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Original article

Determination of the oxidative stability and shelf life of anchovy (*Engraulis engrasicholus* l., 1758) fish burgers supplemented with garlic peel extract during cold storage

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Summary. The effect of garlic peel extract (GPE) on the quality of anchovy (*Engraulis engrasicholus* L., 1758) burgers during cold storage was investigated. GPE was able to prevent lipid oxidation compared to control, since the scores of PV, TBARS and pH of the burgers supplemented with GPE showed lower values. The lowest TVC and PBC values were observed in the burgers treated with GPE, especially in the burgers treated %4 GPE. Sensory results showed that the use of 2% and 4% garlic peel extract extended the shelf life of anchovy burgers for 3 days.

Keywords: Garlic peel extract, anchovy burger, cold storage, lipid oxidation, fish quality

Introduction

Ready-to-cook meat products are in demand in all parts of the world (1). Among ready-to-cook meat products, burgers produced from beef, poultry and fish meat are the most popular in the world. Fishery products such as fish burger, fish fingers, fish cake are developing newly in Turkey and fish burgers are produced commercially.

The most important and popular fish species in Turkey is anchovy (*Engraulis engrasicholus* L., 1758) and it constitutes 58.62% of the fish caught from sea in Turkey. According to TUIK (2) data, total amount of marine fish obtained from catching is 269.676 tons and 158.093 tons of this figure is composed of anchovy. Anchovy is generally classified as fatty fish and contains high essential amino acids and high content of long-chain polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) which make it prone to oxidation (3, 4). On the other hand, the processed meat products such as meat and fish burgers are sensitive to oxidation due to the grinding process (5). Due to lipid oxidation and protein denaturation undesirable changes are observed in storage of seafood products (6). Various natural antioxidants have been used to prevent the lipid oxidation in food systems and among those garlic (*Allium sativum*), belongs to family Alliaceae, has a high antioxidant activity (7, 8, 9, 10).

Although garlic (*Allium sativum*) has been known well, garlic peel has received little attention. Because garlic peel is not edible part of garlic, so they are treating as an industrial waste (11). In fact, some studies showed that garlic peel has a high antioxidant activity (11,12). Ichikawa et al. (12) found that garlic skin has a strong DPPH radical scavenging activity, and identified six phenylpropanoid derivatives from the extract as primary antioxidant constituents. Garlic peel contains proteins, lipids, lignin, mannitol, polysaccharides and high amount of pectin (27%). They also contain p-coumaric acid, ferulic acid, and sinapic acid (13).

Therefore, in this study it was intended to determine the effects of garlic peel extract on the oxidative stability, microbiological and sensorial quality of anchovy fish burgers stored at +4±1˚C for 18 days.

Material and Methods

Extraction of garlic peels

Garlic peels (GPs) were provided from the local markets in Nigde, Turkey. The peels were washed twice in tap water and dried at 45˚C for 48 h. After drying, the peels were ground into powder with a blender. The extraction procedure was conducted according to Ifesan et al. (11). GPs powder and ethanol solvent (80 %) were stirred (1:10, g: mL) in a flask for 24 h. After extraction procedure, the garlic peel extracts (GPE) were filtered and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45˚C under vacuum.

Preparation of fish burgers

Anchovies (*Engraulis encrasicolus*) were purchased from a local market in Nigde, Turkey. Fish were transported to the laboratory in polystyrene ice boxes in 1 hour. Then anchovies were gutted beheaded and washed. The fish were minced with a meat mixer and divided into three groups (Control, G2 and G4). Burger preparation was conducted according to method of Tokur et al. (14) with slight modifications (Table 1). There were twenty-eight fish burgers in each group at a weight of approximately 50 g each.

Table 1 Formulation of fish burger (%)

Ingredients	Percentages
Minced anchovy meat	86.2
Corn flour	7
Wheat flour	5
Salt	1.3
Sugar	0.5
Total	100

Garlic peel extract, along with other ingredients, was added to the G2 and G4 groups in concentration of 2% and 4%, and mixed well by a mixer. Control group did not contain GPE. After mixing, burgers were shaped by shaper. All samples were placed in styrofoam plates and covered with stretch film. Then stored at 4±1˚C for 18 days and subjected to quality analysis during the storage period.

