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The Effect of Olive Leaf Extract on Physicochemical, Microbial and Shelf-Life Characteristics of Chicken Nuggets

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ABSTRACT: The primary objective of this study was to examine the effects of olive leaf extracts on the quality characteristics and shelf-life of chicken nuggets .Chicken nuggets were treated with aqueous and ethanolic extract of olive leaves (at two levels: 0.25 and 0.35%) and the results were compared with chicken nuggets without additives (control). The treatments were stored in the freezer for 6 months and on days 0, 45, 90, 135 and 180, physicochemical characteristics (pH, moisture content, cooking efficiency and color indicators) and microbial qualities (total count, salmonella and total Forms) of the samples were evaluated. The addition of ethanolic extract to nugget formulation increased the pH during storage, and the pH values of all tests gradually increased. By increasing the concentration of olive leaf extract in the samples, the moisture content and cooking efficiency increased significantly. During storage, moisture content and cooking efficiency of nugget samples decreased. By the addition of olive leaf extracts to the nuggets, the color indices were improved. Microbial tests revealed that the salmonella and coliforms in all the tested samples were discent. The addition of aqueous and ethanol extracts of olive leaves to the nugget formulation reduced the microbial load, therefore the bacterial count of the samples containing the extracts was essentially lower than the control. Sensory evaluation showed that the lowest sensory score was related to the sample containing 0.35% ethanol extract, but there was no noteworthy distinction between other tests. It can be concluded that olive leaf extract can be utilized as a normal preservative in chicken nugget formulation.

Keywords: Durability, Microbial Load, Nugget, Olive Leaf Extract.

Introduction

The request of prepared meals has become popular in recent years. One of the foremost imperative foods in this aspect are foods made from dough products. Pastry products such as chicken nuggets are produced and covered with a coating and are affected by the preliminary heat process. Therefore, consumers only need a gentle final cooking (frying, microwave or traditional oven) to consume these kind of foods. (Adedeji *et al*., 2011; Chen *et al*., 2009). Today, in meat industry, many chemical additives are used to process meat products to mitigate the proliferation of pathogenic microorganisms and elongate product durability. As in recent years, there has been a growing concern regarding the safety of chemical additives and there has been a notable focus on natural bioactive compounds derived from traditional and medicinal plant sources.

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Numerous investigations have been directed towards exploring the anti-oxidant and antimicrobial activities exhibited by natural compounds present in plants, with a specific focus on their applicability in the meat and poultry industry. (Davidson *et al*., 2013; Natheer, 2010).

Olive fruit, oil, and leaves have a rich historical background regarding nutritional, medicinal, and traditional applications. The consumption of Olive products holds a significant position within Middle Eastern dietary patterns which have high nutritional and medicinal value due to the presence of polyphenols. (Soni *et al*., 2006; Taamalli *et al*., 2012). Olive leaf polyphenols have garnered significant research attention due to their potential to promote health and exhibit unique properties, including, anti-cancer, anti-arteriosclerosis, anti-inflammatory, and antimicrobial activities (Lee and Lee, 2010; Sudjana *et al*., 2009; Wang *et al*., 2008). Studies have demonstrated that olive leaf extract harbors bioactive compounds that exhibit potent antimicrobial activity against bacterial pathogens. Oleuropein is the primary phenolic compound present in olive leaf extract, responsible for bitter taste observed in both olives and olive oil. It possesses antimicrobial properties and is capable of inhibiting the growth of various foodborne pathogens, including Campylobacter jejuni, Helicobacter pylori, and Staphylococcus aureus (Sudjana *et al*., 2009). Given the confirmed antimicrobial activity of olive leaf extract in various sources, the present research aims to assess the impact of olive leaf extract on the physicochemical, microbial, and shelf life characteristics of chicken nuggets.

Materials and Methods

- **Materials**

Chicken breast, wheat flour and other ingredients needed to make chicken nuggets were purchased from the local market. The culture medium required for this research was purchased from Quelab Company. Ethanol and other chemicals used in the experiments were obtained from Merck Chemical Company, Germany.

- **Preparation of aqueous and ethanol extracts of olive leaves**

After washing the olive leaf with water, it was dried in an oven. The electric mill was employed for the purpose of grinding samples. A quantity of fifty grams of the powdered substance was mixed with one liter of distilled water with a 70% ethanol content (in a 70:30 ratio) and allowed to blend for a duration of 48 hours. The solution underwent agitation for a period of 24 hours at room temperature using a magnetic stirring apparatus. Following the filtration process, the resultant extracts were subjected to concentration in a water bath utilizing reduced pressure via a rotary evaporator, maintained at a temperature of 40°C. Then the resulting extracts were poured into glass containers covered with aluminum foil and kept at 4°C until the next use (Mirzaei *et al*., 2019).

- **Preparation of nugget samples**

Treatments including: control sample (T_0) , chicken nuggets containing 0.25% olive leaf aqueous extract (T_1), chicken nuggets containing 0.35% olive leaf aqueous extract (T_2), chicken nuggets containing 0.25% 0% olive leaf ethanol extract (T_3) and chicken nuggets containing 0.35% olive leaf ethanol extract (T_4) were prepared and analyzed. In order to produce nugget samples, first boneless chicken breast (60%) with ice (23%) was cut for 30 seconds by a cutting machine. Typical ingredients utilized in the production of commercial nuggets include

15% potato flakes, 1% salt, and 1% albumin. The combination of all constituents was carried out conjointly with the aim of achieving a homogeneous blend. Subsequently, chicken nugget samples were meticulously fashioned into predefined forms, measuring 1 x 3 x 5 cm and possessing a mass of 25 grams. These samples were carefully stored in a freezer maintained at the temperature of -18 degrees Celsius. Subsequently, the fragments were submerged in the pre-prepared batter (93.57% wheat flour, 1.17% salt, 0.24% bicarbonate, 2.34% yeast, and 1.17% xanthan gum) for a duration of fifteen seconds. (Teruel *et al*., 2015).

- **Microbial tests**

In order to perform microbial tests, firstly, based on the national standard of Iran No. 1-8923 for the total count of bacteria, the sample solution was prepared with the desired dilutions. In order to count total bacteria (Total count), as per the methodology outlined by the Iranian National Standard No. 5272-1. The presence of Salmonella bacteria in the samples was determined using the Iranian National Standard No. 1810 and the overall count of the nugget samples' forms was assessed through adherence to the methodology delineated by the Iranian National Standard No. 11166.

