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Combined effect of probiotics on prolonging the shelf life of GIFT tilapia fillets

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Abstract

Probiotics are posing diverse applications to modify food constituents for health benefits of humans. There is an increasing trend to get insights into developments of new technologies to improve the food products. The present study explored the preservation effect of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* as potential probiotics for improving the shelf life of GIFT Tilapia (*Oreochromis mossambicus*). The probiotics addition in agar film exhibited a reasonable antioxidant activity and antimicrobial potency against some food-borne pathogens such as H2S-producing *Pseudomonas* sp. and luminescent bacterial colonies at 15°C. During storage, the physicochemical properties (pH) and microbial and sensory properties were evaluated. The study indicated that probiotics addition in agar film was found principal responsible for the inhibitory activity of total bacteria. Probiotics reduced the H2S-producing bacteria due to which reduction in chemical spoilage was observed. The lactic acid bacterial counts were 4.5 log CFU/g in treatment F4 on day 10 and 15. H2S-producing bacteria were 8.5–3 log CFU/g in F4. Enterobacteriaceae were 7–2 log CFU/g on day 10. *Pseudomonas* spp. was 3.5 log CFU/g in F4 on day 15. Total viable bacterial count was 9 log CFU/g in F4 on day 15. The pH was 6.8 in F4 at day 15, which indicate that decreasing in bacterial count was observed from control to treatment F4 which contain both probiotics. The colour of fillet was stable throughout the storage period in treatments. Thus, it may be concluded that films with probiotic could extend shelf life of tilapia fillets up to at least one week.

KEYWORDS

agar, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, sensory properties, shelf life, tilapia fillets

1 | INTRODUCTION

Tilapia is one of the oldest cultured and economically important worldwide fish species (Ispır, Yonar, & Oz, 2011; McAndrew, 2000; Trosvik et al., 2012) and is an important part of diet in the Pakistan due to aquaculture suitability, nutritional quality and delicious taste (Malik, Shah, Kalhoro, & Kalhoro, 2014; Zikria et al., 2012). The food industries are constantly looking for novel preservative methods to protect the perishable foodstuffs like fish fillet from microbial deterioration (Ahmed, Shehata, Abd-Rabou, & El-Menshawy, 2019; Angiolillo, Conte, & Del Nobile, 2018; Korkmaz, Kocaman, & Alak, 2019; Mozaffarzogh, Misaghi, Shahbazi, & Kamkar, 2020; Roobab et al., 2020). Several technologies along with intelligent packaging were developed to protect and attain high-quality food with prolonged shelf life. Monitoring the degree of microbial deterioration is basic requirement for enhancing the

shelf life and controlling food quality through understanding antioxidant activity and antimicrobial potency against some foodborne pathogens such as H2S-producing *Pseudomonas* sp. and luminescent bacterial colonies (Lee, 2010; Lopez De Lacey, Lopez-Caballero, & Montero, 2014).

Previously, numerous techniques were applied by several workers to improve or analyse the shelf life of perishable food stuffs like growth of the naturally occurring bacteria pseudomonads, *Shewanella putrefaciens*, lactic acid bacteria, Enterobacteriaceae and yeast, on gilt-head seabream (*Sparus aurata*) (Koutsoumanis & Nychas, 2000). They used Marjoram and Cumin oils at lower concentrations on meat products and found beneficial effects on quality, protection and shelf life (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; El-Desouky, Bahlol, & Sharoba, 2006; Keokamnerd, Acton, & Han, 2008). Herbal extract of Marjoram is observed as an antioxidant (Badee, Moawd, El-Noketi, & Gouda, 2013; Mohamed & Refat, 2011). Marjoram oil reduced the total aerobic mesophilic bacterial counts and enhance shelf life of minced meat (Kamel, 2013; Shaltout, Salem, Khater, & Lela, 2016) while Cumin-aldehyde essential oil keep antimicrobial potential against gram-positive bacteria (Rezai, Sadeghi, Nateghi, & Mohammadi, 2014). Similar positive effects of edible coatings were observed on seafood (Ambardekar, 2007; Ou, Tsay, Lai, & Weng, 2002), and microorganisms were considered main cause of seafood products spoilage (Gram & Dalgaard, 2002). Andevari and Rezai (2011) described the reduced microbial growth in trout fillets after using cinnamon oil-gelatine coatings. Núñez-Flores et al. (2013) used gelatin and gelatin-lignin composite films which produced plasticizing effect with elongation values. Similar effects were reported previously by Pérez-Mateos, Montero, and Gómez-Guillén, (2009).

The present study was conducted to evaluate the shelf life of *Oreochromis mossambicus* after using two probiotics *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. Moreover, the probiotics reveals high antimicrobial activity by production of antimicrobial substances or competition with spoilage microorganisms (Pereira et al., 2016). Among the probiotic bacteria has gained much attention owing to the high probiotic activity because of high to low pH tolerance and antimicrobial activity (Brachkova et al., 2011). There are inadequate evidences regarding antimicrobial activity of probiotics with agar coatings to prolong shelf life of fillets (Gialamas, Zinoviadou, Biliaderis, & Koutsoumanis, 2010; Pavli et al., 2017; Sanchez-Gonzalez, Saavedra, & Chiralt, 2014; Soukoulis, Singh, Macnaughtan, Parmenter, & Fisk, 2016). *L. acidophilus* and *S. cerevisiae* are considered the main tools for controlling pathogens, improving food safety and prolonging shelf life (Rastall, Fuller, Gaskins, & Gibson, 2000). Referring to previous studies in the literature, short data are available on the application of agar films containing probiotic to enhance the shelf life, particularly reduction in H2S producing *Pseudomonas* sp., Enterobacteriaceae and luminescent bacteria. Therefore, the aim of the present study was to evaluate impact of *L. acidophilus* and *S. cerevisiae* added in agar films on the GIFT Tilapia fillet during refrigerated storage for 15 days to enhance the shelf life by analysing physicochemical, structural, sensory and microbiological parameters.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All the experimental protocols and methods of this study were performed following the guidelines and regulations approved by the animal ethics committee of Government College University, Faisalabad.

2.2 | Bacterial strains and culture conditions

Two species of spoilage bacteria *Photobacterium phosphoreum* CECT 4192 and *S. putrefaciens* CECT 5346 were used for the inoculation of the fish. Both strains were kept in Brain Heart Infusion Broth (Oxoid, Basingstoke, UK) at −80°C with glycerol (25%) (Panreac, Reixac, Moncadai, Barcelona, Spain) until their use. A day before conducting the assay, the strains were incubated by culturing in BHI broth (Oxoid) supplemented with NaCl (1%). Incubation was carried out for 24 hr, and 30 and 15°C temperatures were maintained for *S. putrefaciens* and *P. phosphoreum* strains respectively. A bioactive film was prepared by using two commercially available probiotic bacteria (*S. cerevisiae* and *L. acidophilus*). These bacterial strains were acquired, lyophilized and then frozen at −20°C (Lopez de Lacey et al., 2014).

2.3 | Formulation of films

An agar film was formed by preparing a solution made by dissolving agar (1.5 g) (Gold Agar, Hispanagar, Burgos, Spain), glycerol (1 g) and glucose (2 g) in water (100 ml). Glycerol was used to work as plasticizer. The mixture was stirred well to get homogeneity of ingredients, and the films were made by moulding 40 ml on 144 cm^2-square plates. Then, these were dried at 40°C by using forced-air oven for 16–18 hr so a uniformity of thickness was obtained among all cases (200 mm). These films were hardened in desiccators at 22°C for a period of 2 days; meanwhile relative humidity was maintained at 63% (Gomez-Estaca, Lopez De Lacey, & Lopez-Caballero, 2010). About 100 ml of each probiotic was taken and spread individually on each 12 × 12 cm squared film for preparation of bioactive film.

2.4 | Fish preparation, inoculation and storage

The fish was purchased from fish seed hatchery Mian Channu, Punjab, Pakistan. Five Fish were taken after completion of both 30 and 60 days trials which were conducted to evaluate the effects of probiotics on the growth performance, proximate analysis, haematological parameters and digestive enzymes activity of

GIFT Tilapia (Yasin, Jabeen, Ali, Samiullah, & Hussain, 2018). Every piece was cut around 5 g and was coated with 5 ml of each spoilage bacterial suspensions (properly diluted with 0.9% solution of NaCl) to obtain 10^3–10^4 CFU/g concentration initially (Montero, Gomez-Estaca, & Gomez-Guillen, 2007). Latterly, all pieces of fish were covered with two squared films (4 × 4 cm) and vacuum packed in bags (Cryovac BB-1, Grace, Barcelona, Spain). All covered pieces were packed in separate bags and were stored for 15 days at 4°C.

