



SAGE AND CLOVE AS ANTIOXIDANT AGENTS FOR PROTECTION OF FISH BURGER

Sara A. Saied*; M.A. Abdel-Samie and M.M. Ahmed

Dept. Food and Dairy Sci. and tech., Fac. Environ. Agri. Sci., Arish Uni., Egypt.

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ABSTRACT

Growing concerns about the health effects of synthetic preservatives have increased consumer demand for natural alternatives. On the other hand, medicinal and aromatic plants have shown strong potential for improving food safety and preservation. This study evaluated the antioxidant potential of sage powder (SP) and clove powder (CP) at 1% and 1.5%, as well as their mixtures (SCM); at 0.5% of sage powder and 0.5% of clove powder, and at 0.75% of sage powder and 0.75% of clove powder, in fish burgers stored at $4 \pm 1^\circ\text{C}$ for 12 days. Antioxidant activity was assessed based on total phenolic compounds content, total flavonoid content, and DPPH free radical scavenging capacity. Treated fish burgers were further analyzed for chemical, physical, antioxidant (DPPH), and sensory attributes. Results demonstrated that among the tested medicinal plant powders, CP showed the highest DPPH scavenging activity (90.02%), followed by SCM (85.82%) and SP (83.15%). SP, CP, and SCM effectively reduced lipid oxidation and maintained acceptable sensory quality throughout the storage period. These findings suggest that incorporating sage and clove powders can serve as a promising natural strategy to minimize oxidation and extend the shelf life of fish burgers.

INTRODUCTION

Fish and fish products are essential nutrient sources for humans due to their high-quality proteins, lipid-soluble vitamins (A, E, and D), vitamins, and minerals (P, I, Mg, and Se) (Uçak and Afreen, 2023). Fish products are rich in n-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic (EPA, C20:5) and docosahexaenoic acids (DHA, C22:6). PUFAs are involved in numerous metabolic and physiological processes such as cardiovascular disease and brain health, in addition to immunological response, allergies, pregnancy, and infancy (Gouvêa *et al.*, 2023). Due to the existence of PUFAs, fish products are susceptible to protein and fat oxidation, and they are

prone to the action of endogenous enzymes and the activity of microorganisms, which cause a reduction in safety, lower nutritional quality, and degraded sensory properties, including changes in taste, odor, color, and texture (Ameur *et al.*, 2022). Oxidation of PUFAs in fish is related to the production of off-odor molecules, including the formation of toxic and mutagenic compounds that might lead to the development of chronic diseases in humans (Chi-Cheng *et al.*, 2023).

Due to the increased awareness of consumers in their demand for natural antioxidants instead of synthetic antioxidants, and the fact that synthetic antioxidants are harmful to health and cause many health problems, many researchers have focused on finding alternative natural sources of antioxidants (Ripke Ferreira *et al.*, 2022).

* Corresponding author: E-mail address: alaasara354@gmail.com

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Antioxidant active compounds can maintain the quality of fish and fish products through the relegation of oxidation. The natural antioxidant additives, including plant extracts, like herbs, spices, tea, fruits, vegetables, and seeds, contain high and strong natural bioactive compounds that can enhance oxidative stability and microbial safety in meat and fish products (Chi-Cheng *et al.*, 2023). Bioactive compounds offer potential health benefits. Essential oils, phenolics, alkaloids, flavonoids, and terpenoids are of special important because they have antimicrobial, antioxidant, anti-inflammatory, anticancer, immunomodulatory, antitumor, and antiviral activities (Dar *et al.*, 2023). Sage (*Salvia officinalis* L.) belongs to the family Lamiaceae known as "maramia" in Arabic culture, it has shown various biological activities such as antifungal, antioxidative, and antibacterial.

Sage is used in food industries, pharmaceuticals, and perfumery, it contains phenols, such as carnosol, rosmanol, and rosmarinic acid, which are considered as its bioconstituants, acting as hydrogen donors to reactive oxidative species to prevent oxidation (Ripke Ferreira *et al.*, 2022; Vosoughi *et al.*, 2018). Using sage powder in burgers showed high antioxidant activity and was able to preserve the sensory quality of the cooked beef burger (Mizi *et al.*, 2019).

Clove (*Syzygium aromaticum*) belongs to the Mirtaceae family and is a species known for its antimicrobial and antioxidant characteristics, it has significant applications in pharmaceuticals, cosmetics, food, agriculture, and various medical uses. Clove is considered the highest source of phenolic compounds, including eugenol, eugenol acetate, flavonoid, and gallic acid (Pandey *et al.*, 2024). Clove was applied in cooked ground beef have been shown to decrease lipid oxidation, and levels of volatile compounds after cooking even when compared to BHT (Zahid *et al.*, 2022).

