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Effect of marination with *Eruca vesicaria longirostris* leaves on Turkey meat properties during storage and consumer acceptance.

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Abstract - Marinating of meat with *Eruca vesicaria* fresh leaves and with ethanolic extract were prepared. Physicochemical, color, microbiological and sensorial analyses were evaluated for marinating meat during 8 days at 4°C. Results showed that marinated meat with 1,5% of fresh *Eruca* leaves and with 800 ppm of ethanolic extract has the highest consumer acceptance. Physicochemical analysis showed that meat marinated with 1,5% fresh *E. vesicaria* leaves and 1000 ppm of ethanolic extract presented microbiological, physicochemical color and sensorial properties improved. These findings showed that presence of *Eruca* leaves with an important percentage of addition may improve meat quality. In other hand, results indicated that ethanolic extract was more effectives than fresh leaves for this improvement. Microbiological analysis results of cheese showed that the *E. vesicaria* leaves have inhibitory activity against coliforms, total aerobic mesophilic flora, psychrotropic flora, staphyloccus flora and yeast and molds which highlights the potential of leaves as antibacterial agent. The results of the sensory analysis showed that samples marinated with 800 ppm extract and with 1.5% leaves is the most appreciated by consumer. The Pearson correlation test has shown that there is a significantly positive correlation between moisture, color parameters, descriptors and the overall appreciation of meat. This indicates that when moisture content increase and color of meat is more reddish, descriptors scores and overall appreciation of consumer are improved.

Key words: meat, *Eruca vesicaria*, physicochemical, microbiological, consumer acceptance.

Introduction

Rocket salads include different species like *Eruca vesicaria* belonging to Brassicaceae vegetables (Kyung and Lee, 2001; Brandi *et al.*, 2006). Salad rocket has been grown in the Mediterranean area since Roman times and are being presently extensively cultivated in various places for commercial purposes (Pasini *et al.*, 2012).The major antimicrobial activity of Brassicaceae vegetables has been shown to be due to glucosinolates and other volatile sulfur compounds (Kyung and Lee, 2001). The food-borne bacteria tested were also significantly reduced by isothiocyanates, abundant natural components in the Brassica genus, indicating that isothiocyanates derived from glucosinolate may play a major role in the antimicrobial activity (Brandi *et al.*, 2006).Rocket salad is widely consumed fresh by human , as salad or mix of salad, or prepared as a steamed vegetable or used as a spice or food ingredient in Middle Eastern and European countries (Bouacida *et al.*, 2017).

Previous researches have been carried out to provide evidence that the rocket possesses antibacterial (Gulfraz *et al.*, 2011) and anti-cancer like melanoma properties (Khoobchandani *et al.*, 2011). Phytochemistry analyses revealed that rocket leaves and seeds present high content of human and animal health-promoting compounds, mainly antioxidants and glucosinolates with proven pharmaceutical properties (Bouacida *et al.*, 2017).This study is focused marination with fresh *Eruca vesicaria* leaves and ethanolic extract to improve food quality of turkey meat during storage and consumer acceptance.

The aims of the present study were the evaluation of microbiological, physicochemical, color and sensorial characteristics of turkey meat marinated with Eruca *vesicaria* leaves and ethanolic extract.

Materials and methods

1- Materials and sample preparation

Eruca vesicaria longirostris leaves were harvested from Tunisia Kasserine between 25 March and 15 April 2016. Mature leaves were collected randomly from open field where the plant grows spontaneously (from the fields of olive plants or from roadsides). Samples were immediately used as fresh leaves for marinating meat or dried at 40°C for ethanolic extract.

2- Preparation of ethanolic extracts

The ethanolic extract was carried out according to the method used by (Bouacida *et al.*, 2016a). 2 g of of *E. vesicaria* samples, dried and ground into a fine powder, were extracted with 20 ml of ethanol (80%) at room temperature at 150 rpm, in a shaker, for 72 h. The extract was concentrated to dryness under reduced pressure in a rotary evaporator at 40°C. The dried etanolic extract was used to prepare solutions at different concentrations for marinating of meat.

3- Physicochemical analysis

3.1-Water-holding capacities (WHC)

WHC was determined according to the method used by (Rawdkuen *et al.*, 2013). Minced meat (20 g) was placed in a centrifuge tube containing 30 ml of 0.6MNaCl and was stirred with a glass rod for 1 min. The tube was then kept at 4 ± 1°C for 15 min, stirred again, and then centrifuged at 3000g (Hettich universal 32, D-78532, Germany) for 25 min. The supernatant was measured, and the WHC was expressed as a percentage of initial volume.

3.2- Moisture content

The moisture content of the samples was determined according to the Association of Official Analytical Chemists (AOAC) method No. 950.46 (2000). 2 g of meat were crushed and put in a petri dish previously tarred. Samples were placed in an oven at 103 ° C for 24 hours. The water content is expressed in percentage (Rawdkuen *et al.*, 2013).

3.3-pH

The pH of samples was determined according to (Rawdkuen *et al.*, 2013). To determine pH, 10 g of the sample were homogenised with 50 ml of chilled distilled water. The pH values were measured with a digital pH meter (Consort PH C860, Belgium)

4- Color measurements

Surface color measurements of meat were performed using a colorimeter (Minolta CR-300, Japan) according to (Rodríguez-Carpena *et al.*, 2011). The instrument was calibrated using the standard white file. Color measurements were made on the surface of each sample in triplicate on three randomly selected locations. Color measurements were made at room temperature (≈25 °C). The meat samples color is expressed as chromatic ordinates a*, b* and L*.

5- Microbiological analysis

Ten grams of meat samples were added aseptically to 90 mL of sterile peptone saline diluents and homogenized in a stomacher (Cadun *et al.*, 2008). Total aerobic mesophile bacteria count (TMAB) and psychotropic bacteria (PSY) were determined on plate count agar (PCA, Merck, Germany) after incubation at 30 °C for 48 h to 72 h and at 4°C for 10 days, respectively (Bachtarzi, et al., 2015). Lactic bacteria (LAB) count was carried out in molten de Man Rogosa Sharpe agar (MRS, Biolife 401728). The incubation was carried out at 37 °C for 72 h (Cadun *et al.*, 2008). Total coliforms (TC) was counted on Deoxycholate Lactose agar (DCL, Merck, Germany) incubated at 37 °C during 24 h (Bachtarzi *et al.*, 2015). Yeasts and molds (Y/M) count was were carried out in Potato Dextrose Agar (PDA, Biolife, Milano, Italia) at 30 °C for 3-5 days. Staphylococcus bacteria (STAPH) count was determined on Chapman agar (Chapman, Merck, Germany) after incubation at 37 °C for 48 h (Cadun *et al.*, 2008). Three samples were microbiologically examined for each treatment.