- **pH measurement**

In order to obtain the pH measurements, the samples were introduced into a container with a volume of 150 ml, into which 90 ml of distilled water had been previously added. Subsequently, the mixture was homogenized by stirring with a glass rod. Then, the pH of the sample was read and recorded by a pH meter at ambient temperature (Najafi *et al*., 2012).

- **Measurement of moisture content**

The samples were ground twice by a meat grinder. The empty plate was weighed and then 5-8 grams of the sample was transferred to the plate and weighed again. The sample was subjected to a temperature of 103°C in an oven and left to dry for a duration of two hours. Then the plate with all the contents was cooled in a desiccator until reaching the ambient temperature and weighed approximately 0.001 grams. The operation of heating and cooling and weighing was repeated until the result of two consecutive weightings (with a difference of one hour) is not more than 0.1% of the sample weight. Equation No. 1 was used to calculate moisture percentage by weight (Najafi *et al.*, 2012):

Equation (1)

$$\text{Moisture content} = [(M_1-M_2) / (M_1-M_0)] \times 100$$

where;

M_0: The weight of an empty plate is denominated in grams;

M_1: The pre-drying weight of the plate and the sample;

M_2 The weight of the plate and the sample:

- **Calculation of cooking efficiency**

In order to evaluate the cooking efficacy, the nugget specimens were first weighed and subsequently subjected to frying at a temperature of 180°C for a duration of 5 minutes. Over the course of this process, the internal temperature at the core of each sample attained at 80°C. Subsequent to frying, the specimens were allowed to cool at ambient temperature for a duration of one hour. The fried specimens were then subjected to weighing procedures to determine their post-cooking weight. The calculation of cooking efficiency was performed using Equation 2. (Kim *et al*., 2015).

Equation (2)

Cooking efficiency= [Raw sample weight/ Cooked sample weight] × 100

- **Determination of color indicators**

The colorimetric test was performed using Hunterlab colorimeter. The color indices a* (redness intensity), b* (yellowness intensity) and L* (brightness intensity) were determined by placing the samples inside the tank of the device. (Babuskin *et al*., 2014).

- **Sensory evaluation**

In the current study, sensory evaluation of nugget samples was conducted utilizing a seven-point hedonic test. In order to do this, the nugget samples were fried in frying oil until golden and placed hot in coded containers. The sensory evaluation form was provided to the evaluators and 20 semi-professional evaluators rated the sensory characteristics of the nugget samples. (Carpenter *et al*., 2012).

- **Statistical analysis**

The data obtained were subjected to statistical analysis using the software SPSS 16.0, specifically employing one-way analysis of variance (ANOVA). The disparities among the treatments were analyzed using Duncan's multiple test, with a significance level of 95% ($p < 0.05$) being observed.

Results and Discussion

- **pH Values of nugget samples**

The findings derived from the statistical analysis indicate that both the treatments applied and the duration of storage have a statistical impact on the pH levels of chicken nuggets. The latter evidenced by a p-value of less than 0.05. The alterations in the mean pH readings of the control group and the experimental groups, which consisted of varying concentrations of aqueous and ethanolic extracts of olive leaves, were monitored over a six-month period of storage in a freezer. The results are illustrated in Figure 1. On the day of production, the inclusion of alcoholic olive leaf extract resulted in a notable increase in pH levels ($p<0.05$). Conversely, the integration of aqueous extract led to a reduction in pH levels, although this decrease was deemed insignificant ($p > 0.05$). During the initial 45 days of production, no notable alterations were detected in the pH levels of the nugget samples. However, an evident increase in pH levels was registered between the 45th day and the final day of their frozen state, that attained a notable statistical significance ($p < 0.05$). On the final day of the experiment, the pH levels were observed to be significantly higher in the control sample (6.19 ± 0.03) and the sample containing 0.25% aqueous extract (6.17 ± 0.04), where as the sample containing 0.35% alcoholic extract exhibited the lowest pH. The measured pH value was found to be (6.0 ± 0.04) with an estimated uncertainty.

In Figure 2, the effect of the treatments used on the average pH values of chicken nuggets is presented. As it can be observed, there was no significant difference between the pH values of the control and the levels of 0.25 and 0.35 olive leaf aqueous extract ($p >0.05$) , but the difference between different concentrations of the alcoholic extract was significant ($p <0.05$) .) and had the lowest pH.

The results of the present study align with the findings of the research conducted by Banerjee and colleagues in 2012. Various concentrations of broccoli extract were utilized in the production of goat meat nuggets, where by the consequential pH levels of the nuggets were evaluated. It was observed that the incorporation of said extract resulted in a marked reduction in

the pH value of the nugget. They stated that the reason for this decrease can be related to the relatively lower pH of broccoli extract compared to the normal pH of goat meat (Banerjee *et al*., 2012).

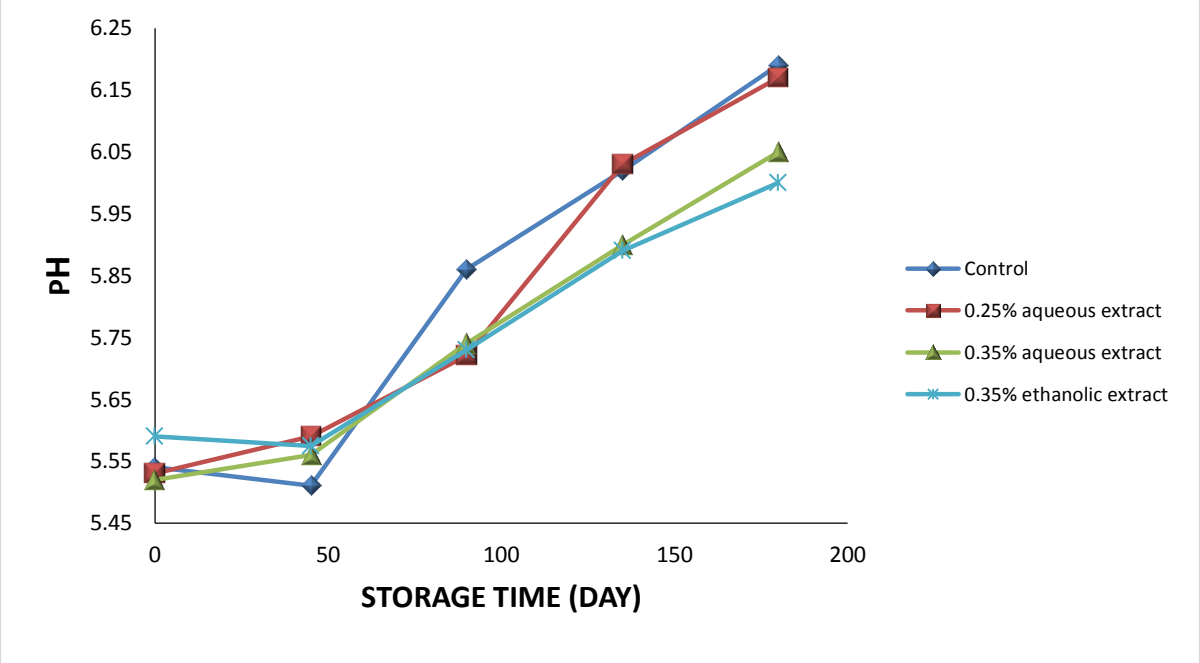

Fig. 1. Changes in the average pH values of different chicken nugget samples during s torage.