Antioxidant active compounds can slow or stop oxidation in foods that causes quality deterioration and spoilage in some cases, even if it happens in low rates. Oxidation inhibition is achieved through processes that include converting free radicals formed during the initiation and propagating phases into stable products and protecting lipids from oxidation initiators (Gouvêa *et al.*, 2023). Several chemical indices are commonly used to monitor oxidation and spoilage, including thiobarbituric acid reactive substances (TBARS), which measure secondary lipid oxidation and are widely used to detect oxidative rancidity (Ameur *et al.*, 2022), and peroxide value (PV), an indicator of the primary oxidation of fats, reflecting the formation of odorless hydroperoxides or their breakdown into secondary oxidation products. PV also reflects the reactivity of hydroperoxides with proteins and is considered a detrimental quality parameter (Ucak and Fadiloğlu, 2020). Another key indicator in fish burger storage experiments is Total Volatile Basic Nitrogen (TVB-N), which is used to evaluate seafood quality and detect spoilage. In addition to chemical assessments, physical parameters such as cooking loss, cooking yield, and shrinkage are essential to reflect the product's behavior during cooking (Chi-Cheng *et al.*, 2023).

The present study aimed to evaluate the addition of sage and clove powders and their mixture as natural antioxidants and to investigate their effects on the chemical, physical, and sensorial properties of fish burgers that are stored under cooling at $4\pm1^{\circ}\text{C}$ for 12 days in the refrigerator.

MATERIALS AND METHODS

Materials

1,1-Diphenyl-2-picryl-hydrazil (DPPH), Folin-Ciocalteu reagent, gallic acid. Thiobarbituric acid (TBA) was obtained from Sigma-Aldrich Chemical, Germany.

Chloroform and acetic acid glacial were obtained from Alpha Chemika, India. Sodium carbonate, ammonium chloride, potassium acetate, hydrochloric acid (HCl), acetic acid, methyl red, boric acid, sulfuric acid, magnesium oxide, potassium iodide, sodium thiosulphate, starch, ethanol, and methanol were purchased from El-Gomhouria Company. Clove buds and sage leaves were obtained from the local market in Arish City, North Sinai, Egypt

Plant Extraction

Plant parts were washed with clean water, dried in a forced air oven (DHG-9140A; Yiheng Instrument Co., Ltd., Shanghai, China) at 40°C to a constant weight, ground into a fine powder using a commercial grinder (Tornado, Elaraby), and passed through 80-mesh sieve to obtain fine powder (Abdel-Wahab *et al.*, 2020). Clove and sage extract solutions were prepared according to (Miliauskas *et al.*, 2004) by dissolving 0.25 g of dry plant powder in 100 ml of methanol, holding it in a shaking water bath at 60°C for 30 min, cooled in an ice bath at 10°C for 5-10 min, and centrifuged (Hettich Zentrifugen, Model 2002, Serial No. 0071868, Germany) at 7000×g for 10 min, the supernatant was then separated and kept at -18°C until further use.

Preparation for Fish Burgers

Mullet Fish was obtained from Bardawil Lake, North Sinai, Egypt, and transferred to the laboratory in an ice box. Fish was cleaned with water; skin, gut, and heads were removed, and fish was then cleaned and cut into fillets, minced in a mixer (Tornado, Elaraby), and divided into seven groups. Fish burger samples were prepared according to Uçak and Afreen (2023), by mixing 87.8% minced fish meat with other dry ingredients, including 0.2% onion powder, 0.2% garlic powder, 1.2% salt, 0.6% sugar, 6% corn flour, and 4% wheat flour, and medicinal plants powders were added based on Table 1. Mixtures of control samples with different additives were divided to 50g weight ball, formed in

cylindric disks using a burger manual press with a 9 cm diameter. Fish burger samples were kept at 4±1°C up to 12 days.

Fish Burger Extraction for Analysis

Fish burger extract was prepared by the homogenization of 10 g of fish burger samples with 50 mL of 80% methanol and kept for 5 minutes under agitation, centrifuged (Hettich Zentrifugen, Model 2002, Serial No. 0071868, Germany) at 7000×g for 10 min, and supernatant was separated and kept at -18°C until further use (Alqurashi and Aldossary, 2021).

Antioxidant Activity

Total phenolic contents were determined using the Folin-Ciocalteu reagent according to the method of (Spinelli *et al.*, 2018). Briefly, 500 µL of the supernatant of plant extract or fish burger sample extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 mL of NaCO₃ solution (7.5% w/v). The mixtures were kept in dark for 1 h at room temperature (25 ± 2°C), and the absorbance of the mixtures was read at 760 nm using a Spectrophotometer (Jenway 6300), blank was prepared using water instead of the extract and treated in the same manners. A standard curve was prepared using different concentrations of gallic acid. Total phenolic contents were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (DW), and the total phenolic contents were calculated using the following formula:

$$y = 0.0056x - 0.0972; R^2 = 0.9982$$

y = absorbance (measured at a specific wavelength, 760 nm for total phenolic content assay). x = concentration of gallic acid equivalent (GAE) in mg/g of extract (calculated based on the calibration curve). R^2 = coefficient of determination, indicating how well the linear regression model fits the data. An R^2 value of 0.9982 represents an excellent fit, meaning 99.82% of the variability in absorbance is explained by the concentration of GAE.

