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Alginate based active edible coating effectiveness in shelf-life enhancement of hot smoked rainbow trout

Alginat-basierte essbare Beschichtungen – Verlängerung der Haltbarkeit von heiß-geräucherten Regenbogenforellen

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Summary

Alginate based active edible coating have significant potential to develop packaging films and coatings for shelf-life extension of seafood products. In this study hot smoked rainbow trout fillets were separated into two groups: uncoated trout fillet (C) and coated with alginate based active edible materials containing 1 % thyme oil (w/w). The effect of alginate coating enriched with thyme oil on the quality of smoked rainbow trout fillets during refrigerated storage (2 ± 1 °C) over a period of 7 weeks was evaluated. Hot smoked trout fillets were analysed for microbiological (mesophilic aerobic, psychrotrophic, anaerobic bacteria count, mould and yeast, *Clostridium* spp.) and chemical (Total volatile basic nitrogen, trimethylamine nitrogen and tiobarbituric acid index values) characteristics. This study demonstrates the effectiveness of edible active alginate coating in preserving hot smoked trout fillets from lipid oxidation and microbial growth.

Keywords: Hot smoked fish, Active edible coating, Shelf life, Quality

Zusammenfassung

Auf Alginat-basierte essbare Beschichtungen besitzen ein hohes Potenzial für die Entwicklung von Verpackungsfolien und Lebensmittel-Beschichtungen zur Verlängerung der Haltbarkeit von Fisch und Fischerzeugnissen. In dieser Studie wurden heißgeräucherte Regenbogenforellenfilets in zwei Gruppen aufgeteilt: unbeschichtete Forellenfilets (C) und Forellenfilets beschichtet mit 1 % Thymianöl angereichertem Alginat (w/w). Die Wirkung der Alginat-Beschichtung mit Thymianöl auf die Qualität der heiß-geräucherten Regenbogenforellenfilets wurde über einen Zeitraum von 7 Wochen bei einer Kühllagerung (2 ± 1 °C) ausgewertet. Die heiß-geräucherten Forellenfilets wurden auf mikrobiologische (mesophile aerobe, Psychrotrophen, Anaerobe Bakterien, Schimmel und Hefe, *Clostridium* spp.) und chemische Eigenschaften (Flüchtigem Basischem Stickstoff, Trimethylamin Stickstoff und Tiobarbituric Säure Indexwerte) untersucht. Diese Studie zeigt die Wirksamkeit von essbarer Alginat-Beschichtung bei heiß-geräucherten Forellenfilets bezüglich der Lipidoxidation und des mikrobiellen Wachstums.