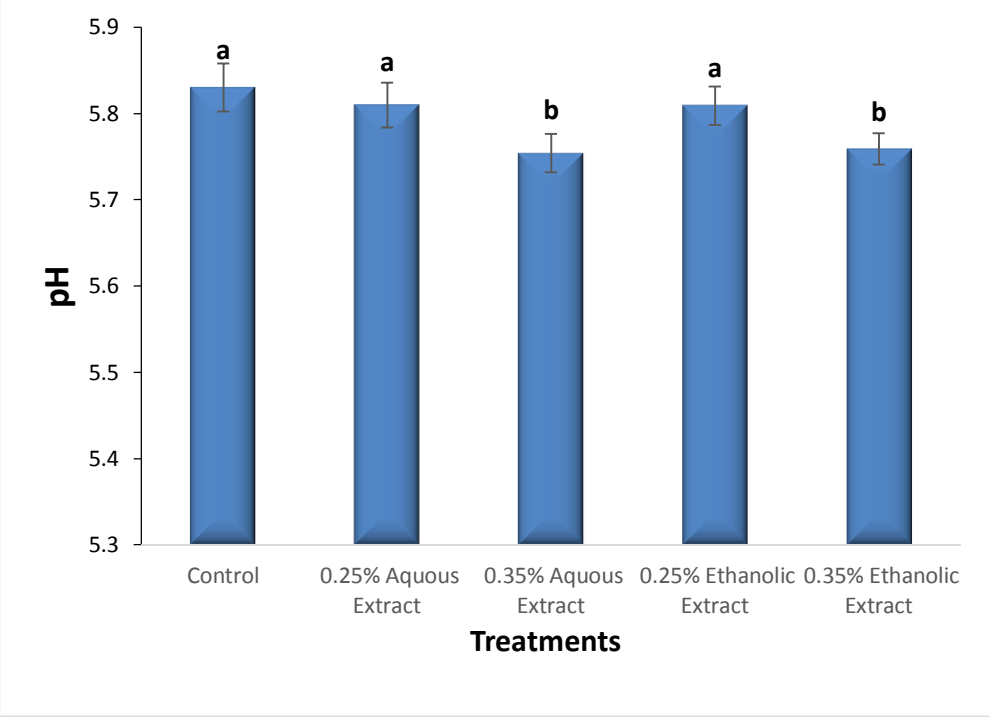

Fig. 2. The effect of the type of treatment used on the average pH values of chicken nuggets**.**
* Similar letters indicate the absence of significant differences between the values (p >0.05).

- **Moisture content of nugget samples**

The findings of the statistical examination of the data have demonstrated a noteworthy impact of the applied treatments and length of storage on the moisture level of chicken nuggets ($p < 0.05$). The alterations observed in the mean levels of moisture content between the control specimen and those incorporating diverse concentrations of aqueous and ethanol extracts of olive leaves over a period of storage are illustrated in Figure 3. During the initial day of experimentation, a marked elevation in the moisture content was observed upon augmenting the dosage of olive leaf extracts in the formulation of the nuggets. Throughout the duration of storage, the moisture content of all nugget samples exhibited a progressive decline. However, it was observed that the control sample and the sample supplemented with 0.25% olive leaf aqueous extract experienced a notably greater rate of moisture loss as compared to the remaining treatments studied ($p < 0.05$). Such findings suggest that the incorporation of olive leaf aqueous extract has the potential to induce a noteworthy influence on the reduction of moisture content in nugget samples during storage. On the final day of storage, the two aforementioned samples exhibited the least amount of moisture, while the sample that contained 0.35% aqueous extract displayed the maximum amount of moisture (46.83% ± 0.52%).

The findings in Figure 4 demonstrate the impact of the treatments administered on the mean moisture level of chicken nuggets. As it is evident from the depicted figure, the control sample exhibited the lowest moisture content. The application of aqueous extract derived from olive leaves resulted in a marginal, non-significant enhancement in moisture content ($p > 0.05$). Nonetheless, a noticeable increase in the total moisture content was observed with a rise in the concentration of the extract ($p < 0.05$). The introduction of an alcoholic extract into chicken nugget samples, alongside an associated increase in concentration, demonstrated a significant effect on the moisture content of the samples ($p < 0.05$).