2.5 | Bioactive film preparation

A bioactive film was prepared, for this purpose agar was incorporated by probiotics strains (*L. acidophilus* and *S. cerevisiae*) to compose bioactive film to apply on the fillet of GIFT tilapia to evaluate the effect on shelf life of fish fillet during storage for 15 days. Different types of films prepared to evaluate shelf life were as follows: agar film (F1); agar + probiotic 1 (LA) film (F2); agar + probiotic 2 (SC) film (F3); agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4). These films were applied on the fillet and their impact was observed after 2, 7, 10 and 15 days according to method described by Lopez de Lacey et al., 2014.

2.6 | Microbiological analysis

Shelf life was determined using following methods of Koutsoumanis and Nychas (2000) and Lopez de Lacey et al. (2014). Briefly, the microbial populations were counted on the sample after inoculation at 2, 7, 10 and 15 days of storage. The microbiological analyses were as follows: a total amount of 3 g of fish (after removal of the film), from at least 3 different packages, were collected and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of buffered 0.1% peptone water (Oxoid, Basingstoke, UK) in a vertical laminar-flow cabinet (mod. AV 30/70 Telstar, Madrid, Spain). After 1 min in a Stomacher blender (model Colworth Stomacher 400), appropriate dilutions were made for the following microorganisms determinations: (a) total bacteria counts on spread plates of Iron Agar NaCl (1%) incubated at 15°C for 72 hr, (b) H_2S-producing bacteria, as black colonies on spread plates of Iron Agar NaCl (1%) incubated at 15°C for 72 hr control, (C) luminescents bacteria on spread plates of Iron Agar NaCl (1%) incubated at 15°C for 5 days as presumptive *P. phosphoreum*, (d) total viable bacteria on pour plates of PCA incubated at 30°C for 72 hr, (e) *Pseudomonas* spp. on spread plates of *Pseudomonas* Agar Base (Oxoid) with added CFC (Cetrimide, Fucidine, Cephalosporine) supplement (Oxoid) incubated at 25°C for 48 hr, (f) lactic acid bacteria on overlay plates of MRS Agar (Oxoid) incubated at 30°C for 72 hr and (g) Enterobacteriaceae on double-layered plates of Violet Red Bile Glucose agar (VRBG, Oxoid) incubated at 30°C for 24 hr. All microbial counts were stated as log CFU/g of sample (detection limits were 1 log CFU/g and 2 logs CFU/g for pour plate and spread plate techniques respectively). All the analyses were conducted in triplicate.

2.7 | Instrumental colour analysis

The colour constraints like redness, yellowness and lightness were checked with the help of colorimeter (Konica Minolta CM-3500d Aquateknica S.A., Valencia, Spain). For this study, 10° observer angle and iluminant D65 were used. The measurements were taken from different portions of fish (Lopez de Lacey et al., 2014; Núñez-Flores et al., 2013).

2.8 | Statistical analysis

Analysis of variance was performed by using the SPSS 14.0 computer program (SPSS Inc., Chicago, IL, USA). One-way analysis and paired comparisons were conducted by using the Duncan and Tamhane tests. The significance level was $p < .05$.

3 | RESULTS

To simulate the spoilage of fish, fillets of GIFT tilapia were inoculated with two spoilage bacteria *S. putrefaciens* and *P. phosphoreum* and then to evaluate the role of the films during storage period. Microbial counts of fish fillet covered with films are described in Figure 1a–h.

3.1 | Luminescent bacteria

Fillets were inoculated with *P. phosphoreum* (luminescent bacteria), which could not be detected during the first days of storage, may be due to recovery of these bacteria. Luminescent colony was observed from day 7, and decreasing trend was observed in treatments. In control group (X), luminescent colony was observed 6.5 log CFU/g on day 7, and increasing trend was observed towards day 10 (7 log CFU/g) and 15 (7.5 log CFU/g). In treatment F1 (agar film), luminescent colony was observed maximum 6.0 logs CFU/g on day 7 while 5.5 logs CFU/g were observed on day 10 and 15. In F2 (agar + probiotic 1 (LA) film), maximum colony 6.0 log CFU/g was observed on day 7 while it was 5.0 log CFU/g on day 10 and 5.5 log CFU/g on day 15. In F3 (agar + probiotic 2 (SC) film), maximum luminescent colony bacteria were observed on day 10 (6.0 log CFU/g) while it was 5.5 log CFU/g on day 7 and 5.0 log CFU/g on day 15. In F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), maximum luminescent colony bacteria were observed 4.0 log CFU/g on day 15 and minimum 3.0 log CFU/g were observed on day 7 while 3.5 log CFU/g were observed on day 10. Overall, decreasing trend in luminescent colony bacteria was observed and minimum bacteria were in the treatment F4, which contain agar along with both probiotic LA and SC (Figure 1a, Table 1).

3.2 | Lactic acid bacteria

Counts in control (X) for lactic acid bacteria were less throughout the storage period as compare to other treatments F1 to F4

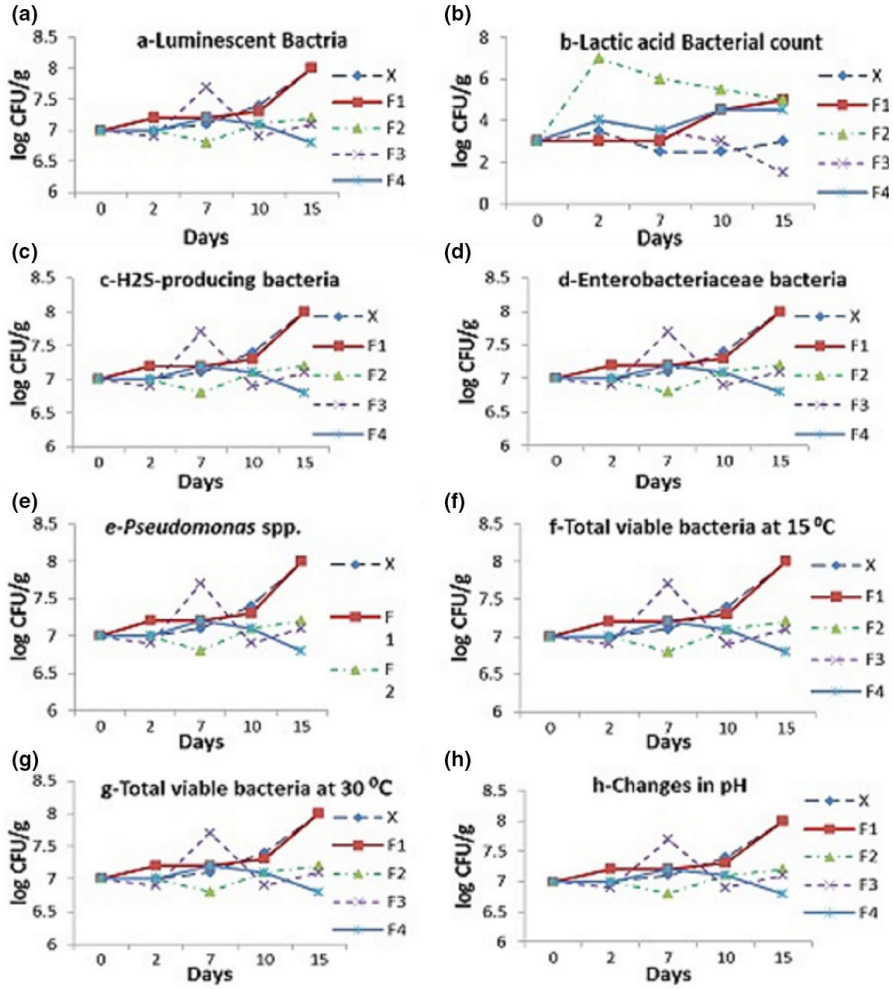

FIGURE 1 (a–h) Shelf life, bacterial counts (log CFU/g) and pH in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage, Control (X), Agar film (F1), Agar + probiotic 1 (LA) film (F2), Agar + probiotic 2 (SC) film (F3) and Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)

TABLE 1 Shelf life, bacterial counts (luminescent bacteria, log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage (4°C)

