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Doctoral thesis

Freshness and shelf-life of fish products

Ruoyi Hao

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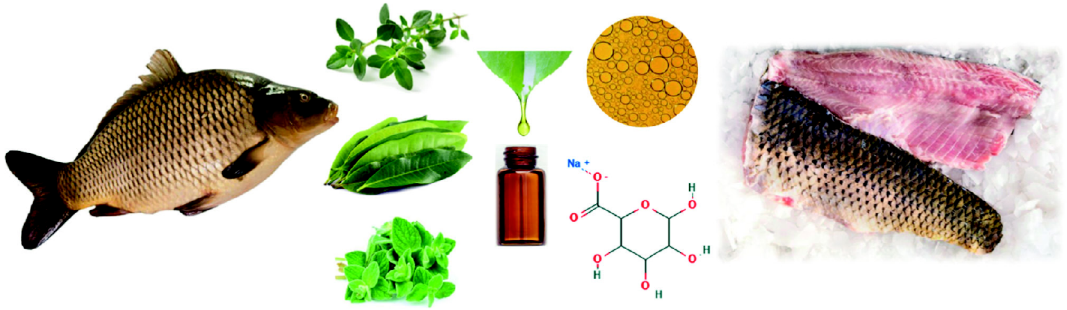
Čerstvost a trvanlivost rybích produktů



Doctoral thesis by
Ruoyi Hao

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CHAPTER 1

GENERAL INTRODUCTION

General introduction

As a source of easily digestible and highly nutritious animal protein, fish plays an important role in the human diet. Globally, fish consumed as food increased from 9.0 kg live weight/ capita to 20.5 kg live weight/capita from 1961 to 2018, accounting for 17.4% of the total animal protein intake by humans (FAO, 2020). In addition, fish is rich in n-3 polyunsaturated fatty acids (n-3 PUFA), mainly eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), which show benefits in preventing and alleviating cardiovascular diseases, cancers, and inflammatory diseases (Briggs et al., 2017). Thus, fish also play a positive role in human health. However, with high moisture, low amount of connective tissues, and neutral pH value, fish muscle is more perishable than other food muscles (Liu et al., 2017; Yu et al., 2020). In Europe, up to 20% of captured fish suffer remarkable waste due to freshness loss and microbial spoilage during post-harvest handling, processing, storage, and distribution (FAO, 2011). How to control the quality of fish or fish products and extend their shelf-life is of crucial importance. Chill-stored fish could effectively maintain the original flavor, texture, freshness of fish. Without time-consuming thawing, chill-stored fish suffer no ice crystal damage and are convenient for cooking (Benjakul et al., 2003a). Thus, chill-stored fish is turning into popular fish food with the encouragement of available cold-chains and promoting healthy consumption concepts of fresh food. Nevertheless, fish quality still deteriorates severely during chilled storage, leading to texture deterioration, off-odor, and shelf-life reduction (Yu et al., 2020). These cause significant quality decline, economic loss and even threaten the health and safety of consumers. Thus, it is necessary to comprehensively understand quality deterioration of chilled stored fish and develop effective preservative methods to maintain fish freshness and shelf-life.

Fish gel foods are new fish products with a unique feature of texture. They are usually produced from surimi or fish muscle mince, which can be converted into fish gel foods with resilience after kneading with sodium chloride and thermal treatment. Since fish gel foods contain high protein content but a low amount of lipid, they are considered healthy food, attracting more and more consumers. Most biochemical and microbial actions are reduced to almost zero due to washing, kneading, and heating in fish gel food. Thus, their shelf-life can be significantly prolonged compared with whole fish or fish fillets. However, the properties and stability of fish gel foods depend much on the freshness and quality of the fish muscle used to prepare them (Benjakul et al., 2003b). The lipids, hemoglobin, metal ions, thermal-resistant enzymes in fish muscle might cause lipid and protein oxidation, muscle autolysis, leading to the deterioration of gel properties and reduction of shelf-life (Park and press, 2013). Additionally, even frozen surimi or fish muscle mince might suffer severe protein and lipid oxidation during storage, lowering fish protein's preserving and gel-forming properties. Therefore, it is important to find solutions to control the metabolism or changes in fish muscle to provide fish proteins with excellent gel-forming properties and stability for gel food production.

1. The fish muscle deterioration and its mechanisms

Generally, fish muscle deterioration depends on three mechanisms: enzymatic autolysis, lipid or protein oxidation, and microbial growth (Kamkar et al., 2014; Liu et al., 2017; Zhu et al., 2015). Autolysis occurs mainly in the first few days of storage, while microbial spoilage usually occurs in the second stage. The deterioration of fish quality is a complex process involving physiological, chemical, and microbiological activities. It could appear as undesirable physical, chemical and microbiological changes in fish, including liquid loss, off-odor, microstructure disarrangement, nucleotides, lipid and protein degradation or oxidation, and microorganism accumulation (Cheng et al., 2015; Prabhakar et al., 2020).

1.1. Enzymatic autolysis

After slaughter, enzymatic autolysis in fish starts immediately. Adenosine triphosphate (ATP) is produced from glycogen and phosphocreatine reserves, keeping fish muscle relaxed during the pre-rigor stage. Once ATP is depleted, actin and myosin in fish muscle will combine to form the inextensible actomyosin, which causes stiffness of the fish body. This is the onset of rigor mortis, and it may last for hours or days. Once rigor is resolved thanks to autolytic enzymes, the fish muscle turns less rigid and is no longer elastic, and this stage is called the post-rigor stage (Roco et al., 2018).

Enzymatic autolysis could lead to physical changes in fish muscle during the initial stage of deterioration (Prabhakar et al., 2020). Glycolytic enzymes break down glycogen to produce ATP with the formation of lactic acid as well, leading to fish muscle tenderization and pH decline (D'Alessandro and Zolla, 2013; He et al., 2018). The changes in pH value could stimulate the activities of endogenous proteinases, e.g., cathepsins L., troponin T, and troponin I, promoting protein hydrolysis to form peptides or free amino acids, which usually leads to fish muscle softening and liquid loss (Ghaly et al., 2010). Meanwhile, triacyl lipase and phospholipase help to convert triglycerides or phospholipids into free fatty acids (FFAs) and accelerate lipid oxidation, which could finally give off-flavor (Ghaly et al., 2010).

Noticeably, nucleotides degradation induced by enzymatic autolysis is an essential biochemical process for fish post-mortem. Glycogen in fish muscle would be metabolized to generate ATP rapidly. The latter could be further catabolized to form other nucleotide products as following steps (Fig. 1): ATP→adenosine diphosphate (ADP)→adenosine monophosphate (AMP)→inosine 5′-monophosphate (IMP)→hypoxanthine riboside (HxR)→hypoxanthine (Hx)→xanthine (Xa)→uric acid (UA) (Hong et al., 2017). The transformation from ATP to IMP usually ends within one day. It mainly depends on endogenous enzymes, e.g., ATPase, ADPase, and AMP deaminase, which could reduce the textural quality and improve fish taste due to the accumulation of IMP, which is a classic taste compound for fish (Shi et al., 2014). After that, IMP continues degradation as a cascade reaction to form HxR and Hx with the action of autolytic and bacterial enzymes (e.g., IMP phosphohydrolase, nucleoside phosphorylase, and inosine nucleosidase) (Zhang et al., 2015b). Hx accumulation would result in the progressive loss of a desirable fish flavor and contribute a bitter taste (Shi et al., 2014). As the degradation continues, Hx would be converted to Xa, UA, and ring cleavage products under the action of spoilage microflora (Shen et al., 2015).

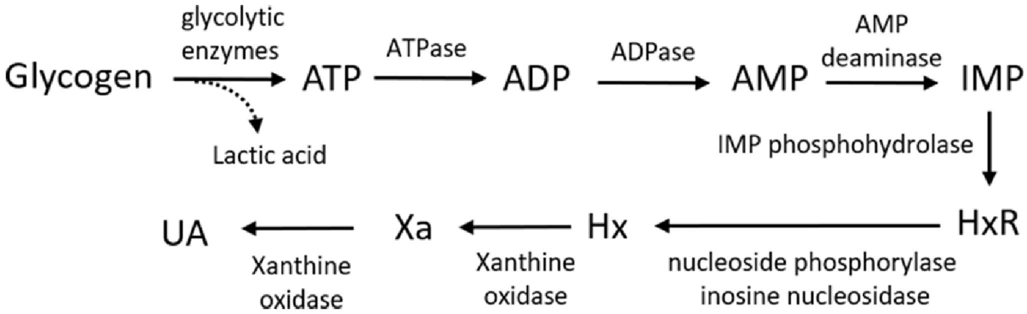

Figure 1. *Nucleotides degradation in fish muscle. Adapted from Hong et al. (2017). ATP, Adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine 5′-monophosphate; HxR, hypoxanthine riboside; Hx, hypoxanthine; Xa, xanthine; UA, uric acid*

The above texts summarize autolytic actions on glycogen, ATP, protein, lipids in fish. These actions induced by endogenous enzymes significantly influence fish freshness and shelf-life. It is noticed that the autolysis process is closely related to the nutrient profile of muscles. The amount of glycogen, nucleic acids, protein, or lipid pattern in fish muscle could intervene with the autolysis speed, lasting length, affecting final fish freshness and shelf-life. As is known, the nutritional profile of cultured fish is decided fairly by the farming system, which involves factors like feed, aquatic environment, temperature, etc. Thus, the farming system might intervene with the quality changes of farmed fish during storage. Common carp (*Cyprinus carpio*) occupied 85.5% (17,945 t) of the total cultured fish (20,968 t) in the Czech Republic in 2019 (Cz-Ryby, 2019). To increase the healthy n-3 PUFA in muscle, a patented system of omega-3 carp was developed in the Czech Republic (Mraz et al., 2011, 2017). The benefits of this carp on human health have been proven, but the knowledge of the potential intervention by nutrient profile modification on carp storage stability is not available yet. It is necessary to explore the influence of modifying nutritional profile on carp freshness and shelf-life to fill the gap of the potential effect of the n-3 carp farming system on the quality deterioration and spoilage progress of carp.

1.2. Oxidation

1.2.1. Lipid oxidation

PUFAs present in fish are susceptible to lipid oxidation, which usually involves the reaction of oxygen with the double bonds of fatty acids (Waraho et al., 2011). Both enzymatic and non-enzymatic reactions could lead to lipid oxidation. Enzymatic lipid oxidation is often promoted by lipoxygenase. Meanwhile, triacyl lipase and phospholipase might accelerate the breakdown of triglycerides or phospholipids into FFAs, resulting in fast lipid oxidation. Non-enzymatic lipid oxidation is a free radical chain reaction, which involves three stages: initiation, propagation, and termination (illustrated by Fig. 2). In the initiation stage, lipid free radicals (L•) are formed by heat, metal ions, irradiation, etc., via the reaction with oxygen, L• is converted into peroxy radicals (LOO•). During propagation, hydroperoxides (LOOH) and a new L• are formed via the reaction of LOO• with other lipid molecules (LH). When non-radical products are formed, lipid oxidation comes to the termination (Waraho et al., 2011). Lipid hydroperoxides are preliminary products of lipid oxidation that do not have an impact on flavor. However, their decomposition could result in the formation of secondary oxidation products, including ketones, aldehydes, alcohols, hydrocarbons, acids, epoxides, amines, etc. Most of these products are volatile and might lead to rancid flavors (Qiu et al., 2014). Noticeably, the level of aldehydes – can be estimated as thiobarbituric acid reactive substances (TBARS) value, which is commonly used as an indicator for monitoring lipid oxidation (Xu et al., 2015).

Previous studies by our research group established an innovative farming system for common carp, enabling the increase of n-3 PUFA in fish muscle (Mráz et al., 2012a,b; 2017; Mráz and Pickova, 2011; Sterniša et al., 2017). In general, n-3; As are more prone to oxidation than saturated FAs or n-6 FAs (Sampels, 2015). On the other hand, the new farming system might provide different categories of bioactive compounds, especially antioxidants such as tocopherol, ascorbic acids, etc., to fish muscle, which implies lipid oxidation in n-3 carp might differ from that in normal carp. Therefore, it comes the question--whether and how the lipid oxidation in common carp will be affected by the modified FA profile and farming system.