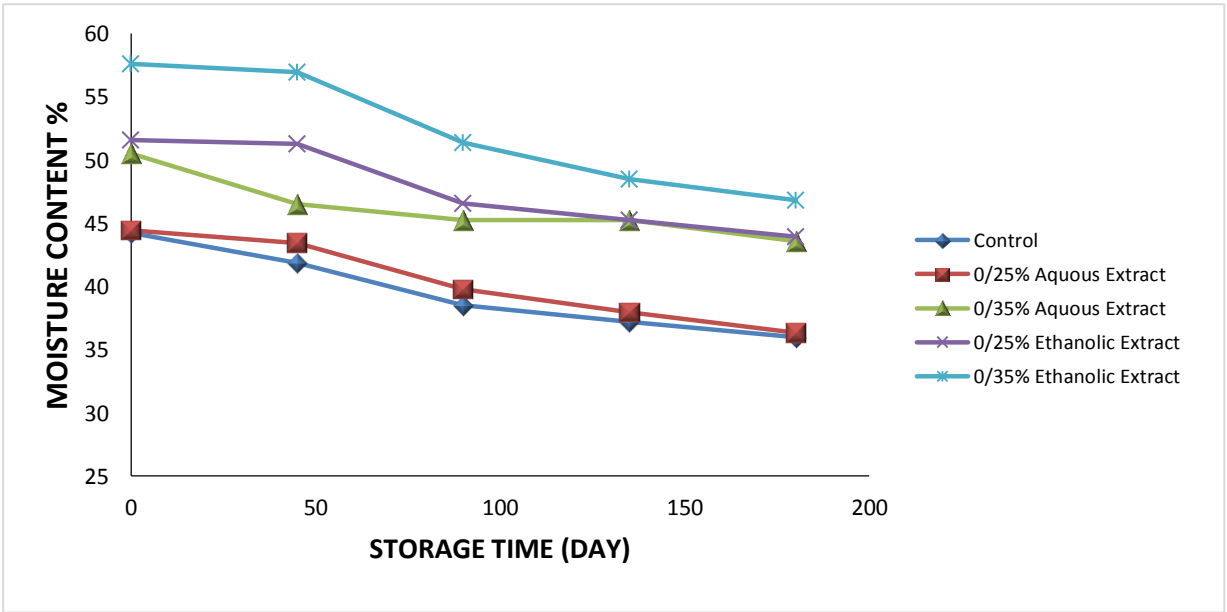

Fig. 3. Changes in the average moisture content of different chicken nugget samples during storage time.

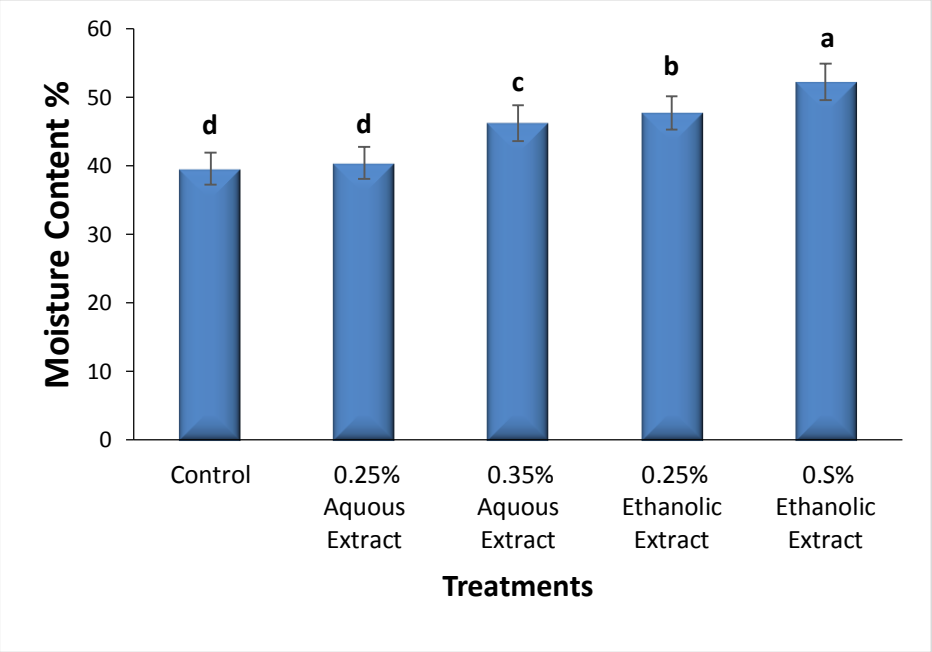

Fig. 4. The effect of the type of treatment used on the average moisture content of chicken nuggets**.**

* Different letters indicate a significant difference between the values ($p < 0.05$).

The enhancement of water retention capacity resulting from the inclusion of olive leaf extracts is hypothesized to be linked to the improvement in oxidative stability and the mitigation of pH variations in meat products throughout the storage period. (Young *et al*., 2003).

- **Cooking efficiency of nugget samples**

The findings derived from the statistical examination of the data have indicated that both the applied treatments and the duration of storage have had a statistically substantial impact on the cooking proficiency of chicken nuggets, with a p-value below 0.05. Figure 5 displays the alterations observed in the mean cooking efficiency values of the control sample and samples containing varying concentrations of aqueous and ethanol extracts derived from olive leaves over the course of storage. On the day of production, the lowest level of cooking efficiency was observed in the control sample (76.22 ± 0.56 percent), while the inclusion of the desired treatments in the formulation of the nuggets resulted in a statistically significant improvement in cooking efficiency ($p < 0.05$). Over the course of time, the cooking efficiency of all samples of nuggets exhibited a decline, with a notably steeper decline observed in the control group compared to the samples containing extracts. On the final day of storage, it was observed that the cooking efficiency of the samples exhibited significant variability. Specifically, the specimen that contained an alcoholic extract concentration of 0.35% demonstrated the highest cooking efficiency, with a mean value of 79.71% ± 0.94%. In contrast, the control sample exhibited the lowest cooking efficiency, with a mean value of 70.78% ± 1.65%.

Figure 6, shows the effect of the treatments used on the average values of chicken nugget cooking efficiency. As stated previousely and can be seen in this figure, the lowest cooking efficiency was related to the sample without additives (control) and the use of aqueous and alcoholic extracts of olive leaves caused a

significant increase in the cooking efficiency. The study revealed a statistically significant increase ($p < 0.05$) in cooking efficiency with higher levels of extracts. The findings suggest that the utilization of alcoholic extract resulted in a greater enhancement of cooking efficiency in chicken nuggets than the utilization of aqueous extract.

