

ORIGINAL ARTICLE

Determination of the protective effects of olive leaf extracts on microbiological and physicochemical properties of pepper paste using the image processing methods

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Abstract

Deterioration due to chemical and microbiological changes is the most important problem encountered in the production and consuming period of the traditional and modern red pepper paste. On the other side, it is stated in the literature that the olive leaf is an effective natural preservative for similar deteriorations. However, a research related to the preservative effect of olive leaf on pepper paste has not yet been presented in the literature. In the study, olive leaf extract (OLE) was added at different concentrations to the industrial sweet pepper paste and preservative effect of OLE was measured to complete the mentioned deficiency in the literature. However, the experimental results were not suitable for modeling since it contained too much local optimum. Therefore, the experimental results corresponding to not tested independent variable combinations were predicted by bicubic interpolation method. Then, the predicted values were transformed into images to visual determination of significant changes associated with OLE concentrations and storage days. According to the findings, the OLE supplementation between 2.5 and 3.5 g/kg provides optimum microbiological preservation along 15 days for the pepper paste stored as cover-open. However, OLE supplementation did not cause any physicochemical or color changes for pepper paste stored as cover-open. Also, there was no significant microbiological, physicochemical or color changes determined for the pepper paste stored as cover-closed. As a result, OLE supplementation between 2.5 and 3.5 g/kg suggested to preserve the canned pepper paste microbiologically after the consumption has started. Thus, the effect of the OLE on pepper paste was determined and presented to the literature.

Practical application

OLE supplementation between 2.5 and 3.5 g/kg inhibits the microbiological growth in pepper paste stored as cover-open. Thus, the canned pepper paste supplemented with OLE can be protected microbiologically after start of consumption. Olive leaf that is originally wasted also can be evaluated.

1 | INTRODUCTION

Red pepper is a spice to colorize and taste the foods in all over the world. It is often consumed as flake pepper, sauce, and paste. Red pepper is consumed as pepper paste especially in the Turkey, Spain, Mexico, Korea, and the geographical regions where citizens of these countries live in large number (Bozkurt & Erkmen, 2005). In countries where red pepper paste is frequently consumed, the production of

red pepper paste is carried out with traditional methods as well as modern industrial methods (Okur, 2011).

However, there are some problems encountered in the production of traditional and modern red pepper paste. The most important of these problems encountered are deterioration due to chemical and microbiological changes. The main cause of these deterioration is that the *Bacillus*, *Lactobacillus*, *Listeria*, *Corynebacterium*, *Kurthia* ve *Streptococcus* bacterias are suitable medium for the yeasts (Uyulaşer &

Başoğlu, 1997). The deterioration can occur from the start of pepper paste production process to consumption. For example, the heat treatment (89–93 °C) applied during the pasteurization process inhibits the growth of lactic acid bacteria, yeast, and molds but some residuals (inactive microflora) can be caused to deterioration of canned pepper paste after the production (Başoğlu & Köşker, 1980). For this reason, in the literature there are some research results that offered to prevent deteriorations leading to economic loss. These researches are usually about determining the degree of deterioration and detecting the microorganisms caused to deteriorations (Bozkurt & Erkmen, 2004). In addition to these detection and reason related researches, some research results suggested to quality improvement and deterioration prevention methods. Addition of starter cultures, pasteurization of fermented red pepper with ohmic heating, microwave assisted extraction are some of them (Bozkurt & Erkmen, 2005; Cho, Yi, & Chung, 2016; Gogus, Ozel, Keskin, Yanık, & Lewis, 2015).

Furthermore, chemical preservatives were also suggested in some previous research results. According to chemical preservatives related these researches, it has been determined that chemical preservatives such as sorbic and benzoic acid have a high antimicrobial effect. Then some producers started to add these chemicals to pepper paste. However, Petkovic et al. had investigated the safety of chemical preservatives such as sodium benzoate and potassium sorbate and they found that 8.8% of beverages containing such chemical preservatives were not safe for health. Thereafter the adverse effects of chemical preservatives on human health have led producers to prefer natural additives (Akgül, 1997). The plants having little sensory effect while having high antimicrobial effect are preferred as natural additives. For example, the hops, daphne leaf, cloves, sage, thyme, samphire, tarragon, wild celery, and wild marjoram plants have high antimicrobial effect and they were preferred (Calo, Crandall, O'Bryan, & Ricke, 2015; Del Nobile, Lucera, Costa, & Conte, 2012; Embuscado, 2015; Gyawali & Ibrahim, 2014; Rakshit & Ramalingam, 2013; Tiwari et al., 2009). In these studies, the effects of mentioned natural preservatives on the meat, fish, dairy products, minimally processed fruit and vegetables, and cereal-based products were determined and the benefits of natural food additives were suggested. Also, the protective effects were modeled and compared in these studies. So, these studies are good reference for future studies.

Although there are some natural preservatives suggested in the literature, new researches are needed to increase the number of natural additives with higher antimicrobial activity and to determine their correct concentration. For example, olive leaf is a healthy, safe, economical, effective, antioxidant, and alternative food additive if used properly. It also has the shelf-life extension property of food products due to the content of oleuropein that is a phenolic component (Ahmed, Rabii, Garbaj, & Abolghait, 2014; Boudhrinou, Bahloul, Slimen, & Kechaou, 2009; Bouaziz, Feki, Ayadi, Jemai, & Sayadi, 2010; Ganje et al., 2016; Gök & Bor, 2012; Jemai, El Feki, & Sayadi, 2009). Moreover, according to result of previous researches, olive leaf and olive barley contain more than 30 phenolic compounds with antibacterial, antiviral and antifungal effects such as phenolic acids, phenolic alcohols, flavonoids, secoiridoids, and lignans (Artajo, Romero, Morello, & Motilva, 2006; Capasso et al., 1995; Soler-Rivas, Espin, &

Wichers, 2000; Sousa et al., 2006). In the literature, even the extraction of phenolic components from olive leaf has been standardized. Accordingly, many chemicals such as ethanol (70%), methanol (80%), isopropanol, ethyl acetate, acetone (80%), and double distilled water are used to extract these phenolics from olive leaves, but most commonly used is the alcoholic solvent (Abaza et al., 2011; Lafka, Lazou, Sinanoglou, & Lazos, 2013). The alcoholic solvent with water is an effective solvent for glycosides since the attached-sugar makes the phenolic compounds more soluble (Abaza et al., 2011).

Olive leaf extract (OLE) has also antimicrobial effect on food-borne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *L. monocytogenes* and therefore it was used to reduce bacteria in shrimp and organic leafy greens. In addition, OLE can enhance the quality and shelf-life of meat products. However, the effect of OLE on food borne pathogens still unclear although it was reported that a concentration of 62.6 mg/ml completely inhibited the growth of *L. monocytogenes* and *S. enteritidis* (Liu, McKeever, & Malik 2017). Olive leaf is also used in the treatment of many diseases since it is unique natural protective in case of the treatment is needed to antioxidant, antimicrobial, anti-HIV, vasodilator, and hypoglycemic effect. Moreover, olive leaf has polyphenols benefits to human health (Vogel et al., 2015). However, the olive leaf that abundant in nature is still agricultural waste for olive growers and olive oil producers although the demand to olive leaf has increased due to the needs of the health and food industries. So, the new benefits of olive leaf should be identified and use of it should be enhanced. Thus, the olive growers can benefit from olive leaf economically.