Types of films	Luminescent bactria colony (log CFU/g)				
	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	0	0	6.5	7	7.5
Agar film (F1)	0	0	6	5.5	5.5
Agar + probiotic 1 (LA) film (F2)	0	0	6	5	5.5
Agar + probiotic 2 (SC) film (F3)	0	0	5.5	6	5
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	0	0	3	3.5	4

but gradually decreased in control group from day 2 to day 15 was observed which may be due to the low storage temperature. Maximum lactic acid bacterial counts (3.5 logs CFU/g) were observed in control (X) on day 2 while 2.5 logs CFU/g were observed on day 7 and 10. After 15 days, the count was 3 log CFU/g in the control group. In F1 (agar film), lactic acid bacteria were observed maximum 5.0 log CFU/g on day 15 while 3 log CFU/g were observed on day 2 and 7, and on day 10 the lactic acid bacterial count was 4.5 log CFU/g. In F2 (agar + probiotic 1 (LA) film), maximum count 7 log CFU/g was observed on day 2 while it was 6 log CFU/g on day 7, 5.5 log CFU/g on day 10 and 5 log CFU/g on day 15. In F3 (agar + probiotic 2 (SC) film), maximum lactic acid bacteria were observed on day 2 (2 log CFU/g) and gradual decrease was observed from day 2 to 15. The bacterial count was 3.5 logs CFU/g on day 7, 3 logs CFU/g on day 10 and 1.5 log CFU/g on day 15, which was minimum bacterial count in this treatment. In treatment F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), maximum lactic acid bacteria were observed 4.5 log CFU/g on day 15 and minimum (3.5 log CFU/g) were observed on day 7 while 4 log CFU/g and 4.5 log CFU/g were observed on day 2 and 10 respectively. Overall, increasing trend in lactic acid bacteria was observed from control to the treatment F4 which contain agar along with both probiotic LA and SC. The increment of lactic acid bacteria observed from control to treatments may be due to the passage of both probiotic bacteria from film to muscle which were used in F4 (Figure 1b, Table 2).

3.3 | H2S-producing bacteria

At the end of the storage period in control group (X), H2S-producing bacteria became dominant as maximum count 8.5 log CFU/g was observed on day 15 and their count found the majority percentage of the total flora during storage. A gradual increase in control was observed from day 2 to day 15. H2S-producing bacteria counts were 4 logs CFU/g, 5 logs CFU/g, 7 logs CFU/g on day 2, 7 and 10 respectively. In F1 (agar film), H2S-producing bacteria were observed maximum 7 logs CFU/g on day 15 while 2.5 logs CFU/g and 4.5 logs CFU/g were observed on day 2 and 7, and on day 10 the H2S-producing bacteria lactic acid bacterial count was 6.5 logs CFU/g. In F2 (agar + probiotic 1 (LA) film), maximum count 8 log CFU/g was observed on day 15 while it was 3 log CFU/g on day 2 and day 7 while 4 log CFU/g H2S-producing bacterial count was observed on day 10. In F3 (agar + probiotic 2 (SC) film), maximum lactic acid bacteria were observed on day 15 (5.5 log CFU/g) and gradual increase was observed from day 2 to 15. The bacterial count was 3.5 logs CFU/g on day 2, 4 logs CFU/g on day 7 and 4.5 log CFU/g on day 10. In treatment F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), H2S-producing bacteria were observed maximum (4.5 log CFU/g) on day 15 and minimum (3 log CFU/g) on day 2 while 4.5 log CFU/g and 5.5 log CFU/g were observed on day 7 and 10 respectively. Overall decreasing trend in H2S-producing bacteria from control to the treatment F4 which contain agar along with both probiotic LA and SC was observed (Figure 1c, Table 3). In the current trial, the films comprise glycerol when formulated. So, due to the use of glycerol reduction in water activity on the surface of fillet was not considered crucial in reduction in microorganisms as they were much lower or even insignificant on films that do not contain probiotics.

3.4 | Detection of Enterobacteriaceae

A delay was observed in the detection of Enterobacteriaceae in lots containing probiotics (F1-F4) at the early stages (2 days) of the fillet storage but these lots resumed their growth on 7 days of storage. Maximum Enterobacteriaceae counts (7 logs CFU/g) were observed in control (X) on day 10 while 6.5 logs CFU/g were observed on day 15 and 2.5 log CFU/g, 3 log CFU/g on day 2 and day 7. In treatment F1 (agar film), Enterobacteriaceae was observed maximum 6 log CFU/g on day 15 and gradual increase was observed from day 2 to day 15. It was observed 2.5 logs CFU/g on day 2, on day 7 the count was 3.5 logs CFU/g and 5.5 logs CFU/g on day 10. In F2 (agar + probiotic 1 (LA) film), maximum count 7 log CFU/g was observed on day 10 while it was 6.5 log CFU/g on day 15, 3 log CFU/g on day 7 while a delay was observed in the detection of Enterobacteriaceae on day 2. In F3 (agar + probiotic 2 (SC) film), maximum Enterobacteriaceae bacteria were observed on day 15 (5.5 log CFU/g) and gradual increase was observed from day 2 to 15. The bacterial count was 2.5 logs CFU/g on day 7, 4.5 log CFU/g on day 10 and delay was observed in the detection of Enterobacteriaceae on day 2. In treatment F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), maximum bacteria were observed 4.5 log CFU/g on day 15 and minimum (2 log CFU/g) were observed on day 7 while 3.5 log CFU/g observed on day 10 while delay was observed in the detection of Enterobacteriaceae on day 2. Overall, decreasing trend in Enterobacteriaceae bacterial count was observed from control to the treatment F4 which contain agar along with both probiotic LA and SC (Figure 1d, Table 4). The decrease observed from control to treatments may be due to the low temperature which may not favour the growth of Enterobacteriaceae bacteria. The films containing probiotics F2-F4 were the most effective to delay the microbial growth.

3.5 | *Pseudomonas* spp. colony count

In *Pseudomonas* spp. colony count, delay was also observed in the detection in lots containing probiotics (F1-F4) along with agar film and control group at the early stages up to 7 days of the fillet storage and all of these lots were resumed their growth on 10 days of storage. *Pseudomonas* spp. Colony counts were observed similar during storage period of control (X) which were 7 logs CFU/g on day 10 and day 15. In treatment F1 (agar film), *Pseudomonas* spp. were observed maximum 7.5 log CFU/g on day 15 and it was observed 6.5 log CFU/g

TABLE 2 Shelf life, bacterial counts (Lactic acid bacteria, log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage (4°C)

	Lactic acid bactria colony (log CFU/g)				
Types of Films	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	3	3.5	2.5	2.5	3
Agar film (F1)	3	3	3	4.5	5
Agar + probiotic 1 (LA) film (F2)	3	7	6	5.5	5
Agar + probiotic 2 (SC) film (F3)	3	4	3.5	3	1.5
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	3	4	3.5	4.5	4.5

TABLE 3 Shelf life, bacterial counts (H2S-producing bacteria, log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage (4°C)

	H2S-producing bacterial colony (log CFU/g)				
Types of films	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	3	4	5	7	8.5
Agar film (F1)	3	2.5	4.5	6.5	7
Agar + probiotic 1 (LA) film (F2)	3	3	3	4	8
Agar + probiotic 2 (SC) film (F3)	3	3.5	4	4.5	5.5
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	3	3	4.5	5.5	4.5

TABLE 4 Shelf life, Bacterial counts (Enterobacteriaceae, log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage (4°C)

Types of films	Enterobacteriaceae bacterial colony (log CFU/g)				
	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	0	2.5	3	7	6.5
Agar film (F1)	0	2.5	3.5	5.5	6
Agar + probiotic 1 (LA) film (F2)	0	0	3	7	6.5
Agar + probiotic 2 (SC) film (F3)	0	0	2.5	4.5	5.5
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	0	0	2	3.5	4.5

TABLE 5 Shelf life, bacterial counts (*Pseudomonas* spp., log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage (4°C)

Types of films	*Pseudomonas* spp. bacterial colony (log CFU/g)				
	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	2.5	0	0	7	7
Agar film (F1)	2.5	0	0	6.5	7.5
Agar + probiotic 1 (LA) film (F2)	2.5	0	0	5.5	6.5
Agar + probiotic 2 (SC) film (F3)	2.5	0	0	3.5	5.5
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	2.5	0	0	3.5	4

on day 10. In F2 (agar + probiotic 1 (LA) film), maximum count 6.5 log CFU/g was observed on day 15 while it was 5.5 log CFU/g on day 10. In F3 (agar + probiotic 2 (SC) film), maximum bacteria were observed on day 15 (5.5 log CFU/g) and the bacterial count was 3.5 log CFU/g on day 10. In treatment F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), maximum *Pseudomonas* were observed 4 log CFU/g on day 15 and minimum (3.5 log CFU/g) were observed on day 10 while delay was observed in the detection of *Pseudomonas* on day 2 and day 7. Overall, decreasing trend in *Pseudomonas* bacterial count was observed from control to the treatment F4 which contain agar along with both probiotic LA and SC (Figure 1e, Table 5). The decrease observed from control to treatments may be due to the low temperature which may not favour the growth of *Pseudomonas* bacteria.