Table 1. Fish burger treatments (addition of medicinal plants)

Treatment Name		Added medicinal plant	Added amount Per 100g
Ctrl		No additions	
SP 1%		Sage powder	1g
SP 1.5%		Sage powder	1.5g
CP 1%		Clove powder	1g
CP 1.5%		Clove powder	1.5g
SCM 1%	Mixture	Sage powder	0.5 g
		Clove powder	0.5 g
SCM 1.5%	Mixture	Sage powder	0.75 g
		Clove powder	0.75 g

Total flavonoid contents (TFC) of medicinal plant extracts were determined according to the method of (Aminzare *et al.*, 2022). The method includes mixing 0.5 ml of the supernatant of plant extract with 1.5 ml of methanol 80%, followed by the addition of 0.1 ml of aluminum chloride 10% solution, 0.1 mL of potassium acetate solution, and 2.8 ml of distilled water, kept for 30 min in dark at room temperature ($25 \pm 2^\circ\text{C}$). The absorbance of the mixture was then taken at 415 nm using a spectrophotometer (Jenway 6300). Quercetin was used to build a standard curve, and the results were expressed as mg quercetin/g DW using the following formula:

$$y = 0.0070x - 0.0968; R^2 = 0.9961$$

Where: y = absorbance (measured at a specific wavelength, 415 nm for total flavonoids assay). x = concentration of Quercetin mg/g of extract (calculated based on the calibration curve). R^2 = coefficient of determination, indicating how well the linear regression model fits the data. An R^2 value of 0.9961 represents an excellent fit, meaning 99.61% of the variability in absorbance is explained by the concentration of Quercetin.

The DPPH scavenging activity percentages of plant extracts and fish burger samples were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method described by Alqurashi and Aldossary (2021). In details, 2 mL of the supernatant of the extract was added to 2 mL of 0.1 mM DPPH working solution (78 mg of DPPH dissolved in 200 mL methanol), methanol was used instead of the sample to from control, and the absorbance was measured after 30 min of incubation at room temperature ($25 \pm 2^\circ\text{C}$) in dark at 517 nm using a spectrophotometer (Jenway 6300). The DPPH scavenging activity was calculated through the following formula:

$$\text{DPPH Scavenged \%} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

Chemical Properties of Fish Burgers

Moisture content of fish burgers was determined according to the official methods (AOAC, 2019). Lipid oxidation of the fish burger samples' extracts was determined using the distillation of 2-thiobarbituric acid (TBARS) method, as mentioned by Ucak and Fadiloğlu (2020). Peroxide value of fish burger sample extracts was determined according to

(Tareq *et al.*, 2018) method. TVB-N values of fish burger sample extracts were determined according to the method of (Ameur *et al.*, 2022).

Physical Properties of Fish Burgers

Physical changes during cooking were assayed through measuring cooking loss and cooking shrinkage according to the methods described by (Parvizi and Moosavi-Nasab, 2021) and cooking yield according to Duman (2022) and were calculated as described in the following formulas:

$$\text{Cooking loss (\%)} = \frac{(\text{weight before cooking} - \text{weight after cooking})}{\text{weight before cooking}} \times 100$$

$$\text{Shrinkage (\%)} = \frac{(\text{Diameter of raw burger} - \text{Diameter of cooked burger})}{\text{Diameter of raw burger}} \times 100$$

$$\text{Cooking yield (\%)} = \frac{\text{Weight of cooked fish burger}}{\text{Weight of uncooked fish burger}} \times 100$$

Sensory Evaluation of Fish Burgers

Sensory evaluation of fish burger samples prepared without and with the addition of different plant powders or their mixtures was performed by 10 panelists of the Department of Food and Dairy Sciences and Technology, Faculty of Environmental Agricultural Sciences, Arish University, and 40 judges according to Ali *et al.* (2019). The sensory parameters of the fish burger included color, odor, texture, flavor, and overall acceptability, and the evaluation was done on a 9-points hedonic scale, with 1 dislike extremely and 9 like extremely, where 4 considered the threshold of acceptability.

Statistical Analysis

All data were done in triplicates and expressed as mean $\pm$ standard deviation. Results were statistically analyzed using one way analysis of variance (ANOVA) and a Post hoc of Tukey s-b using SPSS software, version (27.0.1) (Cui *et al.*, 2023).

RESULTS AND DISCUSSION

Antioxidant Activity of Raw Materials

In our study, sage and clove, individually; sage powder extract (SE), clove powder extract (CE), or combined (SCM) in the ratio of 1:1 w:w as shown in Fig.1, were compared in regards to TPC, TFC, and DPPH scavenging activity, and results were shown in Fig.1.

CE exhibited the highest TPC (341.538 mg GAE/g), followed by SCM (236.9 GAE/g) and SE (208.855 mg GAE/g). The obtained TPC in clove and sage extract was higher than the value reported by Abdel-Wahab *et al.* (2020), which were 317.30 mg GAE/g, and 140.54 mg GAE/g respectively. TPC in sage extract obtained in this study was higher than value reported by (Darwish *et al.*, 2018), which was 88.57 mg GAE/g. In regards to TFC, CE had the highest content (88.143 mg/g) compared to SE and the mixture (66.330 mg/g and 76.926 mg/g, respectively). The results of this study were lower than those reported by Abdel-Wahab *et al.* (2020) for clove and sage powder extract, which were 106.14 and 79.96 mg/g respectively. In regard to DPPH scavenging activity, CE showed the highest value 90.022 %, followed by the SCM 85.828%, while SE showed the lowest value 83.153%. Shi *et al.* (2014) reported that the DPPH activity of clove extract was (94%) and Al-Mashkor (2015) observed similar results, in clove 87.50%. Such DPPH results in clove extract are in accordance with those (Xu *et al.*, 2023), which recorded a value of 94.28%.