If so, the OLE can be used as a natural preservative for pepper paste. However, there is no literature research related to the effect of OLE on pepper paste and inhibition of its microorganisms. For the reasons mentioned above, storage day dependent microbiological, physical, and chemical changes of the OLE supplemented canned pepper paste was investigated in this study. The value changes were determined by visual inspection of images representing the experimental result values predicted using bicubic interpolation method. Thus, a new image processing-based method that can be used in similar food engineering studies has also been tried and offered. According to the results obtained with this new modeling method, the protective effect of OLE on pepper paste was determined and the OLE range providing the optimal effect was proposed for the future researches and applications. Also, it was determined and proposed that the OLE can be protective in which conditions.

2 | MATERIALS

2.1 | Pepper paste and olive leaf extract

Two different canned sweet red pepper pastes were used in this study. The first of these (Type I) was pasteurized pepper paste and supplied directly from a local factory just before canning process. The second one (Type II) is a canned pepper paste of a commercial firm. The Type I pepper paste was used to analyze the effect of OLE on canned pepper paste in the period from end of production to start of consumption. The Type II pepper paste was used to analyze the OLE

effect in other periods (after start of consumption and from start of production to start of canning phase). The Type II pepper paste was also used to analyze the effect of OLE on the domestic pepper paste. The OLE was added to pepper paste samples at different concentrations (2–20 g/kg) just after being obtained in sterile conditions then samples were homogenized. OLE was obtained from a commercial company and ethyl alcohol and water were used as solvents.

3 | METHODS

3.1 | Total phenolic content

About 10 ml of ethanol (70%) was added to 1 g of olive leaf extract and the mixture was left in the dark for 1 day. At room temperature, the extract was centrifuged at 5,000g for 10 min, then the supernatant was filtered using a filter paper. Total phenolics was determined using the Folin–Ciocalteu reagent method (Abaza et al. 2011). About 2.5 ml of Folin–Ciocalteu reagent (diluted 10-fold with double distilled water) was added to 0.5 ml of diluted extract and kept at room temperature for 3 min; then 2 ml of Na_2CO_3 (75 g/L) was added. The sample was incubated for 5 min at 50 °C and then cooled. The absorbance was measured at 760 nm. The results were expressed as mg gallic acid equivalents per g of dry matter (mg GAE/g DM) (Abaza et al., 2011, Lafka et al., 2013).

3.2 | HPLC analysis of olive leaf extract

Compounds contained in the phenolic portion were separated on a UHPLC system (Thermo Dionex, CA), which is composed by a binary solvent delivery pump connected to a diode array detector and an MS spectrometer. About 1 μl of phenolic extract from OLE was separated on a C18 ODS-3 column, (250 \times 4.6 mm \times 5 μm ; Interstil, Japan) by using a gradient elution from 10 to 70% methanol for 9 min, then from 100% methanol for 2 min with a flow rate of 1 ml/min. The mobile phase was 19:1 (v/v^{-1}) mixture of water–formic acid (solvent A) and methanol–formic acid (solvent B). For the determination of phenolic constituents, a photodiode array detector was employed, and 280 and 320 nm were used for determination of phenolic compounds (Bouaziz et al., 2010; Bouarroudj, Tamendjari, & Larbat, 2016).

3.3 | Storage period and method

Pepper pastes were stored at room temperature and along different storage days. The Type I pepper paste was stored in five closed-lid jars and every month one of them was analyzed by opening the jar lid. Type II pepper paste was stored for 15 days in the open-lid jars and they were analyzed on every third day. The analyses for Type II pepper paste were finished at the 15th day because the significant microbial changes in similar analyses were measured up to 15th day (Bozkurt & Erkmen 2005). The extract concentration for Type II pepper was used between 0 and 6% since this range is the best-performance giving in the literature (Pereira et al., 2007). The storage and measurement time and used extract concentrations were more for Type I pepper paste since the microbial change possibility in canned pepper paste is very less as compared with Type II pepper

paste. The characteristics, storage methods, and storage periods of the pepper pastes analyzed in this study can also be seen in Table 1.

3.4 | Physical and chemical analyses

The color values L^* , a^* , b^* were measured by chromameter (CR-400, Konica Minolta-Japan). The pH values of each 10 g pepper paste sample that added to 25 ml of distilled water were measured using Thermo Scientific Orion pH meter. The acidity of the pepper paste sample was titrated by digital burette with 0.1 N NaOH until pH being 8.1 and the total acidity was determined as citric acid for g/100 g. The dry matter of the pepper paste was determined by means of a Krüss Brand refractometer to determine the amount of water-soluble substance (Brix). Distilled water was added to 10 g of pepper paste sample until diluted pepper paste was 100 ml, then 25 ml of this 100 ml was titrated with 10 N NaOH under the control of the phenolphthalein indicator. Thereafter, it was titrated again with 0.1 N AgNO_3 solution with 2 ml of potassium sorbate until a dark red (brown) color was formed and the salt amount was calculated based on the amount of AgNO_3 consumed. The phenolic component content of the OLE was measured at 725 nm using the Shimadzu UV 1200 brand UV spectrophotometer (Cemeroglu & Acar, 1986).

3.5 | Microbiological analyses

Each 25 g pepper paste sample was homogenized in 225 ml of 0.1% buffered peptone water (Merck-Germany) using stomacher. Following the homogenization process, samples were prepared by diluting 1/10 rate then spreading and pouring methods were used. Total mesophilic aerobic bacteria were seeded to PCA (Plate Count Agar-Merck) medium and lactic acid bacteria were seeded to MRS agar-Merck medium. Spreading method was used for both seeding process. Then, colonies were counted by incubation at 30–32 °C for 24–48 hr. Total coliform bacteria were seeded to Violet Red Bile Agar-Merck (VRBA) medium using pouring method. The number of *Staphylococcus aureus* was counted after incubation for 24 hr at 37 °C in Baird Parker Agar (BPA, Merck) medium containing sephixium tellurite and egg yolk. Total number of yeast and mold was counted in Malt Extract Agar (MEA, Merck) medium adjusted to pH 3,5 using 10% tartaric acid by incubation at 25 °C for 72–96 hr.

3.6 | Bicubic interpolation

The experimental results obtained in the study are not suitable for known modeling methods since they contain a large number of instantaneous changing local maxima (Experimental result consists of sharp oscillatory data). Even, all of the known statistical modeling methods in the literature have been tried but the determination coefficients have been calculated as $R^2 < 80\%$. For this reason, the values corresponding to not tested independent variable combinations (not tested coordinates of experimental design) were predicted using bicubic interpolation method then the predicted values were transformed into images. Thus, the statistical significant changes of the values relative to each other were analyzed visually. That is, whether the result values are statistically different were determined according to the

TABLE 1 The characteristics, storage methods, and storage periods of the pepper pastes analyzed in this study

Pepper paste codes	Pepper paste characteristics	Storage time	Storage temperature	Storage methods	OLE concentrations (added)
Type I	Canned as plastic (26–31 Brix)	5 months	20 ± 0.50 °C	In the jar with closed-lid	Control–4–10–20 g/kg
Type II	Canned as tin (28 Brix)	15 days	20 ± 0.50 °C	In the jar with opened-lid	Control–2–6 g/kg

obvious color change in the images obtained from predicted values. The determination coefficients of predicted result values were also calculated as $R^2 = 1$. Although this value is too high, it does not mean that the predicted values are calculated with 100% accuracy, but it is clear from the images obtained that the new proposed method is much more successful as compared with the known modeling methods. The prediction values can be obtained using the bicubic interpolation method as follows (Späth, 1995).