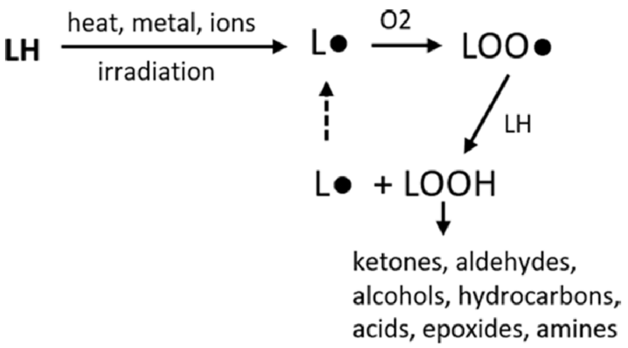

Figure 2. *Lipid oxidation mechanism in fish muscle. Adapted from Olatunde and Benjakul (2018) and Amit et al. (2017). LH, lipid molecules; L•, lipid free radicals; LOO•, peroxy radicals; LOOH, hydroperoxides*

1.2.2. Protein oxidation

Protein deterioration in fish during storage includes protein hydrolysis and protein oxidation. Protein hydrolysis is mainly attributed to the autolytic action by endogenous enzymes (discussed in section 1.1), and its influences on fish texture, taste, and safety have been comprehensively recognized. Similar to lipid oxidation, protein oxidation is a chain reaction induced by free radicals (see Fig. 3). A protein carbon-centered radical (P•) could be generated via reactive oxygen species (ROS) along with a hydrogen atom's abstraction. A peroxy radical (POO•) and an alkyl peroxide (POOH) can be generated consecutively via P• when oxygen occurs. With the presence of peroxide free radical (HO_2•), an alkoxyl radical (PO•) and its hydroxyl derivative (POH) are generated during the further reactions (Lund et al., 2011). Protein oxidation usually leads to the formation of protein carbonyls, loss of sulfhydryl groups, and protein cross-linking. It shows complicated influences on the textural properties of protein foods. Fish fillets suffering heavy protein oxidation could experience increased liquid loss or hardness. Protein oxidation might also lead to a declined gel-forming capability of fish muscle protein (Lu et al., 2017). Noticeably, protein oxidation does not always provide a negative influence on fish or fish products. Appropriate cross-linking by protein oxidation could contribute pleasant gelling properties to myofibrillar protein, improving fish gel products stability (Li et al., 2012). Thus, it is important to develop a strategy to manipulate protein oxidation to balance protein deterioration and gel improvement for producing good fish gel food. Interestingly, lipid oxidation and protein oxidation could interact with each other. It is found that P• could react with L• (Hęś, 2017), and the secondary oxidative products from lipid, e.g., malondialdehyde, could accelerate protein oxidation in fish (Maqsood et al., 2012b). Thus, protein oxidation might be promoted by lipid oxidation. The potential interactions between them raise a hypothesis that the protein stability of n-3 common carp might be affected by the modified FA or antioxidants profile introduced by the innovative farming system.

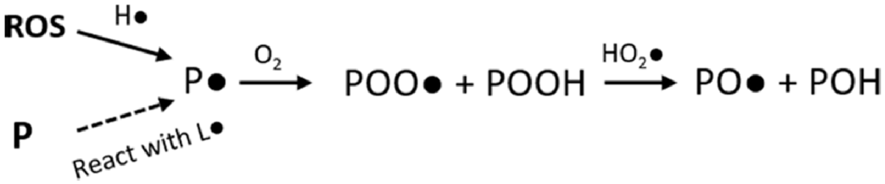

Figure 3. *Protein oxidation mechanism in fish muscle. ROS, reactive oxygen species; P, protein; H•, hydrogen atom; L•, lipid free radicals; P•, carbon-centered radical; POO•, peroxy radical; POOH, alkyl peroxide; HO_2•, peroxide free radical; PO•, alkoxyl radical; POH, hydroxyl derivative.*

1.3. Microbial spoilage

Microorganisms play a vital role in fish quality deterioration during the middle or late storage stage. It determines the freshness and shelf-life of fish or fish products to a large extent. Usually, the original microorganisms in fish are characterized by vast diversity and low relative abundance (Jääskeläinen et al., 2019). Not all can colonize fish or fish products and proliferate to a large population due to their different tolerance against preservation conditions (Yu et al., 2018). Only a small part of these microorganisms can thrive during storage and turn to the dominant spoilage microorganism. These microorganisms are mainly responsible for forming a large amount of off-odor and unpleasant metabolites, leading to a remarkable deterioration of fish freshness. They are termed "specific spoilage organisms (SSOs)". Many studies showed that SSOs are closely associated with undesirable biochemical changes in fish or fish products (Kachele et al., 2017; Kenar et al., 2010; Zhang et al., 2015a).

During storage, protein hydrolysis provides amino acids and peptides to enzymes released by SSOs to form small molecules, including indole, ammonia (NH_3), trimethylamine (TMA), biogenic amines (BAs), organic acids, and sulfur compounds, leading to the change of fish odors and taste (Amit et al., 2017). They can also promote nucleotides catabolism, accelerating the generation of HxR and Hx (Hong et al., 2017).

It is noticed that, among the above microbial metabolites, some chemical substances can pose diverse health threats to humans and animals, which is known as microbial food toxins. It is reported that about 70% of global outbreaks of food-borne diseases are associated with microorganisms and the toxins produced by them (W.H.O., 2008). Several bacterial strains have been recognized as the primary culprits for food-related illnesses, thus making them high priority targets for detection (e.g., *E. Coli* (infectious dosage of 10^1-10^2 CFU), *Listeria monocytogenes* (infectious dosage of 400-10^3 CFU), and *Staphylococcus* (infectious dosage of $>10^6$ CFU)). Such pathogenic microorganisms play an important role in fish food safety, which cause deadly diseases such as diarrhoea, bacteremia, cholecystitis, pneumonia, listeriosis, staph infections, and neonatal meningitis (Kadariya et al., 2014; Chen et al., 2018). Thus, an effective fish preservative strategy should only not inhibit the SSOs but also restrain the growth of pathogenic microorganisms (Gupta et al., 2021).

Many studies investigated the roles of microorganisms in fish freshness and shelf-life or the effects of specific preservation technologies on maintaining fish freshness and shelf-life by measuring microorganism population or single quality-denoting parameter, such as total viable counts (TVC), total volatile basic nitrogen (TVBN), TMA, etc. However, these results cannot provide systematic knowledge to form a whole view of the relations among SSOs, fish freshness, and preservation technologies. Thus, they are not comprehensive enough to provide a good understanding of microorganisms' role in fish freshness, neither to guide to establish a good preservative strategy. A comprehensive understanding of microbial community change during storage and the correlations of SSOs with specific fish quality parameters are necessary to achieve this. Unfortunately, the gap is not well filled due to the requirement of large data analysis by complicated statistical methods.

2. The freshness indicators for fish or fish products

Freshness is crucial to fish quality. Fish freshness depends on physical, chemical, biochemical, and microbiological changes during post-mortem. It is usually quantitatively described by assessing specific quality indicators (Olafsdottir et al., 2004).

2.1. Common freshness indicators

2.1.1. Sensory indicators

Sensory attributes, such as appearance, odor, tactile, etc., are crucial for consumers' acceptance of food, and they can be measured to monitor fish freshness. Usually, well-trained panelists should evaluate sensory properties using a standardized scoring system (Rezaeifar et al., 2020). With analytical technology development, instruments simulating human sense could test sensory properties instead of human sense. For instance, a texture analyzer is widely applied to measure hardness, cohesiveness, gumminess, springiness, chewiness, and resilience of fish muscle (Li et al., 2019). The electronic nose and tongue are used for evaluating the odor and flavor features of fish (Lan et al., 2018). A colorimetric sensor array is also developed for detecting fish appearance properties (Morsy et al., 2016). It must be pointed out that sensory tests by humans might be time-consuming, but some sensory parameters still prefer to be tested by trained panelists. For example, rigor state, a valuable indicator for showing fish freshness during the post-mortem, is often evaluated by finger-pressing test according to a rigor rating system by Erikson and Misimi (2008) to show the status of fish rigor mortis.

2.1.2. Physical indicators

Fish physical properties include textural properties, color properties, electrical properties, spectral properties, etc. Textural properties can be measured by texture analyzer as hardness, tenderness, resilience, etc., while color properties are often detected by colorimeter as L*, a*, b* value. Nowadays, rapid and non-invasive measuring technologies, e.g., infrared hyperspectral imaging system, low-field nuclear magnetic resonance system, and magnetic resonance imaging system, have been used to evaluate color properties (Wu et al., 2012), water stability, and distribution (Li et al., 2018b) in fish or fish products. Interestingly, electrical conductivity is closely associated with freshness indicators, such as pH, K value, TVBN, etc., in fresh water fish, including common carp, grass carp, and bighead carp (Zhang et al., 2011a,b). Thus, it also can be applied to monitor fish freshness.

2.1.3. Chemical indicators

Due to the high precision and stability, chemical indicators are widely measured to suggest fish freshness. In the early stage of post-mortem, before bacterial spoilage occurs, K-value is the most reliable indicator for fish freshness indication. Its measurement is based on the determination of ATP-series products (Olafsdottir et al., 2004). However, K-value might not effectively indicate fish freshness for some species (Prabhakar et al., 2020). Thus, Hx-index, Ki-value calculated by the adapted formulas of K-value can be used instead (Cheng et al., 2015; Dalgaard, 2000; Hong et al., 2017). In the late post-mortem stages, many other chemical parameters can be detected to evaluate fish freshness loss. TMA, a main responsible compound for "fishy" odor, formed from the degradation of trimethylamine oxide by microorganisms, is considered a good biochemical index for fish freshness and shelf-life assessment (Navarro-Segura et al., 2020). TVBN, an indicator closely correlated with the microbial populations, is often measured to estimate fish freshness (Hao et al., 2017). Moreover, peroxide value measuring the primary lipid oxidation products content (Nawaz et al., 2020) and TBARS value reflecting the secondary lipid oxidation products level are commonly assessed to show fish freshness (Yu et al., 2018). Additionally, BAs and VOCs formed from protein, and lipid degradation are widely investigated to describe fish freshness and safety.

2.1.4. Microbiological indicators

As discussed in section 1.3, microbial metabolism could lead to the decomposition of proteins and lipids, resulting in the formation of VOCs with unpleasant off-flavors and the accumulation of deleterious BAs, greatly affecting the freshness and safety of fish or fish products (Ge et al., 2017; Jia et al., 2019; Křížek et al., 2018; Liu et al., 2018a). Thus, the microbial populations are often analyzed to assess the spoilage of fish or fish products. TVC reflects the total number of viable microorganisms in food, and it is widely accepted as a fish freshness indicator. The population of specific microorganisms such as *Pseudomonas, Shewanella, Aeromonas,* etc., could also be investigated to indicate fish spoilage (Fogarty et al., 2019; Huang et al., 2018; Mikš-Krajnik et al., 2016). The specific spoilage microorganisms in fish are usually analyzed by selective media. To better understand the role of microorganisms in fish deterioration, the microbial populations are often plotted against chemical indicators to find correlations. Usually, it shows close correlations with freshness indicators such as TVBN, TMA, BAs, Hx-index, etc. (Wang et al., 2018).

The above indicators could be useful to predict fish freshness and shelf-life. However, it is difficult to claim which one is the most accurate or precise indicator for fish or fish products with specific formulas or specific preserving conditions. Thus, many indicators are often investigated together, and then a comprehensive comparison of them is made to find the most appropriate one to provide a reliable judgment of fish freshness (Öztürk Kerimoğlu et al., 2020; Yu et al., 2017a). The innovative carp farming system provides common carp with the modified nutrient patterns. It is interesting to find reliable indicators for monitoring the freshness and shelf-life of this special fish, which could help understand the effects of innovative farming system and EO preserving method on carp freshness in return.

2.2. Recommended limits

To make a good judgment of freshness or shelf-life, an appropriate limitation of the indicator for fish is needed. The acceptable TVC level of freshwater fish was set as 7.0 log CFU/g by the International Commission of Microbiological Standards for Foods (ICMSF, 1986). Saito et al. (1959) defined fish as fresh, moderately fresh, and not fresh when K-value is <20%, 20%<K-value<50%, and >70%, respectively. Carp is also considered completely spoiled if K-value>70% – (Hong et al., 2017). Nevertheless, the limitation of TVBN for fish is not consistent. A TVBN of 25 mg/100 g is the limitation for spoilage and freshness loss of aquatic food given by Ojagh et al. (2010), while Yu et al. (2017b) recommended a TVBN of 15 mg/100 g as the acceptable threshold for grass carp. Additionally, Duman and Özpolat (2015) set the acceptable level of TVBN for fresh fish at 30 mg/100 g. Thus, the limitations of fish freshness indicators differ in fish species, environment, harvest season, etc. It has to be pointed out that both the threshold based on fish spoilage occurrence and the marginal value for customer rejection need to be considered when an appropriate limitation is tried to be established (Prabhakar et al., 2020).

Above all, fish freshness indicators could be affected by fish species, harvest season, farming environment, feed etc. It is difficult to give a universal value for the limitations of freshness indicators. Thus, investigations of specific freshness indicators and their limitations for specific fish with specific packaging are needed. This will help to obtain a thorough understanding of the influences of innovative farming systems on the storage stability of common carp. A less freshness-loss transportation and storage technology for this common carp might be provided based on this knowledge.

3. The effective preservation methods for fish or fish products

It is known that reducing temperature, oxygen content, water activity, microbial load, or combining several preservatives could inhibit freshness loss and prolong the shelf-life of fish or fish products. Recently, physical, biological, and chemical methods are applied along with each other to maintain fish freshness and extend the shelf-life of fish products.

3.1. Physical preservation methods

Physical preservation technologies usually inhibit microbial population and endogenous enzyme activity in food materials by physical actions, such as light, heat, pressure, electricity, oxygen, etc., to protect the freshness and shelf-life of food. Many physical preserving technologies have been tried to protect fish freshness. However, not all of them are applicable due to unstable effects, high cost, or negative influence on fish quality. Smoking could inhibit enzyme activity and microbiological growth in fish or fish products by lowering water activity and generating antimicrobial phenolic compounds via combustion. However, it might bring potential carcinogenic hydrocarbons to the final product, causing a threat to consumers' health (da Silva Santos et al., 2017). Ozone (Feng et al., 2012), electron-beam (Jung et al., 2018), and UV radiation (Křížek et al., 2018) are 'cold pasteurization' germicidal technologies, showing almost no negative effect on fish nutrients and quality. Unfortunately, their applications are very limited due to insufficient trust and acceptance by consumers. High hydrostatic pressure (HHP) could inactivate microorganisms and autolytic enzymes without scarifying fish quality (Gómez-Estaca et al., 2018), but it requires sophisticated equipment with high cost.

