 ± 0.027 N for C3. OLP supplementation improves cheese firmness and its antioxidant activity, and it can be used in food formulations [23].

Barukčić et al. [21] observed similar trends for physicochemical parameters and antioxidant activity assessed in yogurt fortified with olive leaf extract. It was reported that the extract affected the fermentation process and acidity of yogurt significantly, leading to a lower pH while maintaining the viability of starter cultures. Similarly, in this work, the supplementation of OLP did not significantly affect pH values, suggesting that olive leaf powder does not drastically interfere with acidification in cheese processing. The previous study also reported that water retention and syneresis in yogurt were affected depending on its techno-functional properties. In our case, the moisture content and dry matter of cheese remained stable, indicating that the structural matrix of cheese is more resilient to OLP supplementation than yogurt. Additionally, a significant increase was observed in total phenolic content and antioxidants. This effect was attributed to the richness of olive leaves in phenolic compounds, particularly oleuropein. These bioactive compounds can enhance the shelf life of dairy products by reducing lipid and protein oxidation [21].

Sensory profile: The color, odor, taste, texture, and overall appreciation of the different cheese formulations were assessed using hedonic and descriptive tests. The panel scores for the different cheese samples are presented in Table 3.

The control sample of cheese showed a significantly higher overall appreciation, with a score equal to 6.00 ± 1.93, compared to the three kinds of cheese supplemented with OLP. However, the appreciation decreases with the increase of the percentage of OLP in this order: 4.37 ± 1.41 for C1, 2.87 ± 0.83 for C2, and 1.62 ± 1.06 for C3, compared to the control sample.

The sensory profiles showed a significant difference in color among the four cheese samples. The addition of OLP leads to a change in the typical color of the control cheese sample. In addition, according to the panel's score, supplementation of cheese with 1.5% OLP causes a total change in cheese color since panelists give a high score for the green descriptor (8.25 ± 1.83), and a low score for the white descriptor (1.12 ± 0.64), compared to the other cheese samples (control cheese, C1, and C2). No significant effect was observed on the homogeneity of the experimental cheeses. This result can be explained by the good achievement of the homogenization step during cheese processing. Moreover, for lactic odor, a higher score was attributed to the control sample (8.37 ± 1.30) and C1

(6.62 ± 1.77), whereas, for vegetable odor, the C3 sample had the highest score (3.25 ± 3.06), compared to the other samples.

In addition, the C3 sample had a significantly higher bitterness score (4.37 ± 2.26) than C1 and C2. This result could be explained by the high level of OLP added to cheese (1.5%), which contains a high phenolic content, which is characterized by its bitterness.

Acidity score variations among different cheeses seem to be not significant. This result is in accordance with the results of pH, which were the same (4.95-5) for the four cheese samples. In fact, lactic acid bacteria degrade lactose by forming lactic acid and lowering the pH of milk, thus causing progressive demineralization of casein micelles. Coagulation was improved by adding rennet at an optimal pH activity of 5.5. As expected for a mixed coagulation, the pH of the four cheeses was higher than the casein isoelectric point (4.6). This indicates that the addition of OLP did not interfere with either lactic acid fermentation or rennet coagulation activity. These results suggested that OLP does not alter the key biochemical mechanisms responsible for milk coagulation, preserving the integrity of the cheese texture and the potential use of OLP as a functional ingredient without compromising the technological aspects of cheese manufacturing and the main quality attribute of the final product. However, a decrease in salty taste was detected for samples supplemented with OLP (2.00-2.62), compared to the control sample (3.12 ± 2.23), but it remained statistically insignificant at $p<0.05$.

The three cheese samples received significantly similar scores for texture (melting, sticking, granular, pasty, and viscous) compared to the control sample, except for the granular and unctuous descriptors, which were higher for C3 (Table 3).

The non-significant difference in texture between the different cheese samples is justified by the physicochemical properties, which have not changed too much. This result is consistent with the firmness values, which varied between 0.50 and 0.87 N (Table 2) for all cheese samples. In fact, acidity, moisture, and composition of the cheese affect the cheese texture.

Cheese without OLP and those supplemented with 0.5% OLP were considered the most appreciated formulation by the panelists for the “overall acceptability” parameter (6.00± 1.93 and 4.37 ± 1.41, respectively, for the control sample and C1). The sensory appreciation of cheese supplemented with 1 and 1,5% of OLP decreases due to the color and the taste alteration. The cheese taste became bitter and astringent, and it was not appreciated by the panelists. A negative correlation between phenol content and consumer acceptance was reported for dairy products. Phenol occurrence was reported to be associated with the precipitation of salivary glycoproteins and mucopolysaccharides on the tongue, and this results in roughness and dryness on the palate [33].

Undesirable sensory changes have been reported in the literature for fortified dairy products, primarily involving an untypical green color, off-flavors, bitterness, and astringency, which are associated with lower consumer acceptability.