Physicochemical analysis

For the pH value determination, the probe of the pH-meter (Thermo Scientific Orion 2-star, Germany) was dipped into the fish homogenates prepared with distilled water (1:1, w:v) (15).

Peroxide value (PV) was determined according to method of AOAC (16). 30 mL of chloroform:glacial acetic acid (3:2, v:v) solution was added into 2 g sample. After addition of 1 mL of saturated potassium iodide (KI) solution the mixture was stored in a dark place for 5 min. Then 75 mL of distilled water was added and the mixture was titrated with sodium thiosulfate ($Na_2S_2O_3$) (0.1M) with the addition of starch solution as an indicator. The results were calculated as meq O_2/kg.

Thiobarbituric acid reactive substances (TBARS) analysis was conducted using the method of Tarladgis et al. (17). 10 g of sample was distilled with 2.5 mL HCl and distilled water (1:2, v:v). 5 mL of the distillate was mixed with 5 mL thiobarbituric acid (TBA) reagent in the stoppered test tubes. Then tubes were put in a water bath at 110˚C for 35 min for the color reactions. The absorbance was measured with spectrophotometer (Shimadzu UV 160A, Tokyo, Japan) at 538 nm against a blank solution. The results were expressed as mg malonaldehyde/kg fish flesh.

Microbiological analyses

Fish burger sample (10 g) was mixed with 90 mL pre-chilled sterile ringer solution. Further decimal serial dilutions were used from this homogenate. For the determination of total viable counts (TVC) and total

psychrophilic bacteria (TPB) Plate Count Agar (PCA) was used. Then the plates were incubated at 37°C for 24-48 h and 8°C for 7 days, respectively.

Sensory analysis

Sensory evaluation of fish burgers was performed according to the method of Paulus et al. (18). Fish burgers were grilled at 350°C for 5 min and served to six panelists by using 9-point hedonic-scale. A score of 9 represents 'very good quality', a score of 7-8, 'good quality', a score of 5-6 'acceptable', while a score of 1-4 was regarded as 'bad or unacceptable'.

Statistical analysis

All measurements were performed in triplicate and analysis was carried out using the SAS software (Statistical Analysis System, Cary, NC, USA). Variance analysis (ANOVA) was used to evaluate the data and 5% significance level of Duncan's test was based to compare the differences between means of parameters.

Results and Discussion

pH value

Changes in the pH value of anchovy (*Engraulis engrasicholus* L., 1758) fish burgers during cold storage are shown in Figure 1. Fluctuations were observed in pH values of all anchovy burgers throughout the storage period. Among the treatments, the pH values of the fish burgers containing GPE were lower than those of the control samples ($P<0.05$). The lower pH in anchovy burgers supplemented with GPE may be due to the presence of antimicrobial ingredients in GPE (19), increase in the pH value of the control group may be due to alkaline substances such as ammonia produced from bacteria (20). While the pH value of the control group tended to increase during the storage period, this value increased nine days of storage and then began to decrease in G2 group. Similarly, pH value of the G4 group increased only six days of storage then decreased and remained same as its initial value. Initial and last pH values of GPE added fish burgers were the same and this situation may verify that GPE has the