Antioxidant Activity of Fish Burgers

The DPPH free radical scavenging activity% decreased significantly in all fish burger samples during storage, as shown in Fig.2. The first day of cold storage, Ctrl fish burger samples observed the lowest value (59.2%), while SP1%, SP1.5%, CP1%,

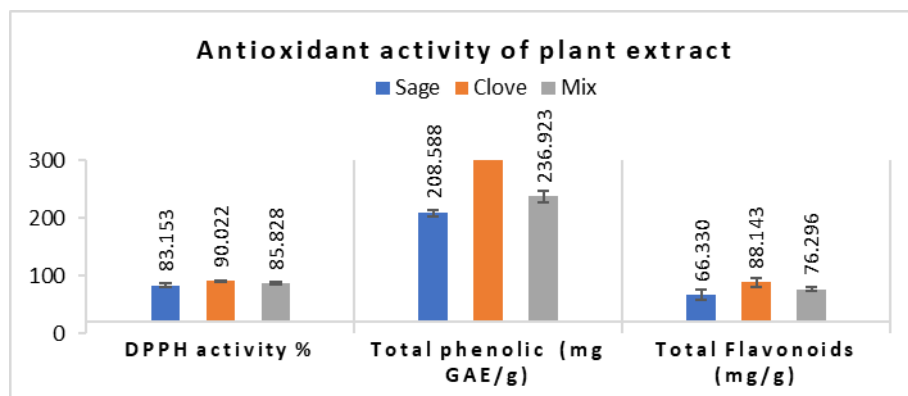


Fig. 1. Antioxidant Activity of Plant Extract

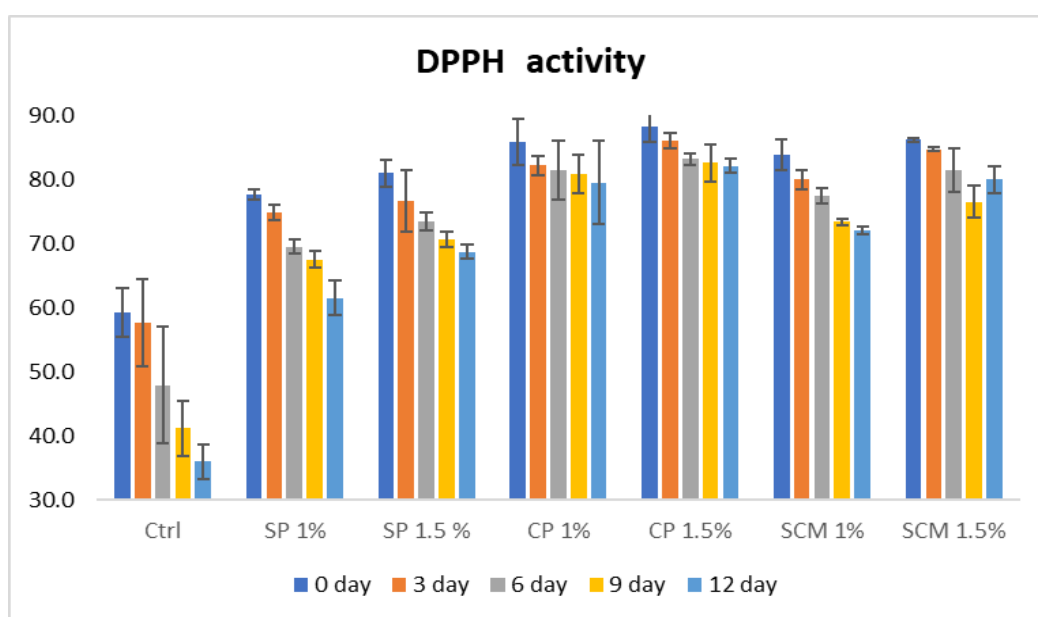


Fig. 2. Effect of sage, clove powders and their mixtures addition to fish burger in different concentrations (1 and 1.5%) on DPPH scavenging activity % during storage for 12days at 4°C

CP1.5%, SCM1% and SCM 1.5% fish burger samples significantly showed higher DPPH scavenging activity% compared to control fish burger samples. Among the medicinal plant samples, CP1.5% exhibited the highest value (88.3%), while SP1% showed the lowest (77.7%). At 12 days of storage, Ctrl continued obtaining the lowest DPPH scavenging activity (36%), while CP1.5% recorded the highest value (82.1%). All medicinal plants showed better DPPH scavenging activity% compared to that of control samples without the addition

of medicinal plants. During storage time, the DPPH scavenging activity% of all tested samples decreased by different rates, as it decreased by 39.2% in control fish burger sample, while in other fish burger samples its decreased by 20.8 and 15.2% in sage added fish burger (1 and 1.5%). The least decrease in DPPH scavenging activity was obtained by clove added fish burger samples, as it showed 7.3 and 7.0% decrease in 1 and 1.5 clove added fish burger samples. SCM showed closer results to the clove containing fish burger samples,

as SCM 1 and 1.5% showed 14.1 and 7.3% decreases in DPPH scavenging activity, respectively. Concluding that cloves individually or in mixture with sage had better protection of fish burgers and maintained good antioxidant activity stability through storage period for 14 days of cold storage. These results were in-line with those found by (Cedola *et al.*, 2017), who reported that the addition of olive paste flour to fish burger samples increased DPPH scavenging activity. Decrease in DPPH scavenging activity might be attributed to the depreciation of the antioxidant bioactive compounds in the applied medicinal plants in protection of fish burger samples against oxidation (Ahmed *et al.*, 2022).