Schlüsselwörter: Obst, Sorten, Makro- und Mikronährstoffe, Schwermetall, ICP-AES

Introduction

Rainbow trout is one of the most economically important aqua cultured freshwater fish species. Its global popularity is due to its fast growth rate, palatability, and the nutritional quality of its flesh. The production of rainbow trout has grown exponentially since the 1950s, especially in Europe and more recently in Chile. This is primarily due to increased inland production in countries such as France, Italy, Denmark, Germany and Spain to supply the domestic markets, and mariculture in cages in Norway and Chile for the export market. Global production of rainbow trout reached nearly 855981 tons in 2010 (FAO, 2015). The total production of Turkey was reported 114024 tonnes in 2014 for rainbow trout (TUIK, 2015). Products for human consumption come as fresh, smoked, canned and frozen trout that are eaten steamed, fried, broiled, boiled, or micro-waved and baked (FAO, 2015). Fish are usually more perishable than other muscle foods and requires a good preservation. Smoking is a traditional fish preservation method with considerable economic importance worldwide (FAO, 2012). Smoking imparts characteristics of flavour and colour to the fish. In addition, smoking increases the shelf life of fish, as a result of the combined effects of dehydration, antimicrobial and antioxidant activity of several of the smoke constituents, which are mainly formaldehyde, carboxylic acids and phenols (Benjakul & Aroonrueng, 1999; Leroi & Joffraud, 2000; Anvari et al., 2015). The preferred fish species for smoking are salmon, mackerel, herring, haddock, eel and rainbow trout. Smoked fish were packaged under vacuum packaging (VP) or modified atmosphere packaging (MAP). The spoilage in smoked fish is determined by the microbial growth and/or activity and related changes in the sensory characteristics. The Food Hygiene Directive (Forsythe, 2010) proposed the following total viable count scale as a basis for determining the microbiological quality of smoked fish: $<10^6$ cfu/g "satisfactory", 10^6 to $<10^7$ cfu/g "acceptable", $>10^7$ cfu/g "unsatisfactory". According to these criteria, should be not consumed, evaluated as unacceptable products. The limit of microbiological acceptability for VP and MAP hot smoked rainbow trout ranged from 2 to 4 weeks (Kolsarıcı & Özkaya, 1998; Çaklı et al., 2006; Erkan et al., 2009; Erkan et al., 2011a; Erkan & Yeşiltaş, 2014). Increasing consumer preference for smoked fish products requires research for new preservation methods. In this context, edible film coating or active coating enriched with natural extract is an alternative food preservation technology, which provides a way to increase fish product shelf life when combined with good refrigeration and handling process (Campos et al., 2011). Bay leaf, rosemary, black cumin seed, lemon oil, sage, grape seed, thyme and garlic extract are amongst the most employed natural extracts as antimicrobial agents against food-borne pathogens and spoilage micro flora in fish and fish products (Fisher and Philips, 2006; Frangos et al., 2010; Erkan et al., 2011a; Erkan et al., 2011b; Erkan, 2012). The advantage of edible coating application includes inactivation of food spoilage micro-organisms at low temperature with minimal effects on flavour and nutritional attributes of the product (Olivas & Barbosa-Cánovas, 2008; Balasubramanian et al., 2009). Natural polysaccharides are good candidates as base ingredients of edible films and coating to provide a shelf-life extender. Edible coatings made of protein or polysaccharides (with the advantage of polysaccharides of being reported as non-allergenic, tasteless and odourless), alone or in combination with biological or non-biological materials (Polyvinylchloride-PVC, gelatine, proteins) seem to represent a valid alternative too and are actively investigated for shelf-life extension of fish (Erkan et al., 2013; Erkan & Yeşiltaş, 2014; Dursun & Erkan, 2014; Volpe et al., 2015). In this study, sodium alginate was used to make an edible coating added with thyme oil (active coating) to extend the shelf-life of hot smoked rainbow trout fillets. To assess the effectiveness of the active coating, changes in pH, TBV-N, bacteria growth and changes in lipid oxidation parameter were studied on hot smoked rainbow trout fillets stored at 2 °C for 7 weeks.

Materials and Methods

Preparation of hot smoked fish samples

Hot smoked fish was prepared from rainbow trout *(Oncorhynchus mykiss)*. Rainbow trout were obtained from the freshwater fish farm of Liman Company (Bilecik, Turkey). The fresh fish samples were packed in polystyrene boxes with crushed ice, and then transferred to the laboratory. The fish were beheaded, gutted manually, and then washed. The samples (25 gutted fishes) were immersed in brine at a ratio of 1:1 (w/w) for 18 h at 2 °C. The brine contained 6.5 % NaCl. After brining, the samples were briefly dipped in chilled tap water for 1/2 h. The smoke was produced from oak sawdust with combustion. The processing time in the kiln was divided into five stages: preliminary drying for 15 min; at room temperature (20 °C), cooking period for 60 min. at 80 °C; smoking and partial cooking period for 120 min. at 80 °C; cooling for 60 min at 20 °C. After cooling for 60 min at 2 °C, hot smoked rainbow trouts were filleted.

Preparation of alginate-based edible coating containing thyme oil, packaging and storage

Food grade sodium alginate was provided by Smart Kimya Company (Izmir, Turkey). Alginate solution powder (3 %, w/v) was prepared by dissolving alginate in distilled water at a controlled temperature (70–80 °C), and was stirred until the mixture became clear. Glycerol was added to sodium alginate solutions at 1 % concentration as a plasticizer. The mixture was heated on a hot plate with constant stirring until completely dissolved and clear, added 1 % thyme oil (w/w) (Erkan & Bilen, 2010) and stirred. This solution then cooled to room temperature. The smoked fish were filleted and divided into two lots. The first group was called control group (C). The second group (T) were treated with alginate 3 % plus 1 % thyme oil solution (w/w). Solutions were added onto the surface (2 sides) of each fillet using a silicon brush for each group. After treatment, fillets were allowed to dry for 40 min on a sterile stainless mesh screen at ambient temperature (20 ºC). Three treated fillets for each group was packaged into a Low Density Polyethylene/Polyamide (LDPE/PA) barrier pouches (Polinas Plastic Company, Manisa, Turkey). The characteristics of the plastic film bags were as follows: thickness: 90 µm, total light transmission: 30 %; O_2 transmission: 160 $cm^3/m^2/day/atm$ at 75 % relative humidity (RH), 25 ºC; vapour permeability: 8.50 $g/m^2/day$ at 100 % relative humidity (RH), 25 ºC. The pouches were heat sealed using a vacuum-sealing machine (Henkovac vacuum packaging machine, ML's Hertogenbosch, Netherlands). Pouches were kept under refrigeration (2 ± 1 ºC) for a period of 7 weeks.