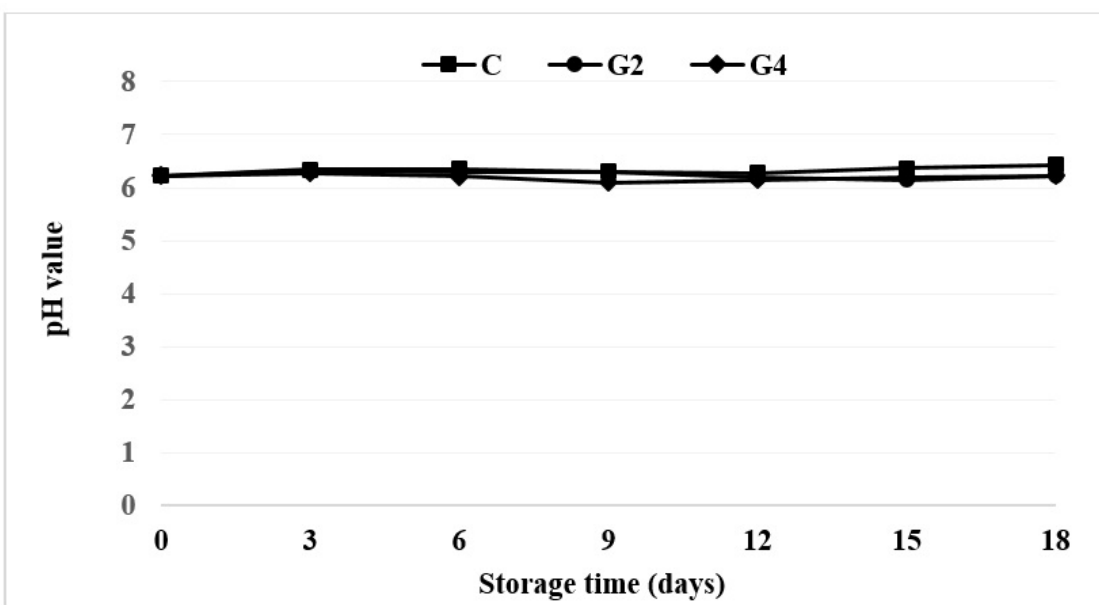

Figure 1. Changes in pH values of anchovy burgers treated with garlic peel extract (GPE) during 18 days of storage at 4°C. * Control: without treatment, G2: 2% GPE treated group; G4: 4% GPE treated group

potential microbial inhibitory activity on the growth of spoilage microorganisms that metabolizing basic nitrogen compounds due to its antimicrobial contents (11, 19). Similar results were observed by Tokur et al. (14) and Guan et al. (20). Fernandes et al. (21) found that lamb burgers containing oregano extracts at different concentrations had lower pH values than the control burgers after 120 days of frozen storage. In contrast, Guan et al. (20) reported that addition of sage, oregano and grape seed extract treatment stabilized the pH value in hairtail fish balls during storage at 4°C.

Peroxide value (PV)

The primary oxidation products of lipid oxidation are peroxides and hydroperoxides break down to aldehydes, ketones and alcohols causing off-flavour in products (22). Initial peroxide values of anchovy samples were determined as 0.2 meq O_2/kg and increased 3.79, 3.34 and 2.65 meq O_2/kg in the control, G2 and G4 samples, respectively (Figure 2) at the end of the storage. The limit value of 8.0-10 meq O_2/kg (23) was not exceeded in anchovy burgers during the storage period. There was a significant difference between the anchovy burgers treated with 2% and 4% GPE ($P<0.05$). Peroxide values of GPE added anchovy burgers were lower than those of the control due to the free radical scavenging activity of GPE. Lowest peroxide values were observed in 4% GPE treated anchovy burgers during storage period ($P<0.05$). It can be concluded that addition of high concentration of GPE can retard the lipid oxidation in anchovy burgers.

The oxidative protection of GPE comes from its flavonoid and diallyl sulfide contents (24, 25). Similarly, Hassanpour et al. (26) reported that minimum peroxide value was found in dill extract added fish burgers during six months of refrigerated storage. Cao et al. (19) found that mixing of ginger, garlic and onion extracts retarded lipid oxidation in stewed-pork. Ucak et al. (27) investigated the effects of rosemary extract on the mackerel fish burgers stored at 4°C for 15 days and they reported that higher peroxide value was obtained from the control group during the storage