6- Sensory evaluation

All cooked meat samples were prepared for sensory evaluation. The sensory analysis was performed by a hedonic test by ranking of appreciation of tested product. This test measures consumer pleasure and / or satisfaction experienced at the sight or consumption / use of a product. Six meat samples with ethanolic extract and four samples with fresh leaves of *Eruca vesicaria* L. were evaluated for appearance, flavor, taste, texture and overall liking by 60 untrained panelists using 7-point hedonic scales, where 7 = extremely like and 1 = extremely dislike (Kaur *et al.*, 2011; Bouacida *et al.*, 2016b)**.**

7- Statistical analysis

A Microsoft Excel 2007 was used for determination of means and standard deviations were determined for all the physicochemical, microbiological and sensory qualities studied. The significant difference of mean values was assessed with one-way analysis of variance (ANOVA) followed by Tukey's test using SPSS software version 23, at a significance level of ($P < 0.05$).

Results and discussion

1- Physicochemical analysis

Water-holding capacities (WHC)

The initial values of water holding capacities (WHC) are between (64.47-74.17%) and (64.47-72.23%) for the meat marinated with ethanolic extract and fresh leaves, respectively (Table 1, 2). These results are similar to those found by Jayasena et al. (2013)on poultry meat, which showed values in the range of (61.36-62.89%). In other hand, (Rawdkuen *et al.*, 2013) have presented lower values of WHC for poultry meat between 20 and 22%. These differences can be due to the overall reduction in the protein reactive group, which is available for water binding or to the difference between samples of meat (Rawdkuen *et al.*, 2013). The one-way ANOVA test shows that the WHC values for each sample are significantly different. Indeed, the WHC decreases according to storage time at 4 ° C to reach values between (30.33-66.47%) and (30.33-64.94%) for meat with ethanolic extract and those with fresh leaves, respectively. The denaturation of meat proteins, which has an important role in determining WHC, could be the reason for decreased WHC (Joo,N S. T., Kauffman, R. G., Kim, B. C., & Park, 1999).Similar results were reported by (Qiao *et al.*, 2001)who presented values between 38.50 and 51.73%. Statistical analysis showed that there is a significant difference between the samples for each storage time. According storage time, samples with 600, 800 and 1000 ppm ethanolic extract have more stable WHC values. Samples with 1.5% fresh leaves have the most stable values (Table 1, 2).Indeed, its shows that *Eruca* leaves preserve the quality of meat proteins, this can be related to previously provide antioxidant activity reported by (Bouacida *et al.*, 2016a).

Moisture content

Moisture values of meat samples are in range of (82.00- 82.60%) and (82.00-82.10%) for marinating with the ethanolic extract and fresh leaves respectively (Table 1, 2). Similar results for poultry meat have been reported by (Qiao *et al.*, 2001) and Rawdkuen et al. (2013) on the order of (76.23 -76.72%) and 82.28% respectively. Moisture decreases with storage time at 4 ° C to reach values of (69.84-74.27%) and (70.01-78.88%) for meat samples with ethanolic extract and fresh leaves, respectively. Statistical analysis showed that there is a significant difference between samples for each analysis time except day 0. During storage, samples with 800 ppm, 1000 ppm extract and 1.5% fresh leaves have the highest values of moisture (Table 1, 2). These results confirm those presented for the water holding capacity. The quality of the meat is preserved with high ethanolic extract or leaves supplementation this can be due to the proteins structure preservation and the antioxidant properties of this plant (Joo,N S. T., Kauffman, R. G., Kim, B. C., & Park, 1999; Bouacida *et al.*, 2016a).

pH analysis

pH values are (5.62-6.37) and (5.62-6.37) for meat samples marinated with ethanolic extract and with fresh leaves, respectively (Table 1, 2). These results are similar with previous studies of poultry meat (Qiao *et al.*, 2001; Jayasena *et al.*, 2013; Rawdkuen *et al.*, 2013; Radha Krishnan *et al.*, 2014) who reported values in the range of (5.72-6.00), (5.81-6.23), 5.5 and 5.5, respectively. The analysis of the variance shows that pH values for each sample are significantly different at the 0.05 threshold. The pH increases according to storage time at 4 ° C to reach values in range of (6.83 - 8.11) and (6.61 -8.11) for meat marinated with ethanolic extract and leaves, respectively (Table 1, 2). Similar results have been reported by Radha Krishnan et al. (2014)who found that pH increased from 5.5 to 7 for 15 days of

storage at 4 ° C. Statistical analysis showed that there is a significant difference at the 0.05 threshold between samples for each analysis time. During storage, the control sample has the highest pH value. On the 8th day, samples with 800 ppm, 1000 ppm extract and 1.5% fresh leaves have the lowest values. These results can be related to antibacterial activity of *Eruca* leaves reported by (Gulfraz *et al.*, 2011). Indeed, *E. vesicaria* prove to be very effective against Gram-positive and Gram-negative bacteria and fungi (Kyung and Lee, 2001; Brandi *et al.*, 2006).

Table 1: Physicochimical analyses of marinated meat with *E. vesicaria* leaves ethanolic extract

Storage time (Days)	0	2	4	6	8
			WHC (%)		
0ppm	64,47±0,45^{Ca}	52,00±0,20^{Bb}	50,01±0,02^{Cb}	45,00±0,25^{Dc}	30,33±2,00^{Fd}
200ppm	65,67±0,20^{Ba}	53,65±0,14^{Bb}	51,65±0,02^{Bc}	46,65±0,14^{Cd}	41,68±0,01^{Ee}
600ppm	73,98±0,14^{Aa}	72,02±0,03^{bA}	69,83±0,29^{Ac}	65,01±0,02^{Bd}	49,98±0,14^{De}
800ppm	73,67±0,57^{Aa}	72,02±0,04^{Ab}	70,00±0,20^{Ac}	65,02±0,04^{Bd}	58,33±0,25^{Ce}
1000ppm	74,00±0,20^{Aa}	71,33±9,81^{Aa}	71,00±1,00^{Aa}	70,01±0,02^{Ba}	63,43±0,17^{Ba}
200 ppm VIT C	74,17±0,29^{Aa}	72,00±0,25^{Ab}	70,00±0,20^{Ac}	65,02±0,02^{Ad}	66,47±0,35^{Ae}
			H (%)		
0ppm	82,00±0,30^{Aa}	78,53±0,14^{Cb}	73,53±0,14^{Ec}	71,53±0,50^{Bd}	70,01±0,40^{Ce}
200ppm	82,10±0,50^{Aa}	78,84±0,50^{BCb}	74,25±0,40^{DEc}	71,84±0,30^{Bd}	69,84±0,15^{Ce}
600ppm	82,60±0,10^{Aa}	79,14±0,30^{BCb}	75,29±0,10^{BCc}	72,14±0,40^{Bd}	70,14±0,10^{Ce}
800ppm	82,00±0,50^{Aa}	79,68±0,50^{ABb}	75,96±0,40^{ABc}	74,68±0,50^{Ad}	72,68±0,30^{Be}
1000ppm	82,30±0,15^{Aa}	80,27±0,14^{Ab}	76,47±0,50^{Ac}	75,27±0,30^{Ad}	74,27±0,14^{Ae}
200 ppm VIT C	82,45±0,10^{Aa}	78,98±0,40^{BCb}	75,00±0,10^{CDc}	71,98±0,14^{Bd}	69,98±0,50^{Ce}
			pH		
0ppm	6,37±0,15^{Ad}	6,64±0,15^{Acd}	6,87±0,15^{Ac}	7,32±0,15^{Ab}	8,11±0,15^{Aa}
200ppm	5,59±0,11^{Bd}	5,86±0,11^{Bcd}	6,09±0,11^{Bc}	6,54±0,11^{Bb}	7,33±0,11^{Ba}
600ppm	5,62±0,04^{Be}	5,89±0,04^{Bd}	6,12±0,04^{Bc}	6,57±0,04^{Bb}	7,36±0,04^{Ba}
800ppm	5,63±0,04^{Be}	5,90±0,04^{Bd}	6,13±0,04^{Bc}	6,43±0,04^{Bb}	6,83±0,04^{Ca}
1000ppm	5,68±0,05^{Be}	5,95±0,05^{Bd}	6,18±0,05^{Bc}	6,48±0,05^{Bb}	6,88±0,05^{Ca}
200 ppm VIT C	5,75±0,14^{Bd}	6,02±0,14^{Bcd}	6,25±0,14^{Bc}	6,70±0,14^{Bb}	7,10±0,14^{BCa}