Assume that the values x and y are tested for the first and second independent variables, respectively, in experiment. Then, assume that the $R(x, y)$ is the dependent variable values corresponding to the values x and y . In this case, the values of $R(x, y)$ is a matrix with y rows and x columns as in Equation (1).

$$R(x, y) = \begin{bmatrix} R_{00} & \dots & R_{0x} \\ \vdots & \ddots & \vdots \\ R_{y0} & \dots & R_{yx} \end{bmatrix} \quad (1)$$

The matrix rows $[R_{y0} \dots R_{yx}]$ corresponding to each value assigned to y can be represented by n th degree polynomials that variable is x . Thus, the values of $R(x, y)$ measured in experiment can be represented by the functions $F_y^n(x)$ as shown in Equation (2).

$$R(x, y) = \begin{bmatrix} R_{00} & \dots & R_{0x} \\ \vdots & \ddots & \vdots \\ R_{y0} & \dots & R_{yx} \end{bmatrix} \approx \begin{bmatrix} F_0^n(x) \\ \vdots \\ F_y^n(x) \end{bmatrix} = \begin{bmatrix} c_{0n}x^n + \dots + c_{00}x^0 \\ \vdots \\ c_{yn}x^n + \dots + c_{y0}x^0 \end{bmatrix} \quad (2)$$

The coefficients c_{yn} can be easily determined since the polynomials $F_y^n(x) = c_{yn}x^n + \dots + c_{y0}x^0$ shown in Equation (2) correspond to the values $[R_{y0} \dots R_{yx}]$ in the rows of the matrix $R(x, y)$. By means of the polynomials $F_y^n(x)$, the values $F_y^n(a)$ can be calculated for the values a that were not tested in the experiment. These $F_y^n(a)$ values are also the prediction of dependent variable values $R(a, y)$ corresponding to independent variable values y that were not tested in experiment.

These prediction values $R(a, y)$ dependent on the independent variable y , can be represented as in Equation (3) with m th degree polynomials $G_x = a^m(y) = G_a^m(y) = d_{am}(y)^m + \dots + d_{a0}(y)^0$ that variable is y while $a = 0 \rightarrow a$.

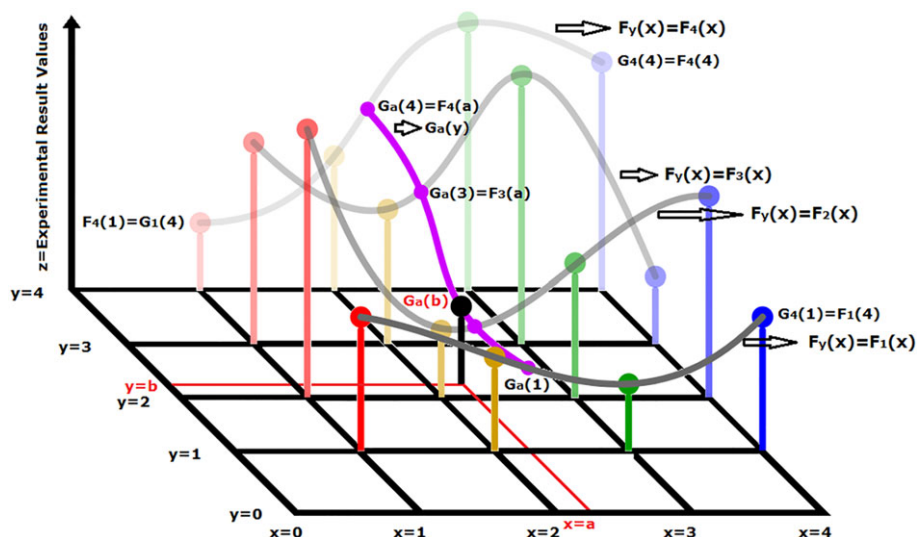
$$R(a, y) \approx [G_0^m(y) \dots G_a^m(y)] \approx \begin{bmatrix} R_{00} & \dots & R_{0a} \\ \vdots & \ddots & \vdots \\ R_{y0} & \dots & R_{ya} \end{bmatrix} \approx \begin{bmatrix} F_0^n(0) & \dots & F_0^n(a) \\ \vdots & \ddots & \vdots \\ F_y^n(0) & \dots & F_y^n(a) \end{bmatrix} \quad (3)$$

As can be seen in Equation (3), since the polynomials $G_a^m(y) = d_{am}x^m + \dots + d_{a0}x^0$ correspond to the column values $[R_{0a} \dots R_{ya}]^T \approx [F_0^n(a) \dots F_y^n(a)]^T$ of the matrix $R(a, y)$, the coefficients d_{am} can be easily determined. If the equation $y = b$ applied to polynomials $G_a^m(y)$, the prediction results $R(a, y) \approx G_a^m(b)$ that correspond to equations $x = a$ and $y = b$ can be calculated.

In the method, only the nearest 16 neighbor values of $R(x, y)$ to the values $G_a^m(b)$ are selected and these 16 neighbor values are used to obtain the polynomials $G_x^m(y)$ and $F_y^n(x)$ that will be used only for each prediction values $G_a^m(b)$. In other words, the polynomials $G_x^m(y)$ and $F_y^n(x)$ are obtained using the closest 16 of measured experimental result values to the values $G_a^m(b)$ that would be predicted, for each prediction value process. Thus, the effect of local maxima can be reduced. Application of the method can also be seen from Figure 1 visually (Keys, 1981).

3.7 | Transforming the predicted values into images

In this study, it was hard to determine the coordinates indicating significant value changes because the prediction matrices obtained by bicubic interpolation were consisting only numerical values and their dimensions were very large. Therefore, the prediction

**FIGURE 1** Prediction of all probable values by Bicubic interpolation method

matrices were transformed into gray-scale and colored image matrices. That is, the similar values that are not statistically different were represented by similar color tones while the values having statistically significant difference were represented by different colors that can be distinguished visually (Akben, 2018). For example, the first value having statistically significant difference according to second value was represented by red color while the second was represented by blue color. Also, two values having no statistically significant difference to each other were represented by two visually similar tones of a color. Thus, the statistical differences of the microbiological and chemical values were visually determined since the visually distinguishable differences between the colors in the images mean that there are also significant differences between the data corresponding to color tones.

Gray-scale image is a matrix that cells consist of gray-toned colors between black and white. Each of the gray-tones in the matrix cells also corresponds to a numerical value. The size of the matrix or the number of cells is called image resolution that the unit name is pixel. The possible largest value corresponding to gray tones in cells is represented by the power of two and the power value is called as bit depth. The resolution determines the size of image while the bit value determines the identity of image with original. Gray-scale images can be colorized by matching the gray tones to the blue-green-red, etc., colors. Colorization process is needed to better distinguish the visually not distinguishable gray-tones. Figure 2 shows a sample 4 bit [the number of possible gray-tones is 2 (Akben, 2018)] and $4 \times 4 = 16$ pixels gray-scale image and its corresponding numerical matrix and colorized image. The scales next to the images are for showing which color-tone corresponds to which value.

The data matrix can be transformed into image matrix as in Equation (4) (Akben, Kalkan, & Çanga, 2017). As a result of this transformation, the gray-tones may not always be distributed homogeneously between black and white. For this reason, some image processing methods such as image normalization or histogram equalization may be needed to determine the optimum region. In this case, the colors and tones are not identical to original image (there may seem to be a lot of fake difference between numerical values in visual analysis of the image). However, the numerical values of color tones should be represented identical to experimental data since the aim of this study is to observe the change of color tones only. This is why only the image conversion method shown in Equation (4) was used in this study.

$$\text{Image matrix} = \left\| \left(2^{\text{bit depth}} - 1 \right) \times \frac{\text{Data matrix}}{\max(\text{Data matrix})} \right\| \quad (4)$$

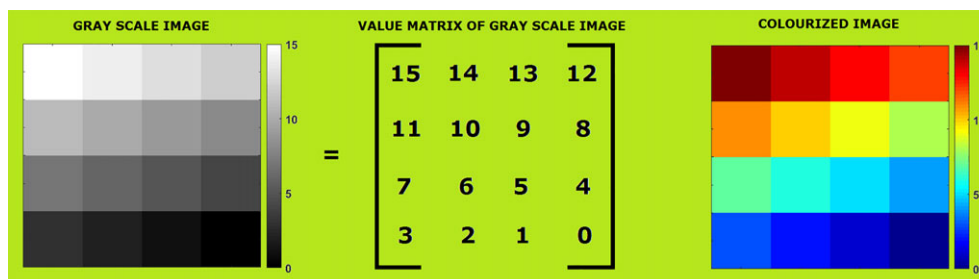


FIGURE 2 The sample grayscale image, its numeric value matrix and its colorized image

In the study, the data were enlarged 100 times by bicubic interpolation and probable data were predicted. Then the predicted data were normalized to numerical values between 0 and 65,535 and transformed into 16-bit images.