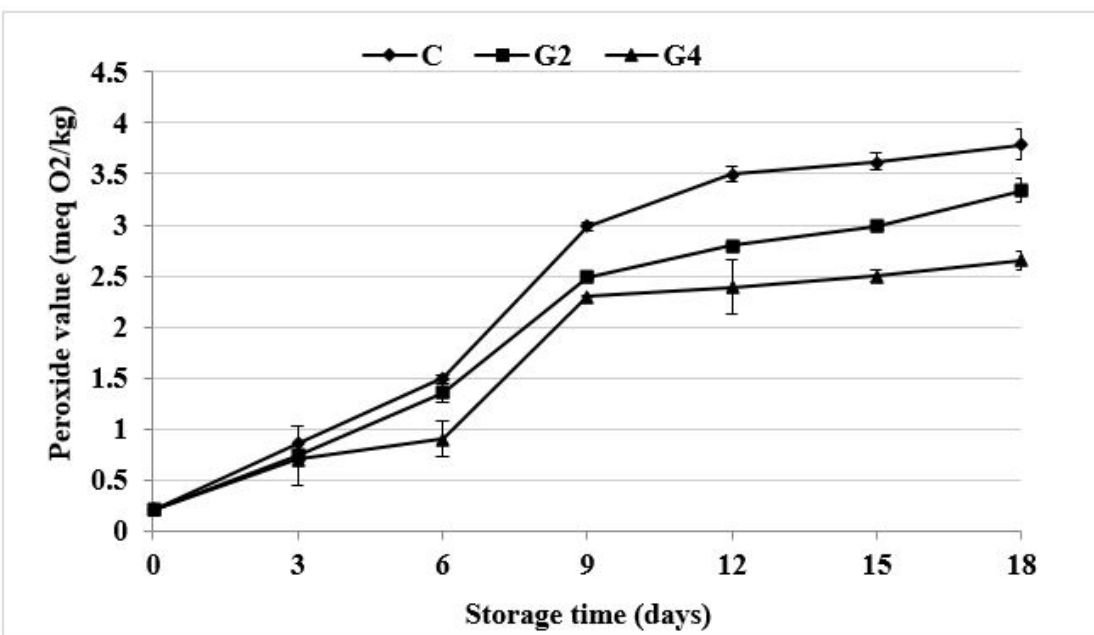

Figure 2. Changes in peroxide values (PV) (meq/kg) of anchovy burgers treated with garlic peel extract (GPE) during 18 days of storage at 4°C. * Control: without treatment, G2: 2% GPE treated group; G4: 4% GPE treated group

period. Al-Bulushi et al. (28) reported that the peroxide value of Arabian Sea meagre fish burgers increased from week 4 till week 8, and then became stable to the end of storage.

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) is widely used as an indicator of lipid oxidation degree and is second breakdown product of lipid oxidation (29). Figure 3 shows the changes in TBARS values of anchovy fish burgers during refrigerated storage. The initial TBARS value of anchovy was 2.72 mg MDA/kg and increased in all samples at the end of the storage. TBARS values of the control was significantly ($P<0.05$) higher than those of the samples supplemented with GPE. At the end of the storage, TBARS values of the anchovy burgers were found as 7.71, 5.78 and 5.56 mg MDA/kg in the control, G2 and G4 samples, respectively. The lowest TBARS values were obtained from G4 samples throughout the storage. High reaction rate of carbonyls with proteins leads to decreasing in malonaldehyde content (30). TBARS values indicated that the limit value of 5 mg MDA/kg sample (31) was exceeded and rancidity developed in the control, G2 and G4 samples after 9, 15 and 18 days of storage, respectively. According to TBARS results, garlic extract able to delay lipid oxidation its high content of organosulphur compounds (11). Our findings were in accordance with results reported in other researches (20, 21, 27, 32). Ucak et al. (27) reported lower TBARS values in 0.4% and 0.8% rosemary extract added mackerel burgers during 15 days of refrigerated storage. Ozogul and Ucar (32) investigated the effects of green tea, oregano and sage extracts on the quality of frozen chub mackerel (*Scomber japonicus*) burgers and they reported that significantly lower TBARS values were obtained from fish burgers treated with green tea extracts. Guan et al. (20) reported that the TBARS value of fish balls treated with sage, oregano and grape seed extract was significantly lower than those of the control. Fernandes et al. (21) reported that burgers containing oregano extract showed higher oxidative stability and oregano extract reduced the TBARS