Moisture Contents, TBARS, Peroxide Values and TVB-N of Fish Burger Samples

The chemical composition of fish burgers without and with the addition of different concentrations (1% and 1.5%) of SP, CP and SCM during storage 4°C for 12 days was analyzed. Assayed chemical composition of fish burger samples included moisture contents, TBARS, peroxide values and TVB-N. Results of chemical composition of fish burgers were presented in Fig. 3.

According to Fig. 3.A, no significant differences ($p < 0.05$) were observed between different fish burger samples (Ctrl, SP1, SP1.5, CP1, CP1.5, SCM1, and SCM1.5) in moisture content at the first day of cold storage ranged around 66.6%. However, after 12 days of cold storage, the moisture content in the control group significantly decreased from 66.6% to 62.5% by rate 6.2%, while SP, CP, and SCM containing burger samples showed more stability in moisture contents, maintaining an average of around 64.2%, as the changes did not exceed 3.6% of their initial moisture contents. Fewer changes in moisture contents in medicinal plants

containing burger samples might be due to the relatively higher fiber contents in the added medicinal plant powders, which retained more moisture and altered its loss. These changes were also reported by (Abou-Taleb, 2022) who found a stability in moisture contents in fish burgers enriched with high fiber pomegranate peel powder.

TBARS of fish burger of different samples without or with the addition of plant powders, including sage, clove, and their mixtures were analyzed for 12 days of storage at 4±1°C. From data presented in Fig. 3.B, it could be merged that, at the first day of cold storage, TBARS values of all samples were statistically the same (0.64-0.70 MA/Kg). TBARS values of control fish burger samples doubled (100.0% increase) from 0.70 to 1.40 MA/Kg. After 12 days of cold storage, fish burger samples containing medicinal plant extracts showed lower increases in TBARS values. Sage resulted in 0.87 and 0.85 mg MDA/kg, clove extract showed 0.87 and 0.85 mg MDA/kg, and the mixtures recorded 0.87 and 0.86 mg MDA/kg for the 1% and 1.5%, respectively. Less TBARS changes in medicinal plants containing fish burger compared to that in control samples indicate less oxidation in fish burger samples with the addition of SP, CP, and SCM as an effect of the higher antioxidant activity in sage and clove, which inhibited the oxidation of lipids, and the higher level of plant powder addition showed proportionally higher levels of inhibition of TBARS changes: 28.8 Vs 33.8% in Sage, 28.8 Vs 31.8%, and 34.4 vs 33.8% in 1.5 Vs 1.0% addition of sage, clove, and their mixtures. The increased TBARS values in fish burgers through the storage period were also found by Abdel-Wahab *et al.* (2020) who reported an increase in TBARS values in fish burger samples from 0.14 to 0.55MA/Kg. Another (Ucak and Fadiloğlu, 2020) reported an increase in TBA value in fish burgers treated with 2 and 4% garlic peel extract, rising from 2.72 mg MDA/kg to 5.78 and 5.56 mg MDA/kg, respectively.