3.6 | Total viable bacteria (log CFU/g) at 15°C

Total viable bacterial were observed maximum (7 log CFU/g) in control (X) on day 15 while 8.5 log CFU/g were observed on day 10 and 4 log CFU/g, 7.5 log CFU/g on day 2 and day 7 respectively which show gradual increase in bacterial counts during storage. In treatment F1 (agar film), total viable bacterial were observed maximum (9 log CFU/g) on day 10 which was similar to maximum bacterial count in control. The count was observed 8.5 logs CFU/g on day 15 and 6.5 log CFU/g was observed on day 7 while it was 4 logs CFU/g on day 2. In F2 (agar + probiotic 1 (LA) film) maximum count 8.5 log CFU/g was observed on day 10 and day 15 while it was 5.5 log CFU/g on day 7 and 4 log CFU/g on day 2. In F3 (agar + probiotic 2 (SC) film), maximum bacteria were observed on day 15 (7 log CFU/g) and gradual increase was observed from day 2 to 15. The bacterial count was 4 logs CFU/g on day 2 and 5 log CFU/g on day 7 while 6 log CFU/g bacterial count was observed on day 10. In treatment F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), maximum bacteria were observed 6 log CFU/g on day 15 and minimum (4 log CFU/g) were observed on day 7 while 5 log CFU/g and 5.5 log CFU/g counts were observed on day 7 and day 10 respectively. Overall, decreasing trend in total viable bacterial count was observed from control to the treatment F4 which contain both probiotic LA and SC (Figure 1f, Table 6).

3.7 | Total viable bacteria (log CFU/g) at 30°C

Total viable bacteria at 30°C were observed maximum in control (X) on day 15 which was 7.5 log CFU/g while 4 log CFU/g were observed on day 2 and 5 log CFU/g bacterial count was observed on 7 days and 5 log CFU/g bacterial count was observed in the control group. Gradual increase in bacterial count was observed in control group during the entire storage period. In F1 (agar film), bacteria count was observed maximum 7 logs CFU/g on day 15 while 4 log CFU/g were observed on day 2 and 7, on day 10 the bacterial count was 6.5 log CFU/g. In F2 (agar + probiotic 1 (LA) film), maximum count of bacteria was 8 log CFU/g on day 15 while it was 5.5 log CFU/g on day 2, 7 log CFU/g bacterial count was observed on day 7 and 7.5 log CFU/g on day 10. So, gradual increase in bacterial count was also observed in this treatment during the entire storage period. In F3

TABLE 6 Shelf life, bacterial counts (total viable bacteria, log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during storage at 15°C

Types of films	Total viable bacteria (log CFU/g) at 15°C				
	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	4	4	7.5	8.5	9
Agar film (F1)	4	4	6.5	9	8.5
Agar + probiotic 1 (LA) film (F2)	4	4	5.5	8.5	8.5
Agar + probiotic 2 (SC) film (F3)	4	4	5	6	7
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	4	4	5	5.5	6

(agar + probiotic 2 (SC) film), maximum were observed on day 15 (5.5 log CFU/g) and the bacterial count was 4 log CFU/g on day 2 and day 7. 4.5 log CFU/g bacterial count was observed on day 10. In treatment F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), maximum lactic acid bacteria were observed 5 log CFU/g on day 15 and minimum (4 log CFU/g) were observed on day 2 and day 7 while 4.5 log CFU/g bacterial count was observed on day 10. Overall, decreasing trend in total viable bacteria at 30°C was observed from control to the treatment F4 which contain agar along with both probiotic LA and SC (Figure 1g, Table 7) while total viable bacteria count was observed maximum (8 log CFU/g) in F2 treatment which contain LA probiotic.

3.8 | pH value

Fillet of tilapia used in the trial pH at initial stage was observed 7. This pH remains 6.8 to 7.2 until day 7 while maximum value of pH was observed 8 in F1 (pH > 7.7) at 15 days (p .05). Same behaviour was observed in F2 and F3 lots but the pH progression in F3 was slower as compared to F2. The fillet covered with probiotic film maintained the initial levels of pH 7–7.3 up to 10 days of the storage period but maximum level of pH up to 7.8 and 8 was observed in control and F1 on the day 15 of the fillet at 4°C. According to the results maximum value of pH was observed in control (X) which was 7 on day 15 while value was 7, 7.1 and 7.4 on day 2, 7 and 10 respectively and increasing trend in pH was observed from day 2 to 15. In agar film (F1); the pH was maximum (8) on day 15 in all over the trial period. It was observed 7 on day 2 but it was decreased (pH 6.8) on day 7 while pH was observed 7.3 on day 10. In F2 (agar + probiotic 1 (LA) film), maximum pH was 7.2 at day 15 of storage while 7, 6.8 and 7.1 pH was observed on day 2, 7 and 10 respectively. In F3 (agar + probiotic 2 (SC) film), the pH was between the range 6.9 to 7.1. It was observed 6.9, 7.1, 6.9 and 7.1 on day 2, 7, 10 and 15 respectively. In F4 (agar + probiotic 1 (LA) + probiotic 2 (SC) film), pH was observed minimum on day 15 which was 6.8 while pH was observed 7, 7.2 and 7.1 on day 2,7 and 10 of storage of fish fillet. The results indicated that the bacterial counts in fillet samples which were treated with probiotic films (F2, F3 and F4) were observed lower due to which bacterial activity was reduced and volatile base compounds were produced which provoked reduction of pH in treatments with probiotic films (F2, F3 and F4) which give help to increase the shelf life of tilapia fillets (Figure 1h, Table 8).

3.9 | Colour, smell and texture

During the shelf life trial, all the samples were preserved at constant values of lightness throughout the storage period but significant small differences were observed in some cases which were not vary more than 10%. The yellowness tendency was observed in FI and F2 lots as compared to F3 and F4. The colour was stable throughout the storage period in the treatments in which probiotics were used (p .05). Odour and overall acceptance of fillet were found better in F4 as compared to other treatments and control. Sensory evaluation criterion of Tilapia fillet is given in Table 9.

The overall results of shelf life trial indicated that probiotic addition in agar film play vital role and were found principal responsible for the inhibitory activity of psychrotrophic organisms like total bacteria at 15°C, H2S-producing bacteria, *Pseudomonas* sp. and luminescent bacterial colonies). The luminescent bacterial colonies (*P. phosphoreum*) were observed most sensitive when probiotics were added to films which retard their growth in fish fillet while Lactic acid bacteria persisted relatively constant, excluding in lot F3 at the end of the storage. For probiotic batches, the reduction in counts of bacteria was more obvious in H2S-producing bacteria and total flora which was reduced up to 4 logarithmic cycles (p .05). Differences of 4 log cycles were also observed between the control and probiotic film fillet for enterobacteria at 7 days (p .05) which was lost over time up to 15 days. In addition, it was observed that the joint presence of probiotic bacteria in the film (F4) produced additive effect on delaying the microbial growth on the coated fillet during storage

TABLE 7 Shelf life, bacterial counts (total viable bacteria, log CFU/g) in GIFT Tilapia (*Oreochromis mossambicus*) fillet during storage at 30°C

	Total viable bacteria (log CFU/g) at 30°C				
Types of films	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	4	4	5	7	7.5
Agar film (F1)	4	4	4	6.5	7
Agar + probiotic 1 (LA) film (F2)	4	5.5	7	7.5	8
Agar + probiotic 2 (SC) film (F3)	4	4	4	4.5	5.5
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	4	4	4	4.5	5

TABLE 8 Shelf life, changes in pH in GIFT Tilapia (*Oreochromis mossambicus*) fillet during chilled storage (4°C)

	Changes in pH				
Types of films	Day 0	Day 2	Day 7	Day 10	Day 15
Control (X)	7	7	7.1	7.4	8
Agar film (F1)	7	7.2	7.2	7.3	8
Agar + probiotic 1 (LA) film (F2)	7	7	6.8	7.1	7.2
Agar + probiotic 2 (SC) film (F3)	7	6.9	7.1	6.9	7.1
Agar + probiotic 1 (LA) + probiotic 2 (SC) film (F4)	7	7	7.2	7.1	6.8

TABLE 9 Sensory evaluation criterion of Tilapia fillet

Quality parameter	Description	Score
Colour	Glossy appearance, bright surface	5
	Slight glossy appearance, bright surface	4
	Slight glossy appearance, dull surface	3
	No glossy appearance, a little yellow surface	2
	No glossy appearance, yellow surface	1
Odour	No fishiness, no earthy smell	5
	Little fishiness, no off-odour	4
	Little freshness and off-odour	3
	Distinct freshness and off-odours	2
	Strong freshness and off-odours	1
Overall acceptance	Fresh, totally acceptable	5
	Little fresh, acceptable	4
	Little fresh, reluctant acceptance	3
	Not fresh, unacceptable	2
	Not fresh, totally unacceptable	1

period. In summary of our experiment on the application of probiotic films on tilapia, fillet showed delayed the growth of microbes in the fish fillet hence reducing the spoilage indexes. It was also observed that probiotic strains may pass inside the fish fillet and increase the production of lactic acid bacterial counts. Probiotics also reduced the H2S-producing bacteria due to which reduction in chemical spoilage was observed. Smell and texture of fish fillet treated with probiotic film (F2 F3 and F4) were observed better as compared to control and F1 throughout entire period of storage. Thus, from the trial, it may be concluded that films with probiotic could extend shelf life of fish (fillet of tilapia) up to at least for a week.