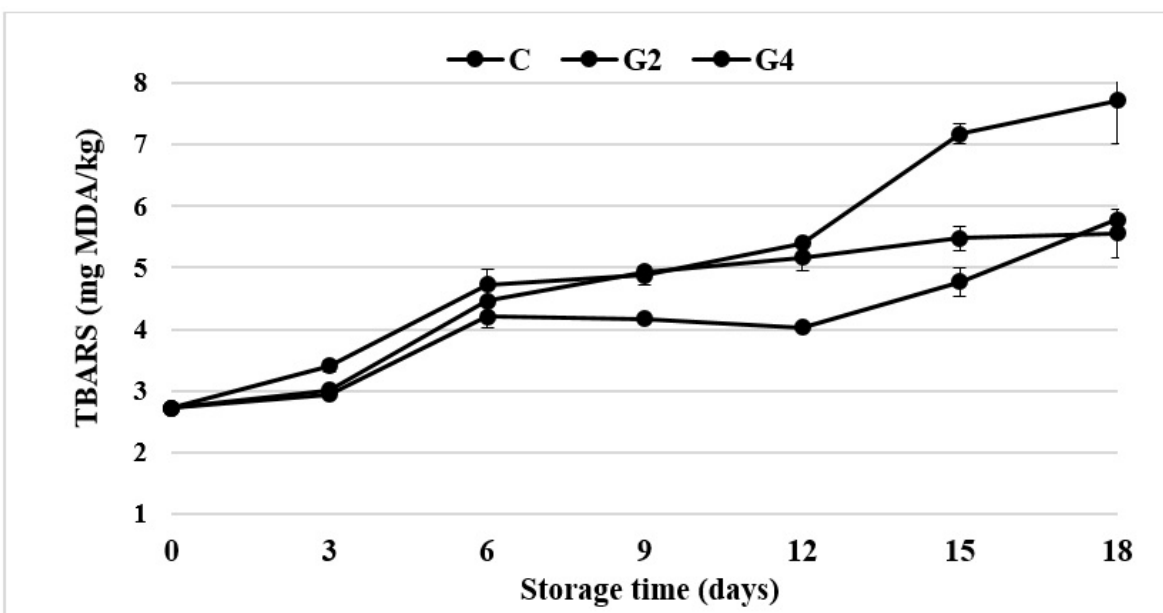

Figure 3. Changes in thiobarbituric acid reactive substances value (TBARS) (mg malonaldehyde/kg) of anchovy burgers treated with garlic peel extract (GPE) during 18 days of storage at 4°C. * Control: without treatment, G2: 2% GPE treated group; G4: 4% GPE treated group

values of lamb burgers. In the present study, fish burgers treated with 4% GPE showed maximum efficiency in delaying lipid oxidation.

Microbiological assessment

Total viable count (TVC) of anchovy burgers supplemented with GPE during refrigerated storage was shown in Figure 4. Initially, the number of bacteria in anchovy was found as 2.25 log CFU/g which is lower than initial TVC value of fresh anchovy observed by other researchers (33, 34, 35). During the storage period, TVC showed increase all samples and reached at 5.45, 5.33 and 4.98 log CFU/g in the control, G2 and G4 groups, respectively. Considering the acceptable limit value for fresh fish (7 log CFU/g) (36), TVC values did not exceeded the limit value in anchovy burgers during the storage. The lowest ($P<0.05$) TVC values were observed in the burgers treated with GPE, especially in the burgers treated %4 GPE. It was reported that GPE showed bacteriostatic activity in the beef samples (11). According to Bakri and Douglas (37), antibacterial effect of garlic resulted from interaction of sulphur compounds, allicin, with sulphur groups of microbial enzymes, leading to an inhibition of microbial growth. The results of present study verify that GPE greatly inhibited microbial growth in anchovy burgers as reported in previous studies.

Gram-negative psychrophilic bacteria group are responsible from aerobic spoilage in chilled stored fresh fish (38). The changes in psychrophilic bacteria count (PBC) of anchovy burgers are presented in Figure 5. At the beginning of the storage, PBC value of the anchovy was 3.30 log CFU/g and showed increase in all groups. The PBC value of G4 samples were lower ($P<0.05$) than the control and G2 samples during the storage period. At the end of the storage PBC values reached 6.46, 6.38 and 5.45 log CFU/g in control, G2 and G4 groups, respectively and PBC values of control and G2 samples exceeded the acceptable limit value of 6 log CFU/g (36).

