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Effect Addition of Lactic Acid and Pomegranate Peel Extract Combined with Paprika Powder on the Safety and Quality of Refrigerated White Grouper Fish (*Epinephelus aeneus*).

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ABSTRACT

White grouper (*Epinephelus aeneus*) is an economically important fish found in the southern Mediterranean Sea. This study was done to assess the effect of lactic acid buffer (LAB), pomegranate peel extract combined with paprika powder (PPP), and their combinations on the safety and quality of white grouper stored at 4 °C. Fresh fish was treated with LAB (2 %, 4 %, 6 %, and 8 %), pomegranate peel extract (100 mg/kg) combined with paprika powder (5 g/kg) (PPP) and combinations of LAB with PPP. Microbiological quality, pH value, and organoleptic characteristics were assessed during refrigerated storage. Results showed that psychotropic aerobic bacteria; H₂S-producing bacteria and *Enterobacteriaceae* were strongly inhibited by all treatments applied, which would enhance safety, quality and prolong the shelf life of fish. The highest inhibition was observed with 8% LAB combined with PPP. pH reductions of 0.64, 1.14, 1.61 and 1.99 were observed by treatment with 2%, 4%, 6%, and 8% LAB, respectively. The sensory evaluation showed that lowering the pH to 4.38 did not influence the organoleptic properties of fish, which was supported by the microbiological changes. Fish treated with either 2% LAB or PPP could be refrigerated safely for 7 days at 4 °C, as compared to 6 days with the untreated samples. Increasing LAB concentration to 4, 6, and 8% increased the shelf life to 8, 9 and 10 days, correspondingly. Additionally, combining LAB (2%, 4%, 6%, and 8%) with PPP could further expand the shelf life to 8, 9, 10 and 11 days, respectively.

Keywords: White grouper fish; Lactic acid; Pomegranate peel extract combined with paprika powder; Quality and Safety; Storage at 4 °C.

INTRODUCTION

The global average consumption of fish has grown from 9.9 kg in the 1960s to 19.7 kg in 2013, with predictions demonstrating further increase exceeding 20.5 kg (FAO, 2020).

A healthy diet should include seafood on a regular basis. The body needs fish to function properly because it has a high content of high value proteins, omega-3 fatty acids, vitamin D, iodine, zinc, and selenium (Hassoun and Karoui, 2017; Minnens *et al.*, 2020; Yu *et al.*, 2020). It is evident that eating more fish will reduce the risk of developing heart disease, stroke, depression, cancer, and mortality (Jayedi and Shab-Bidar, 2020).

White Grouper fish (*Epinephelus aeneus*) is a promising specie to the Mediterranean fisheries because of its remarkable economic significance, high nutrition value and good taste (Hassin *et al.*, 1997; Turan *et al.*, 2017; Mavruk *et al.*, 2018; El-Aiatt, 2021).

Unfortunately, due to microbial activity, natural enzymes, and chemical lipids degradation fish are highly perishable (Dellarosa *et al.*, 2015; Anagnostopoulos *et al.*, 2022). Microbes are the focal cause of deterioration in fish flesh because of its high of non-protein nitrogenous (NPN) content, mild acidity (pH > 6), and high a_w (FAO, 2020; Anagnostopoulos *et al.*, 2022; Nie *et al.*, 2022).

Fish shelf life may be increased while maintaining its high; safety, quality and commercial value if the right processing techniques were used (Tsironi *et al.*, 2020; Prezenza *et al.*, 2023).

The demand for safe, high-quality meals and growing customer concern over the use of chemical preservatives provide substantial problems for the food sector (Davidson *et al.* 2013; Savvaidis and Ayala-Zavala, 2021; Xu *et al.*, 2021; Prezenza *et al.*, 2023).

The major purposes of antimicrobial food preservatives are the elimination or inactivation of pathogenic microorganisms and the inhibition of rotting microorganisms (Davidson and Zivanovic, 2003; Savvaidis and Ayala-Zavala, 2021). Natural antimicrobials are helpful in keeping food preventing food losses and ensuring the safety and quality of food. According to Davidson *et al.*, (2013) and Savvaidis and Ayala-Zavala, (2021) a model natural antimicrobial should be (1) effective at low doses, (2) affordable, (3) not negatively affecting the product's sensory attributes, (4) able to inhibit a large variety of microorganisms that cause disease and deterioration, and (5) nontoxic.

Lactic acid is a safe natural antibacterial food ingredient according to the Food and Drug Administration (FDA) (GRAS; 21 CFR, 172.515). It has substantial antibacterial characteristics due to the direct physical disruption of microbes caused by its free radical scavenging

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action (Mani-López et al., 2012; Upadhyaya et al., 2014; Arshadi et al., 2016; Presenza et al., 2023).

Pomegranate (*Punica granatum* L.) peel extract is an affluent supply of polyphenols, especially tannins and anthocyanins. pomegranate peels are rich in natural antioxidants and antimicrobial agents (Savikin et al., 2018; Kaderides et al., 2021; Al-Moghazy and El-Sayed 2023). According to Baenas et al. (2019) and Oswell et al. (2018) phenolic compounds, Carotenoids, capsaicinoids, capsinoids, vitamins (E, C) and minerals (Potassium, Phosphorus and Magnesium) make up the majority of the bioactive chemicals found in paprika powder of *Capsicum annum* L.

Application of these compounds into food could be a strategy to enhance food safety and quality due to their antioxidant, antimicrobial, flavor enhancer and color (Oswell et al., 2018; Baenas et al., 2019; Mei et al., 2019; De Aguiar et al., 2022).

The aim of this study was to assess the effect of the treated Grouper fish with lactic acid and pomegranate peel extract mixed with paprika powder on the safety and quality during storage at 4 °C.