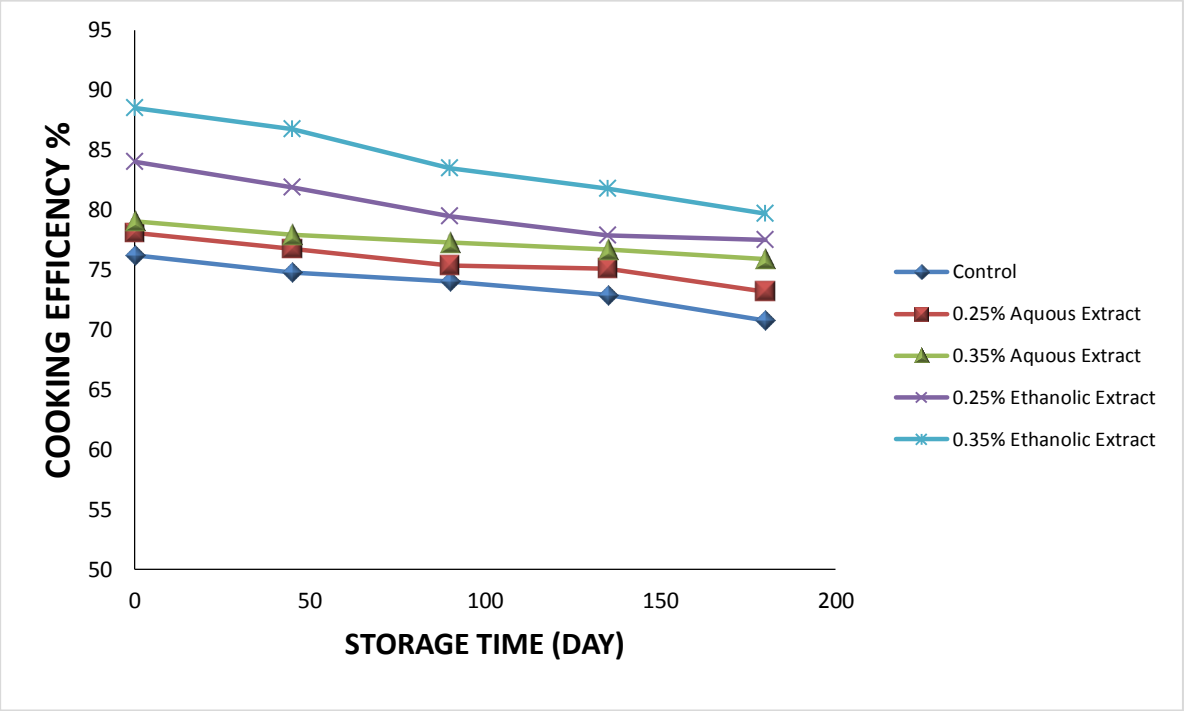

Fig. 5. changes in the average values of percentage cooking efficiency of different samples of chicken nuggets during storage time.

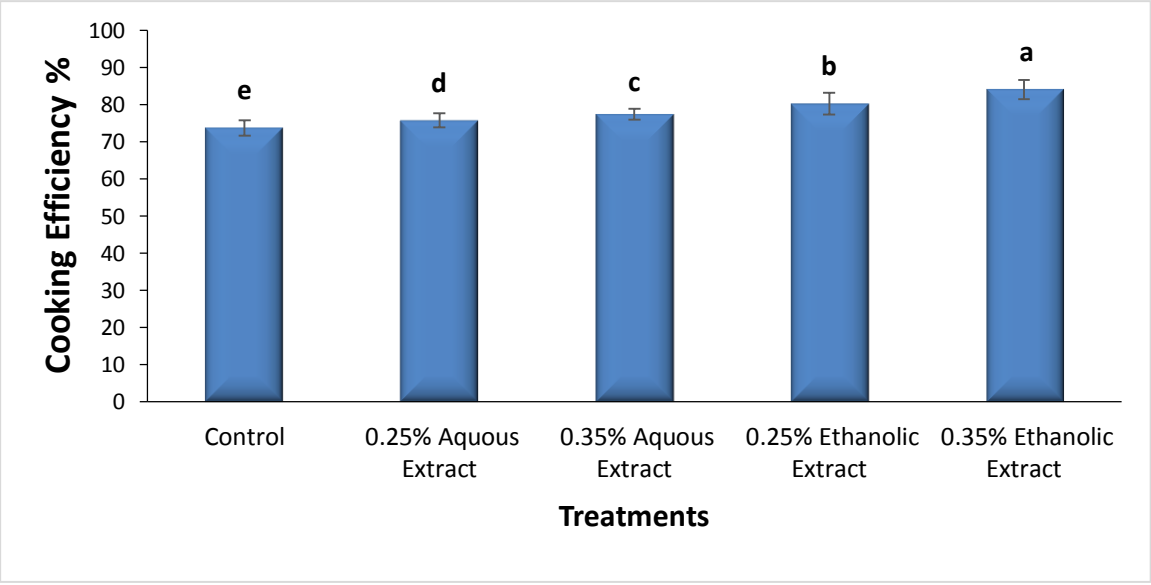

Fig. 6. The effect of the type of treatment used on the average values of the percentage of chicken nugget cooking efficiency
*Different letters indicate a significant difference between the values ($p < 0.05$).

The study of Banerjee *et al.* (2012) was related to the influence of broccoli powder extract on the qualitative characteristics of goat meat nuggets. The findings revealed that the introduction of the broccoli extract did not have a worthly impact on the cooking efficiency of the nuggets. Bani Safar (2013) stated that the incorporation of ethanol extract of olive leaf into veal hamburger resulted in a notable augmentation of cooking efficiency, whereby an increase in the quantity of the extract introduced into the preparation elicited a proportional rise in the cooking efficiency. Furthermore, the augmentation of cooking efficiency attributed to the inclusion of olive leaf extracts was found to correlate with a commensurate increase in water retention capacity, leading to greater moisture retention within the final product. (Bani Safar, 2013).

- **Determination of color indicators of nugget samples**

The statistical analysis of the collected data revealed that both the employed treatments and the duration of storage presented a significant statistical impact on the brightness of the chicken nuggets ($p < 0.05$). The present study illustrates the alterations in the mean values of L* pertaining to both the control group and the experimental treatments incorporating varying concentrations of aqueous and ethanolic extracts derived from olive leaves, over a period of six months of storage within a freezing environment. The graphical representation of these findings is depicted in Figure 7. On the day of production, it was found that the control sample had the lowest quantity of L*. The addition of aqueous and alcoholic extracts of olive leaves was found to markedly enhance the quantity of L* ($p < 0.05$). In the control group, over the course of 45 days from the initial production date, the parameter L* displayed a notable increase followed by a substantial decrease, concluding at the final day of evaluation ($p < 0.05$). Over the course of 90 days following production, the intensity of color brightness in samples consisting of aqueous and alcoholic extracts derived from olive leaves demonstrated an initial increase, followed by a subsequent decrease. On the final day of experimentation, the sample demonstrating the highest and lowest L* values were those containing 0.35% alcoholic extract (70.88 ± 0.77) and the control sample (64.19 ± 1.17), respectively.

In Figure 8, the effect of the treatments used on the average b* values of chicken nuggets is shown. As it is indicated, the lowest intensity of yellow color was related to the control sample and the addition of aqueous and alcoholic extracts of olive leaves to the nugget formulation caused a significant increase in the value of b* . The increase in the concentration of extracts, the amount of b* increased significantly ($p < 0.05$). Among the two types of olive leaf extracts, the amount of b* in the alcoholic extract was higher than the aqueous extract.