Until now, low-temperature preservation (including chilled storage and frozen storage) is still the most common and most effective technology for fish preservation. It could reduce biochemical reactions by deactivating endogenous enzyme activity and alleviate microbial spoilage by decelerating microorganism metabolism. Additionally, chilled storage renders fish original flavor, texture, and good freshness. Without time-consuming thawing, chill-stored fish suffer no ice crystal damage and are convenient for cooking (Benjakul et al., 2003a). With the encouragement of available cold chains and the promotion of healthy consumption concepts of fresh food, chilled storage turns more popular than frozen storage for maintaining fish freshness nowadays.

Packaging is another physical preservation technology that can effectively maintain fish freshness and extend shelf-life. For fish, vacuum packaging (VP) or modified atmosphere packaging (MAP) are usually applied. VP and MAP could retard microorganism proliferation and biochemical reaction in fish or fish products by providing appropriate preserving conditions characterized by low oxygen content or a high ratio of CO_2 or N_2 (Kachele et al., 2017; Li et al., 2018a). It is noticed that though chilled storage is effective for fish freshness protection, protein degradation, lipid oxidation, and microorganism proliferation still occur in fish during chilled storage. That can also lead to remarkable freshness loss and shorten shelf-life (Zhu et al., 2015). Thus, chilled storage is mostly applied coupled with other preserving methods, such as VP, MAP, UV irradiation, ozone treatment, etc., to offer a strengthened preserving role to protect fish or fish product freshness.

3.2. Biopreservation methods

Recently, biopreservation methods have received much attention due to their high safety and acceptance by consumers. The biopreservation method could use secondary metabolites from microorganisms such as nisin and enzymes to inhibit the reproduction of spoilage microorganisms. Nisin and chitosan are the most common ones that have been effectively applied as bio preservatives or preservation film for protecting chill-stored fish freshness (Oner et al., 2021; Zhang et al., 2021). Some microorganisms with probiotic properties and fermentation activities can inhibit the reproduction of other spoilage microorganisms. Therefore, they could be used to extend fish shelf-life. For instance, lactic acid bacteria (LAB) can produce compounds such as lactic acid, acetic acid, bacteriocins, which might inhibit the growth of spoilage microorganisms, and it was successfully used for sardine preservation (Kuley et al., 2018). Fermentation also can be considered as a biopreservation method. It could convert protein, lipids into peptides, free amino acids, acetic acid, etc., providing unique flavor and texture to fish or fish products, and that might improve nutritional value, healthfulness, and digestibility of fish products (Osimani et al., 2019).

3.3. Chemical preservation methods

Food preservation by chemicals is popular in the modern aquatic food industry. It means food ingredients with antibacterial activity such as sugar, salt, or other natural or synthetic preservatives, such as potassium sorbate, sodium benzoate, can be included to improve fish or fish product stability. Salt and sugar are common food ingredients with high safety and acceptance. Their addition in fish or fish products could decrease water activity, which could inhibit the reproduction of microorganisms. At the same time, they could improve the texture and flavor of fish or fish products (Qin et al., 2017; Shi et al., 2017). Thus, the application of sugar and salt for fish freshness protection and shelf-life extension could be a good strategy. However, it goes against the consumption trend of a healthy diet with a low amount of salt and sugar. Thus, careful consideration should be given to their application.

Driven by ‘green consumerism’, foods with few synthetic additives but more natural ingredients are popular nowadays (Hassoun and Çoban, 2017). This novel consumption mind promotes the demand for developing friendly preservatives for fish. Essential oils (EOs) are aromatic liquids derived from plant materials (such as flowers, buds, leaves, stems, bark, and seeds) with strong antibacterial activities (Moosavi-Nasab et al., 2019; Shojaee-Aliabad et al., 2018). EOs are lipophilic, able to penetrate cytoplasm and disturb the phospholipid bilayer of inner membrane and mitochondria, leading to the instability of cellular structure and increasing cellular permeability (Hassoun and Çoban, 2017; Shojaee-Aliabad et al., 2018). Thus, EOs could retard the proliferation of microorganisms. Since EOs are generally considered safe (FDA, 2019), they attract much attention for fish preservation. In the recent 20 years, more than 30 kinds of EOs have been applied to maintain fish freshness and extend shelf life (Cai et al., 2018; Huang et al., 2018; Křížek et al., 2018; Wu et al., 2014). However, the preserving effects of these EOs on fish are inconsistent. It is also difficult to conclude the appropriate application dose of EOs for fish. Moreover, hurdle technology encouraging the combination of EOs with other preservation technologies could amplify their preserving effects on chilled-stored fish.

EO emulsion as an oil-in-water delivery system is colloidal dispersions formed by a combination of two immiscible phases (EO and water) stabilized by a food-grade surfactant (e.g., polysorbates, sugar ester) (Donsì and Ferrari, 2016). Noticeably, EO emulsions exhibit several advantages. It could stabilize EO and enables its long-term release. Furthermore, the emulsion system increases the surface area of EO, facilitating the passive cellular uptake by bacteria. These together could improve the antimicrobial activity of EO, which in return might reduce the applied dose. Additionally, EO emulsion can minimize the organoleptic effects of EO on fish. Establishing economic and efficient preservation strategies for chill-stored n-3 carp by using EO and emulsion technology is one of the core aims of the thesis.

Another type of widely used natural fish products preservatives is phenolic compounds. Phenolic compounds are defined as compounds possessing one or more aromatic rings bearing hydroxyl substituent (Maqsood et al., 2014). They are secondary metabolites commonly existing in herbs and fruits, showing good radical-scavenging capabilities and antibacterial activities. They also exhibit beneficial influences on human health (Maqsood et al., 2014). Their high availability and safety enable the incorporation of them for preserving fish products' freshness a good strategy. Studies reported their antimicrobial activity in fish and fish products (Al-Bandak et al., 2009; Maqsood and Benjakul, 2010). Studies also reported their anti-oxidative roles in chill-stored fish and frozen fish mince (Maqsood and Benjakul, 2010) (Medina et al., 2009). Interestingly, phenolic compounds usually show antioxidative capability in fish, but they might work as prooxidants to promote fish protein crosslinking, improving protein gel properties and stability in some cases. This role of tannic acid and ferulic acid for mackerel surimi gels was reported (Maqsood et al., 2012a). Thus, phenolic compounds can be used as potential antioxidants, antimicrobial and protein cross-linking agents to maintain or improve the quality of fish and fish products. As mentioned previously, fish gel food has great potential. Wisely applying phenolic compounds to improve fish gel foods' properties and prolong their shelf-life is of interest.

4. Research goals

The current studies were devoted to filling these knowledge gaps: 1) in sections 1.1 and 1.2, does the innovative culture system affect the nutrients pattern, spoilage process, and freshness of carp? 2) in sections 2.1 and 2.2, which indicator could be used for denoting n-3 carp freshness? What is their limit? 3) in section 1.3, how to understand microorganisms' role in fish freshness? 4) in sections 3.1 and 3.3, how to establish a good preservation strategy for using EOs-based preservation methods to manipulate microbial spoilage and maintain the freshness of chill-stored fish? 5) in sections 1.2, 3.1, and 3.3, how to improve fish gel product freshness and properties by physical and chemical preserving methods? Therefore, the research goals are:

1. To highlight the differences in the potential nutrients, post-mortem freshness, and shelf life between traditionally cultured common carp and omega-3 carp; to elucidate whether and how the patented farming system could affect carp freshness and shelf-life.
2. To analyze the chemical, physical and microbial properties of n-3 carp that receive EO-coating treatments during chill-storage to find the reliable freshness indicator for it; to obtain a good understanding of the effects of farming system and EO treatment on carp freshness.
3. To investigate the spoilage microorganism in chill-stored fish and their relationships with typical freshness indicators, e.g., TVBN, TVC, and nucleic acid.
4. To manipulate the freshness and shelf-life of chill-stored fish EOs-based preservation methods, providing guides for establishing appropriate preservation strategies by combining EOs with other technologies.
5. To establish intelligent strategies for maintaining fish gel food freshness and properties via phenolic compounds and ultrasound treatment by enhancing protein cross-linking.

References

Al-Bandak, G., Tsironi T., Taoukis P., Oreopoulou V., 2009. Antimicrobial and antioxidant activity of Majorana syriaca in Yellowfin tuna. 44, 373–379.

Amit, S.K., Uddin M.M., Rahman R., Islam S.M.R., Khan M.S., 2017. A review on mechanisms and commercial aspects of food preservation and processing. Agriculture & Food Security 6, 1–22.

Benjakul, S., Visessanguan W., Thongkaew C., Tanaka M., 2003a. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. Food Research International 36, 787–795.

Benjakul, S., Visessanguan W., Tueksuban J., 2003b. Changes in physico-chemical properties and gel-forming ability of lizardfish (*Saurida tumbil*) during post-mortem storage in ice. Food Chemistry 80, 535–544.

Briggs, M.A., Bowen K.J., Kris-Etherton P.M., 2017. Omega-3 polyunsaturated fatty acids and health, Food Lipids: Chemistry, Nutrition, and Biotechnology, pp. 603–625.

Cai, L.Y., Leng L.P., Cao A.L., Cheng X.R., Li J.R., 2018. The effect of chitosan-essential oils complex coating on physicochemical, microbiological, and quality change of grass carp (*Ctenopharyhgodon idella*) fillets. Journal of Food Safety 38, e12399.

Chen, L., Alali, W., 2018. Recent discoveries in human serious foodborne pathogenic bacteria: resurgence, pathogenesis, and control strategies. Frontiers in Microbiology 9, 2412.

Cheng, J.H., Sun D.W., Zeng X.A., Liu D., 2015. Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: a review. Critical Reviews in Food Science and Nutrition 55, 1012–1225.

Cz-Ryby, 2019. Rybářské sdružení Ceské republiky, pp. http://www.cz-ryby.cz/produkce-ryb/produkce-a-trh-ryb

D'Alessandro, A., Zolla L., 2013. Meat science: From proteomics to integrated omics towards system biology. Journal of Proteomics 78, 558–577.

da Silva Santos, F.M., da Silva A.I.M., Vieira C.B., de Araújo M.H., da Silva A.L.C., Carneiro-da-Cunha M.d.G., de Souza B.W.S., de Souza Bezerra R., 2017. Use of chitosan coating in increasing the shelf life of liquid smoked nile tilapia (*Oreochromis niloticus*) fillet. Journal of Food Science and Technology 54, 1304–1311.

Dalgaard, P., 2000. Freshness, quality and safety in seafoods. The National Food Centre, Dublin, Ireland, 31 pp.

Donsì, F., Ferrari G., 2016. Essential oil nanoemulsions as antimicrobial agents in food. Journal of Biotechnology 233, 106–120.

Duman, M., Özpolat E., 2015. Effects of water extract of propolis on fresh shibuta (*Barbus grypus*) fillets during chilled storage. Food Chemistry 189, 80–85.

Erikson, U., Misimi E., 2008. Atlantic salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis, and ice storage. Journal of Food Science 73, C50–C59.

FAO, 2011. Global food losses and food waste – Extent causes and prevention, Global food losses and food waste – Extent causes and prevention, Rome, Italy, pp. 37.

FAO, 2020. FAO Yearbook. Fishery and Aquaculture Statistics, FAO Yearbook. Fishery and Aquaculture Statistics. Food and Agriculture Organization, Roma, Italy, pp. 244.

Feng, L.F., Jiang T.J., Wang Y.B., Li J.R., 2012. Effects of tea polyphenol coating combined with ozone water washing on the storage quality of black sea bream (*Sparus macrocephalus*). Food Chemistry 135, 2915–2921.

Fogarty, C., Whyte P., Brunton N., Lyng J., Smyth C., Fagan J., Bolton D., 2019. Spoilage indicator bacteria in farmed Atlantic salmon (*Salmo salar*) stored on ice for 10 days. Food Microbiology 77, 38–42.

Ge, Y.Y., Zhu J.L., Ye X.F., Yang Y., 2017. Spoilage potential characterization of *Shewanella* and *Pseudomonas* isolated from spoiled large yellow croaker (*Pseudosciaena crocea*). Letters in Applied Microbiology 64, 86–93.

Ghaly, A.E., Dave D., Budge S., Brooks M., 2010. Fish spoilage mechanisms and preservation techniques. American Journal of Applied Sciences 7, 859–877.

Gómez-Estaca, J., López-Caballero M.E., Martínez-Bartolomé M.Á., de Lacey A.M.L., Gómez-Guillen M.C., Montero M.P., 2018. The effect of the combined use of high pressure treatment and antimicrobial edible film on the quality of salmon carpaccio. International Journal of Food Microbiology 283, 28–36.

Gupta, R., Raza, N., Bhardwaj, S. K., Vikrant, K., Kim, K. H., & Bhardwaj, N., 2021. Advances in nanomaterial-based electrochemical biosensors for the detection of microbial toxins, pathogenic bacteria in food matrices. J Hazard Mater, 401, 123379.

Hao, R.Y., Liu Y., Sun L.M., Xia L.N., Jia H., Li Q., Pan J.F., 2017. Sodium alginate coating with plant extract affected microbial communities, biogenic amine formation and quality properties of abalone (*Haliotis discus hannai Ino*) during chill storage. LWT – Food Science and Technology 81, 1–9.

Hassoun, A., Çoban Ö.E., 2017. Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. Trends in Food Science & Technology 68, 26–36.

He, Y.y., Huang H., Li L.H., Yang X.Q., 2018. Label-free proteomics of tilapia fillets and their relationship with meat texture during post-mortem storage. Food Analytical Methods 11, 3023–3033.

Hęś, M., 2017. Protein-lipid Interactions in different meat systems in the presence of natural antioxidants – a review. Polish Journal of Food and Nutrition Sciences 67, 5–17.

Hong, H., Regenstein J., Luo Y.K., 2017. The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: A review. Critical Reviews in Food Science and Nutrition 57, 1787–1798.