MATERIALS AND METHODS

Materials

Raw materials

Fresh white grouper fish (*Epinephelus aeneus*) (24 kg) was procured from the Bahry fish market in Alexandria, and delivered in ice boxes to the Food Science Laboratory at the Faculty of Agriculture, Alexandria University.

Fruits; Pomegranate (*Punica granatum* L.), red Pepper (*Capsicum annum*) pods were purchased from local market Alexandria city, Egypt.

All chemicals and Media used in the Analytical and Microbiological analysis were obtained from Merck and Oxoid companies.

Methods

Preparation of fish samples

Fish was washed with tap water, scales removed, beheaded, eviscerated, washed again and chopped into pieces of 190 ± 10.58 g each, before allowed to drain for 30 minutes at 4 °C.

Treatment with lactic acid buffer (LAB)

Fish was decontaminated with LAB (2%, 4%, 6% and 8% w/v). Three ml of LAB were uniformly sprayed over 1 Kg of fish using spray gun. Samples were then left to drain for 30 min at 4 °C

Treatment with Pomegranate peel extract combined with paprika powder (PPP)

Pomegranate (*Punica granatum* L.) fruit with no symptoms of rotting or external damage was purchased from the local market in Alexandria. The cleaned peel was separated and chopped. It was further pounded and sieved through a 40-mesh sieve (420 µm) after being dried at 50 °C for four days. Twenty grams of dried peel and 200 ml of distilled water were combined for the extraction, which was then shaken at 100 rpm for an entire night in the dark at ambient temperature. The slurry was centrifuged at 2147xg for 30 min and the resulting supernatant was filtered through Whatman number 1 filter papers. The supernatant was then concentrated in a vacuum rotary evaporator and lyophilized at -50 °C (Telstar Model 50, Spain). The freeze-dried powder was used at 100 mg /kg fish

Pepper (*Capsicum annum*) pods, bought from the local market in Alexandria, were scrubbed and dried at 50 °C for three days before being ground and sieved using a 40-mesh screen (420 µm). The powder was applied at 5g/kg of fish. Fish samples were packed separately in polyethylene bags and kept at 4 °C until analysis.

Chemical analysis

Determination of pH

The pH value was determined following the procedure of Wang et al. (2015). Fish samples (5g each) were blended with 45 ml distilled water for one minute. The mixture was read using a pH meter (Mettler Toledo Co., Zurich, Switzerland).

Microbiological assay

Three fish samples (10 g each) were aseptically homogenized for two minutes in 90 ml of sterilized saline (0.85% NaCl, w/v). Decimal dilutions of this homogenate were prepared in sterile physiological saline containing 0.1% peptone.

Colony forming units (CFU) of psychotropic aerobic bacteria were measured in plate count agar (Oxoid CM 325), incubated for 5 days at 20 °C. H₂S-producing CFU were counted on iron agar according to Gram et al. (1987), supplemented with 0.04% L-cystein (w/v) and covered with an overlay of the same agar, incubated for 3 days at 25 °C.

Yeast and mold CFU were determined on Rose Bengal Chloramphenicol agar (Oxoid CM 549) with supplemented with chloramphenicol antibiotic (Oxoid SR 78). The colonies were incubated at 30 °C for 5 days. *Enterobacteriaceae* were counted on Violet Red Bile Glucose agar (VRBG) (Oxoid CM 485) with an overlay of the same agar incubated for 18h at 37 °C. Microbial population of each plate was counted and reported as log₁₀ CFU/g.

Sensory evaluation

Seven trained panel assessors the quality of raw fish (color, odor and texture) and tasted fried samples (170 °C for 12 min) allowed to cool at ambient temperature and introduced to panelists. The hedonic scale has nine points was employed for raw and fried fish (Alcicek, 2011; Zhang et al., 2021).

Statistical analysis:

Assays were conducted in triplicates. Data was analyzed using the two-ways Analysis of variance and Duncan's test to evaluate the significance of correlation between dependent and independent variables. Descriptive statistics and frequency tables were used for data depiction. The difference was considered statistically significant if p-value was less than 0.05.

RESULTS AND DISCUSSION

Effect of treatment with lactic acid buffer and pomegranate peel extract combined with paprika powder on pH values of white grouper fish samples stored at 4 °C is shown in Table 1. The initial pH value of the fresh fish was 6.42. Reductions in pH values of 0.64, 1.14, 1.61 and 1.99 were obtained by treatment with 2%, 4%, 6% and 8% LAB, respectively. The reduction in pH values of white grouper fish is function of increasing concentration in LAB. The pH of fish treated with PPP; 2%LAB; 2%LAB+PPP; 4%LAB; 4%LAB +PPP; 6%LAB; 6%LAB +PPP; 8%LAB and 8%LAB +PPP were significantly reduced (P<0.05) when stored for 3 and 6 days at 4 °C.

Table 1. Effect of treatment with lactic acid buffer system (LAB) and pomegranate peel extract combined with paprika powder (PPP) on pH of white grouper fish samples stored at 4 C°.

Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	6.42 ^{Aa}	6.89 ^{Ba}	7.32 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2%LAB	5.78 ^{Ab}	6.13 ^{Bb}	6.47 ^{Cb}	6.85 ^{Da}	n.d.	n.d.	n.d.	n.d.
4%LAB	5.28 ^{Ac}	5.35 ^{Ae}	5.79 ^{Be}	6.20 ^{Cc}	6.68 ^{Da}	n.d.	n.d.	n.d.
6%LAB	4.81 ^{Ad}	4.89 ^{Af}	5.36 ^{Bg}	5.80 ^{Cd}	5.99 ^{Db}	6.49 ^{Ea}	n.d.	n.d.
8%LAB	4.43 ^{Ae}	4.45 ^{Ag}	4.80 ^{Bi}	5.07 ^{Cf}	5.45 ^{Dc}	5.79 ^{Eb}	6.28 ^{Fa}	n.d.
PPP	6.37 ^{Aa}	6.62 ^{Bc}	6.84 ^{Cc}	7.18 ^{Db}	n.d.	n.d.	n.d.	n.d.
2%LAB+PPP	5.73 ^{Ab}	5.80 ^{Ad}	6.02 ^{Bd}	6.24 ^{Cc}	6.74 ^{Da}	n.d.	n.d.	n.d.
4%LAB+PPP	5.26 ^{Ac}	5.32 ^{Ae}	5.61 ^{Bf}	5.89 ^{Cd}	6.07 ^{Db}	6.53 ^{Ea}	n.d.	n.d.
6%LAB+PPP	4.78 ^{Ad}	4.81 ^{Af}	5.13 ^{Bh}	5.34 ^{Ce}	5.53 ^{Dc}	5.72 ^{Eb}	6.34 ^{Fa}	n.d.
8%LAB+PPP	4.38 ^{Ae}	4.41 ^{Ag}	4.43 ^{Aj}	4.65 ^{Bg}	4.89 ^{Cd}	5.28 ^{Dc}	5.72 ^{Eb}	6.32 ^F

1. In the same horizontal row (A-G) or vertical column (a-i), values with the identical superscripts are not significantly different (P ≥0.05).

2.n.d.=not determined because of spoilage.

3.ppp=Treated with pomegranate peel extract 100mg/kg combined with 0.5% paprika powder.

4.LAB=Lactic acid buffer pH3.

The buffer capacity appears to be adequate to keep the pH of white grouper fish treated with 6% LAB and 8% LAB lower than the initial pH for 8 days of storage at 4 °C. Lowering the pH to 4.38 did not influence the sensory quality of fish (Table6).

The key element reducing fish's shelf life is the activity of microbes. In standards, guidelines, and specifications, an estimation of psychotropic aerobic bacteria is utilized as an acceptability index (Olafsdottir *et al.*, 1997; Nie *et al.*,2022). Microbial spoilage is the chief reason of degraded quality in fresh or minimally preserved fish, which can lead to 25-30% loss of marketable fish (Mei *et al.*, 2019; Tavares *et al.*, 2021; Nie *et al.*, 2022). Effect of treatment with lactic acid buffer and pomegranate peel extract combined with paprika powder on Psychrotrophic aerobic bacteria of white Grouper fish stored at 4 °C is illustrated in Table 2. The initial number of Psychrotrophic bacteria on fresh white

Grouper fish was 3.76 log₁₀ CFU/g. Reductions of 0.72, 0.39, 0.96, 1.08, 1.31, 1.52, 1.79, 1.94 and 2.13 log₁₀ units were caused by the treatment with 2%LAB, PPP, 2%LAB+PPP, 4%LAB, 4%LAB+PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB+PPP, respectively. This reduction would augment the safety and quality of white Grouper fish. The antimicrobial effect increased with increasing LAB concentrations. Similar results were obtained by Heir *et al.*, (2022) who demonstrated that Lactic acid treatment enhanced the microbial and safety quality of poultry meat. On the day 0, 3 and 6 there were a significant difference (P<0.05) between fish treated with PPP, 2%LAB, 2%LAB+PPP, 4%LAB, 4%LAB+PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB+PPP as compared with blank. After 7 days of storage at 4 °C the number of psychotropic bacteria on fish treated with 8%LAB+PPP was still lower than the initial number (day zero) of blank samples.

Table 2. Effect of treatment with lactic acid buffer system and pomegranate peel extract combined with paprika powder on Psychrotrophic aerobic bacteria of white grouper fish samples stored at 4 C°.

Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	3.76 ^{Aa}	4.98 ^{Ba}	6.83 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2%LAB	3.04 ^{Ab}	4.11 ^{Bb}	5.77 ^{Cb}	6.62 ^{Da}	n.d.	n.d.	n.d.	n.d.
4%LAB	2.68 ^{Ae}	3.58 ^{Bd}	5.13 ^{Cc}	6.05 ^{Db}	6.75 ^{Ea}	n.d.	n.d.	n.d.
6%LAB	2.24 ^{Ag}	3.08 ^{Be}	4.49 ^{Cd}	5.22 ^{Dc}	6.00 ^{Eb}	6.71 ^{Fa}	n.d.	n.d.
8%LAB	1.82 ^{Ai}	2.53 ^{Bf}	3.82 ^{Ce}	4.57 ^{Dd}	5.15 ^{Ec}	5.85 ^{Fb}	6.60 ^{Ga}	n.d.
PPP	3.37 ^{Ac}	4.36 ^{Bc}	5.83 ^{Cb}	6.68 ^{Da}	n.d.	n.d.	n.d.	n.d.
2%LAB+PPP	2.80 ^{Ad}	3.62 ^{Bd}	5.18 ^{Cc}	6.12 ^{Db}	6.71 ^{Ea}	n.d.	n.d.	n.d.
4%LAB+PPP	2.45 ^{Af}	3.15 ^{Be}	4.54 ^{Cd}	5.17 ^{Dc}	5.98 ^{Eb}	6.66 ^{Fa}	n.d.	n.d.
6%LAB+PPP	1.97 ^{Ah}	2.56 ^{Bf}	3.85 ^{Ce}	4.63 ^{Dd}	5.21 ^{Ec}	5.93 ^{Fb}	6.64 ^{Ga}	n.d.
8%LAB+PPP	1.63 ^{Ai}	2.14 ^{Bg}	3.15 ^{Cf}	3.68 ^{De}	4.23 ^{Fd}	4.87 ^{Gc}	5.63 ^{Hb}	6.59 ^I

1. In the same horizontal row (A-G) or vertical column (a-i), values with the identical superscripts are not significantly different (P ≥0.05).