Figure 9 presents the impact of the employed treatments on the mean a* values of chicken nuggets. As evident from this study, the minimal concentration of a* was found to be associated with the two variations of alcoholic olive leaf extract, while the aqueous extracts exhibited a higher concentration of a* in comparison to the control group. The findings indicate that variations in the concentration of aqueous and alcoholic extracts were not observed to have a statistically significant influence on the level of a* ($p>0.05$).

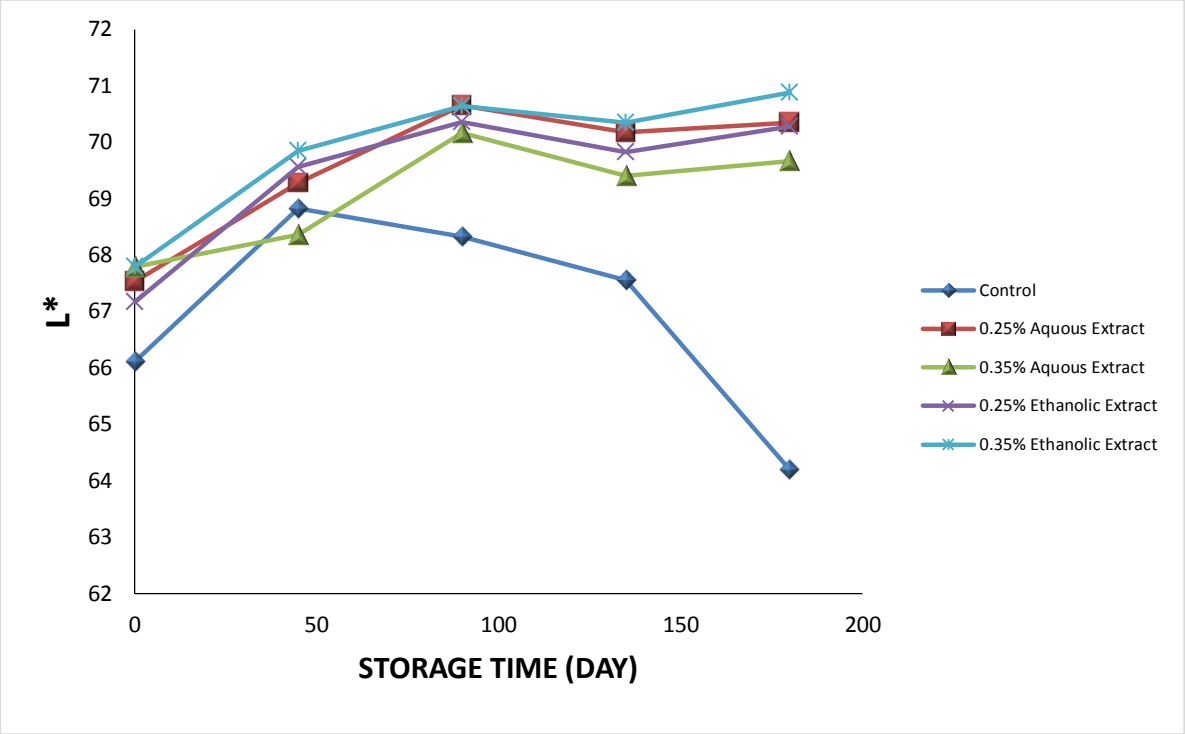

Fig. 7. Changes in the average L* values of different chicken nugget samples during storage time

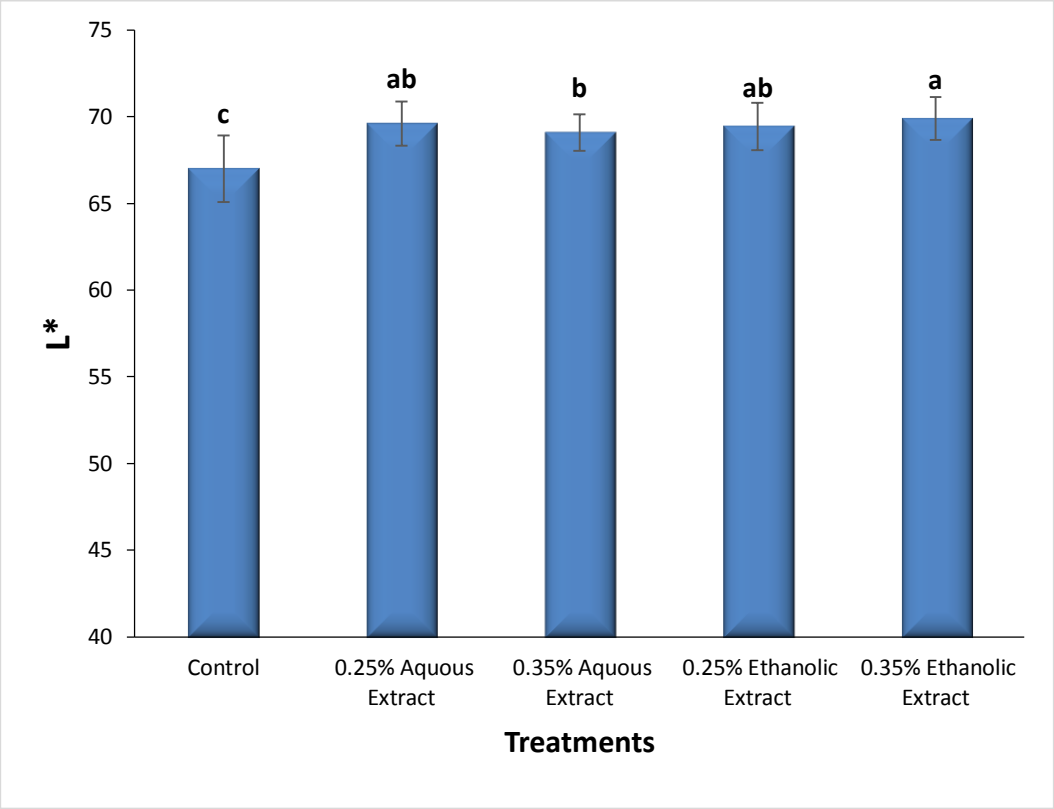

Fig. 8. The effect of the type of treatment used on the average b* values of chicken nuggets

* Different letters indicate a significant difference between the values ($p < 0.05$).