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Microbiological analysis

Sample preparation: Smoked fish (25 g) obtained from smoked fish fillets (5 fillets for each group), were transferred aseptically to a Stomacher bag (Seward Medical, London, UK) containing 225 mL of 0.1 % peptone water (Merck, 107228) and homogenized for 60 s using a Lab Blender 400, Stomacher at high speed (Stomacher, IUL Instrument, Spain).

Microbiological media and count: For microbial count, 0.1 mL samples of serial dilutions (1:10, diluents, 0.1 % peptone water (Merck, 107228, Darmstadt, Germany) of fish homogenates were spread on the surface of agar plates. Total viable counts (TVC) were determined using plate count agar (PCA, Merck, 105463) after incubation for 24–48 h at 37 ºC. Plate count agar was used for psychrotrophic bacteria and incubated at 7 ºC for 10 days. Total anaerobic counts (TAC) were determined by PCA incubated under anaerobic conditions (with 5 % CO_2 incubator, HF 90 model, Shanghai, China) at 30 °C for 24–48 h. Yeasts and molds were enumerated using Dichloran Rose Bengal Chloramphenical (DRBC, Merck 10046) after incubation at 25 °C for 3 days in the dark. Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample (Bell et al., 2005). Thus, the detection limit of total mesophilic, psychrophilic and anaerobic bacterial counts was <1.00 log cfu/g. All the analyses were performed in duplicate. For anaerobic sulphite-reducing Clostridium count, 25 g of sample were homogenized and incubated at 30 °C for 14 days in Differential Reinforced Clostridial Broth (DRCM, Merck 1.11699) under anaerobic condition. Results were expressed as log MPN/g of samples (ICMSF, 1986).

Chemical analysis: The smoked fish skins were removed for the physical and chemical analysis and were homogenized using a food processor. Total volatile basic nitrogen (TVB-N, mg N/100 g fish flesh), trimethylamine nitrogen (TMA-N, mg/100 g) and tiobarbituric acid (TBA) index values (TBA, mg/malondialdehyde (MDA)/kg) was determined according to the method described by Erkan and Bilen (2010).

Sensory analysis: The attributes of hot-smoked fish were evaluated by a panel of five experienced judges on each week of sampling. Panellists were laboratory trained. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity. Smoked fish were assessed on the basis of appearance, odour, taste and texture characteristics using a nine point descriptive scale. A score of 9–7 indicated "very good" quality, a score of 6.9–5.0 "good or acceptable quality", a score of 4.9–1.0 "unacceptable quality". The appearance, odour, taste and colour of the samples were evaluated by five experienced panellists, and the mean values of these attributes were presented (Mohan et al., 2012).

Statistical analysis: Experiments were replicated twice on different occasions with different fish samples. Analyses were run in triplicate for each replicate. Results of microbiological, chemical and sensory analyses were reported as mean values ± standard deviation. Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure of SPSS (SPSS, 1995) was used to test for differences between groups (Sümbüloğlu and Sümbüloğlu, 2002).

Results and Discussion

Effect of the sodium alginate-based edible coating containing thyme oil in bacterial count of hot smoked rainbow trout

The limit of acceptance for total viable count (under the terms of the storage mesophilic, psychrophilic, anaerobic bacteria) is 7 log cfu/g (ICMFS, 1986). Mesophilic, psychrophilic and anaerobic bacteria count of control samples were above this limit on the 3th week of storage (Tab. 1). During the storage, the mesophilic, psychrophilic and anaerobic bacteria counts in treated samples exceeded the permissible limit on 6th and 7th weeks of storage, respectively. Erkan and Yeşiltaş (2014) reported the total bacteria count of hot smoked trout above 7 log cfu/g after 2 weeks at 2 ± 2 °C. Similarly, the mesophilic and psychrophilic aerobic bacteria counts have been reported as 7 log cfu/g in vacuum packaged hot smoked rainbow trout after 2 weeks and 3 weeks of storage (Kolsarıcı & Özkaya, 1998; Dursun & Erkan, 2014). Çaklı et al. (2006), Erkan et al. (2009 and 2011a) reported the mesophilic and psychrophilic aerobic bacteria counts over 7 log cfu/g after 4 weeks of cold storage in vacuum packaged hot smoked rainbow trout. Erkan et al. (2011a) reported that hot smoked trout fillets stored at 2 °C under a combination vacuum packaging and rosemary, black cumin seed, and lemon oil showed total viable counts slightly above the acceptability level after 6 weeks of storage. Erkan & Yeşiltaş (2014) reported that hot smoked trout fillets stored at 2 °C under a combination of VP and alginate coating showed microbial counts slightly above the acceptability level after 5 weeks of storage.