Mean (n = 3) ± SD; Means of WHC, H and pH, with different lower-case superscripts (a-c) in each row or with different capital superscripts (A-F) in each column, are significantly different at α = 0.05 (one-way ANOVA and Tukey's test. a or A—the highest content); Ethanolic extract was used with different content: 200, 600, 800 et 1000 ppm; **WHC**: water holding capacity, H : moisture

Table 2: Physicochimical analyses of marinated meat with *E. vesicaria* fresh leaves

Storage time (Days)	0	2	4	6	8
			WHC (%)		
0%FF	64,47±0,45^{Ca}	52,00±0,20^{Db}	50,01±0,02^{Db}	45,00±0,25^{Dc}	30,33±2,00^{Cd}
0,5%FF	58,98±0,14^{Da}	57,02±0,03^{Cb}	55,00±0,25^{Cc}	53,00±0,25^{Cd}	50,00±0,25^{Be}
1%FF	71,52±0,03^{Ba}	69,51±0,02^{Bb}	67,48±0,14^{Bc}	65,50±0,20^{Bd}	50,07±0,12^{Be}
1,5%FF	72,23±0,17^{Aa}	70,33±0,25^{Aa}	68,23±0,17^{Ab}	66,31±0,14^{Abc}	64,94±1,59^{Ac}
			H (%)		
0%FF	82,00±0,30^{Aa}	78,53±0,14^{Cb}	73,53±0,14^{Dc}	71,53±0,50^{Cd}	70,01±0,40^{De}
0,5%FF	82,06±0,40^{Aa}	80,58±0,30^{Bb}	75,13±0,14^{Cc}	73,58±0,10^{Bd}	71,58±0,14^{Ce}
1%FF	82,04±0,50^{Aa}	81,63±0,50^{Aa}	76,63±0,15^{Bb}	73,79±0,15^{Bc}	72,63±0,15^{Bd}
1,5%FF	82,10±0,40^{Aa}	81,99±0,30^{Aa}	79,88±0,10^{Ab}	78,94±0,30^{Ac}	78,88±0,30^{Ac}
			pH		
0%FF	6,37±0,15^{Ad}	6,64±0,15^{Acd}	6,87±0,15^{Ac}	7,32±0,15^{Ab}	8,11±0,15^{Aa}
0,5%FF	5,62±0,03^{Be}	5,89±0,03^{Bd}	6,12±0,03^{Bc}	6,57±0,03^{Bb}	7,56±0,03^{Ba}
1%FF	5,63±0,09^{Bd}	5,90±0,09^{Bc}	6,13±0,09^{Bc}	6,58±0,09^{Bb}	7,57±0,09^{Ba}
1,5%FF	5,65±0,14^{Bd}	5,92±0,14^{Bcd}	6,15±0,14^{Bbc}	6,45±0,14^{Bab}	6,61±0,14^{Ca}

Mean (n = 3) ± SD; Means of WHC, H and pH, with different lower-case superscripts (a-c) in each row or with different capital superscripts (A-F) in each column, are significantly different at α = 0.05 (one-way ANOVA and Tukey's test. a or A—the highest content); Fresh leaves was supplemented with different content: 0; 0,5; 1; and 1,5 %; **WHC** : water holding capacity, H : moisture.

2- Evaluation of color parameters

The initial values of the L * parameter are between 37.5 and 47. These values are similar to those reported by previous studies of poultry meat (Qiao *et al.*, 2001; Wang *et al.*, 2009; Panea *et al.*, 2014; Radha Krishnan *et al.*, 2014) who have presented values in range to 50, (43-55), 56, (57-54), respectively. L * parameter values are significantly different ($p<0,05$). Indeed, L * increases with storage time to reach values between 45 and 51.5. Comparative results have been reported by (Panea *et al.*, 2014)who found that L * increased from 50 to 54 for 7 days of storage at 4 ° C. In addition, Qiao et al. (2001)showed that L * of poultry meat increases from 43 to 45 during storage. Meat samples of each time of analysis were significantly different. The control sample has the lowest values of the L * parameter. Samples with 1000 ppm of extract and 1.5% fresh leaves present the highest values throughout the storage (Figure 1). Indeed, samples marinating with the highest content of leaves or extract present the lightest-colored.