2.n.d.=not determined because of spoilage.

3.ppp=Treated with pomegranate peel extract100mg/kg combined with 0.5% paprika powder.

4.LAB=Lactic acid buffer pH3.

According to Gram (2009) and Kuuliala *et al.* (2018) the critical fish spoilage level is log CFU/g =7-8 followed by off odors on the next day. According to that limit fish treated with 2%LAB, PPP, 2%LAB+PPP, 4%LAB, 4%LAB+PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB+PPP showed shelf life at 4 °C of 7, 7, 8, 8, 9, 9, 10, 10and 11 days, respectively. Presenza *et al* (2023) revealed that fish shelf life is important point for the industry. This signifies a prolongation of fish shelf life 1, 1, 2, 2, 3, 3, 4, 4 and 5 days respectively as compared with control samples. A synergistic effect was obtained between fish samples treated with PPP and LAB which increased with increasing the concentration

of LAB. These fish treated would substantially augment the quality, safety and added-value to white grouper fish in order to meet customer demand.

In terms of food safety and spoilage, the *Enterobacteriaceae* family plays a significant role (Milijasevic, *et al.*, 2015; Singh *et al.*,2015; Saelens, and Houf, 2022). *Salmonella*, *Cronobacter*, *Yersinia*, *Enterobacter*, *Pantoea*, *Klebsiella*, and *Escherichia coli* are among the bacteria that cause food-borne illness (Singh *et al.*,2015; Saelens, and Houf, 2022). WHO has listed antimicrobial resistance (AMR) as one of the top ten worldwide public health threats (WHO, 2021). AMR causes

inadequate treatment of infectious diseases, resulting in increasing mortality rates, financial losses and food security concerns (WHO, 2021; Phu et al.,2022). Bacterial species from the *Enterobacteriaceae* are included in AMR surveillance schemes around the world (WHO, 2021; Phu et al.,2022). *Enterobacteriaceae* are frequently employed as microbiological safety and quality indicators (Milijasevic, et al., 2015; Saelens, and Houf,2022). The effect of treatment with lactic acid buffer and pomegranate peel extract combined with paprika powder on *Enterobacteriaceae* of white grouper fish stored at 4 °C is illustrated in Table3. The initial number of Enterobacteriaceae on fresh white Grouper (*Epinephelus aeneus*) fish was 2.22 log CFU/g. A reduction of 0.54, 0.96, 1.37 and 1.87 log₁₀ CFU/g were obtained by the treatments with 2%LAB, 4%LAB, 6%LAB and 8%LAB at respectively. The antimicrobial effect increased with increasing LAB concentrations. Heir et al, (2022) also demonstrated that treatment poultry meat with Lactic acid

enhanced the microbial, safety quality and extend shelf life. The number of *Enterobacteriaceae* on blank samples augmented hurriedly and was 6.31 log CFU/g after 6 days of storage at 4 °C. On the day 0, 3 and 6 there were a significant decrease in the log₁₀ CFU/g (P<0.05) between fish treated with 2%LAB, PPP, 2%LAB+PPP, 4%LAB, 4%LAB+PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB+PPP as compared with untreated fish (blank). Also, synergistic effect was obtained between PPP and LAB. Similar trained was obtained for psychotropic aerobic bacteria (Table 2). The number of *Enterobacteriaceae* on samples treated with 8%LAB+PPP was still less than the initial number (day zero) of untreated fish (blank) after 6 days of storage at 4 °C. *Enterobacteriaceae* were effectively inhibited. Our findings imply that treating fresh white grouper fish with LAB and PPP may lessen the possibility of human *Enterobacteriaceae* poisoning while enhancing the safety, quality, and shelf life during storage at 4 °C.

Table 3. Effect of treatment with lactic acid buffer system and pomegranate peel extract combined with paprika powder on *Enterobacteriaceae* of white grouper fish samples stored at 4 °C.

Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	2.22 ^{Aa}	3.45 ^{Ba}	6.31 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2%LAB	1.68 ^{Ab}	2.62 ^{Bb}	5.10 ^{Cb}	6.12 ^{Da}	n.d.	n.d.	n.d.	n.d.
4%LAB	1.26 ^{Ae}	1.98 ^{Be}	4.33 ^{Cc}	5.13 ^{Db}	6.05 ^{Ea}	n.d.	n.d.	n.d.
6%LAB	0.85 ^{Ag}	1.51 ^{Bg}	3.66 ^{Cd}	4.36 ^{Dc}	5.10 ^{Eb}	5.96 ^{Fa}	n.d.	n.d.
8%LAB	0.35 ^{Ai}	0.97 ^{Bi}	2.90 ^{Ce}	3.51 ^{Dd}	4.32 ^{Ec}	5.19 ^{Fb}	6.01 ^{Ga}	n.d.
PPP	1.89 ^{Ac}	2.79 ^{Bc}	5.17 ^{Cb}	6.09 ^{Da}	n.d.	n.d.	n.d.	n.d.
2%LAB+PPP	1.48 ^{Ad}	2.11 ^{Bd}	4.29 ^{Cc}	5.20 ^{Db}	6.08 ^{Ea}	n.d.	n.d.	n.d.
4%LAB+PPP	1.05 ^{Af}	1.72 ^{Bf}	3.59 ^{Cd}	4.28 ^{Dc}	5.14 ^{Eb}	5.93 ^{Fa}	n.d.	n.d.
6%LAB+PPP	0.62 ^{Ah}	1.10 ^{Bh}	2.96 ^{Ce}	3.55 ^{Dd}	4.25 ^{Ec}	5.11 ^{Fb}	5.94 ^{Ga}	n.d.
8%LAB+PPP	10>00 ^{Aj}	0.45 ^{Bg}	2.10 ^{Cf}	2.69 ^{De}	3.30 ^{Ed}	4.05 ^{Fc}	4.89 ^{Gb}	5.68 ^H

1. In the same horizontal row (A-G) or vertical column (a-i), values with the identical superscripts are not significantly different (P ≥0.05).