TABLE 1: *Microbiological analyses results of the samples stored at 2 ± 1 °C.*

Storage weeks	Mesophilic aerobic bacteria count (log cfu/g)		Psychrotrophic bacteria count (log cfu/g)		Anaerobic bacteria count (log cfu/g)		Mould and yeast count (log cfu/g)		*Clostridium* spp. (MNP/g)	
	C	T	C	T	C	T	C	T	C	T
0	1.33±0.46Aa	<1.00±0.00Ba	1.20±0.28Aa	<1.00±0.00Ba	1.00±0.00Aa	<1.00±0.00Ba	2.15±0.21Aa	<1.00±0.00Ba	0.45±0.00 Aa	<0.47±0.00Aa
1	5.93±0.61Ab	2.60±0.09Bb	4.46±0.76Ab	2.50±0.09Bb	5.95±0.49Ab	3.00±0.00Bb	5.35±0.49Ab	2.00±0.00Ba	3.50±0.71Ab	<0.47±0.00Ba
2	**6.77±0.66Ac**	3.60±0.09Bc	**6.27±0.18Ac**	3.72±0.17Bc	**6.80±0.27Ac**	4.28±0.04Bc	6.74±0.08Ac	4.59±0.00Bb	3.78±0.32Ab	<0.47±0.00Ba
3	7.90 ±0.57Ad	4.54±0.09Bd	7.02±0.11Ac	4.75±0.14Bd	7.55±0.49Ac	4.88±0.04Bd	7.15±0.19Ad	4.80±0.01Bb	3.80±0.28Ab	<0.47±0.00Ba
4	8.45±0.07Ae	5.50±0.04Be	8.35±0.07Ae	5.70±0.04Be	7.86±0.22Ad	5.50±0.040Be	7.15±0.07Ad	4.94±0.01Bb	4.80±0.26Ac	0.96±0.00Bb
5	8.64±0.06Af	**6.30±0.14Bf**	8.60±0.14Af	**6.38±0.04Bf**	8.05±0.06Ae	6.05±0.07Bf	7.72±0.82Ad	5.87±0.04Bc	5.70±0.40Ad	1.18±0.00Bc
6	9.05±0.49Ag	7.13±0.08Bg	8.60±0.20Af	7.08±0.11Bg	8.63±0.18Af	**6.51±0.04Bg**	7.93±0.81Ad	6.91±0.05Bd	5.60±0.57Ad	2.32±0.00Bd
7	8.82±0.11Ah	7.81±0.04Bh	9.10±0.28Ag	8.54±0.14Bh	8.75±0.07Af	7.62±0.11Bh	7.00±0.85Ad	7.10±0.03Ad	5.80±0.85Ad	3.04±0.00Be

* bold colour represents acceptable value

Dursun & Erkan (2014) reported microbial counts lower than 7 log cfu/g of hot smoked trout fillets after 6 weeks of storage at 2 °C in presence of collagen, gelatine and fish protein coating.

TABLE 2: *Sensory and chemical analyses results of the samples stored at 2 ± 1 °C.*

Storage weeks	Sensory score		TVB-N (mg/100 g)		TMA-N (mg/100 g)		TBA-i (mg/kg)	
	C	T	C	T	C	T	C	T
0	8.50±0.63Aa	8.78±0.24Aa	17.07±0.02Aa	15.64±0.45Ba	0.64±0.14Aa	0.25±0.01Ba	0.33±0.02Aa	0.22±0.03Aa
1	7.20±0.16Ab	8.56±0.13Ba	18.00±0.97Aa	14.54±0.18Bbc	1.88±0.03Aa	0.15±0.15Ba	1.39±0.32Ab	0.19±0.01Ba
2	6.80±0.18Ab	7.81±0.17Bb	21.72±1.16Ab	15.05±0.09Bac	2.20±0.01Aa	0.32±0.03Ba	**1.93±0.11Ac**	0.75±0.08Bb
3	**5.30±0.23Ac**	7.04±0.24Bc	**22.53±0.66Ab**	15.48±0.02Ba	**3.44±0.09Aa**	0.65±0.09Bb	3.33±0.54Ad	1.08±0.02Bc
4	4.70±0.10Ad	6.03±0.16Bd	34.99±2.42Ac	17.39±0.02Bd	5.41±0.43Aa	1.13±0.05Bc	3.12±0.45Ad	1.28±0.01Bd
5	3.51±0.42Ae	**5.28±0.07Be**	33.33±0.36Ac	19.30±0.17Be	5.72±0.43Aa	1.98±0.11Bd	2.09±0.83Ad	0.82±0.02Be
6	2.80±0.32Af	4.66±0.11Bef	39.20±0.25Ad	**23.58±1.16Bf**	7.05±0.06Aa	2.02±0.11Bd	1.86±0.11Ac	0.69±0.02Bf
7	1.95±0.23Ag	3.98±0.14Bf	41.31±0.44Ae	25.81±0.07Bg	8.93±0.13Aa	2.62±0.17Be	0.97±0.04Ab	1.44±0.11Bg