3.8 | Visual determination of statistically different data using images

If the colors in an image are visually distinguishable the numerical values corresponding to these different colors also having statistical significant difference ($p < .05$). However, it cannot be said that only the numerical values corresponding to the shades (tones) of a color are statistically significant difference between each other ($p > .05$). The colors that can be clearly distinguished visually are between claret red and navy blue. These are red, orange, yellow, cyan, green, blue, etc., especially. For example, the numerical values corresponding to red or its tones have statistically significant difference according to the values corresponding to blue or tones of blue. However, the numerical values corresponding to only tones of red are not much different statistically from each other. This case can also be seen from Figure 3. The scales at the top of images in the Figure 3 indicate the values of data corresponding to colors. The sample dataset in Figure 3 is [0.017, 0.921, 0.204, 0.368, 0.970, 0.237, 0.748, 0.527, 0.857, 0.914, 0.165, 0.257, 3.576, 3.853, 3.195, 3.623, 3.453, 3.509, 3.476, 3.300, 3.762, 3.42, 3.821, 3.184].

As shown in Figure 3, the first 12 data correspond to the blue tones are not statistically different between each other ($p > .37$). In similar, the second 12 data correspond to the red tones are also not statistically different between each other ($p > .41$). However, there is a significant statistical difference between the first 12 data corresponding to blue tones and the second 12 data corresponding to red tones ($p < .0001$). Furthermore, it should be note that the limits of different colors cannot always be distinguished clearly. In such cases, the limits where the colors are clearly visible should be determined while distinguishing the statistically different data or the word "approximate" should be used for the limits.

4 | RESULTS AND DISCUSSION

4.1 | General findings (significant/insignificant effects)

Five major peaks were identified for OLE. These are the oleuropein, apigenin, o-coumaric acid, hydroxytyrosol, and luteolin and the peak

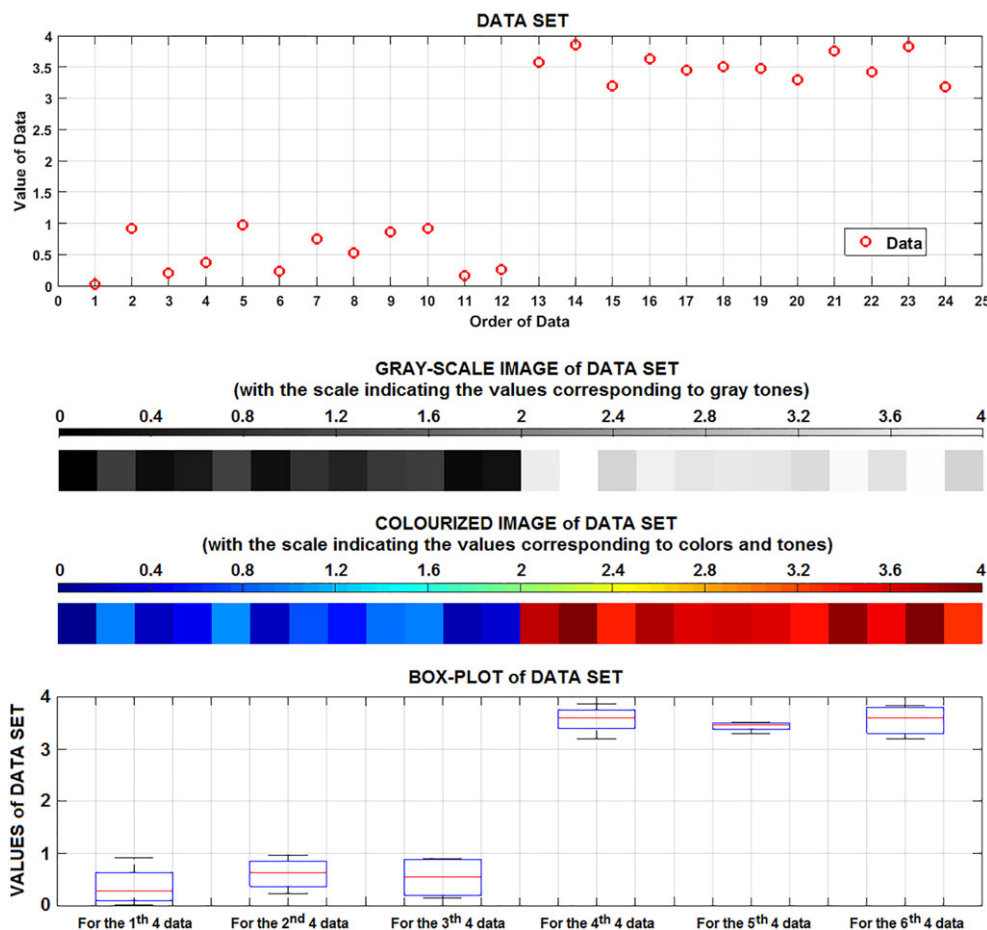


FIGURE 3 Corresponding gray-scale and colorized images of a sample data set and related statistical box-plot

for oleuropein that represents the major component of OLE polyphenols was 74.4% in this study.

According to the findings obtained, there was no microbiological change caused by extract concentration in Type I pepper paste. In addition, storage period and extract concentration dependent *S. aureus* and *coliform* growth were not observed for the Type II pepper paste. However, it was determined that there was a change in count of the LAB, Yeast-Mold, and TMAB. In summary, the OLE supplementation was provided antimicrobial effect only for the LAB, Yeast-Mold, and TMAB values of Type II pepper paste.

For reasons mentioned above, only the LAB, Yeast-Mold, and TMAB values of Type II pepper paste were analyzed using bicubic interpolation and image processing methods and the findings were suggested. The phenolic component value of the OLE added to pepper paste was also measured as 12.8 g/L.

For both Type I and Type II pepper paste, the extract concentration dependent pH and titration acidity changes were not significant statistically (There was the random color-tone changes dependent on extract concentration in images that represents the experimental results). In addition, the color values of the Type I pepper paste have also changed similarly (the extract concentration dependent random color-tone changes in images were not statistically significant). For this reason, the prediction method used to analyze the microbiological values of Type II pepper paste was also used to analyze the pH and titration acidity of Type I and Type II pepper paste and color values of

Type I pepper paste. That is, these random changes were also shown in the study.

Prediction values of the experimental results were obtained by bicubic interpolation and the prediction values were then transformed into images. Detailed analysis of these images and related findings are in the following sections.

4.2 | Findings for type I pepper paste

Salt content of Type I pepper paste used in this study was 7.78% while the dry matter was between 26 and 31 Brix. The measured storage day and extract concentration dependent changes in chemical and physical values of Type I pepper paste is shown in Table 2.

The change of physical and chemical values in Table 2 was predicted using bicubic interpolation method then the predicted values were transformed into images that are shown in Figures 4 and 5.