2.n.d.=not determined because of spoilage.

3.ppp=Treated with pomegranate peel extract100mg/kg combined with 0.5% paprika powder.

4.LAB=Lactic acid

Effect of treatment with lactic acid buffer and pomegranate peel extract mixed with paprika powder on yeast of white grouper fish stored at 4 °C is shown in Table 4. All fish samples were free of molds that mainly due to good hygiene employed. The initial number of yeast (day zero) for control samples was 2.65 log, the number increased rapidly

and was 5.34 log after 6 days of storage. on the meanwhile growth of yeast on all fish samples treated with 6%LAB+PPP, 8%LAB and 8%LAB+PPP were strongly inhibited as compared with blank samples after 6 days of storage at 4 °C.

Table 4. Effect of treatment with lactic acid buffer system and pomegranate peel extract combined with paprika powder on yeast of white grouper fish samples stored at 4 °C.

Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	2.65 ^{Aa}	3.38 ^{Ba}	5.34 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2%LAB	2.43 ^{Ab}	2.77 ^{Ba}	4.28 ^{Cb}	4.73 ^{Da}	n.d.	n.d.	n.d.	n.d.
4%LAB	2.18 ^{Ac}	2.52 ^{Bd}	3.93 ^{Cd}	4.25 ^{Dc}	4.60 ^{Eb}	n.d.	n.d.	n.d.
6%LAB	1.89 ^{Ad}	2.10 ^{Bf}	2.90 ^{Cf}	3.31 ^{De}	4.10 ^{Ed}	4.55 ^{Fa}	n.d.	n.d.
8%LAB	1.40 ^{Ae}	1.68 ^{Bh}	2.15 ^{Ch}	2.64 ^{Dg}	3.30 ^{Ef}	3.84 ^{Fb}	4.40 ^{Ga}	n.d.
PPP	2.58 ^{Aa}	2.92 ^{Bc}	4.53 ^{Cc}	4.98 ^{Db}	n.d.	n.d.	n.d.	n.d.
2%LAB+PPP	2.37 ^{Ab}	2.81 ^{Bb}	3.85 ^{Cd}	4.32 ^{Dc}	4.71 ^{Ea}	n.d.	n.d.	n.d.
4%LAB+PPP	2.12 ^{Ac}	2.37 ^{Be}	3.28 ^{Ce}	3.79 ^{Dd}	4.28 ^{Ec}	4.61 ^{Fa}	n.d.	n.d.
6%LAB+PPP	1.84 ^{Ad}	1.95 ^{Bg}	2.41 ^{Cg}	2.92 ^{Df}	3.48 ^{Ec}	3.90 ^{Fb}	4.45 ^{Ga}	n.d.
8%LAB+PPP	1.32 ^{Ae}	1.53 ^{Bi}	1.82 ^{Ci}	2.21 ^{Dh}	2.79 ^{Eg}	3.25 ^{Fc}	3.73 ^{Gb}	4.28 ^H

1. In the same horizontal row (A-G) or vertical column (a-i), values with the identical superscripts are not significantly different (P ≥0.05).

2.n.d.=not determined because of spoilage.

3.ppp=Treated with pomegranate peel extract100mg/kg combined with 0.5% paprika powder.

4.LAB=Lactic acid buffer pH3.

The effect of PPP and LAB on growth of yeast were slightly lesser than those obtained for psychotropic aerobic bacteria (Table2), *Enterobacteriaceae* (Table3) and H₂S-Producing bacteria (Table 5).

Hydrogen sulphide (H₂S) production is regarded as prominent characteristic of fish spoilage bacteria (Gram

(2009); Wu et al., (2019). Effect of treatment with lactic acid buffer and pomegranate peel extract combined with paprika powder on H₂S-Producing bacteria of white Grouper fish stored at 4 °C is illustrated in Table 5. The initial number on blank sample was 1.64 log CFU/g and increased rapidly rich 6.15 log after 6 days of storage at 4 °C. Meanwhile significant

decreases were obtained by all the treatments ($P < 0.05$) as compared with blank samples at day zero. After 6 days of storage at 4 °C reduction of 1.13, 1.20, 2.02., 2.07, 2.75, 2.83, 3.11, 3.17 and 3.76, log CFU/g were obtained with the treatments 2%LAB, PPP, 2%LAB+PPP, 4%LAB,

4%LAB+PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB+PPP respectively as compared with blank. Again, synergistic effect was identified between PPP and LAB, which increased as LAB concentration increased. Similar findings are shown in tables 2 and 3.

Table 5. Effect of treatment with lactic acid buffer system and pomegranate peel extract combined with paprika powder on H₂S-Producing bacteria of white grouper fish samples stored at 4 °C.

Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	1.64 ^{Aa}	3.38 ^{Ba}	6.15 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2%LAB	1.21 ^{Ab}	2.82 ^{Bb}	5.02 ^{Cb}	5.93 ^{Da}	n.d.	n.d.	n.d.	n.d.
4%LAB	0.81 ^{Ae}	2.04 ^{Bd}	4.08 ^{Cc}	4.95 ^{Db}	5.88 ^{Ea}	n.d.	n.d.	n.d.
6%LAB	0.54 ^{Af}	1.57 ^{Be}	3.32 ^{Cd}	4.08 ^{Dc}	5.22 ^{Eb}	5.92 ^{Fa}	n.d.	n.d.
8%LAB	0.20 ^{Ah}	1.21 ^{Bf}	2.98 ^{Ce}	3.52 ^{Dd}	4.35 ^{Ec}	4.79 ^{Fb}	5.71 ^{Ga}	n.d.
PPP	1.42 ^{Ac}	2.90 ^{Bb}	4.95 ^{Cb}	5.98 ^{Da}	n.d.	n.d.	n.d.	n.d.
2%LAB+PPP	1.04 ^{Ed}	2.23 ^{Bc}	4.13 ^{Cc}	4.87 ^{Db}	5.91 ^{Ea}	n.d.	n.d.	n.d.
4%LAB+PPP	0.61 ^{Af}	1.60 ^{Be}	3.40 ^{Cd}	4.12 ^{Dc}	5.19 ^{Eb}	5.89 ^{Fa}	n.d.	n.d.
6%LAB+PPP	0.42 ^{Ag}	1.28 ^{Bf}	3.04 ^{Ce}	3.59 ^{Dd}	4.28 ^{Ec}	4.84 ^{Fb}	5.79 ^{Ga}	n.d.
8%LAB+PPP	10>00 ^{Ai}	0.95 ^{Bg}	2.39 ^{Cf}	2.96 ^{De}	3.67 ^{Ec}	4.18 ^{Fc}	4.80 ^{Gb}	5.74 ^H

1. In the same horizontal row (A-G) or vertical column (a-i), values with the identical superscripts are not significantly different ($P \geq 0.05$).

2.n.d.=not determined because of spoilage.

3.ppp=Treated with pomegranate peel extract100mg/kg combined with 0.5% paprika powder.

4.LAB=Lactic acid buffer pH3.

After 3 days of storage at 4 °C the log CFU/g of H₂S-Producing bacteria on fish treated with 8%LAB+ PPP,8% LAB, 6%LAB+PPP, 6%LAB and 4%LAB+PPP were still lower than the initial number (day zero) of blank sample. Likewise, after 11 days of storage the number of H₂S-Producing bacteria on samples treated with 8%LAB+PPP were still lower than the number of blank samples at day 6. The results obtained for H₂S-Producing bacteria confirmed those obtained for microbiological Table 2, 3,4 and sensory quality Table 6. interaction of a lactic acid buffer (LAB)and pomegranate peel extract combined with paprika powder (PPP) were responsible for the hurdle technology developed. The obtained hurdle technology will increase safety, quality, shelf life and economic of white grouper fish (*Epinephelus aeneus*) during storage at 4 °C.

The scientific field of sensory evaluation is used to elicit, quantify, analyze, and interpret fish traits as perceived by the senses including color, texture, odor and taste (Olafsdottir *et al.*, 1997; Wu *et al.*, 2019; Prabhakar *et al.*, 2020).

A significant factor in the quality of fish or fishery products is freshness, it is crucial to the end product's quality for all products (Olafsdottir *et al.*, 1997; Wu *et al.*, 2019; Prabhakar *et al.*,2020). The evaluation of sensory quality of white Grouper fish treated with lactic acid buffer (LAB) and pomegranate peel extract mixed with paprika powder (PPP) during storage at 4 °C is shown in Table 6. The sensory evaluation of Grouper fish revealed that color at day zero of storage was the highest for all the fish samples treated with PPP. Significant differences were observed in the fish color on day 3 and 6 between control and each of PPP, 2%LAB, 2%LAB+PPP, 4%LAB, 4%LAB +PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB, +PPP treatments samples. Odor, texture and taste are important sensory attributes for fish, likewise a similar trained was also observed for odor, texture and taste. The variations in bacterial numbers were closely followed by changes in smell. The sensory evaluation revealed that the white Grouper fish's sensory

quality was enhanced by increasing the concentration of LAB.

Comparable findings were reported by Heir *et al.* (2022) who found that the organoleptic quality of chicken meat was improved by treatment with lactic acid. Similar results were reported by Kanatt *et al.* (2010) for treating chicken with pomegranate peel extract.

According to Allai *et al.* (2022) and Bigi *et al.* (2023), non-thermal innovation technologies are becoming common in the food processing sector since they increase the shelf life of goods while maintaining their safety, quality, freshness, and sensory attributes.

The sensory evaluation revealed that color, odor, texture and taste quality of all PPP and LAB treatment were significantly improvement ($P < 0.05$) as compared with blank samples. Fish treated with PPP, 2%LAB, 2%LAB+PPP, 4%LAB, 4%LAB +PPP, 6%LAB, 6%LAB+PPP, 8%LAB and 8%LAB, +PPP have a shelf life at 4 °C of 7, 7, 8, 8, 9, 9, 10, 10and 11 days respectively. This signifies a prolongation of shelf life at 4 °C of 1, 1, 2, 2, 3, 3, 4,4 and 5 days respectively as compared with blank samples. The results obtained for sensory evaluation confirmed those obtained for microbiological and chemical quality of all white Grouper (*Epinephelus aeneus*) treatments during storage at 4 °C.

Pisosch *et al.* (2018), Zhuang *et al.* (2019), Baptista *et al.* (2020) and Savvaidis & Ayala-Zavala. (2021) noted that natural antimicrobial and antioxidant agents can improve sensory quality and protect food against oxidation and decomposition.

According to Baptista *et al.* (2020), FAO, (2020) and Anagnostopoulos *et al.* (2022) seafood industry is one of the most booming food sectors, since global consumption is growing dramatically every year, However, seafood is one of the most perishable meals. Rapid trade expansion makes it necessary to more closely align Egyptian local standards and laws with the global regime supported by widely accepted food safety and quality concepts.

Table 6. Evaluation of sensory quality of fresh (color, odor and texture) and fried (taste) white grouper fish treated with lactic acid buffer (LAB) and pomegranate peel extract combined with paprika powder (PPP) stored at 4 C° (A=colour,B=odour,C=texture,D=taste).