Huang, Z., Liu X.C., Jia S.L., Zhang L.T., Luo Y.K., 2018. The effect of essential oils on microbial composition and quality of grass carp (*Ctenopharyngodon idellus*) fillets during chilled storage. International Journal of Food Microbiology 266, 52–59.

ICMSF, 1986. Microorganisms in foods. University of Toronto Press Toronto, pp.

Jääskeläinen, E., Jakobsen L.M.A., Hultman J., Eggers N., Bertram H.C., Björkroth J., 2019. Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage. International Journal of Food Microbiology 293, 44–52.

Jia, S.L., Li Y., Zhuang S., Sun X.H., Zhang L.T., Shi J., Hong H., Luo Y.K., 2019. Biochemical changes induced by dominant bacteria in chill-stored silver carp (*Hypophthalmichthys molitrix*) and GC-IMS identification of volatile organic compounds. Food Microbiology 84, 103248.

Jung, S., Ko B.S., Jang H.J., Park H.J., Oh S.W., 2018. Effects of slightly acidic electrolyzed water ice and grapefruit seed extract ice on shelf life of brown sole (*Pleuronectes herzensteini*). Food Science and Biotechnology 27, 261–267.

Kachele, R., Zhang M., Gao Z.X., Adhikari B., 2017. Effect of vacuum packaging on the shelf-life of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4 °C. LWT – Food Science and Technology 80, 163–168.

Kadariya, J., Smith, T.C. D, 2014. Thapaliya, *Staphylococcus aureus* and *staphylococcal* foodborne disease: an ongoing challenge in public health. BioMed Research International.

Kamkar, A., Jebelli Javan A., Nemati G., Falahpour F., Partovi R., 2014. Effects of mentha pulegium water extract dipping on quality and shelf life of silver carp (*Hypophthalmichthys molitrix*) during superchilled storage. Iranian Journal of Fisheries Sciences 13, 341–353.

Kenar, M., Özogul F., Kuley E., 2010. Effects of rosemary and sage tea extracts on the sensory, chemical and microbiological changes of vacuum-packed and refrigerated sardine (*Sardina pilchardus*) fillets. International Journal of Food Science & Technology 45, 2366–2372.

Křížek, M., Dadáková E., Vácha F., Pelikánová T., Matějková K., 2018. The effects of two essential oil and UV-light irradiation treatments on the formation of biogenic amines in vacuum packed fillets of carp (*Cyprinus carpio*). LWT – Food Science and Technology 95, 268–273.

Kuley, E., Durmus M., Ucar Y., Kosker A.R., Aksun Tumerkan E.T., Regenstein J., Ozogul F., 2018. Combined effects of plant and cell-free extracts of lactic acid bacteria on biogenic amines and bacterial load of fermented sardine stored at 3 ± 1 °C. Food Bioscience 24, 127–136.

Lan, W.Q., Che X., Xu Q.L., Wang T., Du R.Y., Xie J., Hou M., Lei H., 2018. Sensory and chemical assessment of silver pomfret (*Pampus argenteus*) treated with *Ginkgo biloba* leaf extract treatment during storage in ice. Aquaculture and Fisheries 3, 30–37.

Li, C., Xiong Y.L., Chen J.J.J.o.A., Chemistry F., 2012. Oxidation-induced unfolding facilitates Myosin cross-linking in myofibrillar protein by microbial transglutaminase. 60, 8020.

Li, D.P., Zhang J.B., Song S.J., Feng L.G., Luo Y.K., 2018a. Influence of heat processing on the volatile organic compounds and microbial diversity of salted and vacuum-packaged silver carp (*Hypophthalmichthys molitrix*) fillets during storage. Food Microbiology 72, 73–81.

Li, N., Shen Y., Liu W.r., Mei J., Xie J., 2018b. Low-field NMR and MRI to analyze the effect of edible coating incorporated with MAP on qualities of half-smooth tongue sole (*Cynoglossus semilaevis günther*) fillets during refrigerated storage. Applied Sciences 8, 1391

Li, N., Shen Y., Liu W.r., Mei J., Xie J., 2018c. Low-field NMR and MRI to analyze the effect of edible coating incorporated with MAP on qualities of half-smooth tongue sole (*Cynoglossus semilaevis günther*) fillets during refrigerated storage. Applied Sciences 8, 1391.

Li, D.P., Qin N., Zhang L.T., Li Q., Prinyawiwatkul W., Luo Y.K., 2019. Degradation of adenosine triphosphate, water loss and textural changes in frozen common carp (*Cyprinus carpio*) fillets during storage at different temperatures. International Journal of Refrigeration 98, 294–301.

Liu, X.C., Zhang Y.M., Li D.P., Luo Y.K., 2017. Characterization of the microbiota in lightly salted bighead carp (*Aristichthys nobilis*) fillets stored at 4 °C. Food Microbiology 62, 106–111.

Liu, X.C., Huang Z., Jia S.L., Zhang J.B., Li K.F., Luo Y.K., 2018. The roles of bacteria in the biochemical changes of chill-stored bighead carp (*Aristichthys nobilis*): Proteins degradation, biogenic amines accumulation, volatiles production, and nucleotides catabolism. Food Chemistry 255, 174–181.

Lu, H., Wang H., Luo Y.K., 2017. Changes in protein oxidation, water-holding capacity, and texture of bighead carp (*Aristichthys nobilis*) fillets under chilled and partial frozen storage. Journal of Aquatic Food Product Technology 26, 566–577.

Lund, M.N., Heinonen M., Baron C.P., Estévez M., 2011. Protein oxidation in muscle foods: A review. Molecular Nutrition & Food Research 55, 83–95.

Maqsood, S., Benjakul S., 2010. Synergistic effect of tannic acid and modified atmospheric packaging on the prevention of lipid oxidation and quality losses of refrigerated striped catfish slices. Food Chemistry 121, 29–38.

Maqsood, S., Benjakul S., Balange A.K., 2012a. Effect of tannic acid and kiam wood extract on lipid oxidation and textural properties of fish emulsion sausages during refrigerated storage. Food Chemistry 130, 408–416.

Maqsood, S., Benjakul S., Kamal-Eldin A., 2012b. Haemoglobin-mediated lipid oxidation in the fish muscle: A review. Trends in Food Science & Technology 28, 33–43.

Maqsood, S., Benjakul S., Abushelaibi A., Alam A., 2014. Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: a detailed review. Comprehensive Reviews in Food Science and Food Safety 13, 1125–1140.

Medina, I., González M.J., Iglesias J., Hedges N.D., 2009. Effect of hydroxycinnamic acids on lipid oxidation and protein changes as well as water holding capacity in frozen minced horse mackerel white muscle. Food Chemistry 114, 881–888.

Mikš-Krajnik, M., Yoon Y.-J., Ukuku D.O., Yuk H.-G., 2016. Volatile chemical spoilage indexes of raw Atlantic salmon (*Salmo salar*) stored under aerobic condition in relation to microbiological and sensory shelf lives. Food Microbiology 53, 182–191.

Moosavi-Nasab, M., Mirzapour-Kouhdasht A., Oliyaei N., 2019. Application of essential oils for shelf-life extension of seafood products. in: El-Shemy H. A. (Ed), Essential Oils-Oils of Nature. IntechOpen, London, UK.

Morsy, M.K., Zór K., Kostesha N., Alstrøm T.S., Heiskanen A., El-Tanahi H., Sharoba A., Papkovsky D., Larsen J., Khalaf H., Jakobsen M.H., Emnéus J., 2016. Development and validation of a colorimetric sensor array for fish spoilage monitoring. Food Control 60, 346–352.

Mráz, J., Pickova J., 2011. Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. Neuro endocrinology letters 32 Suppl 2, 3–8.

Mraz, J., Pickova J., Kozak P., 2011. Feed for common carp and culture of common carp with increased content of omega 3 fatty acids, Krmivo pro kapra obecného a způsob chovu kapra obecného se zvýšeným obsahem omega.

Mráz, J., Máchová J., Kozák P., Pickova J., 2012a. Lipid content and composition in common carp – optimization of n-3 fatty acids in different pond production systems. Journal of Applied Ichthyology 28, 238–244.

Mráz, J., Zajic T., Pickova J., 2012b. Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy. Neuro endocrinology letters 33 Suppl 2, 60–67.

Mráz, J., Zajic T., Kozák P., Pickova J., Kacer P., Adámek V., Lesna I.K., Lanska V., Adámková V., 2017. Intake of carp meat from two aquaculture production systems aimed at secondary prevention of ischemic heart disease-a follow-up study. Physiological Research 66, S129–S137.

Navarro-Segura, L., Ros-Chumillas M., Martínez-Hernández G.B., López-Gómez A., 2020. A new advanced packaging system for extending the shelf life of refrigerated farmed fish fillets. Journal of the Science of Food and Agriculture 100, 4601–4611.

Nawaz, T., Fatima M., Shah S.Z.H., Afzal M., 2020. Coating effect of rosemary extract combined with chitosan on storage quality of mori (*Cirrhinus mrigala*). Journal of Food Processing and Preservation 44, e14833.

Ojagh, S.M., Rezaei M., Razavi S.H., Hosseini S.M.H., 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry 120, 193–198.

Olafsdottir, G., Nesvadba P., Di Natale C., Careche M., Oehlenschläger J., Tryggvadóttir S.a.V., Schubring R., Kroeger M., Heia K., Esaiassen M., Macagnano A., Jørgensen B.M., 2004. Multisensor for fish quality determination. Trends in Food Science & Technology 15, 86–93.

Olatunde, O.O., Benjakul S., 2018. Natural preservatives for extending the shelf-life of seafood: A revisit. Comprehensive Reviews in Food Science and Food Safety 17, 1595–1612.

Oner, B., Meral R., Ceylan Z., 2021. Determination of some quality indices of rainbow trout fillets treated with nisin-loaded polyvinylalcohol-based nanofiber and packed with polyethylene package. LWT 149, 111854.

Osimani, A., Ferrocino I., Agnolucci M., Cocolin L., Giovannetti M., Cristani C., Palla M., Milanović V., Roncolini A., Sabbatini R., Garofalo C., Clementi F., Cardinali F., Petruzzelli A., Gabucci C., Tonucci F., Aquilanti L., 2019. Unveiling hákarl: A study of the microbiota of the traditional Icelandic fermented fish. Food Microbiology 82, 560–572.

Öztürk Kerimoğlu, B., KavuŞAn H.S., SerdaroĞLu M., 2020. The impacts of laurel (*Laurus nobilis*) and basil (*Ocimum basilicum*) essential oils on oxidative stability and freshness of sous-vide sea bass fillets. Turkish Journal of Veterinary & Animal Sciences, 101–109.

Park, press J.J.c., 2013. Surimi and Surimi Seafood, Third Edition.

Prabhakar, P.K., Vatsa S., Srivastav P.P., Pathak S.S., 2020. A comprehensive review on freshness of fish and assessment: Analytical methods and recent innovations. Food Research International 133, 109157.

Qin, N., Zhang L.T., Zhang J.B., Song S.J., Wang Z.Y., Regenstein J., Luo Y.K., 2017. Influence of lightly salting and sugaring on the quality and water distribution of grass carp (*Ctenopharyngodon idellus*) during super-chilled storage. Journal of Food Engineering 215, 104–112.

Qiu, X.J., Chen S.J., Dong S.Y., 2014. Effects of silver carp antioxidant peptide on the lipid oxidation of sierra fish fillets (*Scomberomorus niphonius*) during frozen storage. Journal of Food Biochemistry 38, 167–174.

Rezaeifar, M., Mehdizadeh T., Mojaddar Langroodi A., Rezaei F., 2020. Effect of chitosan edible coating enriched with lemon verbena extract and essential oil on the shelf life of vacuum rainbow trout (*Oncorhynchus mykiss*). Journal of Food Safety 40, e12781.

Roco, T., Torres M.J., Briones-Labarca V., Reyes J.E., Tabilo-Munizaga G., Stucken K., Lemus-Mondaca R., Pérez-Won M., 2018. Effect of high hydrostatic pressure treatment on physical parameters, ultrastructure and shelf life of pre- and post-rigor mortis palm ruff (*Seriolella violacea*) under chilled storage. Food Research International 108, 192–202.

Saito, T., Arai K.-i., Matsuyoshi M., 1959. A New method for estimating the freshness of fish. Nippon Suisan Gakkaishi 24, 749–750.

Sampels, S., 2015. The effects of storage and preservation technologies on the quality of fish products: a review. Journal of Food Processing and Preservation 39, 1206–1215.

Shen, S., Jiang Y., Liu X.C., Luo Y.K., Gao L., 2015. Quality assessment of rainbow trout (*Oncorhynchus mykiss*) fillets during super chilling and chilled storage. Journal of Food Science and Technology 52, 5204–5211.

Shi, C., Cui J.Y., Luo Y.K., Zhou Z.Y., 2014. Effect of lightly salt and sucrose on rigor mortis changes in silver carp (*Hypophthalmichthys molitrix*) stored at 4 °C. International Journal of Food Science & Technology 49, 160–167.

Shi, C., Cui J.Y., Qin N., Luo Y.K., Lu H., Wang H., 2017. Effect of ginger extract and vinegar on ATP metabolites, IMP-related enzyme activity, reducing sugars and phosphorylated sugars in silver carp during postslaughter storage. International Journal of Food Science & Technology 52, 413–423.

Shojaee-Aliabad, S., Hosseini S.M., Mirmoghtadaie L., 2018. Antimicrobial activity of essential oil, Essential Oils in Food Processing: Chemistry, Safety and Applications. John Wiley & Sons Ltd, USA, pp. 191–216.

Sterniša, M., Mraz J., Smole Možina S., 2017. Common carp-still unused potential. Meso: prvi hrvatski časopis o mesu 19, 434–438.