* bold colour represents acceptable value

Effect of the sodium alginate-based edible coating containing thyme oil in sensory quality of hot smoked rainbow trout

The results of the sensory assessment of samples are in Table 2. In our study, control samples had a lower sensory quality ($p < 0.05$) than coated samples during the storage period, and they were regarded as unacceptable after 3 weeks, while coated samples were acceptable until the 5th week. Erkan et al. (2011b) and Erkan (2012) reported positive effect of bay, rosemary, black cumin seed, lemon, thyme and garlic oil, on sensory quality of hot smoked fish, similar to our result. Dursun and Erkan (2014) studied the preserving effect of gluten and collagen film coating for extending the shelf life of hot smoked rainbow trout. Results of sensory, analyses showed that the shelf life of stored fish in cold storage with gluten and collagen edible film coating had extended by 5 weeks compared to the control samples. The sodium alginate treatment is an effective method to protect food from the degradation in sensory quality (Lu et al., 2009; Song et al., 2011).

Effect of the sodium alginate-based edible coating containing thyme oil in chemical properties of hot smoked rainbow trout

According to the European regulation (European Commission Decision, 95/149/EC) the highest TVB-N limit is set at 35 mg N/100 g of fish flesh. Since no limit of acceptability has been specifically established for rainbow trout, there is a general consensus that 25 mg N/100 g of trout flesh is the highest acceptable level as proposed by Erkan (2012). On the 4th and 7th week of storage, the TVB-N values of control and coated samples exceeded this limit ($p > 0.05$). Erkan et al. (2011b) and Erkan (2012) reported that hot smoked trout fillets stored at 2 °C under a combination of VP-rosemary/lemon and garlic oil showed TVB-N values slightly above the acceptability level after 7 weeks of storage. Erkan & Yeşiltaş (2014) reported TVB-N values lower than 25 mg/100 g of hot smoked rainbow trout fillets after 6 weeks of storage at 2 °C in presence of sodium alginate coating.

Trimethylamin nitrogen is another quality indicator for marine fish and fish products. A wide range of TMA-N values have been reported to set the acceptability limit: 1 mg N/100 g (Kyrana et al., 1997); 2–3 mg N/100 g (Goulas & Kontominas, 2007); 5 mg N/100 g (Chytiri et al., 2004). Control samples contained TMA-N above 5 mg/100 g on the 4th week of storage. In this study, the TMA-N content of coated hot smoked trout was slowly increased throughout the cold storage, however, remained within the limits of acceptability.

According to Goulas & Kontominas (2005), TBA-i values of 2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an intolerable odour/taste. The TBA values of the present hot smoked rainbow trout samples exceeded the value of 2 mg MDA/kg after 2 weeks of storage period for control samples. However, the TBA-i content of coated samples (Tab. 2) remained below the acceptable level during the 7 weeks of storage. Similar TVB-N, TMA-N and TBA findings have been reported for many other fish species coated with chitosan and sodium alginate (Chidanandaiah et al., 2009; Duan et al., 2010; Fan et al., 2009; Günlü and Koyun, 2013; Mohan et al., 2012).

Conclusions

The sensory evaluation results were correlated with microbial and chemical analyses. Due to microbial growth and lipid oxidation, the control samples of hot smoked fish fillet showed spoilage, appearing as off-odour after 3 weeks of storage. The antioxidant, antimicrobial and gas barrier effects of sodium alginate containing thyme oil treatment had been shown to prolong the fish shelf life by 2 weeks as compared to the control samples.

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Conflict of interest

I certify that there is no potential conflict of interest in relation to this article.

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