If the Figure 4 is analyzed according to the horizontal axis (storage time), the acidity values (pH) are represented only by the shades of red corresponding to about 4.26 for the first 3 months. After third month, the colors in image are changing toward orange that corresponds to about 3.85. If the images are analyzed according to the vertical axis (extract concentration), it is obvious that the extraction concentration does not cause any color change in image. That is, the acidity value (pH) of Type I pepper paste remained constant around 4.26 for the first 3 months and decreased to 3.85 thereafter. Extract

TABLE 2 The measured chemical values of pepper paste Type I corresponding to combinations of the extract concentrations and storage period

Dependent variables (experimental result variables)	Extract concentration	Storage period				
		1 month	2 month	3 month	4 month	5 month
pH	0 g/kg (control)	4.02	4.12	3.87	3.45	3.08
	4 g/kg	4.07	4.19	3.92	3.49	3.10
	10 g/kg	4.08	4.18	3.90	3.47	3.09
	20 g/kg	4.08	4.22	3.93	3.49	3.12
Titration acidity	0 g/kg (control)	1.15	1.29	1.17	1.37	1.53
	4 g/kg	1.12	1.18	1.05	1.37	1.47
	10 g/kg	1.06	1.20	1.15	1.35	1.44
	20 g/kg	1.10	1.13	1.10	1.33	1.37

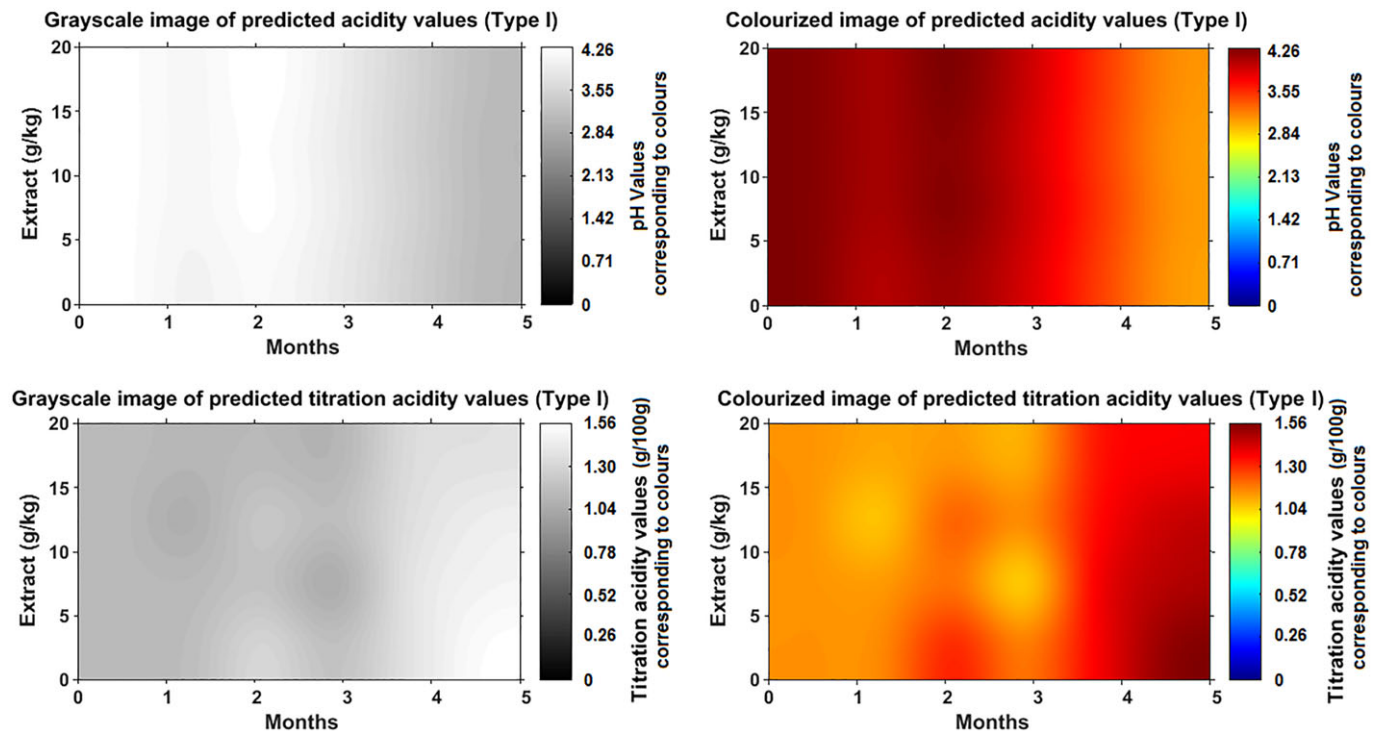
addition did not cause any effect on acidity since the color or tone change due to the extract supplementation was not observed in the image. It should be remembered that the change of color means that the change of corresponding value is statistically significant, and the change of color shade is not significant statistically.

If the images in Figure 4 were also analyzed for titration acidity, the colors were orange along the first 3 months and changed to red and its tones thereafter. That is, it is seen that there is a gradual increase of 0.56 after 3 months since the orange color correspond to about 1 and the dark red color corresponds to about 1.56 in the image related to titration acidity. However, it is observed that the extract supplementation not causes meaningful change of color as in the acidity image. That is, titration acidity did also not cause any statistically significant change.

There was a significant storage time dependent increase/decrease for the titration acidity and pH values of the Type I pepper paste. However, this increase/decrease has also mentioned in some studies

in the literature and the previous studies stated that this is a normal situation and not related to extract supplementation. So, the value changes related to storage days can be neglected for this study (Yassihöyük, 2012; Şentürk, 1986; Bağcı, 1974; Bozkurt & Erkmen, 2004). The images representing the change of color values (L^* , a^* , b^*) for the Type I pepper paste can be seen in Figure 5.

If the images of the L^* , a^* , and b^* values in Figure 5 are observed, some random color changes are observed. However, they are not statistically meaningful since the coordinates of these changes is ambiguous (The images have red and its tones that are statistically insignificant in general while orange color is appearing in random coordinates). Thus, it can be said that the extract supplementation did not cause any statistically significant change in the L^* , a^* , and b^* of Type I pepper paste along 5 months. In summary, the chemical and physical values of Type I pepper paste have not changed except for some statistically negligible random changes (there are not extract supplementation related color changes that coordinates are obvious).

**FIGURE 4** Images representing the storage period and extract concentration dependent chemical values of the Type I pepper paste