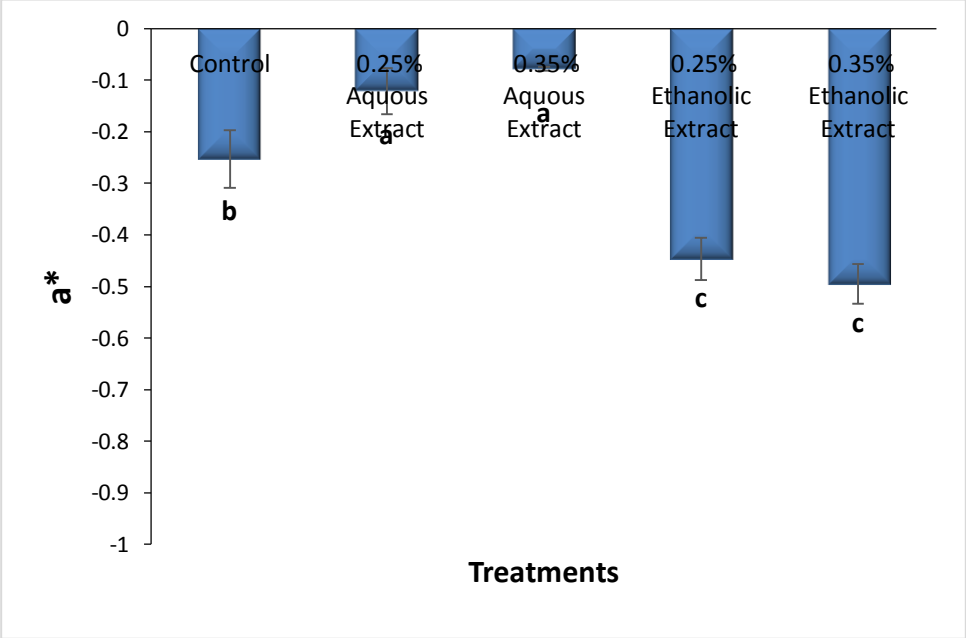

Fig. 9. The effect of the type of treatment used on the average a* values of chicken nuggets

* Similar letters indicate the absence of significant differences between the values ($p > 0.05$).

Tzurier *et al.* (2007) in their investigation discovered that phenolic extracts were efficacious in obstructing the metamorphosis of myoglobin and its conversion to ferrilmyoglobin state. This outcome serves as a compelling indication of the reaction occurring between certain phenolic compounds and heme protein reduction reactions (Tesoriere *et al.*, 2007). Zhang *et al.* (2006) treated raw chicken meat with extracts of different spices and observed that during the storage time, the brightness of the color of the samples containing the extracts increased, while the color of the control sample became darker. Singh and colleagues. The impact of incorporating clove powder, garlic, and ginger on the qualitative characteristics of raw chicken meat was investigated in a study by Singh *et al.* (2014). The outcomes revealed that the inclusion of ginger had a negative effect on the brightness of the meat, while the incorporation of garlic and cloves had a positive effect, resulting in an increase in the brightness of the meat samples. The addition of ginger and garlic also reduced the intensity of the red color of the samples.

- **Microbial evaluations**

The statistical analysis of the data indicates that both treatments employed and the duration of storage exerted a statistically significant impact on the total bacterial count in chicken nuggets ($p < 0.05$). Figure 10 displays the alterations in the mean logarithmic values of the overall bacterial count for the control group, as well as for the samples infused with diverse concentrations of aqueous and ethanol extractions of olive leaves over the course of storage. On the initial day of experimentation, the control sample indicated to have the greatest logarithmic value of total bacterial count per gram at 2.454 ± 0.064 log CFU/g. The inclusion of both aqueous and alcoholic extracts of olive leaf in the nugget formulation demonstrated a significant reduction in bacterial count ($p < 0.05$). The outcome of the present study indicates that the

administration of alcoholic extract exhibited a statistically significant decrease in the microbial burden of the nugget, as compared to the aqueous extract. Throughout the period of storage, a substantial increase in the logarithmic value of the concentration of bacteria per gram of the analyzed samples was observed. However, it was observed that the rate of increase in the control sample surpassed that of the samples containing various extracts. It was therefore noted on the final day of analysis that the microbial load of the control sample was the most elevated. (log CFU/g 4.435 ± 0.134).

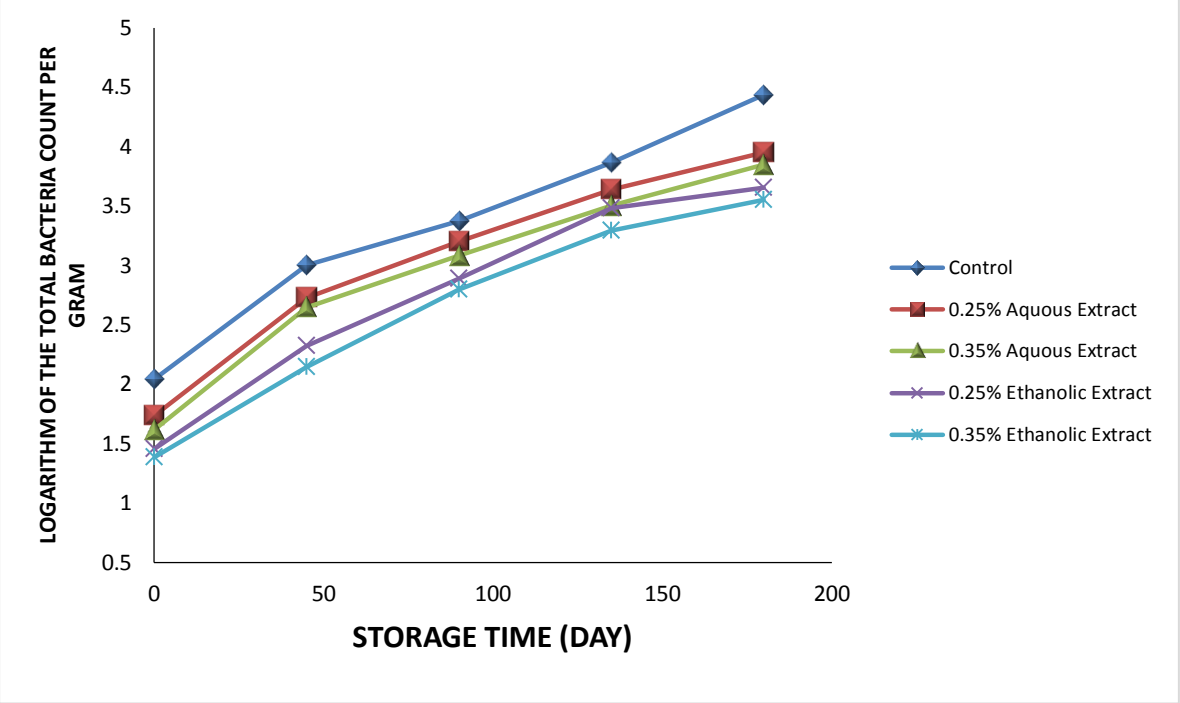

Fig. 10. Changes in the average logarithm of the total bacterial count per gram of different nugget samples during storage time.