4 | DISCUSSION

Microbial deterioration is known as an important quality criterion for shelf life of the perishable foodstuffs due to its relationship to food spoilage ability. Several technologies along with intelligent packaging were developed to protect and attain high-quality food with prolonged shelf life. Monitoring the microbial deterioration is basic requirement for controlling the shelf life and food quality through understanding deterioration mechanisms (Lee, 2010). Numerous techniques were applied by several workers to improve or analyse the shelf life of perishable foodstuffs like growth of the naturally occurring bacteria pseudomonads, *S. putrefaciens*, lactic acid bacteria, Enterobacteriaceae and yeasts, on gilt-head seabream (*S. aurata*) (Koutsoumanis & Nychas, 2000) were evaluated for shelf life, and pseudomonads were identified as a good spoilage index. Shaltout et al. (2016) treated minced meat with the Marjoram and Cumin oils for the shelf life, and their results were similar to El-Desouky et al. (2006); Chouliara et al., (2007) and Darwish-Soumia, El-Geddawy, Khalifa, and Mohamed (2012), who describe that their use in lower concentrations on meat products had beneficial effects on quality, protection and shelf life. It has been observed that lactic acid bacteria can retard the growth of Enterobacteriaceae in the food models (Alves et al., 2019; Espitia, Batista, Azeredo, & Otoni, 2016; Hosseini et al., 2013; Mozaffarzogh et al., 2020; Zhang, Lin, & Nie, 2013). The bacterial cells survival in edible films may be associated to the adhesion forces, physicochemical and osmotic conditions (Burgain, Gaiani, Cailliez-Grimal, Jeandel, & Scher, 2013). The use of probiotics with sodium alginate was also found an active way to bio-preserve the fish fillets. Its combination with glycerol was found more effective to retard microbial deterioration and to improve sensory properties in fillets (Mozaffarzogh et al., 2020).

Alak (2011) reported the increasing number of psychrotrophic bacteria like *Leuconostoc* spp., *Carnobacterium* spp. and *Lactobacillus* spp. in cold water fish which is confirmed by different film-coating studies (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008). It is also observed that biofilm additives and solvent materials are effective to inhibit lactic acid bacteria (Volpe et al., 2015; Yıldız & Yangılar, 2016). Various solvents and films are found effective to inhibit *Pseudomonas* (Alak & Aras Hisar, 2012a, Alak & Aras Hisar, 2012b; (Yu, Jiang, Xu, & Xia, 2017). Edible films or coating with 2% thyme, 1.5% oregano and 1% lemon were found effective against Enterobacteriaceae in rainbow trout fillets (Jouki, Yazdi, Mortazavi, Koocheki, & Khazaei, 2014; Kazemi & Rezaei, 2015; Korkmaz et al., 2019; Volpe et al., 2015; Yıldız & Yangılar, 2016).

Our study show notable inhibitory effect of coating and is parallel with the results of several studies where different materials were used by adopting the same storage process or different film techniques. Various researchers confirmed the antimicrobial effect of these coatings by using different materials from our study like Marjoram oil and Cumin oil (Alak, Aras Hisar, Hisar, Kaban, & Kaya, 2010; Bahram et al., 2016; Qiu, Chen, Liu, & Yang, 2014; Shokri & Ehsani, 2017; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009; Yıldız & Yangılar, 2016; Zhou, Liu, Xie, & Wang, 2011). The results about breakdown of proteins were in agreement with El-Desouky et al., (2006); Chouliara et al., (2007); Keokamnerd et al., (2008) and Badee et al., (2013), which indicated antimicrobial activity of oils (Sachindra, Sakhare, Yashoda, & Rao, 2005). Marjoram oil reduced the total aerobic mesophilic bacterial counts and enhance shelf life of minced meat (Kamel, 2013; Shaltout et al., 2016) while Cumin-aldehyde essential oil keep antimicrobial potential against gram-positive bacteria (Iacobelis, Cantrop, Capasso, & Senatore, 2005; Rezai et al., 2014). Özyurt, Özkütük, Şimşek, Yeşilsu, and Ergüven (2015) used protein-based biodegradable coatings on rainbow trout (*Oncorhynchus mykiss*) fillet to evaluate quality and shelf life. Similar positive effects of edible coatings were observed on seafood (Ambardekar, 2007; Ou et al., 2002), and microorganisms were considered main cause of seafood products spoilage (Gram & Dalgaard, 2002). Andevari and Rezai (2011)

described the reduced microbial growth in trout fillets after using cinnamon oil-gelatine coatings. Núñez-Flores et al. (2013) used gelatin and gelatin-lignin (GeL) composite films which produced plasticizing effect with elongation values. Similar effects were reported previously in different fish species (Carvalho et al., 2008; Núñez-Flores et al., 2013; Pérez-Mateos et al., 2009; Vengal & Srikumar, 2005).

The present study was conducted to evaluate the shelf life of *O. mossambicus* after using two probiotics *L. acidophilus* and *S. cerevisiae* and the results about shelf life indicated that probiotics addition in agar film play vital role and were found principal responsible for the inhibitory activity of psychrotrophic organisms like total bacteria at 15°C, H2S-producing bacteria, *Pseudomonas* sp. and luminescent bacterial colonies. The luminescent bacterial colonies (*P. phosphoreum*) were observed most sensitive when probiotics were added to films which retard their growth in fish fillet while Lactic acid bacteria persisted relatively constant, excluding in percentage F3 at the end of the storage. For probiotic batches, the reduction in counts of bacteria was more obvious in H2S-producing bacteria and total flora which were reduced. Differences were also observed between the control and probiotic film fillets for *enterobacteria* at 7 days ($p > .05$) which was negligent over time up to 15 days. In addition, it was observed that the joint presence of probiotic bacteria in the film (F4) produced additive effect on delaying the microbial growth on the coated fillet during storage period. The results are testifying the previous work of several workers like (Lopez de Lacey et al., 2014), who used an agar film coating having green tea extract along with probiotic bacteria to observe the extension of shelf life in hake fillets. The application of films on hake fillet containing green tea, reduced microbial spoilage in fish (Núñez-Flores et al., (2013). The films containing green tea extract along with probiotics *Lactobacillus paracasei* and *Bifidobacterium lactis* were found most effective for microbial degradation and delay their growth (Lopez de Lacey et al., 2014). Similar results for green tea extract and their antimicrobial activity were observed on gram-negative and gram-positive bacteria (Chiu & Lai, 2010; López de Lacey, 2012) but the effects were species specific (Almajano, Carbó, Jiménez, & Gordon, 2008; Cushnie & Lamb, 2011; Ku, Hong, & Song, 2008; Sivarooban, Hettiarachchy, & Johnson, 2008).

Changes in pH of tilapia fillets in present study revealed that bacterial activity was reduced and volatile base compounds were produced which provoked reduction of pH in treatments with probiotic films (F2, F3 and F4, Table 8) which gave help to increase the shelf life. The results are allied with previous observation of Lopez de Lacey et al. (2014). The increase in pH is due to accumulation of volatile nitrogen bases like trimethylamine and ammonia while decrease in pH may be due to CO2 dissolution in the fillets which produce carbonic acid (Chaijan, Benjakul, Visessanguan, & Faustman, 2005). Our results were confirmed by previous studies (Lopez de Lacey et al., 2014; Shokri & Ehsani, 2017; Shokri, Ehsani, & Jasour, 2015). Change in pH values was also due to the activation of microbial load which was also observed in fish meat with natural essential oils (El-Desouky et al., 2006; Keokamnerd et al., 2008; Shalaby, MoawSpoiled, Emam, & Mohamed, 2013).