Izci et al. (35) reported the initial PBC value of anchovy as 3.61 log CFU/g which is higher than the initial value of present study. In another study, Kose et al. (39) found the PBC value of anchovy as 4.39 log CFU/g at the beginning of the storage.

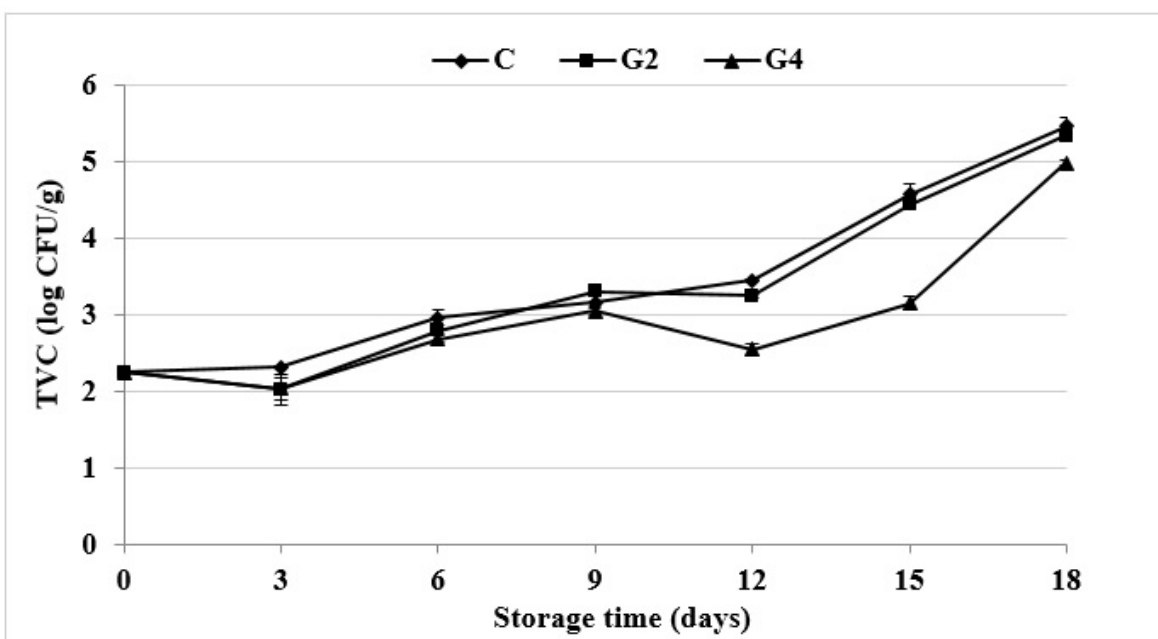

Figure 4. Total viable count of anchovy burgers treated with garlic peel extract (GPE) during 18 days of storage at 4°C. * Control: without treatment, G2: 2% GPE treated group; G4: 4% GPE treated group