Wang, H., Wang H.Y., Li D.P., Luo Y.K., 2018. Effect of chitosan and garlic essential oil on microbiological and biochemical changes that affect quality in grass carp (*Ctenopharyngodon idellus*) fillets during storage at 4 °C. Journal of Aquatic Food Product Technology 27, 80–90.

W.H. Organization, Foodborne disease outbreaks: guidelines for investigation and control, World Health Organization, 2008

Wu, D., Sun D.W., He Y., 2012. Application of long-wave near infrared hyperspectral imaging for measurement of color distribution in salmon fillet. Innovative Food Science & Emerging Technologies 16, 361–372.

Wu, J.L., Ge S.Y., Liu H., Wang S., Chen S.F., Wang J.H., Li J.H., Zhang Q.Q., 2014. Properties and antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. Food Packaging and Shelf Life 2, 7–16.

Xu, J.L., Riccioli C., Sun D.W., 2015. An overview on nondestructive spectroscopic techniques for lipid and lipid oxidation analysis in fish and fish products. Comprehensive Reviews in Food Science and Food Safety 14, 466–477.

Yu, D.W., Jiang Q.X., Xu Y.S., Xia W.S., 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.

Yu, D.W., Regenstein J., Zang J.H., Jiang Q.X., Xia W.S., Xu Y.S., 2018. Inhibition of microbial spoilage of grass carp (*Ctenopharyngodon idellus*) fillets with a chitosan-based coating during refrigerated storage. International Journal of Food Microbiology 285, 61–68.

Yu, D.W., Wu L.Y., Regenstein J., Jiang Q.X., Yang F., Xu Y.S., Xia W.S., 2020. Recent advances in quality retention of non-frozen fish and fishery products: A review. Critical Reviews in Food Science and Nutrition 60, 1747–1759.

Zhang, J., Li Y., Yang X., Liu X., Hong H., Luo Y., 2021. Effects of oregano essential oil and nisin on the shelf life of modified atmosphere packed grass carp (*Ctenopharyngodon idellus*). LWT 147, 111609.

Zhang, L., Shen H., Luo Y.J.E.F.R., Technology, 2011a. A nondestructive method for estimating freshness of freshwater fish. 232, 979–984.

Zhang, L.N., Luo Y.K., Xue L.I., Shen H.X.J.J.o.C.A.U., 2011b. Research on relationship between electric conductivity and freshness indicators of Grass carp.

Zhang, Y.M., Li Q., Li D.P., Liu X.C., Luo Y.K., 2015a. Changes in the microbial communities of air-packaged and vacuum-packaged common carp (*Cyprinus carpio*) stored at 4 °C Food Microbiology 52, 197–204.

Zhang, Y.M., Qin N., Luo Y.K., Shen H.X., 2015b. Changes in biogenic amines and ATP-related compounds and their relation to other quality changes in common carp (*Cyprinus carpio var. Jian*) stored at 20 and 0 °C. Journal of Food Protection 78, 1699–1707.

Zhu, S.C., Zhou Z.Y., Feng L.G., Luo Y.K., 2015. Postmortem changes in physicochemical properties of songpu mirror carp (*Cyprinus carpio*) during iced storage. Food Bioscience 9, 75–79.

CHAPTER 2

POST-MORTEM QUALITY CHANGES OF COMMON CARP (*CYPRINUS CARPIO*) DURING CHILLED STORAGE FROM TWO CULTURE SYSTEMS

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Post-mortem quality changes of common carp (*Cyprinus carpio*) during chilled storage from two culture systems

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Abstract

BACKGROUND: Omega-3 common carp (OCC) raised by patented culture systems have higher level of n-3 fatty acids and n-3/n-6 ratio than normal common carps (NCCs) from traditional culture system. Whether the patented farming system and modified fatty acid profile will influence OCC storage stability is unclear. This study aimed to expose the differences of post-mortem quality changes between NCC and OCC.

RESULTS: NCC and OCC have similar rigor mortis patterns, only a higher level of lactic acid was observed in NCC after 96 h. Adenosine triphosphate (ATP) related compounds had no major differences, but slightly higher inosine monophosphate in OCC was found at 36 h. The *K*-value, Ki-value and Hx-index demonstrated high cohesiveness (Pearsons two-tailed, $r = 0.968$–0.984, $P < 0.05$) during storage, with statistically comparable ($P > 0.05$) temporal progress of change in NCC and OCC. The indices were lower in OCC than in NCC. Attenuation of myosin heavy chain in OCC was not as distinct as in NCC, coincided with its higher salt-soluble protein level at 144 h. Before 96 h, thiobarbituric acid value (TBA), total viable count (TVC), cooking loss (CL), drip loss (DL), and hardness in NCC and OCC were similar. However, at 144 h, higher TBA, TVC, CL and DL while lower hardness in NCC than in OCC were observed. Principle component analysis showed good separation of NCC and OCC in biplot at 0 and 144 h.

CONCLUSION: Patented culture system has a slightly positive influence on post-mortem quality of common carp. It can be used for producing OCC without compromising storage stability.

Supporting information may be found in the online version of this article.

Keywords: common carp; rigor mortis; ATP related compounds; lipid oxidation; protein degradation

INTRODUCTION

Fish is considered healthy food, because it provides a high content of proteins with good amino acid composition, lipids with high proportion of n-3 polyunsaturated fatty acids (n-3 PUFA) and a certain amount of minerals and vitamins. Among the nutrients, n-3 PUFA, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is often considered the most featured nutrition factor, showing many benefits on human health, such as preventing cardiovascular disease and inhibiting cancers and inflammatory diseases.[1] The n-3 PUFA content of fish depends on various factors, for example fish species, age, size and diet which is a crucial one.[2,3] Generally, marine fish are enriched with n-3 PUFA but freshwater fish are not.

Common carp (*Cyprinus carpio*) is a freshwater fish species that is farmed worldwide owing to its easy cultivation, fast growth rate and high feed efficiency ratio.[4] In 2017, the global production of common carp was approximately 4.13 million tonnes.[5] Common carps farmed in traditional semi-intensive culture system are fed with a diet containing high level of cereals, which accumulates high level of oleic acid but low level of n-3 PUFA in muscle.[6] Recently, a patented system by Mraz *et al.*[7] for farming 'omega-3 common carp' (OCC) was established. Common carps produced by this system showed higher level of n-3 fatty acids and n-3/n-6 ratio than normal common carps (NCCs) from traditional semi-intensive culture system. Studies showed that these OCC

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had positive effects on alleviating ischemic heart disease symptoms.[8,9] The high healthy and economic value are driving more aquaculture companies to adopt this system for producing OCC.

As perishable food, fish experiences protein denaturation or degradation, lipid oxidation or hydrolysis and microorganism proliferation by the actions of endogenous enzymes and microorganism during storage, suffering texture deterioration, off-odour and shelf-life reduction.[10] The fish farming environment, diet and physiological status prior to slaughter closely relate with the earlier-mentioned post-mortem changes, affecting final fish quality. Since OCC are farmed in a system different from the traditional one, their storage stability might differ from NCC. Freshness is one of the most important attributes of fish quality. And is considered as the degree of various physical, chemical, biochemical and microbiological changes occurring post-mortem in fish.[11] The metabolic rate of nucleic acid is a good proxy for checking the progress of biochemical changes in fish. Adenosine triphosphate (ATP) related compounds are studied to expose the quality of fish and *K*-value is commonly applied. However, limitations exist surrounding the sole use of *K*-value in rendering a freshness verdict of aquatic products. The reliability of *K*-value as a freshness index is dependent on species and season, and it cannot represent freshness well after significant microbial spoilage as reviewed by Cheng *et al.*[12] Moreover, the decomposing rate of ATP could be very fast after slaughtering, which could lead to a high *K*-value in the fish received special processing but maintaining high freshness. It is reported that *K*-value can be above 20% even for newly processed cold-smoked salmon of high sensory quality.[13] The concentrations of ATP and the products obtained from its breakdown such as adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), hypoxanthine riboside (HxR) and hypoxanthine (Hx) are often calculated as a Hx-index, *K*-value or Ki-value for indicating fish freshness.[12,14] It is reasonable to doubt whether OCC has a different metabolic rate of ATP related compounds from NCC.

However, n-3 PUFAs are susceptible to oxidation. Their secondary oxidation products, for example malondialdehyde, might also promote protein oxidation, resulting in quality deterioration.[15] Therefore, it is necessary to investigate the patented farming system and n-3 PUFAs roles on OCC storage stability.

Until now, whether and how the patented farming system and modified fatty acid profile will influence OCC perseverative stability have not been investigated. This study was aimed to highlight the differences of post-mortem quality changes between NCC and OCC to elucidate the influences of patented farming system on carp storage stability (briefly showed as Fig. S2) . Rigor mortis, *K*-value compounds, protein degradation, lipid oxidation and other quality indices of NCC and OCC during chilled storage were investigated. With scientific rationales, results of the current study will provide a platform to encourage application of the patented carp farming system.

MATERIALS AND METHODS

Fish information

Four-year-old NCC and OCC of marketable size (2929 ± 515 g and 3211 ± 504 g) raised by traditional culture system and patented culture system were obtained from ponds in Vodňany, Czech Republic, during October 2018. Fish were transported to the laboratory at the Institute of Aquaculture and Protection of Waters in České Budějovice. Twenty-two carps from each group were killed by blow on head. Five whole fish were packed individually in plastic bags and used for rigor mortis analysis. Seventeen fish were descaled and filleted into parts as the scheme shows in The Supporting Information (Fig. S1). All the small pieces were kept in plastic bags separately and stored in refrigerator at 4.0 ± 0.5 °C. One small piece of sample was taken out on each sampling (time) point for analysis. To analyse ATP, glycogen and lactic acid at 11 time points, part D in Fig. S1 was divided into two parts, thus there was enough sample to be used for performing all the analyses, as each of the pieces was designated for the analysis of specific parameters. Drip loss was measured continuously by using the same piece of fillet.

Lipid content and fatty acid profile analysis

Lipid content and fatty acid profile of fresh carp muscle were extracted and analysed as by Hematyar *et al.*[16] Fatty acids were methylated with boron trifluoride and analysed using a Trace Ultra FID GC (Thermo Scientific, Milan, Italy) equipped with flame ionization detector and PVT injector. BPX 70 column (SGE Inc., Austin, Texas) with length, 50 m; i.d., 0.22 mm; film thickness, 0.25 μm was used.

Rigor mortis analysis

Rigor mortis of whole fish stored in a straight horizontal position at 4.0 ± 0.5 °C was studied over 180 h. Rigor state was evaluated according to the rigor rating system by Erikson *et al.*[17] The pH of whole fish and fish fillet were measured using a 206 digital pH meter (Testo AG, Germany) by inserting the probe into muscle.

Glycogen and lactic acid analysis

Muscle was homogenized with water (*m/v*, 1:10) and incubated at 60 °C for 30 min to extract glycogen. Glycogen were measured using Amplex® Red Glucose/Glucose Oxidase Assay Kit A22189 (Invitrogen, Carlsbad, CA, USA). Result was expressed as g kg^{-1} muscle. Lactic acid was extracted with acetonitrile (*m/v*, 1:10), dissolved in 0.03 mol L^{-1} phosphoric acid (H_3PO_4) and determined using an UltiMate3000 HPLC (Thermo Scientific, Waltham, MA, USA) equipped with RS diode array detector and LiChroCART column (4.6 mm × 250 mm, 5 μm) (Merck, Darmstadt, Germany). Detection wavelength was 210 nm; injection volume was 20 μL. Separation was achieved using mobile phase composed of 80% 0.03 mol L^{-1} H_3PO_4–8% acetonitrile–12% water at 0.5 mL min^{-1}. Result was expressed as μmol lactic acid g^{-1} muscle.

ATP-related compounds analysis and related indices

ATP-related compounds were extracted as reported by Li *et al.*[18] and analysed using an UltiMate 3000HPLC equipped with a RS diode array detector and a Luna C18 column (5 μm, 100 Å, 4.6 mm × 250 mm) (Phenomenex, Torrance, CA, USA). A gradient elution with 0.8 mL min^{-1} flow rate was applied: 0 min, 100% 0.05 mol L^{-1} phosphate-buffered saline (PBS, pH 6.8); 5 min, 92% PBS + 8% acetonitrile; 18 min, 92% PBS + 8% acetonitrile; 20 min, 100% PBS. Column temperature was 35 °C; injection volume was 20 μL. Samples were detected at 254 nm. Standards (Sigma-Aldrich, St Louis, MO, USA) were used for identifying and calculating the content of targeted compounds. K-value, Ki-value, Hx-index[13] were calculated as:

$K\text{-value}\ (\%) = (HxR + Hx) \times 100/(ATP + ADP + AMP + IMP + HxR + Hx)$.

$Ki\text{-value}\ (\%) = (HxR + Hx) \times 100/(IMP + HxR + Hx)$.

$Hx\text{-index} = \log_{10}(Hx + 5)$.

Muscle protein analysis

Protein solubility analysis

Water-soluble protein (WSP) and salt-soluble protein (SSP) were extracted as described by Pan *et al.*[19] Briefly, muscle was homogenized with 0.1 mol L^{-1} sodium chloride (NaCl, *m/v*, 1:10) and centrifuged (10 000 × *g*, 10 min, 4 °C). Supernatant was collected as WSP. Sediment was homogenized with 10 mL 0.6 mol L^{-1} NaCl and centrifuged again. The obtained supernatant was collected as SSP. Protein content was measured using a protein quantification Rapid-Kit (Sigma-Aldrich). SSP content was expressed as g kg^{-1} muscle.