A: Colour								
Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	8.35 ^{Aa}	6.78 ^{Ba}	5.19 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2% LAB	8.32 ^{Aa}	7.11 ^{Bb}	5.82 ^{Cb}	5.35 ^{Da}	n.d.	n.d.	n.d.	n.d.
4% LAB	8.36 ^{Aa}	7.50 ^{Be}	6.24 ^{Ce}	5.83 ^{Dc}	5.36 ^{Ea}	n.d.	n.d.	n.d.
6% LAB	8.34 ^{Aa}	7.60 ^{Bg}	6.63 ^{Cf}	6.24 ^{Dd}	5.89 ^{Eb}	5.39 ^{Fa}	n.d.	n.d.
8% LAB	8.38 ^{Aa}	7.76 ^{Bi}	7.05 ^{Ch}	6.81 ^{De}	6.45 ^{Ec}	6.02 ^{Fb}	5.49 ^{Ga}	n.d.
PPP	8.70 ^{Ab}	7.40 ^{Bc}	5.91 ^{Cc}	5.38 ^{Da}	n.d.	n.d.	n.d.	n.d.
2% LAB+PPP	8.69 ^{Ab}	7.65 ^{Bd}	6.45 ^{Cd}	5.97 ^{Db}	5.41 ^{Ea}	n.d.	n.d.	n.d.
4% LAB+PPP	8.75 ^{Ab}	7.90 ^{Bf}	6.70 ^{Cf}	6.30 ^{Dd}	5.97 ^{Eb}	5.45 ^{Fa}	n.d.	n.d.
6% LAB+PPP	8.72 ^{Ab}	8.03 ^{Bh}	7.19 ^{Gg}	6.89 ^{De}	6.51 ^{Ec}	6.08 ^{Fb}	5.55 ^{Ga}	n.d.
8% LAB+PPP	8.77 ^{Ab}	8.28 ^{Bj}	7.65 ^{Ch}	7.42 ^{Df}	7.11 ^{Ed}	6.74 ^{Fc}	6.33 ^{Gb}	5.71 ^H

B: odour								
Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	8.50 ^{Aa}	6.57 ^{Ba}	5.22 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2% LAB	8.62 ^{Ab}	7.14 ^{Bb}	5.81 ^{Cb}	5.34 ^{Da}	n.d.	n.d.	n.d.	n.d.
4% LAB	8.64 ^{Ab}	7.32 ^{Bd}	6.16 ^{Cc}	5.79 ^{Db}	5.36 ^{Ea}	n.d.	n.d.	n.d.
6% LAB	8.60 ^{Ab}	7.59 ^{Bf}	6.54 ^{Cd}	6.27 ^{Dc}	5.79 ^{Eb}	5.36 ^{Fa}	n.d.	n.d.
8% LAB	8.63 ^{Ab}	7.55 ^{Bh}	6.95 ^{Cf}	6.75 ^{Dd}	6.32 ^{Ec}	5.84 ^{Fb}	5.38 ^{Ga}	n.d.
PPP	8.78 ^{Ac}	7.21 ^{Bb}	5.88 ^{Cb}	5.37 ^{Da}	n.d.	n.d.	n.d.	n.d.
2% LAB+PPP	8.80 ^{Ac}	7.46 ^{Bc}	6.20 ^{Cc}	5.85 ^{Db}	5.31 ^{Ea}	n.d.	n.d.	n.d.
4% LAB+PPP	8.81 ^{Ac}	7.71 ^{Be}	6.62 ^{Cd}	6.33 ^{Dc}	5.75 ^{Eb}	5.30 ^{Fa}	n.d.	n.d.
6% LAB+PPP	8.84 ^{Ac}	7.83 ^{Bg}	7.10 ^{Ce}	6.78 ^{Dd}	6.31 ^{Ec}	5.87 ^{Fb}	5.35 ^{Ga}	n.d.
8% LAB+PPP	8.80 ^{Ac}	8.02 ^{Bi}	7.46 ^{Cg}	7.19 ^{De}	6.85 ^{Ed}	6.51 ^{Fc}	6.10 ^{Gb}	5.54 ^H

C=texture								
Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	8.63 ^{Aa}	6.75 ^{Ba}	5.47 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2% LAB	8.63 ^{Aa}	7.08 ^{Bb}	6.10 ^{Cb}	5.52 ^{Da}	n.d.	n.d.	n.d.	n.d.
4% LAB	8.63 ^{Aa}	7.50 ^{Bc}	6.62 ^{Cc}	6.28 ^{Db}	5.59 ^{Ea}	n.d.	n.d.	n.d.
6% LAB	8.63 ^{Aa}	7.64 ^{Bd}	6.80 ^{Cd}	6.49 ^{Dc}	6.17 ^{Eb}	5.69 ^{Fa}	n.d.	n.d.
8% LAB	8.63 ^{Aa}	7.79 ^{Be}	7.08 ^{Ce}	6.89 ^{Dd}	6.68 ^{Ec}	6.25 ^{Fb}	5.62 ^{Ga}	n.d.
PPP	8.63 ^{Aa}	7.14 ^{Bb}	6.15 ^{Cb}	5.49 ^{Da}	n.d.	n.d.	n.d.	n.d.
2% LAB+PPP	8.63 ^{Aa}	7.48 ^{Bc}	6.59 ^{Cc}	6.23 ^{Db}	5.61 ^{Ea}	n.d.	n.d.	n.d.
4% LAB+PPP	8.63 ^{Aa}	7.67 ^{Bd}	6.78 ^{Cd}	6.51 ^{Dc}	6.13 ^{Eb}	5.60 ^{Fa}	n.d.	n.d.
6% LAB+PPP	8.63 ^{Aa}	7.82 ^{Be}	7.13 ^{Ce}	6.86 ^{Dd}	6.65 ^{Ec}	6.28 ^{Fb}	5.67 ^{Ga}	n.d.
8% LAB+PPP	8.63 ^{Aa}	8.05 ^{Bi}	7.49 ^{Ct}	7.24 ^{De}	6.97 ^{Ed}	6.72 ^{Fc}	6.24 ^{Gb}	5.71 ^H