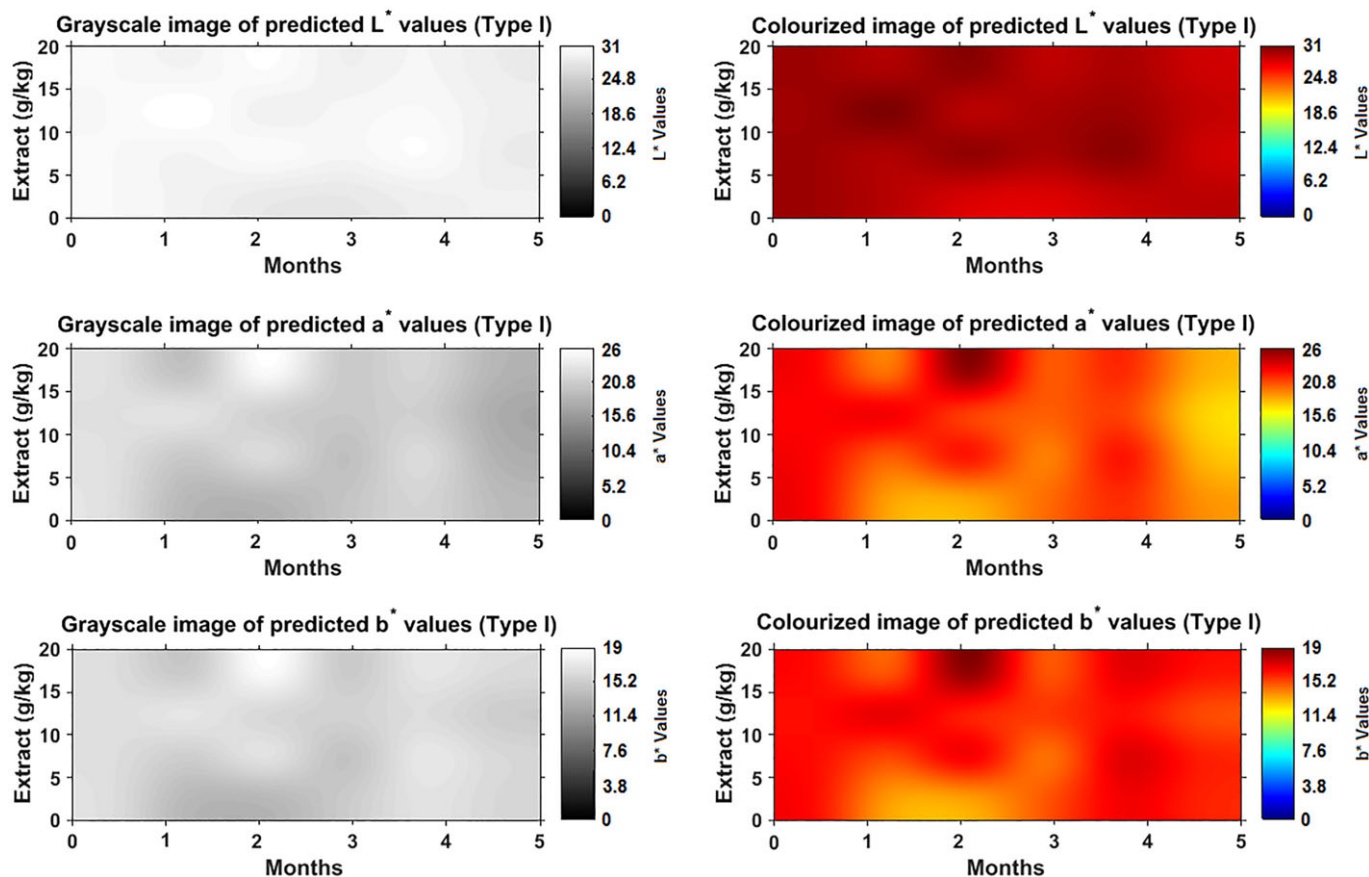


FIGURE 5 Images representing the storage-day and extract-concentration dependent color values (L^* , a^* , b^*) of the Type I pepper paste

The random coordinated color changes in images can also be due to the measurement environment, the method of measurement, the image transformation process or the bicubic interpolation method.

4.3 | Findings for Type II pepper paste

The salt content of Type II pepper paste was 0.26% and the dry matter amount was 28 Brix. Table 3 shows the extract concentration and

storage period related chemical and microbiological values of Type II pepper paste.

In the study, the not measured microbiological values of Type II pepper paste were predicted by bicubic interpolation then the predicted values were transformed into images. Figure 6 shows the images that represent the predicted microbiological values of Type II pepper paste.

TABLE 3 The chemical and microbiological values of Type II pepper paste that measured according to the extract concentration and storage period

Dependent variables (experimental result variables)	Extract concentration	Storage day				
		Start	3th day	6th day	9th day	12nd day
pH	0 g/kg (control)	4.58	4.55	4.35	4.35	4.34
	2 g/kg	4.58	4.53	4.42	4.44	4.36
	6 g/kg	4.58	4.51	4.39	4.44	4.48
Titration acidity	0 g/kg (control)	1.05	1.18	1.27	1.32	1.38
	2 g/kg	1.05	1.14	1.22	1.26	1.34
	6 g/kg	1.05	1.18	1.26	1.28	1.31
TMAB (log cfu/g)	0 g/kg (control)	0.00	4.79	5.83	6.64	7.23
	2 g/kg	1.33	0.00	0.00	5.96	3.07
	6 g/kg	1.33	4.32	5.21	5.16	4.95
LAB (log cfu/g)	0 g/kg (control)	0.00	4.30	5.83	6.46	6.88
	2 g/kg	0.00	0.00	0.67	0.00	3.14
	6 g/kg	0.00	5.06	4.89	4.89	4.95
Yeast-mold (log cfu/g)	0 g/kg (control)	0.00	4.36	5.66	6.61	7.11
	2 g/kg	0.00	0.67	2.30	1.49	2.93
	6 g/kg	0.00	4.12	5.05	4.94	5.09

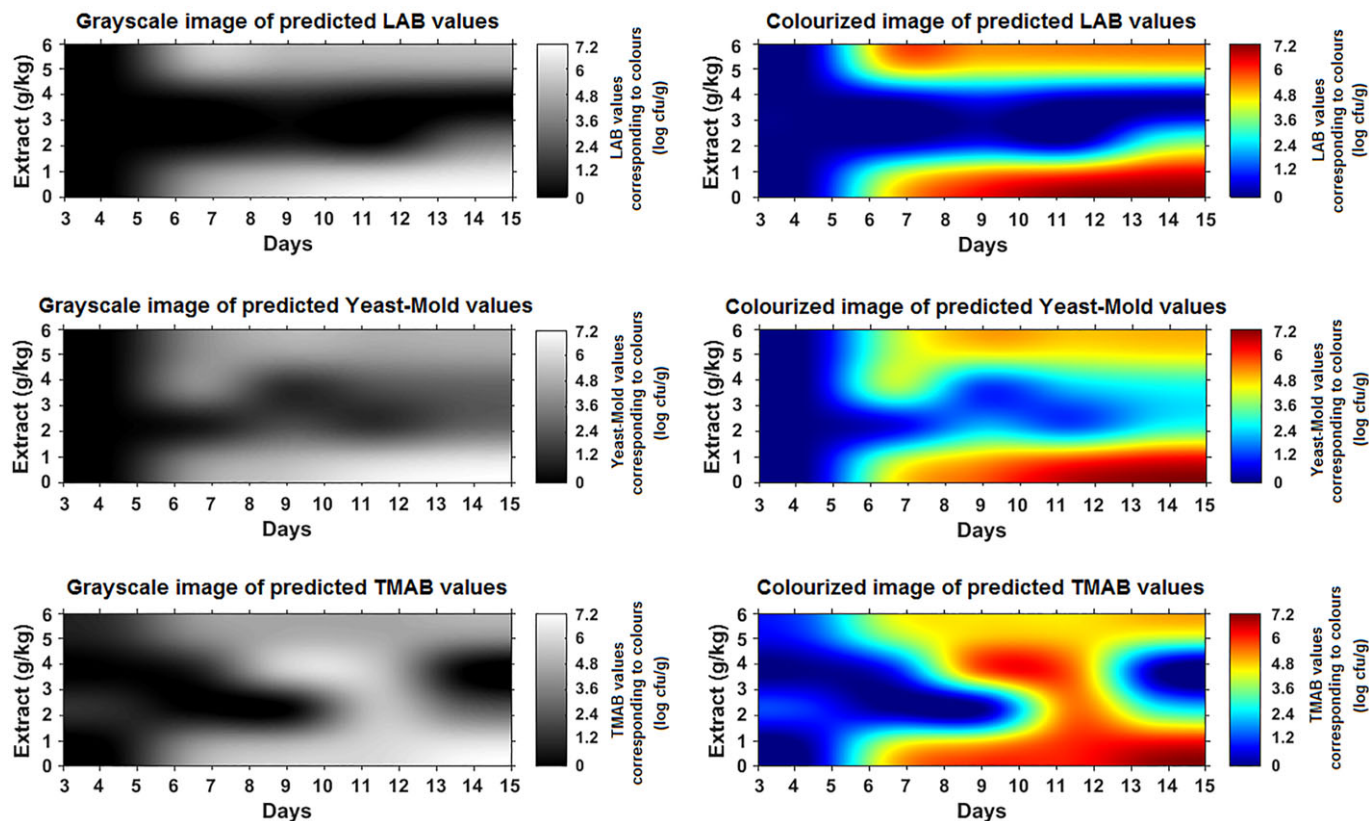


FIGURE 6 Images representing the storage-day and extract-concentration dependent microbiological values (log cfu/g) of the Type II pepper paste