Colour is considered significant properties for acceptance of fish products due to direct effect on consumer's selection (Lawless & Heymann, 2010). No any significant difference and effect on colour were reported (Özyurt et al., 2015; Sathivel, 2005) except low values of redness in hake fillet (Lopez de Lacey et al., 2014; Sánchez-Zapata, Pérez-Alvarez, Fernández-López, & Barber-Valles, 2010) which resembles our study and indicated that the colour was stable throughout the storage period in the treatments in which probiotics were used. The fish composition may influence the sensory characteristics like colour, odour and texture during storage (Abdollahi, Rezaei, & Farzi, 2014). Therefore, the current trial on the application of probiotic films on tilapia fillets showed delay in the growth of microbes in the fish fillets. It was also observed that probiotic strains may pass inside the fish fillets and increase the production of lactic acid bacterial counts. Probiotics also reduced the H2S-producing bacteria due to which reduction in chemical spoilage was observed. Smell and texture of fish fillet treated with probiotic film (F2, F3 and F4) were observed better as compared to control and F1 throughout entire period of storage. Thus, from the trial it may be concluded that films with probiotic could extend shelf life of tilapia fillet up to at least one week.

5 | CONCLUSIONS

Probiotics addition in agar film play vital role and were found principal responsible for the inhibitory activity of psychrotrophic organisms like H2S-producing bacteria, *Pseudomonas* sp. and luminescent bacterial colonies. The luminescent bacterial colonies were observed most sensitive when probiotics were added to films which retard their growth in fish fillet while Lactic acid bacteria persisted relatively constant, excluding in lot F3 at the end of the storage. It was observed that the joint presence of probiotic bacteria in the film (F4) produced additive effect on delaying the microbial growth on the coated fillet. Changes in pH of tilapia fillets in present study revealed that bacterial activity was reduced and volatile base compounds were produced which provoked reduction of pH in treatments with probiotic films (F2, F3 and F4). The probiotic strains may pass inside the fish fillet and increase the production of lactic acid bacterial counts. Probiotics also reduced the H2S-producing bacteria due to which reduction in chemical spoilage was observed. The colour was stable throughout the storage period in the treatments. Therefore, the current trial showed delay in the growth of microbes in the fish fillet by reducing the spoilage index. Smell and texture of fish fillet treated with probiotic film were observed better throughout entire period of storage. Thus, it may be concluded that films with probiotic could extend shelf life of fish (fillet of tilapia) up to at least one week.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

RY, KS and RMF conceived the study. KS supervised the work. RY, SH and RMF participated in experimental work. SM, KA and ZA analysed the data. RMF, RY and KS developed a first draft of the manuscript. FA and SM participated in uplifting the draft manuscript. SM, KA, FA and ZA helped in funding acquisition.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

Abdollahi, M., Rezaei, M., & Farzi, G. (2014). Influence of chitosan/clay functional bionanocomposite activated with rosemary essential oil on the shelf life of fresh silver carp. *International Journal of Food Science and Technology*, *49*(3), 811–818. https://doi.org/10.1111/ijfs.12369

Ahmed, E. S. S., Shehata, M. G., Abd-Rabou, H. S., & El-Menshawy, H. (2019). Extend shelf-life of vacuum-packaged herring fish fillets using garlic and ginger extracts. *Journal of Pure and Applied Microbiology*, *13*(3), 1571–1581. https://doi.org/10.22207/JPAM.13.3.30

Alak, G. (2011). *Effects of probiotics and prebiotics on some physical, chemical and microbiological properties of rainbow trout* (Oncorhynchus mykiss*) intestinal flora and fillets* (Ph.D. Thesis). Atatürk University, Erzurum, Turkey.

Alak, G., & Aras Hisar, S. (2012). The effect of chitosan prepared in different solvents on the quality parameters of brown trout fillets (*Salmo trutta fario*). *Journal of Aquaculture*, *2*(3), 11–14. https://doi.org/10.4236/fns.2012.39172

Alak, G., & Aras Hisar, S. (2012). The effects of probiotics and prebiotics on rainbow trout (*Oncorhynchus mykiss*) intestinal flora. *International Journal of Aquaculture*, *2*(3), 11–14. https://doi.org/10.5376/ija.2012.02.0003

Alak, G., Aras Hisar, S., Hisar, O., Kaban, G., & Kaya, M. (2010). Microbiological and chemical properties of bonito fish (*Sarda sarda*) fillets packaged with chitosan film, modified atmosphere and vacuum. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *16*(Suppl. A), 73–80. https://doi.org/10.9775/kvfd.2009.1475

Almajano, M. P., Carbó, R., Jiménez, J. A. L., & Gordon, M. H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, *108*(1), 55–63. https://doi.org/10.1016/j.foodchem.2007.10.040

Alves, D., Marques, A., Milho, C., Costa, M. J., Pastrana, L. M., & Cerqueira, M. A. (2019). BacteriophageφIBB-PF7A loaded on sodium alginate-based films to prevent microbial meat spoilage. *International Journal of Food Microbiology*, *291*, 121–127. https://doi.org/10.1016/j.ijfoodmicro.2018.11.026

Ambardekar, A. A. (2007). Potential use of arrowtooth flounder (*Atherestes stomias*) protein as edible coating in food industry. *Asian Fish Sciences*, *203*, 83–393.

Andevari, G. T., & Rezai, M. (2011). Effects of gelatine coating incorporated with cinnamon oil on the quality of fresh rainbow trout in cold storage. *International Journal of Food Sciences and Technology*, *46*, 2305–2311. https://doi.org/10.1111/j.1365-2621.2011.02750.x

Angiolillo, L., Conte, A., & Del Nobile, M. A. (2018). A new method to bio-preserve sea bass fillets. *International Journal of Food Microbiology*, *271*, 60–66. https://doi.org/10.1016/j.ijfoodmicro.2018.01.010

Badee, A. Z. M., Moawd, R. K., El-Noketi, M. M., & Gouda, M. M. (2013). Improving the quality and shelf-life of refrigerated chicken meat by Marjoram essential oil. *Journal of Applied Sciences Research*, *9*(57), 18–29. Retrieved from http://www.aensiweb.com/old/jasr/jasr/2013/5718-5729.pdf

Bahram, S., Rezaie, M., Soltani, M., Kamali, A., Abdollahi, M., Ahmadabad, M. K., & Nemati, M. (2016). Effect of whey protein concentrate coating cinamon oil on quality and shelf life of refrigerated Beluga Sturegeon (*Huso huso*). *Journal of Food Quality*, *39*(6), 743–749. https://doi.org/10.1111/jfq.12227

Brachkova, M. I., Marques, P., Rocha, J., Sepodes, B., Duarte, M. A., & Pinto, J. F. (2011). Alginate films containing *Lactobacillus plantarum* as wound dressing for prevention of burn infection. *Journal of Hospital Infection*, *79*, 375–377. https://doi.org/10.1016/j.jhin.2011.09.003

Burgain, J., Gaiani, C., Cailliez-Grimal, C., Jeandel, C., & Scher, J. (2013). Encapsulation of *Lactobacillus rhamnosus* GG in microparticles: Influence of casein to whey protein ratio on bacterial survival during digestion. *Innovative Food Science & Emerging Technologies*, *19*, 233–242. https://doi.org/10.1016/j.ifset.2013.04.012

Carvalho, R. A., Sobral, P., Thomazine, M., Habitante, A., Giménez, B., Gómez-Guillén, M. C., & Montero, P. (2008). Development of edible films based on differently processed Atlantic halibut (*Hippoglossus hippoglossus*) skin gelatin. *Food Hydrocolloids*, *22*(6), 1117–1123. https://doi.org/10.1016/j.foodhyd.2007.06.003

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2005). Changes of pigments and color in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. *Food Chemistry*, *93*(4), 607–617. https://doi.org/10.1016/j.foodchem.2004.10.035

Chiu, P. E., & Lai, L. S. (2010). Antimicrobial activities of tapioca starch/decolorized hsian-tsao leaf gum coatings containing green tea extracts in fruit-based salads, romaine hearts and pork slices. *International Journal of Food Microbiology*, *139*(1–2), 23–30. https://doi.org/10.1016/j.ijfoodmicro.2010.01.010

Chouliara, E., Karatapanis, A., Savvaidis, I. N., & Kontominas, M. G. (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiology*, *24*, 607–617. https://doi.org/10.1016/j.fm.2006.12.005

Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, *38*(2), 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

Darwish-Soumia, M. I., El-Geddawy, M. A. H., Khalifa, R. M. B., & Mohamed, R. A. A. (2012). Antioxidant activities of some spices and herbs added to frozen chicken burger. *Frontiers in Science*, *2*, 144–152. https://doi.org/10.5923/j.fs.20120206.03

El-Desouky, A. I., Bahlol, H. E. M., & Sharoba, A. M. A. (2006). Effect of some essential oils and preservatives on the growth of *E. coli* 0157 and quality of refrigerate minced meat. *Annals of Agricultural Sciences*, *44*, 1675–1695.