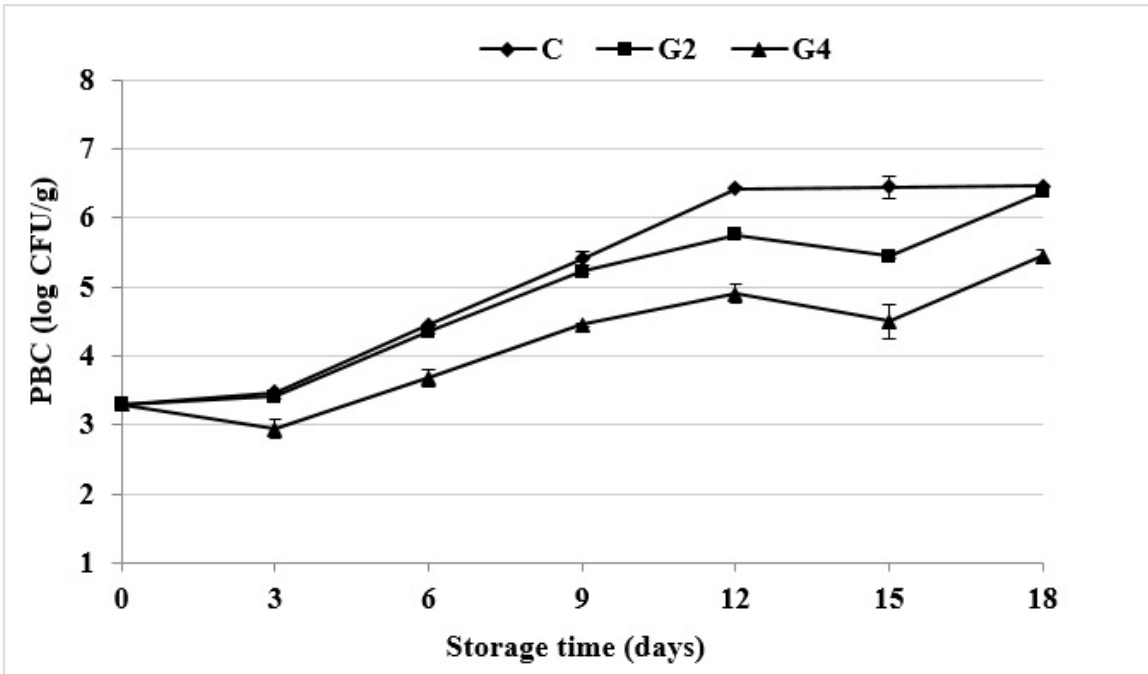

Figure 5. Psychrophilic bacteria count of anchovy burgers treated with garlic peel extract (GPE) during 18 days of storage at 4°C. * Control: without treatment, G2: 2% GPE treated group; G4: 4% GPE treated group

Sensory analysis

The changes of sensory properties scores including color, odor, flavor, texture and overall acceptability of anchovy burgers supplemented with different concentrations of GPE during storage at 4°C are presented in Table 2. According to sensory results, sensorial parameters of all groups decreased significantly during the storage period ($P<0.05$) and there were no significant difference between 2% and 4% GPE treated fish burgers.

There was an accordance between TBA values and taste panel results. Taste scores of the control, G2 and G4 samples decreased during the storage period ($P<0.05$). There was no significant differences in terms of taste scores between GPE treated burgers. Control samples reached unacceptable scores at day 12, while G2 and G4 samples reached at day 15 of the storage.

Significant differences ($P<0.05$) were observed in texture scores of the control and GPE added anchovy burgers from day 9 until at the end of the storage period. Sensory results showed that no significant differences were found in color scores of all groups during six days of storage. Color and odor scores of GPE added samples received higher values than those of the control samples but significant differences were observed between 9 and 18 days of storage.

According to overall acceptance results, the control group was rejected at day 15 by the panelists and considered as unacceptable. 2% and 4% GPE added anchovy burgers were still acceptable for consumption at the end of the storage period. The increase in shelf life of G2 and G4 groups compared to the control may be attributed due to the antibacterial effect of garlic peel. It can be concluded that addition of GPE prevented the formation of off-odor and off-color in anchovy burger samples. Addition of garlic extract did not have any negative effect on sensory attributes of garlic extract added anchovy burgers rather than having positive effects on odor, color and overall acceptance.

Bavitha et al. (40) reported fish burgers treated with 0.5% and 1% ginger have been acceptable for 17 days, while the control showed 15 days shelf life. Coban (41) found that the *Sarda sarda* fish fingers contained

Table 2. Changes in sensory properties of anchovy burgers treated with garlic peel extract (GPE) during 18 days of storage at 4°C. * Control: without treatment, G2: 2% GPE treated group; G4: 4% GPE treated group