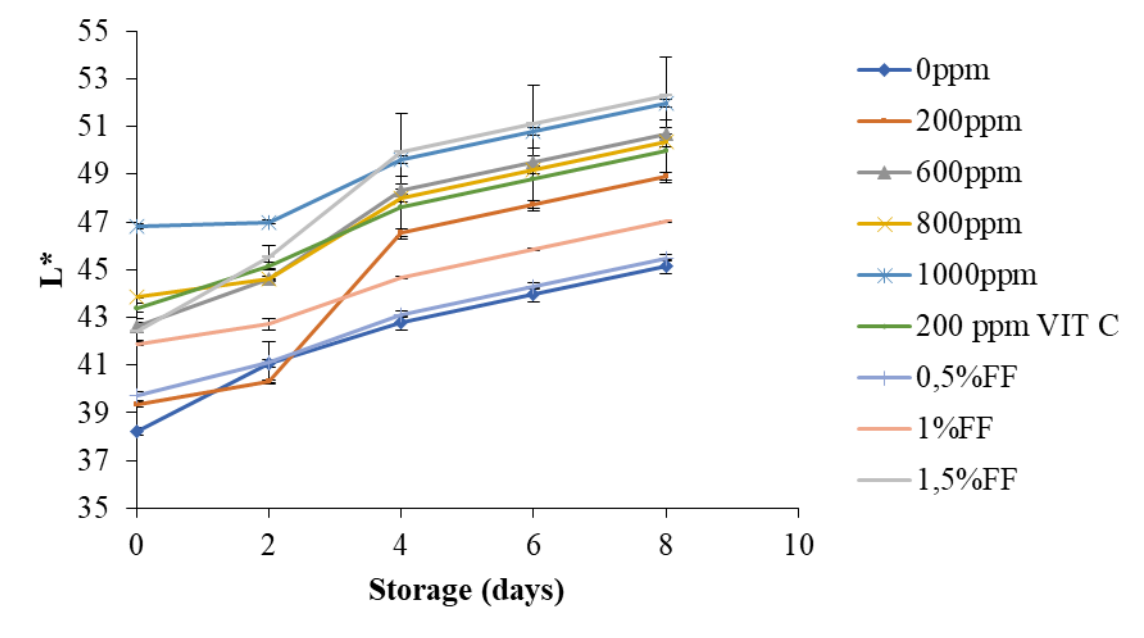

Figure 1 : *E. vesicaria* marination effect on L* according to storage time at 4 °C with fresh leaves (FF) and ethaolic extract with different content (0-1000 ppm).

Values of a * parameter are positive which gives meat samples a reddish coloring. The values of the b * component, which shows the change in color from yellow to blue, are also positive, indicating a yellowish coloration of the meat samples. The combination of the two parameters a * and b * on the a * b * plane of the CIE LAB system gives rise to a light red coloration. The initial values of the parameter a * are of the order of (5.68-5.91). These values are higher than those reported by (Qiao *et al.*, 2001; Wang *et al.*, 2009; Panea *et al.*, 2014)which ranged 0.8; 4 and (2.18-2.98), respectively. a * increases according to the storage time to reach values between 6 and 10. A similar evolution of the parameter a * during storage time at 4 ° C has been observed in different previous studies (Qiao *et al.*, 2001; Panea *et al.*, 2014).The control sample has the lowest values of the parameter a *. Values of parameter b * are ranged from (2) to (4-8) at the 8th day of storage. These values are significantly different according time of storage. Comparative results were reported by Wang et al. (2009).In other hand, Qiao et al. (2001) found that b * increased from 3 to 4 during 24 h storage at 4 ° C. The control sample (no added leaves or extract) has the highest b * values except for day 0. Ethanolic extract and fresh leaves with high level of supplementation may have a color stabilizing effect. In fact, the samples marinated with 800 and 1000 ppm of ethanolic extract followed by the sample with 1.5% fresh leaves have the lowest values of a* and b* during all the storage time (Figure2,3).

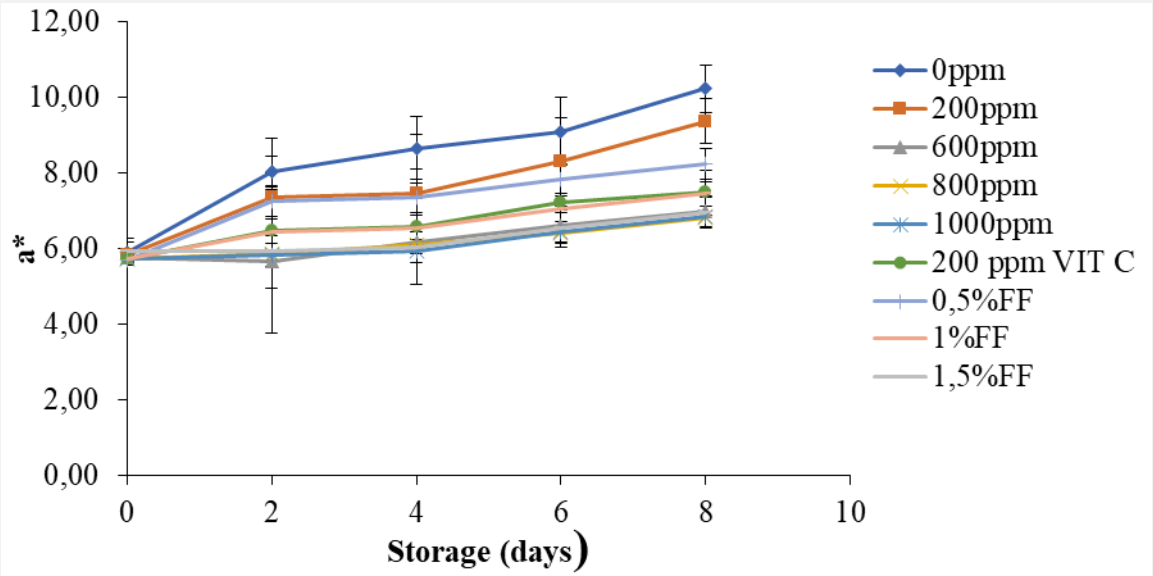

Figure 2 : *E. vesicaria* marination effect on a* according to storage time at 4 °C with fresh leaves (FF) and ethaolic extract with different content (0-1000 ppm).

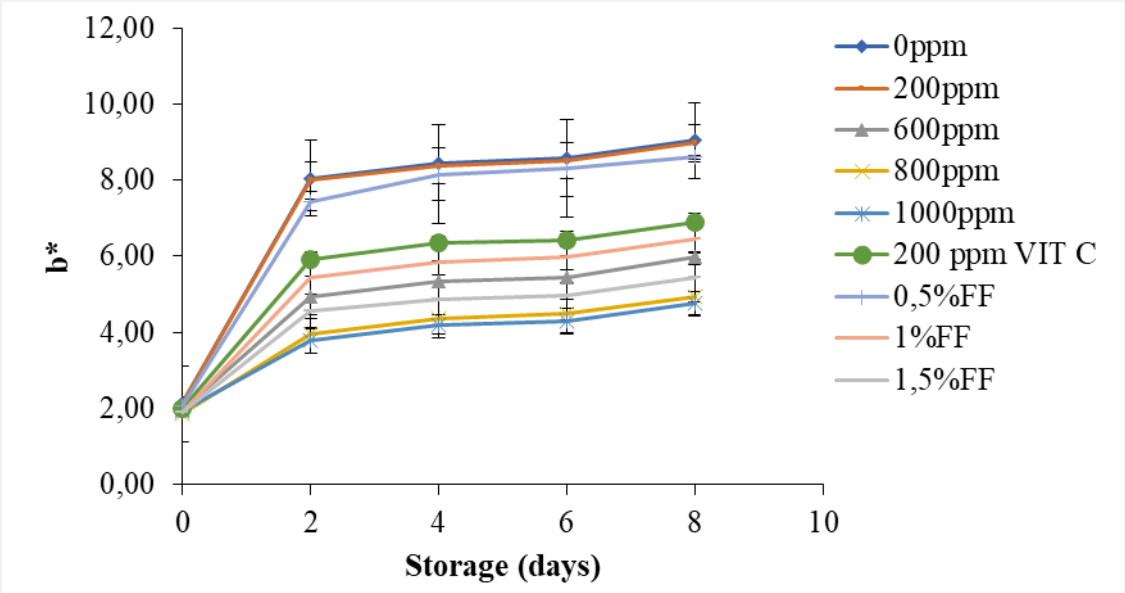

Figure 3 : *E. vesicaria* marination effect on b* according to storage time at 4 °C with fresh leaves (FF) and ethaolic extract with different content (0-1000 ppm).