As can be seen in Figure 6, the colors corresponding to extract supplementation between 0 and 2 g/kg are changing from dark blue to red that means the change of microbiological values between 0 and 7.2 log cfu/g along the storage time. If the extract supplementation is between about 2–4 g/kg the colors of image change between only tones of blue that corresponding to 0–1.5 log cfu/g. If extract supplementation is between 4 and 6 g/kg, the microbiological values increase from 0 to 5.5 log cfu/g while

corresponding colors change from blue to orange. It means that the extract supplementation between 2 and 4 g/kg causes the statistically insignificant microbiological change while other extract concentration ranges causing the statistically significant change. It should be note that the change in tones of blue means the statistically insignificant change of microbiological values but change of colors from blue to red or orange means the statistically significant change.

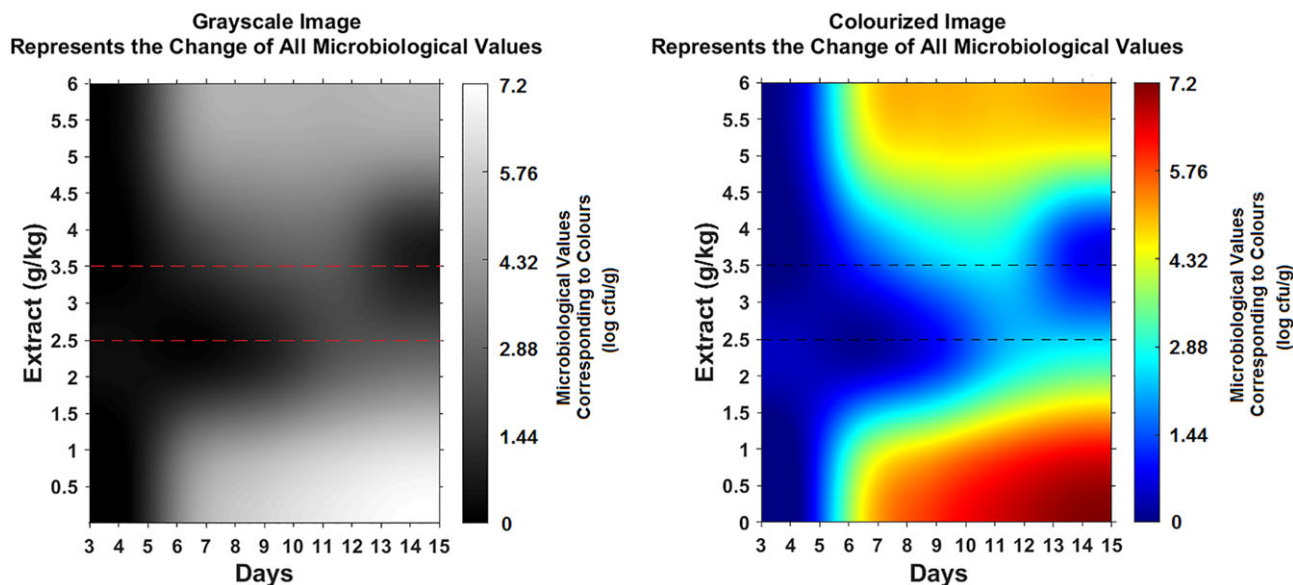


FIGURE 7 Images representing the average of the all microbiological value changes of Type II pepper paste

According to these results, it can be said that the extract supplementation between about 2–4 g/kg optimally inhibits the increase of microbiological values in pepper paste. Even, until the fifth day of storage the microbiological values can be stationary by extract supplementation between 2 and 4 g/kg. If 4–6 g/kg of extract is supplemented, the microbiological growth can be inhibited 25% as compared with control group. However, this 25% inhibition effect is less than the effect of extract supplementation between 2–4 g/kg.

Although these findings indicate that the inhibition effect is ordered by $2-4 \frac{\text{g}}{\text{kg}} > 4-6 \frac{\text{g}}{\text{kg}} > 0-\frac{2\text{g}}{\text{kg}}$ according to the extract concentration, it is necessary to determine the optimal extract supplementation clearer. Due to the clearer determination of the optimal range from three different images is difficult, the ranges should be determined with an image corresponding all microbiological values. All microbiological values in Figure 6 are between 0 and 7.2 log cfu/g along the storage time. That is, all maximum and minimum microbiological values are approximately the same. If so, the analysis can be done more clearly by taking the average of the images in Figure 6 as seen in Figure 7. Thus, the extract providing the optimum inhibition can be determined with precise range for all microbiological values.

The values in Figure 6 can be seen more detailed in Figure 7. According to image in Figure 7, the extract supplementation between 2.5 and 3.5 g/kg caused the shades of blue corresponding to the microbiological values between 0 and 1.5 log cfu/g. If there is only color shade observed in image the increase/decrease of corresponding microbiological values is also not statistically significant. So, it can be said that the extract supplementation between 2.5 and 3.5 g/kg inhibits LAB, TMAB, and Yeast-Mold increase in Type II pepper paste optimally. On the other side, adding the extract concentrations except 2.5–3.5 g/kg caused the cyan, green, yellow, orange, and red colors in image corresponding to the microbiological growth from 2 to 7.2 log

cfu/g. That is, the supplementation of OLE except concentration range of 2.5–3.5 g/kg was not ensured the desired microbiological inhibition because the color change means that the corresponding microbiological growth is statistically significant.

The chemical analysis results of Type II pepper paste are as shown in Figure 8. As can be seen from the images in Figure 8, there is only shades of red color corresponding to acidity value (pH value) of pepper paste. It is therefore clear that the extract added to the Type II pepper paste did not cause a statistically significant pH change during storage days and the pH value remained constant around 4.55 that corresponding to the shades of red.

If the Figure 8 is examined in terms of titration acidity, the image has orange and red shades corresponding to values ranging from 1 to 1.35. However, the transition of image from orange to red depends only on the storage day and this means that there is no statistically significant change depending on extract concentration. In addition, although there is lighter red in image for the extract supplementation between 2 and 4 g/kg until the 10 storage day, it is not remarkable change again since the shade difference means that corresponding value change is statistically not significant. This red tone differences in the image can also be caused by acceptable prediction error of the methods or some neglectable effects in the measurement. Also, some researches in the literature approve that this change is normal and can be omitted (Bağcı, 1974; Bozkurt & Erkmen, 2004; Şentürk, 1986; Yassihöyük, 2012). In summary, the extract supplementation did not cause any significant effect on the chemical values.