D=taste								
Treatment	Storage Days							
	0	3	6	7	8	9	10	11
Control	8.54 ^{Aa}	6.86 ^{Ba}	5.43 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.
2% LAB	8.55 ^{Aa}	7.38 ^{Bb}	6.04 ^{Cb}	5.51 ^{Da}	n.d.	n.d.	n.d.	n.d.
4% LAB	8.57 ^{Aa}	7.80 ^{Bd}	6.82 ^{Cc}	6.14 ^{Db}	5.58 ^{Ea}	n.d.	n.d.	n.d.
6% LAB	8.51 ^{Aa}	7.92 ^{Be}	7.04 ^{Cd}	6.57 ^{Dc}	6.12 ^{Eb}	5.63 ^{Fa}	n.d.	n.d.
8% LAB	8.56 ^{Aa}	8.13 ^{Bf}	7.28 ^{Ce}	6.94 ^{Dd}	6.61 ^{Ec}	6.19 ^{Fb}	5.63 ^{Ga}	n.d.
PPP	8.81 ^{Ab}	7.50 ^{Bc}	6.11 ^{Cb}	5.55 ^{Da}	n.d.	n.d.	n.d.	n.d.
2% LAB+PPP	8.82 ^{Ab}	7.87 ^{Bd}	6.85 ^{Cc}	6.09 ^{Db}	5.60 ^{Ea}	n.d.	n.d.	n.d.
4% LAB+PPP	8.83 ^{Ab}	8.00 ^{Be}	7.11 ^{Cd}	6.63 ^{Dc}	6.09 ^{Eb}	5.61 ^{Fa}	n.d.	n.d.
6% LAB+PPP	8.84 ^{Ab}	8.15 ^{Bf}	7.33 ^{Ce}	6.96 ^{Dd}	6.58 ^{Ec}	6.14 ^{Fb}	5.67 ^{Ga}	n.d.
8% LAB+PPP	8.87 ^{Ab}	8.35 ^{Bg}	7.64 ^{Cf}	7.37 ^{De}	7.16 ^{Ed}	6.78 ^{Fc}	6.41 ^{Gb}	5.84 ^H

1. In the same horizontal row (A-G) or vertical column (a-i), values with the identical superscripts are not significantly different (P ≥ 0.05).

2.n.d.=not determined because of spoilage.

3.ppp=Treated with pomegranate peel extract 100ppm/kg combined with 0.5% paprika powder.

4.LAB=Lactic acid buffer pH3.

In conclusion, the treatment of white Grouper fish with lactic acid and pomegranate peel extract combined with paprika powder will exert an antimicrobial and antioxidant effect, enhance safety, quality and prolong the shelf life of fresh white grouper fish, would be economically advantageous as the Grouper have a higher market value for export.

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تأثير اضافة حامض اللاكتيك ومستخلص قشر الرمان مع مسحوق البابريكا علي سلامة وجودة سمك الهامور الأبيض المبرد

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المخلص

الهامور الأبيض سمك ذو قيمة اقتصادية كبيرة وينتمي للعائلة السردينية ويتواجد في شرق المحيط الأطلسي وجنوب البحر الأبيض المتوسط. أجريت هذه الدراسة لتقييم تأثير محلول حامض اللاكتيك ومستخلص قشر الرمان مع البابريكا وتوليفتهما علي سلامة وجودة سمك الهامور الأبيض المخزن علي درجة حرارة 4 °م. تم معاملة الأسماك الطازجة بمحلول حامض اللاكتيك (2% و4% و6% و8% LAB) ومستخلص قشر الرمان (100 ملجم/كجم) مع مسحوق الفلفل (5 جم/كجم PPP) والتوليفات ما بين حامض اللاكتيك LAB ومستخلص قشر الرمان مع البابريكا PPP. أجريت هذه الدراسة لتقييم الجودة الميكروبيولوجية والجودة الحسية ورقم الـ pH. أظهرت النتائج أن العدد الكلي للبكتيريا المحبة للبرودة الهوائية والأنتيروبيكترياسي والمنجدة للـ H₂S تم تثبيطهم بشدة من قبل جميع المعاملات وذلك يؤدي إلي تحسين السلامة والجودة وإطالة فترة الصلاحية للسمك. أعلي تثبيط تم الحصول عليه بواسطة المعاملة 8% LAB+PPP تم الحصول على انخفاض في رقم الـ pH مقاربه 0.64 و 1.14 و 1.61 و 1.99 بواسطة المعاملة 2% و 4% و 6% و 8% LAB على التوالي. أظهر التقييم الحسي ان انخفاض رقم الـ pH إلي 4.38 لم يؤثر على الخواص الحسية كما دعمت نتائج الخواص الحسية النتائج الميكروبيولوجية. الأسماك التي تم معاملةها بواسطة 2% LAB أو PPP يمكن تخزينها مبردة بأمان لمدة 7 أيام في حين الأسماك الغير معاملة تم تخزينها لمدة 6 أيام. زيادة تركيز LAB إلى 4% و 6% و 8% أدى إطالة فترة الصلاحية 9 و 10 و 10 أيام على التوالي. بالإضافة لذلك فإن الجمع ما بين، LAB (2%، 4%، 6%، و 817%) مع PPP أدى لإطالة مدة الصلاحية الي 8 و 9 و 10 و 11 يوم علي التوالي.