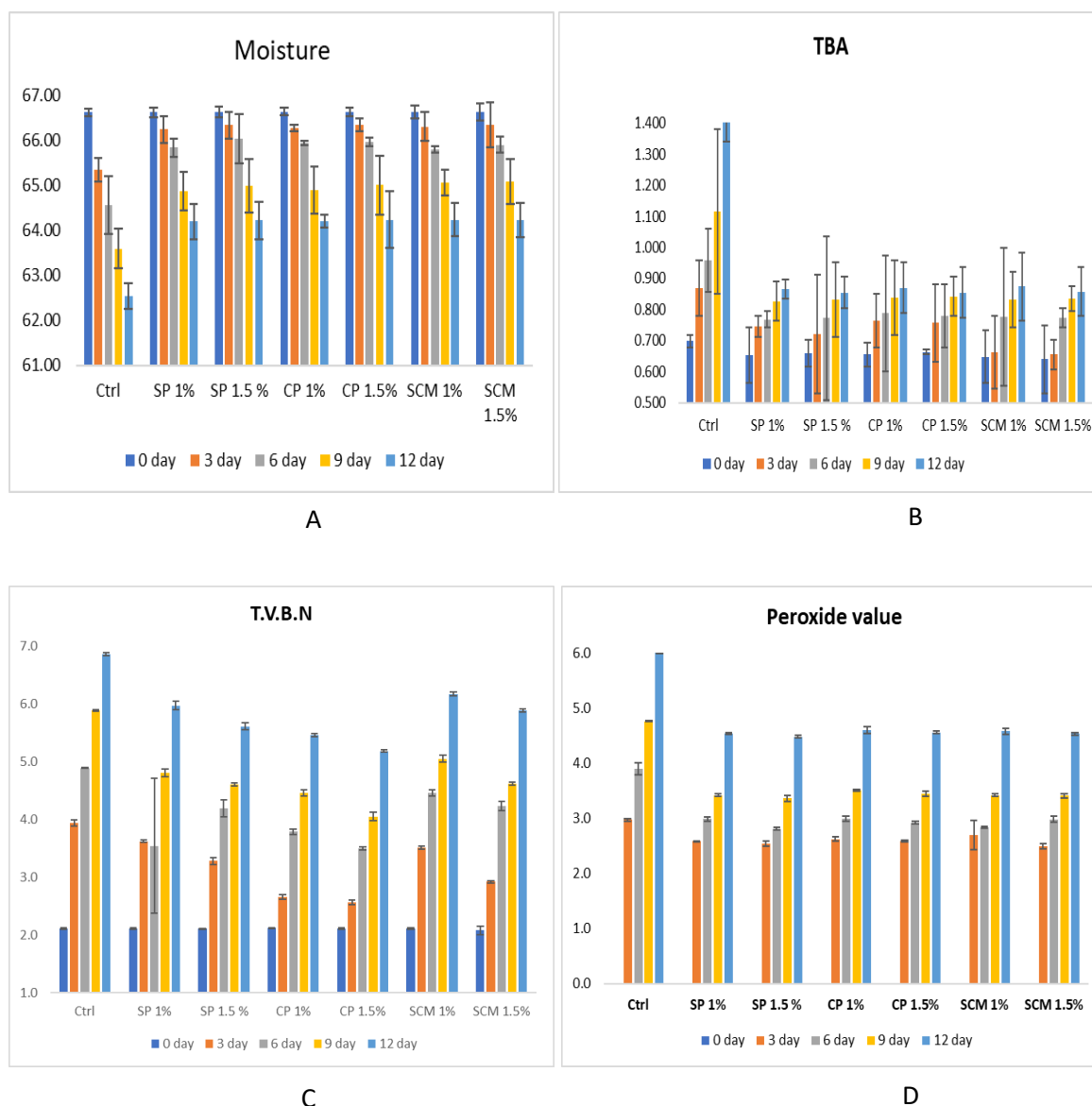


Fig. 3. Effect of sage, clove powder and their mixture at different concentrations on (A) Moisture content, (B) TBA, (C) TVB-N. and (C) Peroxide value of fish burger samples during storage at 4°C

TVBN of fish burger of different samples without or with the addition of plant powders, including sage, clove, and their mixtures were analyzed for 12 days of storage at $4 \pm 1^\circ\text{C}$. During the storage periods, control sample exhibited the highest increased in TVB-N, raising by 228.6%, (from 2.1 to 6.9 mg/100g), while fish burger samples containing medicinal plant powders (SP 1 and 1.5%, CP 1 and 1.5%, and SCM 1 and 1.5%) showed lower rates of increase,

ranging from 147.6 to 195.2% of its initial TVB-N concentration. The lowest increase was observed in CP1.5% sample (147.6%, from 2.1 up to 5.2mg/100g), while the highest increase among medicinal plant treated was recorded in SP1% (185.6% from 2.1 to 6.0 mg/100g). Previous studies have reported similar results, as TVB-N increased from 2.2 to 4.8 mg/100g in fish burgers stored for 17 days under cooling (Vanitha *et al.*, 2015). Same results were

also found by (Abdel-Wahab *et al.*, 2020), who managed to suppress the changes in TVB-N in fish fingers using sage and clove and kiwi peel powder.

Peroxide value (PV) as could be merged from Fig. 3.D., at the first day of storage, PV was not detected because none of the oxidation processes had started yet. Through storage period, PV increased in all fish burgers samples. In control sample, PV rose by 3.5% from its initial value, reaching 3 meq O₂/kg. In contrast, samples containing medicinal plant powders showed a lower increase, ranging from 2.5% to 2.7%, with PV values between 2.5 and 4.6 meq O₂/kg. After 12 days of storage PV of control sample was maximal among all other samples (6.0 meq O₂/Kg), while lower PV values were scored by other samples with a minimal value (4.6 meq O₂/Kg) which was obtained by CP 1.5%. Our trend of changes were in line with Parvizi and Moosavi-Nasab (2021) who reported that PV of fish burgers increased dramatically with time of storage (freezing for 90 days), and the spiking of burger using some preservatives relegated the increasing rate (Parvizi and Moosavi-Nasab, 2021). Same trends were also obtained by Gomaa *et al.*, (2019) who concluded that sage might decrease the rate of PV increase in fish burgers (Gomma *et al.*, 2019).

Physical Properties

Physical properties of fish burger samples' ctrl, SP1, SP1.5, CP1, CP1.5, SCM1, and SCM1.5 for stored 12 days at 4±1°C were presented in Table 2.