3- Microbiologic Analyses

The initial TMAB count is ranged 10^3 CFU / ml. Similar results have been reported by Panea et al. (2014)and Higueras et al. (2014). Statistical analysis showed that there was a significant difference ($p < 0.05$) between TMAB counts for each samples according storage time. Indeed, the mesophilic flora count increases during storage time. At the 8th day it is between 3.80×10^5 and 3.34×10^6 CFU / ml for the samples with extract and between 1.34 and 2.34×10^6 CFU / ml for those marinated with fresh leaves (Table 3 and 4). Panea et al. (2014)have reported comparative results so they showed that TMAB count increases from 10^3 to 10^5 CFU / ml in 8 days of storage at 4 ° C. In addition, Higueras et al. (2014) showed that the load of TMAB increases over storage time in refrigerator to reach 10^7 CFU / ml. In other hand results showed that samples marinated with different percentage of ethanolic extract were significantly different at the 0.05 level, except for day 0 and 8 (Table 3). Indeed, the sample with 1000 ppm of ethanolic extract has the lowest load of TMAB, so its development is slowed down according to storage time. In fact, this bacterial load reaches 10^5 on the 6th day and on the 2nd day for the sample with 1000 ppm and the control respectively (Table 3). Statistical analysis (Table 4) shows that the

sample marinated with 1.5% of fresh leaves has the lowest TMAB load ranged 1.34×10^6 CFU / ml at the end of storage. This sample reached 10^5 CFU / ml on the fourth day, while the others samples reached this bacterial load in the second day. The slow development of these bacteria is more important in the presence of 1000 ppm of extract than 1.5% of fresh leaves.

The initial load of lactic bacteria (LAB) is between 10^3 and 10^4 CFU/ml. Similar results have been presented in previous studies on poultry meat ((Radha Krishnan *et al.*, 2014) ; (Higueras *et al.*, 2014)). Statistical analysis showed that the load of lactic bacteria increases during the storage time (Tables 3 and 4). On the 8th day, values of LAB were ranged between (6.00 - 6.78 log CFU / ml) and (5.00 - 6.78 log CFU / ml) for samples marinating with ethanolic extract and fresh leaves, respectively (Tables 3 and 4). Radha Krishnan et al. (2014) have showed, in a previous research about poultry meat that the LAB loading increases from 4.25 to 5.25 log CFU / ml in 8 days of storage at 4 ° C. In addition, Higueras et al. (2014) have showed that LAB loading increases according to storage time at 4°C to reach 10^5 CFU / ml. In other hand, sample with 1000 ppm of the ethanolic extract and 1.5% of fresh leaves have the lowest load of LAB at the 8th day, with values ranged 6.00 and 5.00 log CFU / ml (Tables 3 and 4), respectively. These samples were followed by those with 800 ppm and 600 ppm of ethanolic extract (6.48 log CFU / ml) (Tables 3 and 4). These results can be explained by the antimicrobial activity of *Eruca* leaves (Gulfraz *et al.*, 2011).

Total coliforms (TC) have an initial load ranged (2.75-3.82 log CFU / ml). (Javanmard *et al.*, 2006)showed a higher initial load of 10^7 CFU / ml for poultry meat stored at -18 ° C for 9 months with different irradiation doses. Panea et al. (2014)showed that enterobacteria values increased from 10^2 to 10^4 after eight days of storage at 4°C for poultry. TC load increases significantly according storage time from (2.74- 3.82 log CFU / ml) to (5.48 - 5.11 log CFU / ml) and from (2.75 -2. 90 log CFU / ml) at (5.20-5.48 log CFU / ml), for samples with ethanolic extract and fresh leaves respectively (Tables 3 and 4). Statistical analysis of the samples in each time of storage showed that there is a significant difference, especially for day 2 and 4. At the 4th day samples with 1000 ppm of ethanolic extract and 1.5% of fresh leaves have the lowest TC count ranged to 4.08 and 4.40 Log CFU / ml, respectively. These results showed the inhibition effect of *Eruca* leaves and ethanolic extract. This inhibition effect increases with concentration of ethanolic extract or percentage of fresh leaves added. Indeed, the load of TC for sample with 1000 ppm reaches 10^5 CFU / ml at the 8th day, whereas the other samples with extract and control reach this load at the 6th day and the second day, respectively. On the other hand, samples with 1% and 1.5% of fresh leaves added reach this same bacteria count on the 6th day (Tables 3 and 4).

The initial staphylococcus (STAPH) count was (3.32 - 3.88 Log UFC / ml) and (3.11 - 3.88 Log UFC / ml) for samples with fresh leaves and ethanolic extract, respectively. The load of STAPH increases during the storage time to reach, at the 8th day, values between (4.85 - 5.77 log CFU / ml) and (5.23 - 5.77 log CFU / ml) for samples with ethanolic extract and fresh leaves, respectively (Tables 3 and 4). There is a significant difference, between samples for all times except for day 0 and 4 (Tables 3 and 4). In fact, sample with 1000 ppm of ethanolic extract and 1.5% of fresh leaves have the lowest STAPH count at the 8th day, 4.85 and 5.23 log CFU / ml, respectively. Ethanolic extract slows the development of staphylococci more than fresh leaves. In fact, the inhibition of STAPH count with ethanolic extract increase with quantity of extract or leaves added.

Initial psychrotropic count (PSY) is between 10^2 and 10^3 CFU / ml. Similar results were presented by Higueras et al. (2014)for poultry kept for 9 days at 4 ° C. Statistical analysis showed that the load of psychrotropic bacteria increases during storage time to reach, at the 8th day, values between (5.41 - 6.78 Log CFU / ml) and (6.02 - 6.78 Log CFU / ml) for the samples with ethanolic extract and fresh leaves, respectively (Tables 3 and 4). These results are comparative to those of Higueras et al. (2014) who showed that the psychrotrophic load increased from 10^2 to 10^6 CFU / ml in 9 days. Statistical analysis showed that there is a significant difference between samples according concentration of ethanolic extract added. Ethanolic extract at 1000, 800 and 600 ppm present the important inhibition activity. For the 8th day, sample with 200 ppm of vitamin C has the lowest load in psychrotrophs followed by samples with 1000 ppm, 800 ppm and 600 ppm (Tables 3and 4). Samples with 1.5% fresh leaves had the lowest psychrotrophic bacteria count at the 8th day 6.02 log CFU / ml (Table 4). The slowing down of psychrotrophic development is more important for the sample with 1000 ppm which reaches the load of 5.94 log UFC / ml at the 8th day.