Espitia, P. J., Batista, R. A., Azeredo, H. M., & Otoni, C. G. (2016). Probiotics and their potential applications in active edible films and coatings. *Food Research International*, *90*, 42–52. https://doi.org/10.1016/j.foodres.2016.10.026

Gialamas, H., Zinoviadou, K. G., Biliaderis, C. G., & Koutsoumanis, K. P. (2010). Development of a novel bioactive packaging based on the incorporation of *Lactobacillus sakei* into sodium-caseinate films for controlling *Listeria monocytogenes* in foods. *Food Research International*, *43*(10), 2402–2408. https://doi.org/10.1016/j.foodres.2010.09.020

Gomez-Estaca, J., Lopez De Lacey, A., & Lopez-Caballero, M. (2010). Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology*, *27*(7), 889–896. https://doi.org/10.1016/j.fm.2010.05.012

Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria problems and solutions. *Current Opinion in Biotechnology*, *13*, 262–266. https://doi.org/10.1016/s0958-1669(02)00309-9

Hosseini, S., Hamzeh, A., Moslemi, M., Lashkan, A., Iglesias, A., & Feás, X. (2013). Effect of delayed icing on biogenic amines formation and bacterial contribution of iced common carp (*Cyprinus carpio*). *Molecules*, *18*(12), 15464–15473. https://doi.org/10.3390/molecules181215464

Iacobelis, N. S., Cantrop, L. O., Capasso, F., & Senatore, F. (2005). Antibacterial activity of *Cuminum cyminu* and *Carum carvi* essential oil. *Journal of Agriculture and Food Chemistry*, *53*, 57–61. https://doi.org/10.1021/jf0487351

Ispır, U., Yonar, M. E., & Oz, O. B. (2011). Effect of dietary vitamin E supplementation on the blood parameters of Nile tilapia (*Oreochromis niloticus*). *Journal of Animal and Plant Sciences*, *21*(3), 566–569. Retrieved from http://thejaps.org.pk/docs/21-3/32.pdf

Jouki, M., Yazdi, F. T., Mortazavi, S. A., Koocheki, A., & Khazaei, N. (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf-life extension of refrigerated rainbow trout fillets. *International Journal of Food Microbiology*, *174*, 88–97. https://doi.org/10.1016/j.ijfoodmicro.2014.01.001

Kamel, S. M. (2013). The use of some herbs for improving the refrigerated storage stability of minced camel meat. *Journal of Microbiology*, *2*, 95–103. https://doi.org/10.14196/SJMI.V2I5.777

Kazemi, S. M., & Rezaei, M. (2015). Antimicrobial effectiveness of gelatin-alginate film containing oregano essential oil for fish preservation. *Journal of Food Safety*, *35*(4), 482–490. https://doi.org/10.1111/jfs.12198

Keokamnerd, T., Acton, J. C., Han, I. Y., & Dawson, P. L. (2008). Effect of commercial rosemary oleoresin preparations on ground chicken thigh meat quality packaged in a high oxygen atmosphere. *Poultry Science*, *87*, 170–179. https://doi.org/10.3382/ps.2007-00066

Korkmaz, F., Kocaman, E. M., & Alak, G. (2019). Using of quinoa based film to extend the shelf life of rainbow trout fillets under cold storage (4 ± 1°C) condition. *Marine Science and Technology Bulletin*, *8*(2), 76–84. https://doi.org/10.33714/masteb.651262

Koutsoumanis, K., & Nychas, G. J. E. (2000). Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. *International Journal of Food Microbiology*, *1*(60), 171–184. https://doi.org/10.1016/s0168-1605(00)00309-3

Ku, K. J., Hong, Y. H., & Song, K. B. (2008). Mechanical properties of a *Gelidium corneum* edible film containing catechin and its application in sausages. *Journal of Food Science*, *73*(3), 217–221. https://doi.org/10.1111/j.1750-3841.2008.00700.x

Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: Principles and practices*, *Spring Science*, *technology*, *medicine*, *business* (2nd ed., pp. 1–587). Retrieved from https://www.springer.com/gp/book/9781441964878

Lee, D. S. (2010). *Packaging and the microbial shelf life of food*, (pp. 55–74). London, New York: CRC Press, Taylor & Francis Group, Boca Raton. https://doi.org/10.1201/9781420078459

López de Lacey, A. M. (2012). *Diseño, desarrollo y aplicación de envases comestibles potencial mentebioactivos* (Doctoral thesis). Universidad Complutensede, Madrid, Spain. Retrieved from https://eprints.ucm.es/17857/

Lopez De Lacey, A. M., Lopez-Caballero, M. E., & Montero, P. (2014). Agar films containing green tea extract and probiotic bacteria for extending fish shelf-life. *Food Science and Technolology*, *55*, 559–564. https://doi.org/10.1016/j.lwt.2013.09.028

Malik, A., Shah, S. A., Kalhoro, I. B., & Kalhoro, H. (2014). Growth performance and adaptation of imported strain of Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758) culture at Fish Hatchery Chilya Thatta. *Sindh-Pakistan*, *Lasbela*, *University Journal Science Technology*, *3*, 5–9.

McAndrew, B. J. (2000). Evolution, phylogenetic relationships and biogeography. In M. C. M. Beveridge & B. J. McAndrew (Eds)., *Tilapias: Biology and exploitation* (Vol. *25*, pp. 1–32). Dordrecht, The Netherlands: Kluwer Academic Publishers, Fish and Fisheries Series.

Mohamed, M. H., & Refat, N. A. G. A. (2011). Pathological evaluation of probiotic, *Bacillus Subtilis*, against *Flavobacterium columnare* in Tilapia (*Oreochromis Niloticus*) Fish in Sharkia Governorate, Egypt. *Journal of American Science*, *7*(2), 244–256. Retrieved from http://www.jofamericanscience.org/journals/am-sci/am0702/32_4672am0702_244_256

Montero, P., Gomez-Estaca, J., & Gomez-Guillen, M. C. (2007). Influence of salt, smoke, and high pressure on growth of *Listeria monocytogenes* and spoilage microflora in cold smoked dolphin fish (*Coryphaena hippurus*). *Journal of Food Protection*, *70*(2), 399–404. https://doi.org/10.4315/0362-028x-70.2.399

Mozaffarzogh, M., Misaghi, A., Shahbazi, Y., & Kamkar, A. (2020). Evaluation of probiotic carboxymethyl cellulose-sodium caseinate films and their application in extending shelf life quality of fresh trout fillets. *LWT-Food Science and Technology*, *126*, 1–14. https://doi.org/10.1016/j.lwt.2020.109305

Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M. E., Montero, P., & Gómez-Guillén, M. C. (2013). Physical and functional characterization of active gelatin films incorporated with lignin. *Food Hydrocolloids*, *30*(1), 163–172. https://doi.org/10.1016/j.foodhyd.2012.05.017

Ou, C., Tsay, S., Lai, C., & Weng, Y. (2002). Using gelatin-based antimicrobial edible coating to prolong shelf-life of tilapia fillets. *Journal of Food Quality*, *25*, 213–222. https://doi.org/10.1111/j.1745-4557.2002.tb01020

Özyurt, G., Özkütük, A. S., Şimşek, A., Yeşilsu, A. F., & Ergüven, M. (2015). Quality and shelf life of cold and frozen rainbow trout (*Oncorhynchus mykiss*) fillets: Effects of fish protein-based biodegradable coatings. *International Journal of Food Properties*, *18*(9), 1876–1887. https://doi.org/10.1080/10942912.2014.971182

Pavli, F., Kovaiou, I., Apostolakopoulou, G., Kapetanakou, A., Skandamis, P., Nychas, G.-J., … Chorianopoulos, N. (2017). Alginate-based edible films delivering probiotic bacteria to sliced ham pretreated with high pressure processing. *International Journal of Molecular Sciences*, *18*(9), 18–67. https://doi.org/10.3390/ijms18091867