Cooking loss of fish burgers in all samples increased during storage period. The control sample recorded the highest rate of change as it increased by 59.0% (from 16.1 to 25.6%), and all treatments followed the same trend of changes, but in lower ratios in medicinal plants added burger. SCM 1.5% showed the lowest rate of increase, at 19.7% (rising from 14.7 to

17.6%) during the storage periods. This was followed by SP and CP (1 and 1.5%) as well as SCM 1%, which recorded values ranging from 14.5% to 18.8%. Reduced shrinkage in the medicinal plants containing fish burger samples might be because of their relatively higher fiber contents (determined as 27.9% in sage and 14.4% in clove as mentioned in the study of Gengatharan and Rahim (2023) and Vlaicu *et al.* (2022), which can retain more water and prevented its evaporation during cooking, thus decrease cooking loss. These results were found to be in the same trends obtained in chicken sausages with the addition of clove powders (Kamel Moawad *et al.*, 2020).

Cooking yield of fish burger samples through storage period were shown in Table 2. The control fish burger samples showed a decline in cooking yield over time, ranging by (18.2% from 83.9 to 68.6%). CP1.5% and SCM1.5% observed similar value and lower cooking yield of 88% and 88.1%, respectively at zero time, while other treatment ranged from 84.9 to 87.9%. By the 12 days of storage, fish burgers containing medicinal plants showed a cooking yield ranging from 78.8% to 84.6%. Among treatments, CP 1% exhibits the lowest cooking yield change rate during storage, which reached 3.4%. (Kamel Moawad *et al.*, 2020) noticed a decrease in cooking yield in chicken sausage treated with clove extract (0.05%) from (85.4 to 78.7%).

Cooking shrinkage of fish burger samples without and with the addition of sage, clove, their mixtures were presented in Table 2. Statistically, there were no significant differences between shrinkage rates of different fish burger samples in the beginning of burger preparation. Ctrl obtained the highest increase of 5.6%, while all medicinally treated fish burgers recorded lower values ranging from (4.5 to 4.8%). After storage of burgers, shrinkage increased by 53.6% in fish burger samples without any medicinal plant added from

Table 2. Effect of sage, clove powder and their mixture with different concentrations on cooking loss, cooking yield and shrinkage in fish burger during storage at 4±1°C

Physical Properties	Storage Periods	Treatment						
		Ctrl	SP 1%	SP 1.5%	CP 1%	CP 1.5%	SCM 1%	SCM 1.5%
Cooking loss	first day of cold storage	16.1± 0.0 ^c	15.1± 0.1 ^b	14.7± 0.1 ^a	14.8± 0.0 ^a	14.5± 0.2 ^a	14.8±0.2 ^a	14.7 ±0.1 ^a
	6 days	20.9± 0.2 ^c	16.8± 0.1 ^b	16.7± 0.1 ^b	16.6± 0.1 ^b	15.8± 0.1 ^a	16.7±0.1 ^b	15.9 ± 0.2 ^a
	12 days	25.6± 0.2 ^d	18.8± 0.1 ^c	18.3± 0.1 ^b	18.3± 0.0 ^b	18.1± 0.1 ^b	18.2±0.2 ^b	17.6 ± 0.1 ^a
Cooking yield	first day of cold storage	83.9± 3.5 ^a	84.9± 0.1 ^a	85.4± 0.1 ^a	85.6± 0.1 ^a	88.0± 2.0 ^a	87.2± 1.0 ^a	88.1± 0.2 ^a
	6 days	74.2± 0.1 ^a	83.3± 0.2 ^c	83.6± 0.1 ^d	83.2± 0.1 ^c	86.5± 0.0 ^f	79.3± 0.1 ^b	85.5 ± 0.2 ^c
	12 days	68.6±0.1 ^a	81.7± 0.1 ^c	82.1± 0.1 ^c	82.7± 0.1 ^d	84.6± 0.1 ^f	78.8± 0.6 ^b	83.4 ± 0.1 ^c
Shrinkage	first day of cold storage	5.6±0.0 ^f	4.8±0.0 ^e	4.6±0.0 ^d	4.6±0.0 ^{cd}	4.5±0.0 ^{ab}	4.5±0.0 ^{bc}	4.5 ± 0.0 ^a
	6 days	7.1±0.0 ^e	5.6±0.0 ^d	5.3±0.0 ^b	5.3±0.0 ^b	5.3±0.0 ^b	5.5±0.0 ^b	5.0 ± 0.0 ^a
	12 days	8.6±0.0 ^e	6.7±0.0 ^d	6.6±0.0 ^c	6.5±0.0 ^b	6.5±0.0 ^a	6.5±0.0 ^{bc}	6.4± 0.0 ^a

* Values are expressed as Mean ± standard deviation (n= 3 replicates). ** Lowercase letters (a, b, c, d, f) within the same storage time indicate significant differences between treatments (horizontal comparison) at $p < 0.05$.

(5.6 to 8.6%), while the addition of sage decreased this rate to 39.6 and 43.5% in SP1 and SP 1.5% respectively and to 41.3 and 44.4% in CP1 and CP 1.5% and to 44.4 and 42.2% SCM1 and SCM 1.5%, respectively. These results were also obtained by **Parvizi and Moosavi-Nasab (2021)** who noticed an increase in fish burger shrinkage from 7 to 8.47% after freezing for 3 months. **Chi-Cheng *et al.* (2023)** also reported an increase in sardine patti treated with oregano, cloves, cinnamon, turmeric, and green tea, ranging from 8.4 to 11.4%.