	Storage days	Control	G2	G4
Taste	**0**	8.50±0.58^{Aa}	8.50±0.50^{Aa}	8.75±0.50^{Aa}
	3	8.00±0.00^{Aa}	7.75±0.98^{Aab}	7.50±0.58^{Aab}
	6	5.75±1.89^{Bb}	7.75±0.96^{ABab}	8.00±0.82^{Aa}
	9	5.75±0.96^{Bb}	7.00±0.00^{Ab}	8.00±0.82^{Aa}
	12	3.75±0.96^{Bc}	7.25±0.50^{Aab}	6.25±1.26^{Ab}
	15	3.00±1.41^{Acd}	3.25±1.71^{Ac}	3.25±1.26^{Ac}
	18	1.00±0.00^{d}	1.00±0.00^{d}	1.00±0.00^{d}
Texture	**0**	7.75±0.50^{Aa}	8.75±0.50^{Aa}	8.75±0.50^{Aa}
	3	7.00±2.00^{Aa}	7.50±0.58^{Aab}	7.75±0.50^{Ba}
	6	6.50±0.58^{Aa}	7.75±0.96^{Aa}	7.75±0.96^{Aa}
	9	6.50±0.58^{Ba}	7.50±0.58^{Aab}	8.25±0.50^{Aa}
	12	4.50±0.58^{Bb}	6.25±0.96^{Ab}	5.75±0.96^{ABb}
	15	4.00±0.82^{Ab}	3.75±1.26^{Ac}	4.25±1.71^{Ac}
	18	2.50±0.58^{Ac}	3.75±0.96^{Ac}	2.75±0.96^{Ad}
Color	**0**	7.75±0.50^{Ba}	8.75±0.50^{Aa}	8.50±0.58^{ABa}
	3	6.50±1.73^{Aa}	8.25±0.50^{Aab}	7.00±0.82^{Aa}
	6	6.75±0.50^{Aa}	8.00±0.82^{Aab}	7.75±0.96^{Aa}
	9	6.75±0.96^{Ba}	7.25±0.50^{ABbc}	8.25±0.96^{Aa}
	12	4.50±0.58^{Cb}	6.50±0.58^{Ac}	5.50±0.58^{Bb}
	15	3.50±1.29^{Ab}	4.25±0.96^{Ae}	4.50±1.29^{Ab}
	18	2.00±0.00^{Bc}	5.50±0.58^{Ad}	5.00±1.41^{Ab}
Odor	**0**	7.75±0.50^{Bab}	9.00±0.00^{Aa}	8.75±0.50^{Aa}
	3	8.00±0.82^{Aa}	8.50±0.58^{Aab}	7.75±0.50^{Aa}
	6	7.00±0.82^{Aab}	7.50±1.29^{Abc}	7.50±1.29^{Aab}
	9	6.50±0.58^{Bb}	7.25±0.96^{Bbc}	8.50±0.58^{Aa}
	12	4.25±0.50^{Bc}	7.00±0.82^{Ac}	6.25±0.50^{Ab}
	15	3.50±1.73^{Acd}	4.25±1.26^{Ad}	4.00±1.41^{Ac}
	18	2.50±1.00^{Bd}	4.75±0.96^{Ad}	3.00±0.82^{Bc}
Overall acceptance	**0**	7.75±0.50^{Ba}	8.75±0.50^{Aa}	8.50±0.58^{Aa}
	3	6.50±1.73^{Bb}	8.25±0.50^{Aab}	7.00±0.82^{Aa}
	6	6.75±0.50^{Bb}	8.00±0.82^{Ab}	7.75±0.96^{Aa}
	9	6.75±0.96^{Ab}	7.25±0.50^{ABb}	8.25±0.96^{Aa}
	12	4.50±0.58^{Cc}	6.50±0.58^{Ab}	5.50±0.58^{Bb}
	15	3.50±1.29^{Ad}	4.25±0.96^{Ac}	4.50±1.29^{Ac}
	18	2.00±0.00^{Be}	5.50±0.58^{Ac}	5.00±1.41^{Abc}

1% ginger oil can be stored for 17 days at 4°C without undesirable changes of sensory and chemical quality. In another study, it was observed that the addition of oregano extract prevented the loss of sensory quality in sheep burgers up to 15 days of storage.

Conclusions

The results of the study indicated that garlic peel extract has potential to prevent lipid oxidation and to maintain the initial product quality. The use of GPE in anchovy burgers also reduced the microbial growth, especially in %4 GPE treated group. The sensory quality of anchovy burgers was improved by addition of GPE. The use of 2% and 4% garlic peel extract prolonged the shelf life of anchovy burgers for 3 days.

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