Protein pattern analysis

Protein patterns of WSP and SSP were evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were incubated with loading buffer (0.02 mol L^{-1} dithiothreitol, *v/v*, 1:1) at 90 °C for 2 min. About 30 μg of proteins were loaded onto 12% Criterion Precast Protein Gel (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis was performed using Criterion™ vertical electrophoresis cell (Bio-Rad Laboratories) at 200 V, 45 min. Gel was stained with Coomassie brilliant blue and destained with methanol.

Lipid oxidation analysis

Lipid oxidation was analysed as thiobarbituric acid (TBA) value according to Sampels *et al.*[20] Briefly, fish muscle (1 g) was homogenized with 0.6 mol L^{-1} (9.1 mL) trichloroacetic acid and filtered. The filtrate was incubated with equal volume of 0.02 mol L^{-1} TBA at 80 °C for 30 min in darkness. Absorbance at 530 nm was measured using an AF2200 PlateReader (Eppendorf AG, Hamburg, Germany). TBA value was expressed as g kg^{-1} muscle.

Total viable count (TVC) analysis

Total viable count (TVC) was analysed as conveyed by Hao *et al.*[21] with modifications. Briefly, 10 g of muscle was homogenized with 90 mL 0.85% sterile physiological saline in Stomacher Classic Panoramic IU500 (IUL Instruments, Spain) for 90 s. Obtained solution was used for TVC analysis. TVC was determined by plate count agar (Sigma-Aldrich) after aerobic incubation at 30 °C for 72 h. Enumeration of microbial communities was recorded as logCFU g^{-1} muscle.

Fish quality analysis

Colour properties

Colour properties of fish fillet were evaluated using a CM-600d Colorimeter (Konica Minolta, Tokyo, Japan). The L^*, a^* and b^* values of fillet surface were recorded. Each fillet was analysed at six points.

Texture

Texture profile analysis was applied for textural properties investigation by using a texture analyser (TA-XT. Plus, UK) equipped with a P/50 probe by following specifications: pressed depth 50%, test speed 1 mm s^{-1}, two 5 mm consecutive cycles with 5 s holding time in between. Hardness and springiness were calculated.

Drip loss (DL) and cooking loss (CL)

For drip loss (DL), initial weight of fillet was recorded as W_0. Each 24 h, weight of the fillet was recorded as W_i. DL was calculated as: $DL = [(W_0 - W_i)/W_0] \times 100\%$. Cooking loss (CL) was determined according to Hong *et al.*[22] A fish muscle cube of 1.5 × 1.5 × 1 cm^3 was prepared and scaled as W_b. The sample was steamed with boiling water on a rack for 3 min and cooled at 22 °C for 10 min. The sample was wiped with paper to remove water and scaled as W_c. CL was calculated as: $CL = 100\% \times (W_b - W_c)/W_b$.

Statistical analysis

Data were expressed as mean ± standard deviation. A *t*-test was used for comparison of means between NCC and OCC. A comparison of means at different times was performed by Duncan's multiple range test using Statistic Package for Social Science 16.0 (SPSS Inc, Chicago, IL, USA). If $P < 0.05$, difference was significant. Data of NCC and OCC were subjected to principle component analysis (PCA) using SIMCA13.0 (Umetrics, Umeå, Sweden) to obtain a comprehensive understanding of the differences.

RESULTS AND DISCUSSION

Lipid content and fatty acid profile of fish

Lipid content of OCC was lower than that of NCC (4.2% *versus* 10.8%) (Table 1). Saturated fatty acids, characterized by high proportion of C16:0, accounted for 26% of total fatty acid in the two groups, close to that reported by Lu *et al.*[23] But its absolute amount was higher in NCC than in OCC due to the higher lipid content in the former. C18:1n-9 was primary fatty acid in common carp[16] and it showed a higher percentage in NCC (50.8%) than in OCC (32.6%). NCC had both higher proportion and absolute amount of monounsaturated fatty acids (64.8% and 699.8 mg kg^{-1} muscle) than OCC (47% and 197.4 mg kg^{-1} muscle). C18:2n-6, C20:4n-6 and n-6 PUFA exhibited a higher percentage in OCC than in NCC. However, their absolute amounts were opposite due to the lower lipid content in OCC. The proportions of C18:3n-3, DHA and EPA in OCC were higher than those in NCC. Though lipid content in OCC was lower, the absolute contents of n-3 PUFA and EPA + DHA were higher in OCC (65.6 and 22.6 mg kg^{-1} muscle) than in NCC (18.9 and 9.8 mg kg^{-1} muscle). As a result, n-3/n-6 ratio of OCC was 5.6 times higher than that of NCC. The information of fatty acids and lipid content of OCC was consistent with that reported by Mraz *et al.*,[8] indicating that OCC had lower lipid content along with an improved fatty acid profile featured with higher content of n-3 PUFA, EPA + DHA, n-3/n-6 ratio compared with NCC. The beneficial effects of n-3 PUFAs on human mental and bone health, as well as in the control and treatment of different diseases such as heart disease, diabetes, arthritis, cancer and obesity have been extensively reported.[1,24] Therefore, OCC could be a potential for changing the negative Western diet that is deficient in n-3 PUFA with low n-3/n-6 and improved human health.

Rigor mortis and pH

In Fig. 1(A), rigor score started to increase at 24 h in NCC and OCC, indicating rigor onset, and it reached the maximum at 96 h, denoting full rigor. After that, it gradually returned to the lowest until 180 h. Glycogen experiences anaerobic glycolysis after slaughtering together with phosphor creatine, forming ATP and lactic acid or H_3PO_4. The declined pH value could destroy sarcoplasmic reticulum by releasing endogenous enzymes to hydrolyse myofibril structure, resulting in soft texture.[25] The above processes closely relate with textural properties of muscle. Full rigor in silver carp and grass carp stored at 4 °C was found to occur at 48 h and 24 h, respectively.[26,27] Postponed rigor mortis could extend the shelf life of fish. Results indicate that NCC and OCC might have higher stability than other carp species. The rigor

Table 1 Fatty acid profile as absolute amount (mg kg^{-1} muscle) and percentage (% of total fatty acid) of normal common carp (NCC) from the traditional farming system group, and omega-3 common carp (OCC) from the patterned farming system ($n = 4$)

	Absolute amount (mg kg^{-1} muscle)		Percentage (% of total fatty acid)	
Fatty acids	NCC	OCC	NCC	OCC
C14:0	11.1 ± 1.9^{a}	6.3 ± 0.5	1.0 ± 0.0	1.11 ± 0.10
C16:0	211.8 ± 39.0^{a}	83.3 ± 1.9	19.5 ± 1.1	19.8 ± 1.7^{a}
C18:0	58.5 ± 5.6^{a}	19.6 ± 2.9	5.4 ± 0.2^{a}	4.7 ± 0.1
SFA	285.0 ± 46.4^{a}	109.5 ± 31.1	26.3 ± 0.9	26.1 ± 1.6
C16:1	96.5 ± 18.7^{a}	36.0 ± 8.8	8.9 ± 0.6^{a}	8.6 ± 0.4
C18:1n-7	34.6 ± 5.0^{a}	17.5 ± 7.5	3.2 ± 0.1	4.2 ± 0.4^{a}
C18:1n-9	548.4 ± 66.1^{a}	136.6 ± 1.5	50.8 ± 0.8^{a}	32.6 ± 1.1
C20:1n-9	18.4 ± 3.1^{a}	6.8 ± 0.2	1.70 ± 0.1	1.6 ± 0.2
MUFA	699.8 ± 92.6^{a}	197.4 ± 9.9	64.8 ± 0.0^{a}	47.0 ± 0.5
C18:2n-6	66.9 ± 5.4^{a}	39.6 ± 3.0	6.2 ± 0.5	9.4 ± 0.9^{a}
C20:4n-6	9.4 ± 0.7	7.7 ± 0.2	0.9 ± 0.1	1.8 ± 0.4^{a}
n-6 PUFA	76.3 ± 5.8^{a}	47.3 ± 4.9	7.1 ± 0.6	11.2 ± 1.0^{a}
C18:3n-3	7.3 ± 0.6	37.1 ± 6.3^{a}	0.7 ± 0.1	8.8 ± 0.2^{a}
C20:5n-3(EPA)	3.7 ± 0.6	12.3 ± 0.3^{a}	0.4 ± 0.1	2.9 ± 0.3^{a}
C22:6n-3 (DHA)	5.2 ± 0.4	10.4 ± 0.0^{a}	0.5 ± 0.1	2.5 ± 0.5^{a}
EPA + DHA	8.9 ± 1.0	22.6 ± 3.5^{a}	0.8 ± 0.2	5.4 ± 0.7^{a}
n-3 PUFA	18.9 ± 1.6	65.6 ± 12.0^{a}	1.8 ± 0.3	15.6 ± 0.6^{a}
PUFA	95.2 ± 6.1	113.1 ± 13.4	8.9 ± 0.9	26.9 ± 1.4^{a}
Total lipid	1080.0 ± 142.9^{a}	420.0 ± 31.1	—	—
n-3/n-6 ratio	0.3 ± 0.0	1.4 ± 0.1^{a}	—	—

Note: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; —, no detection.

[a] Significant higher level than the other group.

mortis patterns of NCC and OCC were similar, implying the patented culture system has no distinct influence on this process.

Initial pH of OCC and NCC in whole fish was 6.88 and 6.85, respectively (Fig. 1(B)). They decreased to 6.55 and 6.50 after 36 h and then remained stable, but fish fillets took a longer time (48 h) to decline to a stable value (Fig. 1(C)). Slow pH drop is an indicator of delayed rigor onset, which allows high muscle stability.[28] The lagged pH decline in fillet suggests that fish fillet might be more stable than whole fish, which could be due to less intensive biochemical reactions. Endogenous proteolysis enzymes can degrade protein into amino acids to expose NH_2, leading to pH increase. However, pH of our carps fluctuated between 6.44 and 6.61 with large variations until the end, differed from pH of chilled-stored blunt-snout bream and silver carp which first showed a sharp decrease and then increased slowly.[26,29] The pH value of whole fish showed no difference between NCC and OCC, but slightly lower pH in NCC was observed in fillet at 120 h.

Glycogen and lactic acid

Initial glycogen content in NCC and OCC was 1.44 and 1.18 mg g^{-1} muscle, respectively. Both declined to < 0.5 mg g^{-1} muscle in 84 h (Fig. 2(A)), indicating similar metabolic rate, and then glycogen level stabilized. Lactic acid in NCC and OCC quickly climbed to 52.2 and 44.9 μm g^{-1} muscle after 84 h and remained until 120 h (Fig. 2(B)). The decrease of glycogen showed close correlation with the increase of lactic acid. This pattern for changes of them was also reported in silver carp[26] and grass carp.[27] It is noticed that lactic acid content in NCC was higher than it is in OCC after 96 h, which coincided with the lower pH in NCC than in OCC at 120 h (Fig. 1(C)). It could be owing to the slightly higher initial glycogen content in NCC (not significant) that formed more lactic acid via glycolysis, leading to lower pH. Results indicate that OCC and NCC might have slightly different initial glycogen content, but they could have a similar metabolic rate to form lactic acid.

ATP-related compounds

Initial ATP content were 2.40 and 4.01 μmol g^{-1} in NCC and OCC, respectively. They declined to < 0.1 μmol g^{-1} after 36 h and remained stable (Fig. 3(A)). ADP and AMP content in the two groups showed similar trends (Fig. 3(B, C)). IMP content of OCC and NCC increased to a maximum (5.07 and 4.26 μmol g^{-1}) at 36 and 48 h, and gradually decreased till the end (Fig. 3(D)). Temporary accumulation of IMP indicates rapid degradation of ATP, ADP and AMP to IMP during the first 36 h or 48 h, confirming the conclusion by Alasalvar *et al.*[30] that the degradation of ATP to IMP in fish muscle usually occurs in 1 or 2 days. IMP is an umami taste compound. Its accumulation can improve fish flavour. Initial IMP content in NCC and OCC were similar. However, slightly higher IMP content in OCC was observed at 36 h, but no difference was observed after that. This suggests that OCC might have a slightly higher IMP level than NCC, which is favourable for OCC quality. IMP can be further converted to HxR and Hx by autolytic and bacterial enzymes.[31] Their accumulation is considered as progressive loss of good flavour and freshness.[32] HxR increased constantly and peaked at 120 h in the two groups, showing a slight decrease afterwards (Fig. 3(E)). Hx content kept increasing throughout storage and achieved 1.71 and 2.03 μmol g^{-1} at 144 h (Fig. 3(F)). There was no significant difference of HxR and Hx between NCC and OCC.