Table 3. Sensory attribute ratings of cheese supplemented with the different levels of olive leaves (scores from 12 panelists)

Attributes	*Control*	*C1*	*C2*	*C3*
Overall acceptability	6.00 ± 1.93^{a}	4.37 ± 1.41^{ab}	2.87 ± 0.83^{bc}	1.62 ± 1.06^{c}
Color				
White	9.25 ± 1.75^{a}	4.37 ± 2.72^{b}	3.37 ±0.74^{bc}	1.12 ± 0.64^{c}
Green	0.00±0.00^{d}	2.50 ± 0.92^{c}	5.00 ± 2.07^{b}	8.25 ± 1.83^{a}
homogeneity	8.50 ± 1.69^{a}	6.75 ± 1.83^{a}	6.50 ± 1.93^{a}	6.25 ±2.05^{a}
Odor				
Lactic	8.37 ± 1.30^{a}	6.62 ±1.77^{ab}	4.12 ±2.47^{bc}	3.50 ± 2.45^{c}
Vegetable	0.00±0.00^{b}	2.62 ± 1.99^{ab}	2,62 ± 2.67^{ab}	3.25 ± 3.06^{a}
Olive	0.00±0.00^{b}	2.12 ± 1.80^{a}	2.00 ± 1.31^{a}	2.12 ± 2.59^{a}
Taste				
Acidity	5.75 ± 2.76^{a}	5.87 ± 1.81^{a}	5.12 ± 2.17^{a}	5.25 ± 1.98^{a}
Bitterness	0.00 ± 0.00^{b}	1.87 ± 1.46^{b}	2.12 ± 1.46^{b}	4.37 ± 2.26^{a}
Sweetness	5.50 ± 1.31^{a}	4.00 ± 1.31^{b}	2.87 ±0.99^{bc}	2.25 ± 1.39^{c}
Salty	3.12 ± 2.23^{a}	2.00± 1,85^{a}	2.62 ± 1.77^{a}	2.12 ± 1.73^{a}
Olive	0.00±0.00^{b}	1.75 ± 2.55^{a}	1.75 ± 2.37^{a}	2.62 ± 3.42^{a}
After taste	1.37 ± 1.68^{c}	3.00 ± 2.20^{bc}	4.75 ± 2.25^{b}	7.50 ± 2.45^{a}
Texture				
Melting	5.75 ± 2.60^{a}	5.62 ± 3.16^{a}	5.00 ± 2.98^{a}	4.75 ± 3.24^{a}
Sticky	4.75 ± 2.96^{a}	4.25 ± 2.81^{a}	4.75 ± 2.87^{a}	4.62 ± 3.07^{a}
Granular	0.50 ± 0.92^{b}	1.50 ± 1.93^{ab}	2.37 ± 2.26^{ab}	3.25 ± 2.71^{a}
Smooth	4.00 ± 2.20^{a}	5.44 ± 2.80^{a}	3.62 ± 1.68^{a}	2.75 ± 2.55^{a}
Pasty	1.62 ± 2.56^{a}	1.87 ± 2.36^{a}	1.50 ± 2.20^{a}	1.50 ± 2.00^{a}
Viscous	2.62 ± 3.20^{a}	2.37 ± 2.20^{a}	2.25 ± 2.60^{a}	2.00 ± 2.14^{a}

Control: cheese not supplemented with olive leaves powder; *C1*: cheese supplemented with 0.5% of olive leaves powder; *C2*: cheese supplemented with 1.0% of olive leaves powder; *C3*: cheese supplemented with 1.5% of olive leaves powder. Means ± standard deviation (SD) of three separate determinations. Values with the same letter are not considered significantly different at $p<0.05$.

This effect has been observed not only with olive leaf powder but also with green tea, coffee, and basil, which caused noticeable color alterations and bitter notes [34]. High concentrations were deemed unacceptable, whereas lower OLP inclusion maintained acceptable sensory characteristics without significant bitterness or off-odors. Further optimization may be needed to balance functionality and consumer preference.

As a solution for the bitterness and astringency induced by the high level of OLP (1%) in cheese, microencapsulation can be a good alternative to mask the astringent taste of OLP but also the granular appearance of cheeses. It can be used to protect phenolic compounds of the OLP against the harsh conditions prevailing during food processing and storage, as well as to avoid the alteration of the quality/sensory characteristics of the supplemented cheese.

CONCLUSION

Olive leaf powder possesses good water and oil retention capacities (1.963 ± 0.730 g water/g DM; 2.066 ± 0.160 g oil/g DM, respectively) and a noticeably high milk retention capacity (2.479 ± 0.292 g milk/g DM). Olive leaves are also rich in total phenols (3.235 ± 0.247 g GAE/100 g of powder), which gives them a percentage of inhibition of DPPH of 82.91 ± 0.79%. The characterization of the formulated cheeses has shown that the addition of OLP led to a decrease in fat content and a slight change in ash contents compared to that of the control. The pH, dry matter, moisture, and protein content have remained the same for the formulated cheeses. The supplemented formulations are rich in phenols which increase with the increase of the percentage of OLP: 54.17%, 59.26%, and 64.52%; for cheeses supplemented with 0.5, 1, and 1.5%, respectively. Sensory analysis has shown that some sensory descriptors have been significantly changed, such as color, odor, taste, and aftertaste. According to sensory analysis, the fresh cheese supplemented with 0.5% olive leaf powder seems to be the most appreciated supplemented cheese. In future studies, it would be valuable to assess the effect of olive leaf powder on the shelf life of the final product compared to the control, to better understand its potential as a natural preservative.

List of Abbreviations: OLP, olive leaves powder**;** OL, Olive leaves; WRC, Water retention capacity; MRC, milk retention capacity; ORC oil retention capacity; TPC, Total phenol contents; RSA, Radical Scavenging Activity; GAE, gallic acid equivalent; ΔE, total color difference.

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