The initial yeast and mold load (Y/M) is between 1.98 and 2.15 log CFU / ml. Higueras et al. (2014) showed a similar initial load 2.5 log CFU / mL for poultry meat kept in the refrigerator for 9 days. The load of Y/M increases according storage time to reach, on the 8th day, values of (5.04 - 6.70 log CFU / ml) and (4.30 - 6, 70 log CFU / ml) for the samples with ethanolic extract and fresh leaves, respectively

(Tables 3 and 4). These results are comparative to those of presented by Higueras et al. (2014) who showed that the load in Y/ M increases from 2.5 to 5.00 Log CFU / ml in 9 days of storage at 4 ° C. In other hand, statistical analysis showed that there is a significant difference, between samples for all time except for day 0 (Tables 3 and 4). Samples with 1000 ppm of ethanolic extract and 200 ppm of vitamin C have the lowest Y/M bacteria count 5.53 and 5.04 log CFU / ml, respectively (Table 3). The sample with 1.5% fresh leaves has the lowest load at the 8th day, ranged of 4.30 log CFU / ml (Table 4).
In this study, results showed that the increase of slowdown of development of all bacteria tested can be related with the increase of ethanolic extract or leaves supplementation in marination of meat. In other hand, statistic analysis showed that the ethanolic extract may be more effective as anti-microbial ingredient supplemented on meat (Tables 3 and 4). This can be explained by the fact that antimicrobial compounds, such as phenolic compounds, present in the plant, are more effective in protecting meat when using the ethanolic extract than minced leaves. Indeed, phenolic acids are found in plants as metabolic intermediates and accumulate in vacuoles (Chism and Haard, 1996)and their release requires a mechanical action to burst plant cells or a thermal cellular aggression (Oh *et al.*, 2010; Memon *et al.*, 2012).

Table 3: Microbiological analyses for meat marinating with ethanolic extract according to storage time

	Storage time (Days)	**0**	**2**	**4**	**6**	**8**
	Echantillons					
TMAB (log UFC/ml)	0ppm	3,67±0,10^{Ac}	5,51±0,20^{Ab}	5,60±0,20^{Ab}	6,34±0,20^{Aa}	6,37±0,10^{Aa}
	200ppm	3,57±0,10^{Ac}	5,03±0,10^{Bb}	5,26±0,02^{Bb}	6,21±0,10^{ABa}	6,25±0,50^{Aa}
	600ppm	3,54±0,15^{Ac}	4,96±0,01^{Bb}	5,23±0,01^{Bb}	6,17±0,50^{ABa}	6,20±0,40^{Aa}
	800ppm	3,52±0,18^{Aa}	4,62±0,10^{Cb}	5,08±0,02^{Bb}	5,88±0,01^{ABa}	5,94±0,50^{Aa}
	1000ppm	3,41±0,23^{Ab}	4,08±0,20^{Db}	4,95±0,01^{Ba}	5,41±0,50^{Ba}	5,58±0,40^{Aa}
	200ppm VitC	3,59±0,08^{Ac}	5,55±0,10^{Ab}	5,64±0,20^{Ab}	6,21±0,30^{ABa}	6,25±0,10^{Aa}
LAB (log UFC/ml)	0ppm	4,00±0,10^{Ac}	6,36±0,10^{Ab}	6,40±0,01^{Aab}	6,68±0,10^{Aab}	6,78±0,30^{Aa}
	200ppm	3,90±0,10^{Ab}	6,18±0,10^{ABa}	6,23±0,30^{Aa}	6,58±0,05^{Aa}	6,60±0,30^{ABa}
	600ppm	3,87±0,15^{Ac}	6,04±0,01^{BCb}	6,11±0,20^{Ab}	6,45±0,01^{Aa}	6,48±0,11^{AB}
	800ppm	3,95±0,18^{Ac}	5,78±0,10^{Cb}	5,90±0,01^{ABb}	6,45±0,20^{Aa}	6,48±0,10^{ABa}
	1000ppm	3,83±0,21^{Ac}	4,30±0,20^{Dbc}	5,00±0,20^{Cb}	4,30±0,30b^{Cc}	6,00±0,50^{Ba}
	200ppm VitC	3,92±0,08^{Ad}	5,00±0,10^{Cc}	5,48±0,30^{BCb}	5,90±0,10^{Bab}	6,00±0,11^{Ba}
TC (log UFC/ml)	0ppm	2,90±0,10^{Ab}	5,02±0,20^{Aa}	5,09±0,01^{Aa}	5,42±0,50^{Aa}	5,48±0,12^{Aa}
	200ppm	2,80±0,10^{Aa}	4,49±0,10^{Bb}	4,71±0,20^{ABb}	5,27±0,10^{Aa}	5,36±0,11^{Aa}
	600ppm	2,77±0,15^{Ac}	4,20±0,01^{BCb}	4,56±0,30^{ABCb}	5,23±0,30^{Aa}	5,32±0,12^{Aa}
	800ppm	2,75±0,18^{Ad}	3,85±0,10^{Cc}	4,43±0,20^{BCb}	5,16±0,30^{Aa}	5,26±0,10^{Aa}
	1000ppm	2,74±0,21^{Aa}	2,48±0,10^{Da}	4,08±0,30^{Cb}	4,94±0,05^{Aa}	5,11±0,50^{Aa}
	200ppm VitC	3,82±0,08^{Ac}	4,15±0,10^{BCb}	4,53±0,02^{ABCab}	5,09±0,50^{Aa}	5,21±0,50^{Aa}
STAPH (log UFC/ml)	0ppm	3,88±0,10^{Ad}	5,20±0,10^{Ac}	5,32±0,30^{Abc}	5,70±0,50^{Aab}	5,77±0,11^{Aa}
	200ppm	3,38±0,10^{Ad}	4,94±0,10^{Abc}	5,14±0,20^{Abc}	5,59±0,30^{Bab}	5,68±0,4^{Aa}
	600ppm	3,34±0,15^{Ab}	4,85±0,01^{Ba}	5,08±0,20^{Aa}	5,26±0,01^{ABa}	5,43±0,50^{ABa}
	800ppm	3,11±0,18^{Ad}	3,79±0,10^{Bc}	5,05±0,02^{Abc}	5,11±0,05^{ABab}	5,34±0,11^{ABa}
	1000ppm	3,31±0,21^{Ab}	4,20±0,10^{Ca}	4,82±0,01^{Aa}	4,84±0,50^{Ba}	4,85±0,40^{Ba}
	200ppm VitC	3,39±0,08^{Ad}	4,97±0,20^{Abc}	5,16±0,20^{Abc}	5,45±0,10^{ABab}	5,57±0,11^{ABa}
PSY (log UFC/ml)	0ppm	2,78±0,10^{Ac}	5,62±0,20^{Ab}	5,72±0,20^{Ab}	6,78±0,10^{Aa}	6,78±0,40^{Aa}
	200ppm	2,68±0,10^{Ac}	5,15±0,10^{Bb}	5,38±0,20^{Ab}	6,50±0,01^{Aa}	6,51±0,50^{Aa}
	600ppm	2,64±0,15^{Ac}	5,17±0,01^{Bb}	5,39±0,30^{Ab}	6,34±0,50^{Aa}	6,36±0,11^{ABa}
	800ppm	2,63±0,18^{Ae}	3,48±0,10^{Cd}	4,63±0,02^{Bc}	5,11±0,05^{Bb}	6,30±0,04^{ABa}
	1000ppm	2,61±0,21^{Ac}	3,30±0,20^{Cc}	4,34±0,30^{Bb}	4,90±0,30^{Bb}	5,94±0,40^{ABa}
	200ppm VitC	3,69±0,08^{Ab}	5,39±0,20^{ABa}	5,54±0,20^{Aa}	5,20±0,10^{Ba}	5,41±0,40^{Ba}
Y/M (log UFC/ml)	0ppm	2,15±0,10^{Ac}	5,78±0,20^{Ab}	5,85±0,20^{Ab}	6,69±0,10^{Aa}	6,70±0,11^{Aa}
	200ppm	2,05±0,10^{Ac}	5,41±0,10^{ABb}	5,56±0,20^{ABab}	5,78±0,10^{Ba}	5,85±0,10^{Ba}
	600ppm	2,01±0,15^{Ac}	5,40±0,01^{Ba}	5,55±0,30^{ABa}	5,69±0,50^{Ba}	5,78±0,11^{Ba}
	800ppm	2,00±0,18^{Ab}	5,19±0,10^{Ba}	5,41±0,02^{ABa}	5,46±0,01^{BCa}	5,60±0,40^{Ba}
	1000ppm	1,98±0,21^{Ac}	2,30±0,20^{Cc}	5,00±0,30^{Bb}	5,36±0,05^{BCab}	5,53±0,10^{BCa}
	200ppm VitC	2,06±0,08^{Aa}	5,41±0,10^{ABab}	5,56±0,20^{ABa}	5,00±0,20^{cC}	5,04±0,04^{Cbc}