5 | DISCUSSION

In the literature, antimicrobial activity of commercial OLE against 122 microorganisms was reported and it was accepted that OLE has

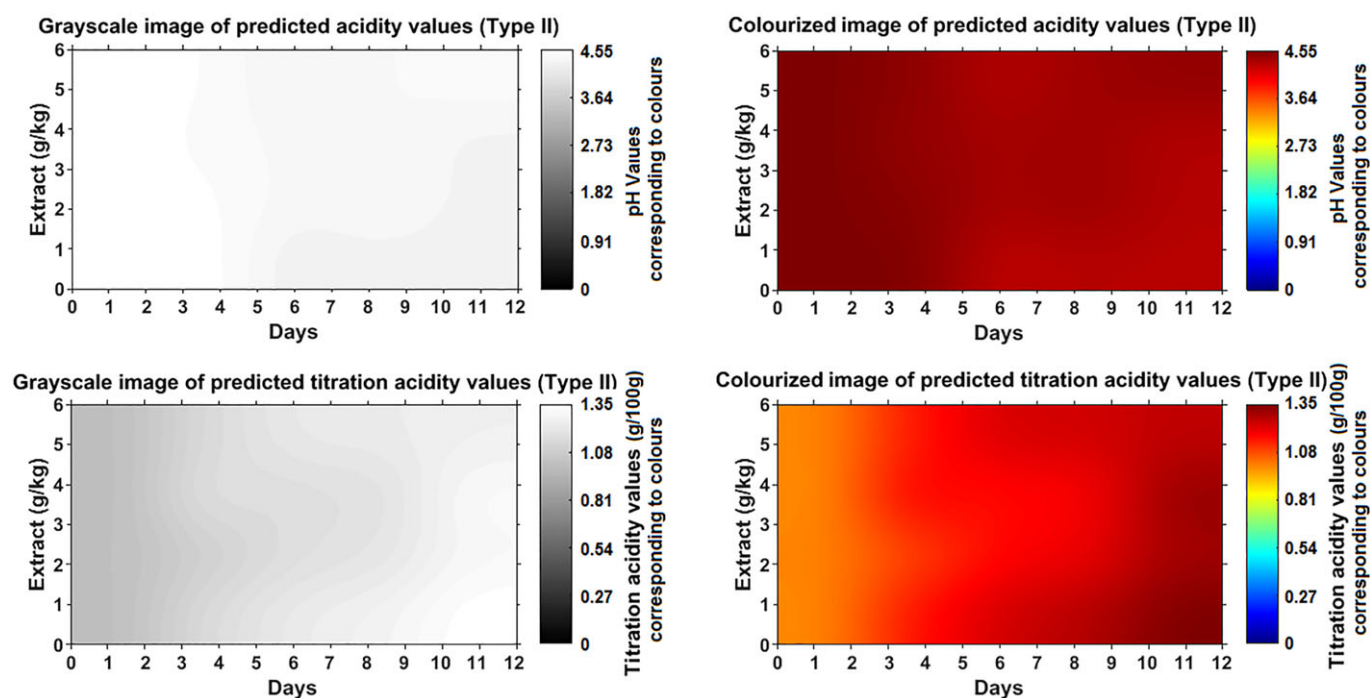


FIGURE 8 Images representing the storage-day and extract-concentration dependent chemical values of the Type II pepper paste

significant antimicrobial effect on *Helicobacter pylori*, *Campylobacter jejuni*, and *Staphylococcus aureus* (Sudjana et al., 2009). Also, some researchers have suggested that the OLE is an important inhibitor for pathogenic bacteria (Aliabadi et al., 2012). As a result of this current study, it was determined that the OLE has antimicrobial effect for the pepper paste stored without cover and inhibits the microbial growth along 15 days. If so, it can be said that the literature findings also support the findings obtained in this study. This antimicrobial effect of OLE that determined in this study may result from the phenolic components of the OLE damages the bacterial cell structure by inhibiting the hydroxyl groups (Gyawali & Ibrahim, 2014). Moreover, the recommended optimal concentration of OLE for microbiological inhibition is low enough that does not affect the sensory properties of pepper paste (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2010). So, the findings of this study can also be used in future studies aiming the antimicrobial activity with the OLE supplementation.

In this study, the total phenols of OLE obtained using ethanol (70%) was 12.8 mg GAE/g DM. This result can be compared with the previous results reported that were 13.37 mg GAE/g for Chetoi olive leaves and 27.3 mg GAE/g DM for wild olive leaves (Abaza et al., 2011; Lafka et al., 2013). If the oleuropein is 74.4%, 25 mg/ml of oleuropein can inhibit the growth of *L. monocytogenes* as 94%, *E. coli* O157:H7 as 58%, and *S. enteritidis* as 36% (Liu et al. 2017). Our findings are consistent with the fact that such microorganisms were sensitive to the polyphenols since the bacterial membranes interact with hydrophobic component of the polyphenols, especially oleuropein.

Olive leaf is used as a natural preservative for many foods and foodstuffs such as minced beef patties, sunflower oil, meatball, raw peeled undeveined shrimp, and tomato paste (Frag, Mahmoud, & Basuny, 2007; Hayes et al., 2010; Ahmed et al., 2014; Liu et al., 2017; Gök & Bor, 2012). OLE is used at different concentrations in these foods or foodstuffs since it provides optimum effect at different concentrations in each food item. It is, therefore, not possible to directly compare the extract concentration range proposed in this study with the concentration ranges proposed in the previous studies that used OLE for other foods. The most finding is that the added extract concentration does not cause the sensory effect.

In other studies of the literature, antimicrobial effects of OLEs in solvents such as water, acetone, ethyl acetate and ethyl alcohol have been tested on gram (+) bacteria and the inhibition effects were almost same even though acetone was slightly better (Korukluoğlu, Şahan, Yiğit, Özer, & Güçer, 2004). In this study, ethyl alcohol and water were used as solvent for the OLE then antimicrobial effect was obtained. If so, the findings of current study are verified by the studies of the literature since the solvents are consistent. It is also clear that the antimicrobial effect of OLE for pepper paste can be improved using different solvents thus the findings of this study may be reference to future studies.

The olive leaves used in this study originate from the olives that grow in the southern parts of Turkey. Since qualitative composition and the total phenol content of the OLEs vary depending on the olive variety, geographical region, collection period, collection method, pre-processing, etc., factors (Jimenez, Masson, Barriga, Chávez, & Robert, 2011; Tsimidou & Papoti, 2010), it was more appropriate to suggest

the optimal extract value as range rather than a concentration. The results of the antimicrobial activity determined in this study can be improved by testing olive leaf extracts obtained from different regions and processed by different methods. This study may be a useful reference for future research.

Although it has been determined in this study that the consumption life of pepper paste can be increased with the olive leaf supplementation, the consumption life can be more increased by the combined use of other additives. Also, more protective OLE can be obtained with different extraction methods. In summary, more effective results can be obtained by trying some modifications. Therefore, this study may be a good reference for future studies.

6 | CONCLUSION

In this study, it was determined that the OLE supplementation between 2.5 and 3.5 g/kg can inhibit the microbiological growth along 15 days in the pepper paste that was stored as cover-open. Also, it was determined that the microbiological values of pepper paste supplemented with OLE stored in same conditions were stationary along first 5 days. However, the OLE supplementation did not cause statistically significant change in the microbiological, color and chemical value of the pepper paste. In addition, it was determined that the OLE supplementation did not affect the chemical and physical values of pepper paste stored as cover closed. In summary, the OLE supplementation inhibits the microbiological values of canned pepper paste in the period from start of production to canning phase and after start of consumption.

The preservative effect of OLE on pepper paste was determined in this study and it is a suggestion that this natural preservative should be used instead of chemical preservatives. The concentration of OLE added to the pepper paste should be within the range of 2.5–3.5 g/kg, as determined in this study. The increase of the antimicrobial effect using more effective olive leaf processing and growing methods is also possible. Thus, the findings of this study can be improved.

To summarize, the method based on the transformation of data predicted by bicubic interpolation into corresponding image proved to be very successful in providing the visual interpretation. This method would be useful for the future studies where experimental results are difficult to model.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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