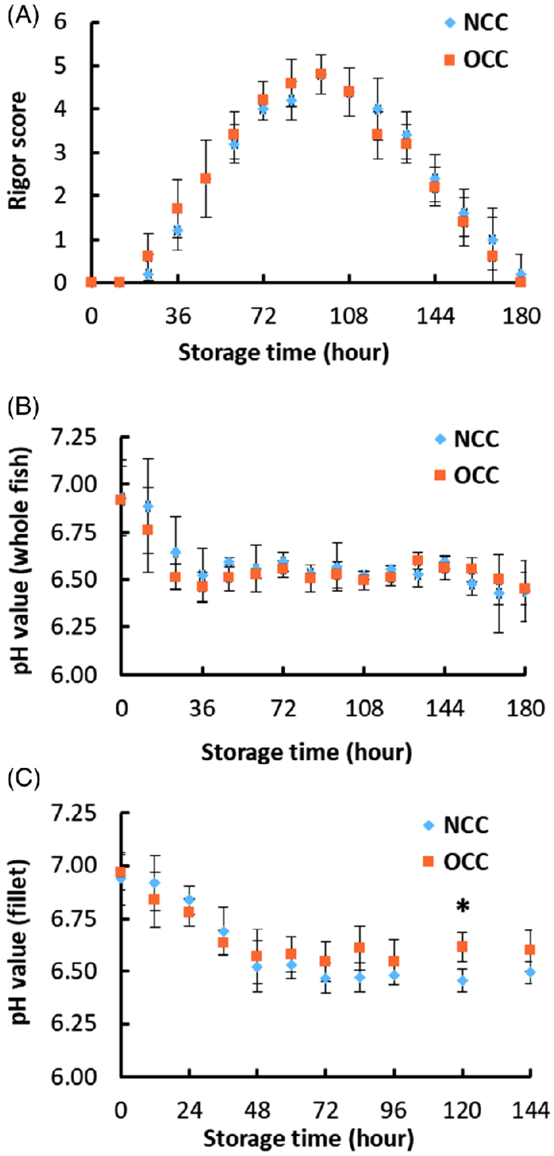

Figure 1 Rigor score (A) and pH (B, C) of whole fish or fillet of normal common carp (NCC) and omega-3 common carp (OCC) during post-mortem under chilled storage. Error bars represented the standard deviation of the mean ($n = 5$). *Denotes significant difference between two groups at the same time.

As stated earlier, although *K*-value is a commonly used indicator to measure freshness the Ki-value and Hx-index are also reported as alternative supplemental indicators for evaluating fish freshness.[13,33] Therefore, we analysed all three recommended indices for a better and biased-free understanding of spoilage and its progress in our experimental subjects. In the current study, initial *K*-value of NCC and OCC was < 8% (Fig. 3(G)), indicating high freshness. The *K*-value of NCC climbed to 22% at 36 h, while OCC achieved 24% at 48 h. After 84 h, the *K*-value of NCC and OCC increased to 53% and 49% and they climbed to 75% and 69% at 120 h. Fish are often classified as fresh (*K*-value < 20%), moderately fresh (20% < *K*-value < 50%) and not fresh (*K*-value > 70%).[14] It is noticed that Ki-value (Fig.3(H)) and Hx-index (Fig. 3(I)) showed similar change as *K*-value during storage, exhibiting high correlation with each other (*K*-value *versus* Ki-value, $r = 0.980$–0.984; *K*-value *versus* Hx-index, $r = 0.968$–0.981, Pearsons two-tailed, $P < 0.05$).

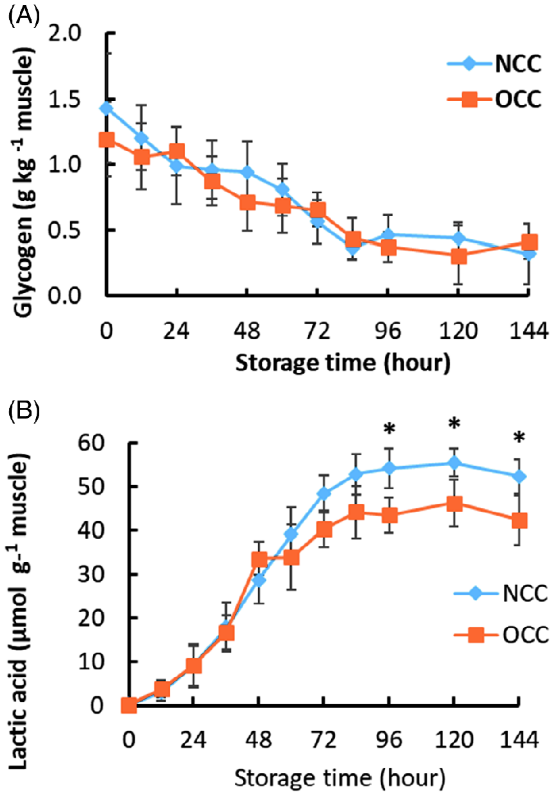

Figure 2 Changes in glucose (A) and lactic acid (B) content of normal common carp (NCC) and omega-3 common carp (OCC) fillet during chilled storage. Error bars represented the standard deviation of the mean ($n = 6$). *Denotes significant difference between two groups at the same time.

Results indicate that NCC and OCC were very fresh before 36 h but not fresh after 96 h. This conclusion is similar to that reported by Qin *et al.*,[27] but different from that given by Li *et al.*,[34] who found carp was very fresh at 48 h and moderately fresh after 192 h. The differences could be due to different size and physiological status before slaughtering. No significant difference of *K*-value, Ki-value and Hx-index between the two groups was observed during the whole period.

Protein solubility and profile

Myofibrillar protein (MP), the main component of muscle protein, is salt-soluble and can be decomposed by endogenous enzymes.[35] In Fig. 4(A), SSP content of NCC and OCC were 112 and 117 mg g^{-1}muscle, respectively. They declined as storage time prolonged, showing no difference before 96 h. At 144 h, SSP of OCC was 90 mg g^{-1}muscle, slightly higher than that

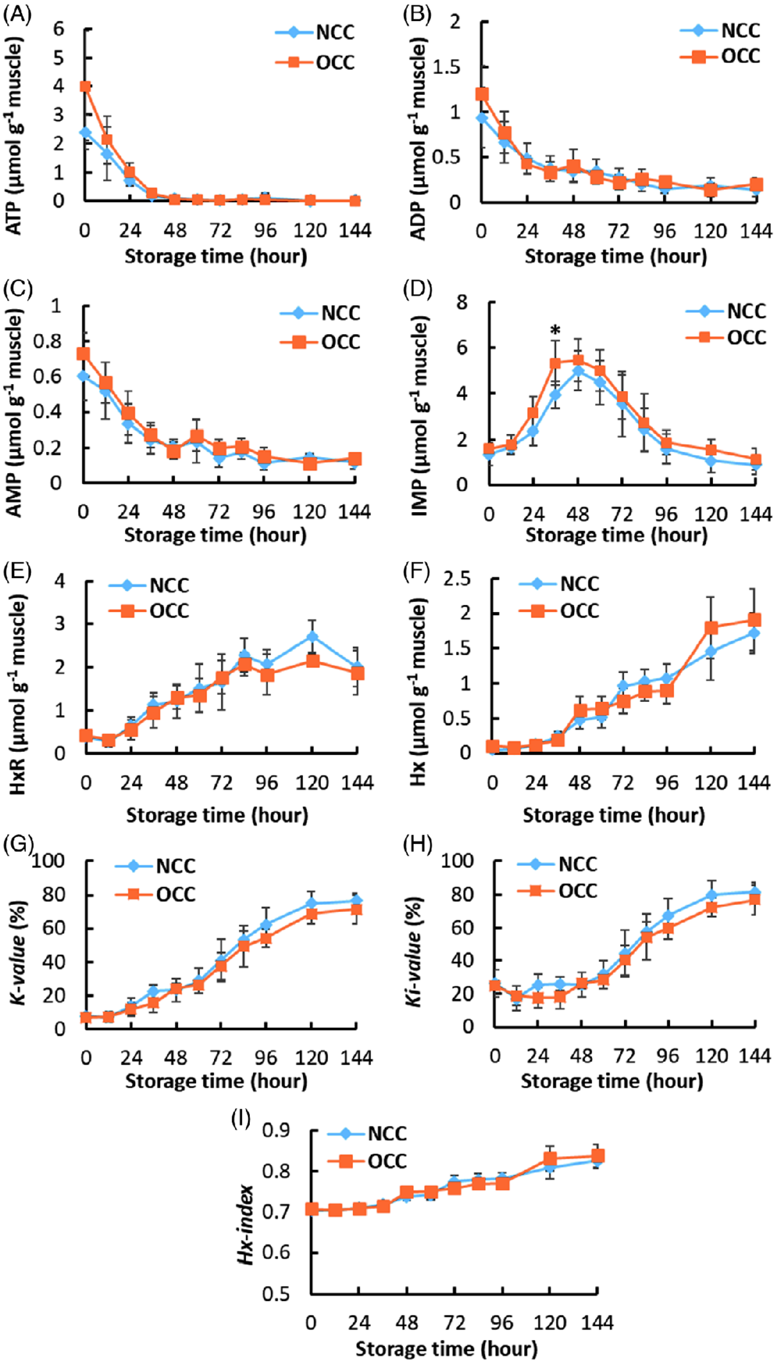

Figure 3 Changes in adenosine triphosphate (ATP) related compounds (A) ATP, (B) adenosine diphosphate (ADP), (C) adenosine monophosphate (AMP), (D) inosine monophosphate (IMP), (E) hypoxanthine riboside (HxR), (F) hypoxanthine (Hx) and freshness indices (G) *K*-value, (H) Ki-value, (I) Hx-index of normal common carp (NCC) and omega-3 common carp (OCC) fillet during chilled storage. Error bars represented the standard deviation of the mean ($n = 6$). *Denotes significant differences between two groups at the same time.

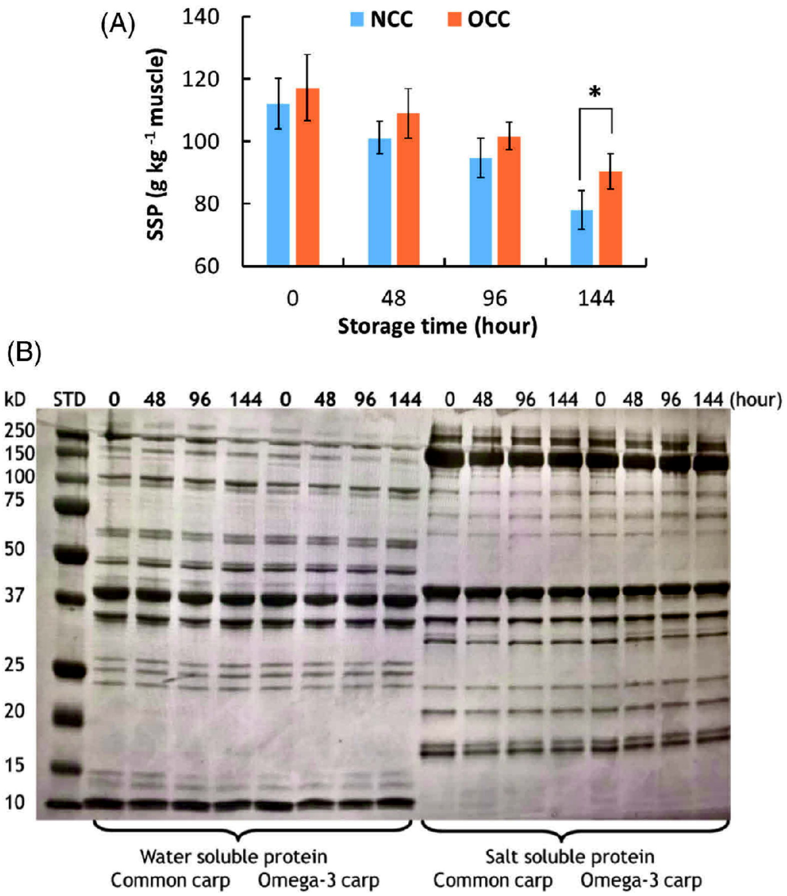

Figure 4 Changes in salt soluble protein (SSP) content (A), water soluble protein (WSP) and SSP pattern (B) of normal common carp (NCC) and omega-3 common carp (OCC) muscle during chilled storage. Error bars represented the standard deviation of the mean ($n = 6$). *Denotes significant difference between two groups at the same time.

of NCC (80 mg g^{-1}muscle). Decrease of SSP indicates muscle protein degradation, which was commonly observed in chilled-stored fish. Slightly higher SSP content in OCC indicates more integrated muscle protein in OCC than NCC at the end of storage.

WSP profile of NCC and OCC were characterized by 19 bands with molecular weight (MW) ranging 10–250 kDa (Fig. 4(B)). There were no big differences in protein patterns of WPS in NCC and OCC. However, two bands at MW between 150 and 250 kDa in NCC showed higher intensity than those in OCC, indicating heavier WSP loss. SSP profile of NCC and OCC showed similar patterns with typical myosin heavy chain (MHC, 220 kDa), actin (43 kDa), tropomyosin (36 kDa) and troponin T (35 kDa). MHC and actin of samples at 48, 96 and 144 h attenuated compared with those of fresh samples in NCC and OCC, indicating degradation of MP and actin. Nevertheless, the change in OCC was not as distinct as that in NCC, implying that the alleviated hydrolysis of them in OCC, coincided with its higher SSP content. Additionally, the intensity of a band with MW of 60 kDa turned weak after 96 h in OCC, while it remained stable in NCC. Results suggest that NCC and OCC had similar protein patterns, but the changes of them differed slightly.

Lipid oxidation

Initial TBA values were 0.075 and 0.095 mg kg^{-1} in NCC and OCC, respectively (Table 2). They kept increasing and appeared as 0.320 and 0.250 mg kg^{-1} in NCC and OCC at 144 h, suggesting lipid oxidation. No difference of TBA was found between NCC and OCC during storage, only slightly higher TBA in NCC was observed at 144 h. PUFA is susceptible to oxidation, but the absolute content of it in NCC and OCC were similar. Thus, it could be a higher content of antioxidants from higher proportion of natural food in OCC that inhibited their oxidation, giving a lower TBA value. This indicates that carp cultured in the patented system enriched with n-3 PUFA should have similar or higher oxidative stability to normal carp.