Mean (n = 3) ± SD; Means of bacteria count, with different lower-case superscripts (a-e) in each row or with different capital superscripts (A-C) in each column, for different samples are significantly different at α = 0.05 (one-way ANOVA and Tukey's test. a or A—the highest content); Ethanolic extract was used with different content: 200, 600, 800 et 1000 ppm; **TMAB:** Total aerobic mesophile bacteria; LAB : Lactic bacteria ; TC : Total coliform; STAPH : staphylococcus bacteria; PSY : psychrotrooic bacteria ; Y/M : Yeast and molds. Vit C :vitamine C°

Table 4: Microbiological analyses for meat marinating with fresh leaves according to storage time

Storage time (days)		0	2	4	6	8
	Samples					
TMAB (log UFC/ml)	0%	3,67±0,10 ^{Ac}	5,51±0,20^{Ab}	5,60±0,20^{Ab}	6,34±0,20^{Aa}	6,37±0,10^{Aa}
	0,5%FF	3,56±0,12^{Ac}	5,51±0,10^{Ab}	5,60±0,30^{Ab}	6,27±0,01^{Aa}	6,30±0,40^{Aa}
	1%FF	3,55±0,14^{Ac}	5,45±0,10^{Ab}	5,56±0,30^{Ab}	6,21±0,05^{Aa}	6,24±0,12^{Aa}
	1,5%FF	3,52±0,19^{Ac}	4,91±0,10^{Bb}	5,20±0,20^{Ab}	6,09±0,30^{Aa}	6,13±0,04^{Aa}
LAB (log UFC/ml)	0%	4,00±0,10^{Ac}	6,36±0,10^{Ab}	6,40±0,01^{Aab}	6,68±0,10^{Aab}	6,78±0,30^{Aa}
	0,5%FF	3,89±0,12^{Ac}	5,60±0,10^{Bb}	5,78±0,01^{Bb}	6,45±0,01^{ABa}	6,48±0,40^{ABa}
	1%FF	3,87±0,14^{Ac}	5,30±0,10^{Cb}	5,60±0,30^{BCab}	5,90±0,10^{Ba}	6,00±0,12^{Ba}
	1,5%FF	3,84±0,19^{Ab}	5,00±0,10^{Da}	5,30±0,02^{Ca}	5,00±0,50^{Ca}	5,00±0,00^{Ca}
TC (log UFC/ml)	0%	2,90±0,10^{Ab}	5,02±0,20^{Aa}	5,09±0,01^{Aa}	5,42±0,50^{Aa}	5,48±0,12^{Aa}
	0,5%FF	2,79±0,12^{Ab}	5,02±0,10^{Aa}	5,09±0,20^{Aa}	5,28±0,20^{Aa}	5,37±0,11^{Aa}
	1%FF	2,78±0,14^{Aa}	4,15±0,20^{Bb}	4,53±0,02^{Bb}	5,11±0,01^{Aa}	5,23±0,40^{Aa}
	1,5%FF	2,75±0,19^{Ad}	3,70±0,10^{Cc}	4,40±0,20^{Bb}	5,08±0,30^{Aa}	5,20±0,04^{Aa}
STAPH (log UFC/ml)	0%	3,88±0,10^{Ad}	5,20±0,10^{Ac}	5,32±0,30^{Abc}	5,70±0,50^{Aab}	5,77±0,11^{Aa}
	0,5%FF	3,36±0,12^{Ad}	5,10±0,10^{AcB}	5,25±0,30^{Abc}	5,67±0,20^{Aab}	5,75±0,10^{Aa}
	1%FF	3,35±0,14^{Ac}	4,85±0,20^{Bb}	5,08±0,20^{Aab}	5,30±0,30^{ABab}	5,46±0,04^{Ba}
	1,5%FF	3,32±0,19^{Ac}	4,08±0,10^{Cb}	4,79±0,01^{Aa}	4,90±0,30^{Ba}	5,23±0,04^{Ca}
PSY (log UFC/ml)	0%	2,78±0,10^{Ac}	5,62±0,20^{Ab}	5,72±0,20^{bA}	6,78±0,10^{Aa}	6,78±0,40^{Aa}
	0,5%FF	2,66±0,12^{Ac}	5,44±0,10^{Ab}	5,58±0,01^{bA}	5,49±0,20^{Aa}	6,50±0,04^{ABa}
	1%FF	2,65±0,14^{Ac}	5,36±0,20^{Ab}	5,52±0,20^{Aa}	6,46±0,01^{Aa}	6,47±0,04^{ABa}
	1,5%FF	2,62±0,19^{Ad}	5,31±0,10^{Ac}	5,48±0,20^{Abc}	5,97±0,30^{Bab}	6,02±0,10^{Ba}
Y/M (log UFC/ml)	0%	2,15±0,10^{Ac}	5,78±0,20^{Ab}	5,85±0,20^{bA}	6,69±0,10^{aA}	6,70±0,11^{Aa}
	0,5%FF	2,03±0,12^{Ac}	5,71±0,10^{Ab}	5,78±0,01^{abAB}	6,58±0,50^{abA}	6,59±0,50^{Aa}
	1%FF	2,02±0,14^{Ac}	5,48±0,20^{ABa}	5,60±0,20^{aAB}	4,30±0,30^{bB}	5,11±0,04^{Ba}
	1,5%FF	1,99±0,19^{Ac}	5,12±0,10^{Ba}	5,37±0,20^{aB}	4,26±0,20^{bB}	4,30±0,40^{Bb}