Sensory evaluation

The sensory characteristics evaluated in our study included color, odor, texture, flavor, and general acceptability of fish burger samples without and with the addition of medicinal plant powders and their scores were shown in Table 3.

There were no significant differences in sensory evaluation parameters of all fish

burger samples. CP 1.5% had a lower flavor score (6.1) due to the strong flavor of clove and lower general acceptability score (6.3). All treatments remained within acceptable sensory limits (5). Medicinal plants in this study have effectively mitigated the fishy odor due to their strong aroma. These findings are aligned with **Chi-Cheng *et al.* (2023)**, who reported that clove yielded lower results for flavor and overall acceptability in sardine patties. In contrast, **Gomma *et al.* (2019)** reported higher scores for all sensory attributes in anchovy fish burgers treated with sage essential oil at concentrations of 2,4% and 6%. The same findings were also obtained by **Zahid *et al.* (2020)**, who reported an increased overall acceptability in cooked beef patties with the addition of clove extract. The unaffected sensory properties might be due to the variability in the preferences of panelists and also because the clove and sage is preferred to the local consumers as they are extensively used as a spice.

Table 3. Effect of sage, clove powder and their mixture with different concentration on sensory evaluation in fish burger at the first day of cold storage

Treatment	Color	Odor	Texture	Flavor	General acceptability
Ctrl	6.9±2.1*	7.0±1.5	7.6± 0.9	7.7 ± 1.3	7.8± 1.2
SP 1%	7.3± 1.1	7.4±1.4	6.6±0.8	6.3± 1.5	6.9 ±1.6
SP 1.5 %	8.1±1.2	7.2±1.1	7.3±1.1	7.4± 1.1	7.7 ±1.1
CP 1%	8.0 ± 0.9	7.4±0.9	7.0±0.8	7.3± 1.1	6.9±1.7
CP 1.5%	7.9±0.7	7.2± 1.3	7.0±1.6	6.1± 1.9	6.3±1.7
SCM 1%	8.2 ±0.9	7.8±1.3	7.8±1.4	7.9± 1.4	7.9±1.4
SCM 1.5%	7.3± 0.9	7.1± 1.6	7.0±1.3	7.1± 1.6	7.3±1.1

* No significant differences were obtained within the means of sensory evaluation scores.

Conclusion

The results indicate that sage and clove powders effectively reduce lipid and protein oxidation in fish burgers during storage. Although moisture content and DPPH antioxidant activity declined over time, TBARS, PV, and TVBN levels, while increasing, remained lower than in the control and within acceptable limits. Consequently, sage and clove powders show promise for extending shelf life and preserving sensory quality of fish burgers.

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الملخص العربي

المريمية والقرنفل كمضادات للأكسدة في برجر السمك

ساره علاء سعيد، محمد عبدالشافى عبدالسميع، محمد مصطفى أحمد

قسم علوم وتكنولوجيا الأغذية والألبان، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.

تزايدت المخاوف بشأن التأثيرات الصحية للمواد الحافظة الصناعية، مما أدى إلى زيادة طلب المستهلكين على البدائل الطبيعية. من ناحية أخرى، تُظهر النباتات الطبية والعطرية إمكانات قوية في تحسين سلامة الأغذية وحفظها. هدفت هذه الدراسة إلى تقييم القدرة المضادة للأكسدة لكل من مسحوق المريمية ومسحوق القرنفل بنسبة ١٪ و ١,٥٪، بالإضافة إلى مزيجهما بنسبة ٠,٥٪ أو ٠,٧٥٪ لكل منهما، في برجر السمك المخزن عند ٤ درجات مئوية لمدة ١٢ يومًا. تم تقييم النشاط المضاد للأكسدة بناءً على محتوى المركبات الفينولية الكلية، ومحتوى الفلافونويدات الكلية، وقدرة التخلص من الجذور الحرة. من بين المعاملات المختبرة، أظهر مسحوق القرنفل أعلى نشاط في التخلص من الجذور الحرة (٩٠,٠٢٪)، تلاه المزيج بنسبة (٨٥,٨٢٪)، ثم مسحوق المريمية بنسبة (٨٣,١٥٪). تم تحليل برجر السمك المعالج من حيث الخصائص الكيميائية والفيزيائية والنشاط المضاد للأكسدة والصفات الحسية. وأظهرت النتائج أن المرمية والقرنفل ومزيجهما ساهمت بشكل فعال في تقليل أكسدة الدهون والحفاظ على جودة حسية مقبولة طوال فترة التخزين. تشير هذه النتائج إلى أن إضافة مساحيق المريمية والقرنفل يمكن أن تكون استراتيجية طبيعية واعدة لتقليل الأكسدة وإطالة العمر الافتراضي لبرجر السمك.

الكلمات الإسترشادية: النشاط المضاد للأكسدة، النباتات الطبية، المريمية، القرنفل، برجر السمك.

REVIEWERS:

Dr. Ramadan Habiba

Dept. Food Technology, Fac. Agric., Suez Canal Univ., Egypt.

| ramadan.habiba@yahoo.com

Dr. Abbas Omar Toliba

Dept. Food sci., Fac. Agric., Zagazig Univ., Egypt.

| beso14omar@yahoo.com