The results of checking the presence of salmonella bacteria and also the total forms during six months storage in all samples were negative.

Baker (2014) examined the impact of olive leaf extract as an antimicrobial agent on lamb patties while undergoing refrigerated storage. The findings revealed that the incorporation of olive leaf extract conferred a significant delay in microbial proliferation throughout the refrigerated preservation of patties. The results of the observation demonstrate that the total bacterial count in the control samples exceeded that of the samples treated with the extract. Furthermore, as the storage period progressed, the number of bacteria increased. Aliabadi *et al*. (2012) reported that the use of olive leaves had a beneficial effect on the control of microbial infections.

- **Sensory evaluations**

The statistical analysis of the data indicates that the administered treatments had a significant impact on the collective acceptance score of chicken nuggets ($p < 0.05$). Figure 11 presents a comparative analysis has been presented regarding the mean acceptance scores of the control

group and the groups treated with varying concentrations of aqueous and ethanol extracts of olive leaves. The sample containing 0.35% alcoholic olive leaf extract demonstrated the lowest overall acceptance score of 5.95 ± 0.31, while the overall acceptance scores of other treatments showed no significant difference ($p > 0.05$).

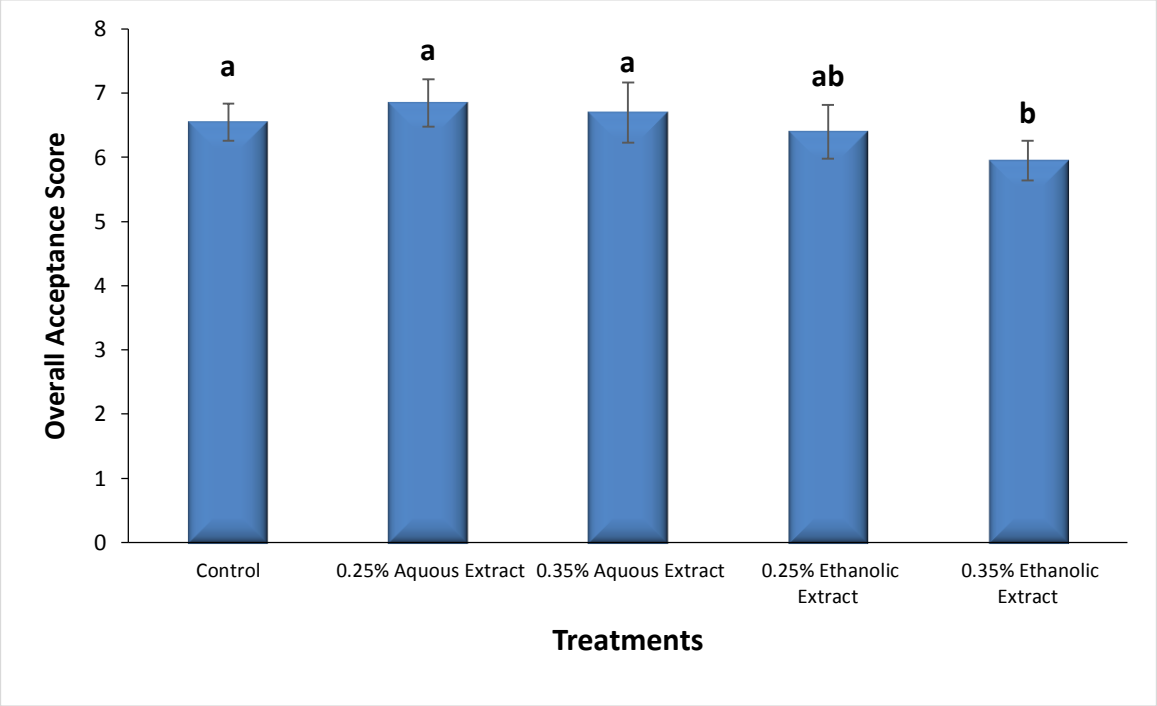

Fig. 11. Average overall acceptance scores of different samples of chicken nuggets.

* Similar letters indicate the absence of significant differences between the values ($p > 0.05$).

A study was conducted by Zhang *et al*. (2016) on the influence of various spice extracts on the sensory characteristics of chicken meat. The research revealed that the samples that incorporated the spice extracts scored superior sensory scores, in contrast to the control sample. Bani Safar (2013) reported that incorporating both aqueous and ethanolic extracts of olive leaves at a concentration of 0.15% resulted in a significant improvement in the overall acceptance of hamburgers. As these treatments had the highest texture, taste and color scores, it is expected that further investigations be carried out for this applications in the chicken nuggets production.

Conclusion

The addition of olive leaf extracts caused a significant increase in pH ($p < 0.05$) . During storage time, the pH of the nugget samples increased. The incorporation of olive leaf extracts into the nugget formulation resulted in an augmentation of the moisture content and cooking efficacy of the specimens. Over time, the moisture content and cooking efficacy of all nugget samples exhibited a gradual decline. The employment of aqueous and ethanolic olive leaf extracts in nugget samples demonstrated a noteworthy impact on color indicators, as evidenced by a substantial increase in the brightness and yellowness of the nugget ($p < 0.05$). During the course of production,

it was observed that the samples infused with extract exhibited lower levels of a* as compared to the control group. Analysis conducted during the storage period indicated that the levels of a* continued to decrease albeit at a slower rate in the samples containing extract. In contrast, the control group displayed a higher rate of decrease in a*. Furthermore, it was found that the extract contributed towards enhancing the color stability of the chicken nuggets. Over the course of storage period, there was a significant increase in the total bacterial count of the analyzed specimens. Nevertheless, the rate of escalation in the control sample was found to be significantly greater than the samples that had been supplemented with the extract. Ethanol extract of olive leaf had higher antimicrobial activity than aqueous extract. The results of the sensory evaluation indicated that the exclusive use of a 0.35% ethanol extract derived from olive leaves resulted in a significant reduction in sensory attributes. However, the degree of acceptability in relation to the score was upheld. In summary, the results indicate that the application of olive leaf extracts had a positive effect on the qualitative and microbial characteristics of chicken nuggets.

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