Mean (n = 3) ± SD; Means of bacteria count, with different lower-case superscripts (a-e) in each row or with different capital superscripts (A-C) in each column, for different samples are significantly different at α = 0.05 (one-way ANOVA and Tukey's test. a or A—the highest content); **TMAB:** Total aerobic mesophile bacteria; LAB : Lactic bacteria ; TC : Total coliforms; STAPH : staphylococcus bacteria; PSY : psychrotrooic bacteria ; Y/M : Yeast and molds. Vit C :vitamine C ; FF : *E. vesicaria* fresh leaves with different percentage.

4- Sensory evaluation

The results of the sensory analysis based on the hedonic test of sensory characteristics (color, flavor, texture, taste, aftertaste and overall appreciation) of the six samples of meat with extract and four samples with fresh leaves (Figures 4 and 5) .For samples with ethanol extract, ANOVA results revealed significant differences between all descriptors except color and overall assessment ($p < 0.05$). Samples marinated with 800 ppm extract and with 1.5% leaves is the most appreciated by consumer.

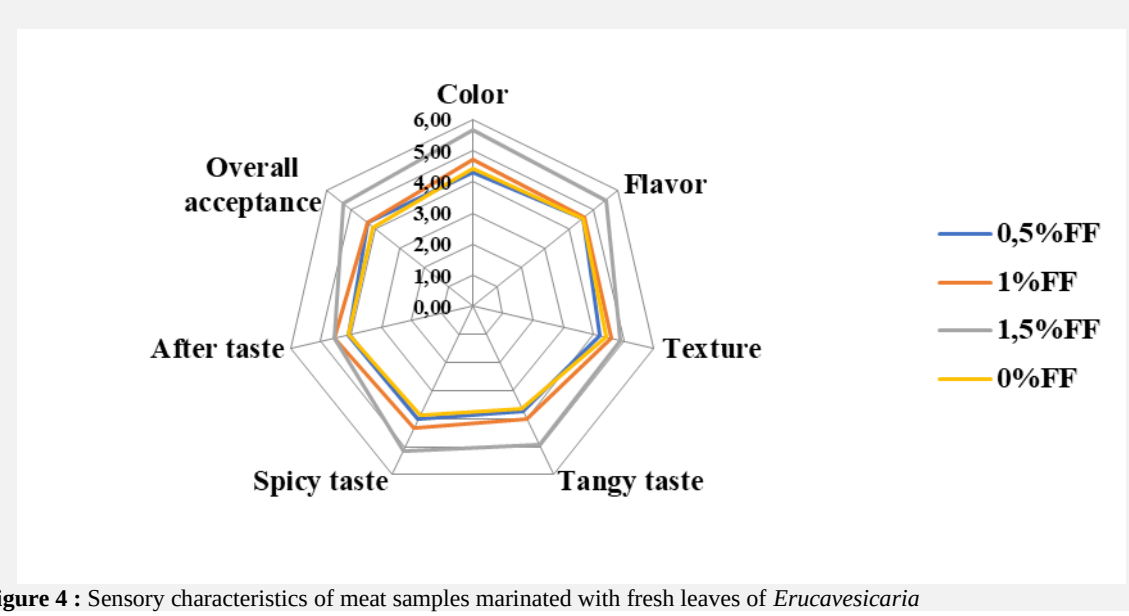

Figure 4 : Sensory characteristics of meat samples marinated with fresh leaves of *Erucavesicaria*

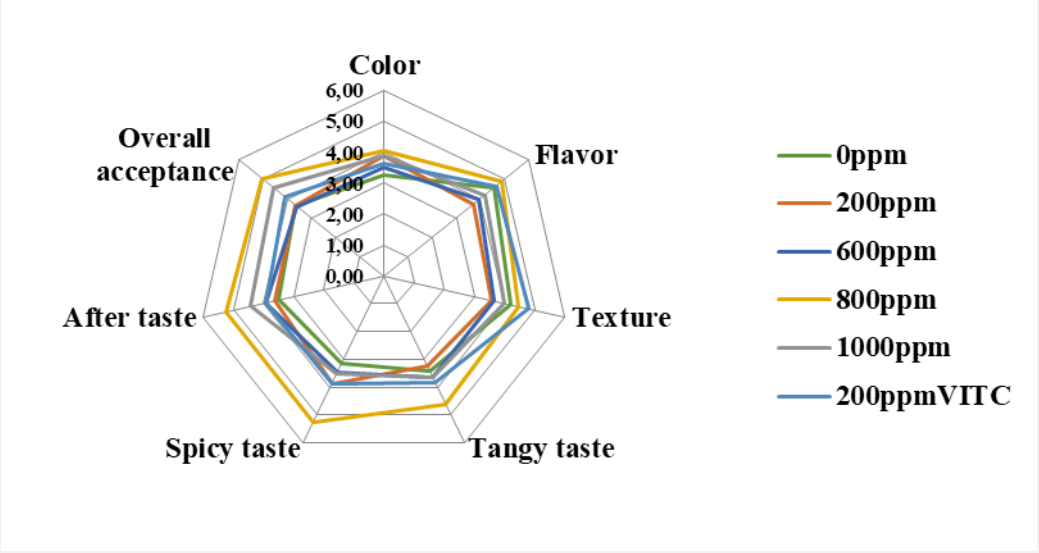

Figure 5 : Sensory characteristics of meat samples marinated with ethanolic extract of *Erucavesicaria* leaves

This result can be correlated with the results obtained for the color parameters where the differences were significant. Indeed, the Pearson correlation test has shown that there is a significantly positive correlation between moisture, descriptors and the overall appreciation of meat. This indicates that when moisture content increase, the juicy of meat is important and sensory descriptors scores and the overall appreciation of consumer are improved. It has been shown that there is a positive and significant correlation between the a * parameter and the color descriptor and the overall ranking. This shows that when meat value of color (light red color) is high, color descriptor scorers is important and the overall appreciation increases.

Conclusion

The results of the sensory, microbiological and physicochemical analyses show that the fresh leaves and the ethanolic extract of *Eruca vesicaria* can be used in the poultry meat marinating with 1.5% of fresh leaves and 800 ppm of ethanolic extract supplementation with a significant improvement in the microbiological quality, which can extend the shelf life of the product, stabilise the physicochemical properties (color, water retention and humidity) with an important consumer overall acceptance.

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