Pereira, J. O., Soares, J., Sousa, S., Madureira, A. R., Gomes, A., & Pintado, M. (2016). Edible films as carrier for lactic acid bacteria. *LWT-Food Science and Technology*, *73*, 543–550. https://doi.org/10.1016/j.lwt.2016.06.060

Pérez-Mateos, M., Montero, P., & Gómez-Guillén, M. C. (2009). Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. *Food Hydrocolloids*, *23*(1), 53–61. https://doi.org/10.1016/j.foodhyd.2007.11.011

Qiu, X., Chen, S., Liu, G., & Yang, Q. (2014). Quality enhancement in the Japanese sea bass (*Lateolabrax japonicas*) fillets stored at 4°C by chitosan coating incorporated with citric acid or licorice extract. *Food Chemistry*, *162*, 156–160. https://doi.org/10.1016/j.foodchem.2014.04.037

Rastall, R. A., Fuller, R., Gaskins, H. R., & Gibson, G. R. (2000). Functional foods. In R. Gibson & C. M. Williams (Eds.), *Colonic functional foods* (pp. 1–365). Cambridge, UK: Woodhead Publishing Limited. Retrieved from http://ssu.ac.ir/cms/fileadmin/user_upload/Daneshkadaha/dbehdasht/behdasht_imani/book/Functional_Foods.pdf

Rezai, R., Sadeghi, E., Nateghi, L., & Mohammadi, M. (2014). The effect of *Cuminum cyminum* essential oil on growth and survival of *Staphylococcus aureus* during storage of hamburger. *International Journal of Biosciences*, *5*(4), 18–26. Retrieved from https://revues.imist.ma/index.php/RHAZES/article/view/14587

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., & Aadil, R. M. (2020). Sources, formulations, advanced delivery and

health benefits of probiotics. *Current Opinion in Food Science*, *32*, 17–28. https://doi.org/10.1016/j.cofs.2020.01.003

Sachindra, N. M., Sakhare, P. Z., Yashoda, K. P., & Rao, D. N. (2005). Microbial profile of buffalo sausage during processing and storage. *Food Control*, *16*, 31–35. https://doi.org/10.1016/j.foodcont.2003.11.002

Sanchez-Gonzalez, L., Saavedra, J. I. Q., & Chiralt, A. (2014). Antilisterial and physical properties of biopolymer films containing lactic acid bacteria. *Food Control*, *35*(1), 200–206. https://doi.org/10.1016/j.foodcont.2013.07.001

Sánchez-Zapata, E., Pérez-Alvarez, J. A., Fernández-López, J., & Barber-Valles, J. X. (2010). Descriptive study of reflectance spectra of Hake (*Merluccius australis*), Salmon (*Salmo salar*) and light and dark muscle from Tuna (*Thunnus thynnus*). *Journal of Food Quality*, *33*(3), 391–403. https://doi.org/10.1111/j.1745-4557.2010.00318

Sathivel, S. (2005). Chitosan and protein coatings affect yield, moisture loss, and lipid oxidation of pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen storage. *Journal of Food Science*, *70*(8), 455–459. https://doi.org/10.1111/j.1365-2621.2005.tb11514

Shalaby, A. R., MoawSpoiled, R. K., Emam, W. H., & Mohamed, G. F. (2013). Quality and shelf life of cold stored mullet fish (*Mugil Cephalus*) pretreated by Sorbate/Thyme oil with emphasis on biogenic amines. *Current Nutrition and Food Science*, *9*, 328–334. https://doi.org/10.2174/15734013113099990003

Shaltout, F. A., Salem, A. M., Khater, D. F., & Lela, R. A. (2016). Impact of some natural preservatives on bacterial profile of minced meat in Egypt. *Benha Veterinary Medical Journal*, *31*(1), 35–42. Retrieved from https://bvmj.journals.ekb.eg/article_31215_2ebb152712bc1b067909162ecbcdf2b6

Shokri, S., & Ehsani, A. (2017). Efficacy of whey protein coating incorporated with lactoperoxidase and α-tocopherol in shelf life extension of Pike-Perch fillets during refrigeration. *LWT-Food Science and Technology*, *85*, 225–231. https://doi.org/10.1016/j.lwt.2017.07.026

Shokri, S., Ehsani, A., & Jasour, M. S. (2015). Efficacy of lactoperoxidase system-whey protein coating on shelflife extension of rainbow trout fillets during cold storage (4°C). *Food and Bioprocess Technology*, *8*(1), 54–62. Retrieved from https://link.springer.com/article/10.1007/s11947-014-1378-7

Sivarooban, T., Hettiarachchy, N. S., & Johnson, M. G. (2008). Transmission electron microscopy study of *Listeria monocytogenes* treated with nisin in combination with either grape seed or green tea extract. *Journal of Food Protection*, *71*(10), 2105–2109. https://doi.org/10.4315/0362-028x-71.10.2105

Soukoulis, C., Singh, P., Macnaughtan, W., Parmenter, C., & Fisk, I. D. (2016). Compositional and physicochemical factors governing the viability of *Lactobacillus rhamnosus* GG embedded in starch-protein based edible films. *Food Hydrocolloids*, *52*, 876–887. Retrieved from https://pubag.nal.usda.gov/catalog/1198730. https://doi.org/10.1016/j.foodhyd.2015.08.025

Trosvik, K. A., Rawles, S. D., Thompson, K. R., Metts, L. A., Gannam, A., Twibell, R., & Webster, C. D. (2012). Growth and body composition of Nile tilapia, *Oreochromis niloticus*, fry fed organic diets containing yeast extract and soybean meal as replacements for fish meal, with and without supplemental lysine and methionine. *Journal of the World Aquaculture Society*, *43*(5), 635–647. https://doi.org/10.1111/j.1749-7345.2012.00595

Vásconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. *Food Research International*, *42*(7), 762–769. https://doi.org/10.1016/j.foodres.2009.02.026

Vengal, J. C., & Srikumar, M. (2005). Processing and study of novel lignin-estarch and ligninegelatin biodegradable polymeric films. *Trends in Biomaterials and Artificial Organs*, *18*(2), 237–241.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2008). Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *International Journal of Food Science and Technology*, *43*(3), 526–531. https://doi.org/10.1111/j.1365-2621.2006.01489

Volpe, M., Siano, F., Paolucci, M., Sacco, A., Sorrentino, A., Malinconico, M., & Varricchio, E. (2015). Active edible coating effectiveness in shelf-life enhancement of trout (*Oncorhynchus mykiss*) fillets. *LWT-Food Science and Technology*, *60*(1), 615–622. https://doi.org/10.1016/j.lwt.2014.08.048

Yasin, R., Jabeen, F., Ali, M., Samiullah, K., & Hussain, S. M. (2018). Effects of yeast (*Saccharomyces cerevisiae*) on the intestinal microbiota of GIFT Tilapia (*Oreochromis mossambicus*). *International Journal of Biosciences*, *12*(4), 283–291.

Yıldız, P. O., & Yangılar, F. (2016). Effects of different whey protein concentrate coating on selected properties of rainbow trout (*Oncorhynchus mykiss*) during cold storage (4°C). *International Journal of Food Properties*, *19*(9), 2007–2015. https://doi.org/10.1080/10942912.2015.1092160

Yu, D., Jiang, Q., Xu, Y., & Xia, W. (2017). The shelf life extension of refrigerated grass carp (*Ctenopharyngodon idellus*) fillets by chitosan coating combined with glycerol monolaurate. *International Journal of Biological Macromolecules*, *101*, 448–454. https://doi.org/10.1016/j.ijbiomac.2017.03.038

Zhang, Q., Lin, S., & Nie, X. (2013). Reduction of biogenic amine accumulation in silver carp sausage by an amine-negative *Lactobacillus plantarum*. *Food Control*, *32*(2), 496–500. https://doi.org/10.1016/j.foodcont.2013.01.029

Zhou, R., Liu, Y., Xie, J., & Wang, X. (2011). Effects of combined treatment of electrolysed water and chitosan on the quality attributes and myofibril degradation in farmed obscure puffer fish (*Takifugu obscurus*) during refrigerated storage. *Food Chemistry*, *129*(4), 1660–1666. https://doi.org/10.1016/j.foodchem.2011.06.028

Zikria, N., Khan, N., Mughal, M. S., Ashraf, M., Khalique, A., Alam, S., & Tayyab, R. (2012). Effect of different ration levels of artificial feed on the growth performance of genetically improved farmed tilapia (GIFT) fry. *Pakistan Journal of Science*, *64*(3), 219. Retrieved from https://www.yumpu.com/en/document/read/29323639/effect-of-different-ration-levels-of-artificial-feed-on-the-paascompk

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