Total viable count (TVC)

Initial TVC were 2.77 and 2.47 logCFU g^{-1} in NCC and OCC (Table 2), lower than that (3.69 log CFU g^{-1}) reported by Li *et al.*,[34] suggesting good sanitary status of the fish. TVC increased as storage time extended. After 144 h, TVC of NCC increased to 6.47 logCFU g^{-1}, higher than that of OCC, 5.85 logCFU g^{-1}. Neither exceeded the maximum acceptable level of microorganism for

Table 2 Changes in thiobarbituric acid (TBA) value (TBA), total viable count (TVC), color properties (*L**, *a** and *b** values), drip loss (DL), cooking loss (CL) and textural properties (hardness and resilience) of normal common carp (NCC) and omega-3 common carp (OCC) fillet during chilled storage ($n = 6$)

		Storage time (hours)				
Indexes	Groups	0	24	48	96	144
TBA (mg MDA kg^{-1})	NCC	0.075 ± 0.023c	—	0.112 ± 0.025bc	0.166 ± 0.028b	0.320 ± 0.032a[a]
	OCC	0.095 ± 0.046c	—	0.103 ± 0.016c	0.150 ± 0.025b	0.250 ± 0.021a
TVC (logCFU g^{-1})	NCC	2.77 ± 0.14d	—	3.37 ± 0.14c	4.75 ± 0.30b	6.47 ± 0.27a[a]
	OCC	2.47 ± 0.18d	—	3.37 ± 0.39c	4.36 ± 0.26b	5.85 ± 0.28a
*L**	NCC	51.66 ± 1.79a	—	50.14 ± 1.53a	50.36 ± 1.45a[a]	49.07 ± 1.41a[a]
	OCC	48.24 ± 1.80a	—	47.86 ± 1.09a	46.98 ± 1.42a	45.58 ± 1.85a
*a**	NCC	0.16 ± 0.37c	—	0.32 ± 0.50bc	0.60 ± 0.39b	1.50 ± 0.39a
	OCC	0.38 ± 0.32c	—	0.51 ± 0.59bc	0.66 ± 0.43b	1.74 ± 0.54a
*b**	NCC	5.16 ± 0.87c	—	6.71 ± 1.19bc	7.60 ± 1.35b	9.76 ± 0.95a
	OCC	5.68 ± 0.96b	—	6.30 ± 0.72b	7.32 ± 1.53ab	9.46 ± 1.43a
DL	NCC	—	1.13 ± 0.19d	2.05 ± 0.24c	3.27 ± 0.37b	6.82 ± 0.69a[a]
(%)	OCC	—	1.22 ± 0.17d	1.83 ± 0.31c	2.94 ± 0.32b	5.57 ± 0.51a
CL (%)	NCC	12.0 ± 1.2ab	—	11.7 ± 0.8b	11.9 ± 0.8b	20.2 ± 1.9a[a]
	OCC	11.9 ± 1.4b	—	13.4 ± 1.6b	10.3 ± 0.9bc	16.5 ± 1.5a
Hardness	NCC	1318 ± 180b	1505 ± 202b	1379 ± 140b	1231 ± 140b	874 ± 94a
(g)	OCC	1492 ± 107bc	1668 ± 226c	1509 ± 173bc	1317 ± 106b	1074 ± 98a[a]
Resilience (%)	NCC	37.20 ± 2.51d	27.62 ± 1.64c	20.50 ± 1.70b	16.25 ± 1.10a	15.93 ± 1.24a
	OCC	35.56 ± 1.99d	29.07 ± 2.48c	21.94 ± 2.28b	18.49 ± 0.96a[a]	18.40 ± 1.18a[a]

Lowercase letters in the same row indicate the significant difference during storage time.
Note: —, no detection.
[a] Significant difference between two groups at the same time.

raw freshwater fish (7.0 logCFU g^{-1}).[36] TVC of our fish at 144 h was lower than that of common carp that experienced the same length of chilled storage,[34] probably due to the lower initial TVC. Muscle protein degradation could enable the release of more soluble nutrients and promote microorganism reproduction. In mentioned earlier, higher SSP content and less distinct degradation of MHC in OCC than in NCC was observed. This might explain the slightly lower TVC in OCC at the end.

Colour and textural properties

The *L** value of NCC and OCC declined from 51.7 and 48.2 to 49.1 and 45.6 (Table 2). The *L** value of NCC was slightly higher than that of OCC after 96 h, indicating its higher lightness. Reduction in *L** value was also found in songpu mirror carp[10] and silver carp.[37] Both *a** and *b** value increased during storage in NCC and OCC, but no difference between them was observed. Results suggest the an increase of redness and yellowness of fish muscle during chilled storage. This could be owing to the decomposition of muscle structure that releases blood to muscle tissue and enhances redness. This is contrary to the declined redness in silver carp, which was attributed to haemoglobin oxidation and brownish met-haemoglobin accumulation.[37] Protein and lipid oxidation were reported to strengthen yellowness of muscle by oxidation products that can modify the absorption and scattering of light.[38] Coincidently, increased TBA value was found with elevated yellowness in NCC and OCC.

Initial hardness in NCC and OCC were 1318 and 1492 g, respectively (Table 2). They increased to 1505 and 1668 g after 24 h and then decreased gradually. Changes in hardness differed from rigor score in whole fish, which showed maximum rigor at 96 h. This inconsistency might be due to the unknown reasons that gives rise to rigor onset in fish fillet. Compared with whole fish, fish fillet was more exposed to microorganism, which might promote the degradation of muscle protein via enzymes by microorganism, resulting in a soft and less elastic texture.[11] No significant difference of hardness between NCC and OCC was found before 96 h, only at 144 h, slightly lower hardness in NCC was observed, which might be attributed to the faster protein degradation or slightly higher lipid content in NCC. For springiness, it decreased in both NCC and OCC during storage, showing no difference. Martinez *et al.*[39] considered that fish texture depended much on the microstructure of fish muscle, which greatly related to the breakdown of extracellular matrix and proteolysis of the intracellular MP. Our results of texture and SSP were in accordance with each other, indicating that OCC muscle microstructure might possess higher stability than NCC.

Drip loss (DL) and cooking loss (CL)

DL and CL denote the loss of water and water-soluble nutrients during storage or after cooking.[40] They closely relate with sensory properties and weight loss of muscle food, thus are of concern by consumer and industry. DL in NCC and OCC kept increasing during storage (Table 2). After 144 h, DL was 5.71% and 6.75%, respectively. Similarly, CL of NCC and OCC increased from 12.0% and 11.9% to 20.2% and 16.5%. Duun *et al.*[41] pointed out that water less tightly entrapped in myofibrils protein lost as drip was mainly attributed to the changes of protein structure or pattern. Heating causes denaturation of myosin and shrinkage of myofibrils, leading to a subsequent water expulsion; the more integrated the muscle protein structure, the lower CL usually is.[42] The slightly lower DL and CL in OCC coincided with its more stable MP with higher SSP content and more integrated MHC bands than those of NCC.

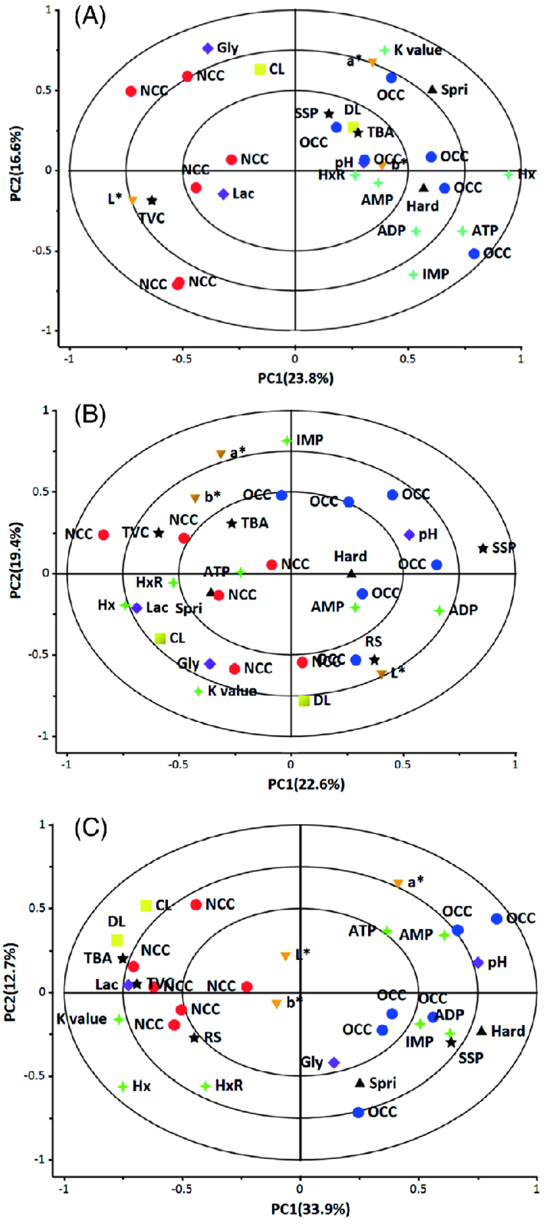

Figure 5 Principal component analysis (PCA) of *K*-value compounds, textural properties and physiochemical properties of normal common carp (NCC) and omega-3 common carp (OCC) chill-stored for 0 h (A), 96 h (B) and 144 h (C) (*n* = 6).

PCA of physiochemical properties

Data of quality parameters of NCC and OCC were collected and subjected to PCA to study their differences during storage. Figure 5(A) shows a biplot of NCC and OCC properties at initial storage (0 h). Two components explained 40.4% variability. NCC clustered at the left with *L**, TVC and glycogen while OCC distributed on the right close to Hx, ATP, *K*-value, IMP, springiness and *a**, indicating a high initial level of *L**, TVC, glycogen and CL in NCC, and a high initial value of the other eight indices in OCC. Results suggest that the two groups might differ slightly in *K*-value compounds, textural and colour properties initially. A biplot explaining 42.0% variability was obtained for properties of NCC and OCC at 96 h (Fig. 5(B)). The separation of them was not distinct, indicating no big difference between them at middle storage, which could be due to the deterioration of quality in both. However, NCC and OCC exhibited good separation in a biplot with 46.6% variability explanation at 144 h (Fig. 5(C)). NCC gathered on the left with *K*-value, lactic acid, TBA, Hx, DL and CL while OCC assembled on the right with hardness, pH, *a** and SSP. OCC showed better texture along with lower DL and CL, higher freshness and oxidative stability than NCC at 144 h. Improved separation of NCC and OCC in third biplot indicates that the differences between NCC and OCC became more distinct as storage time grew. Results denote that NCC and OCC differed slightly at the beginning and their differences turn clearer at the end, which implies that the storage stability of OCC might be slightly higher than that of NCC. It was noticed that the error of data was big, and all of the three models could explain only 40.4–46.6% variations. Thus, if there were differences between OCC and NCC, they could be quite limited.

CONCLUSION

There were no big differences in rigor mortis, *K*-value compounds, lipid oxidation, TVC, textural and colour properties between NCC and OCC during chilled storage. However, muscle protein degradation might occur at a slightly lower rate in OCC than in NCC. As storage extended, OCC exhibited slightly higher preservative stability and better muscle quality than NCC. Therefore, the patented culture system might have quite limited positive influences on post-mortem quality changes of common carp. It could be applied to produce OCC containing low content of lipid with high proportion of n-3 PUFA and n-3/n-6 ratio.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Briggs MA, Bowen KJ and Kris-Etherton PM, Omega-3 polyunsaturated fatty acids and health, in *Food Lipids: Chemistry, Nutrition, and Biotechnology*, ed. by Akoh CC and Min DB, Boca Raton: CRC Press, pp. 603–625 (2017).

2 Mock TS, Francis DS, Drumm DW, Versace VL, Glencross BD, Smullen RP *et al.*, A systematic review and analysis of long-term growth trials on the effect of diet on omega-3 fatty acid levels in the fillet tissue of post-smolt Atlantic salmon. *Aquaculture* **516**:734643 (2020).

3 Sprague M, Dick JR and Tocher DR, Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. *Sci Rep* **6**:21892 (2016).

4 Tokur B, Ozkütük S, Atici E, Ozyurt G and Ozyurt CE, Chemical and sensory quality changes of fish fingers, made from mirror carp (*Cyprinus carpio* L., 1758), during frozen storage (−18 °C). *Food Chem* **99**: 335–341 (2006).

5 FAO, FAO Yearbook. Fishery and Aquaculture Statistics, in *FAO Yearbook Fishery and Aquaculture Statistics*. p 108. Food and Agriculture Organization, Rome (2019)

6 Csengeri I, Dietary effects on fatty acid metabolism of common carp. *Arch Tierernahr* **49**:73–92 (1996).

7 Mraz J, Pickova J and Kozak P, *Feed for Common Carp and Culture of Common Carp with Increased Content of Omega 3 Fatty Acids*, PCT/IB2011/002998 (2011). https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2012046148.

8 Mraz J, Zajic T, Kozak P, Pickova J, Kacer P, Adamek V *et al.*, Intake of carp meat from two aquaculture production systems aimed at secondary prevention of ischemic heart disease - a follow-up study. *Physiol Res* **66**:S129 (2017).

9 Adámková V, Kacer P, Mraz J, Suchanek P, Pickova J, Králova LI *et al.*, The consumption of the carp meat and plasma lipids in secondary prevention in the heart ischemic disease patients. *Neuro Endocrinol Lett* **32**:17–20 (2011).

10 Zhu S, Zhou Z, Feng L and Luo Y, Postmortem changes in physicochemical properties of songpu mirror carp (*Cyprinus carpio*) during iced storage. *Food Biosci* **9**:75–79 (2015).

11 Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschläger J, SaV T *et al.*, Multisensor for fish quality determination. *Trend Food Sci Technol* **15**:86–93 (2004).

12 Cheng JH, Sun DW, Zeng XA and Liu D, Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: a review. *Crit Rev Food Sci Nutr* **55**:1012–1225 (2015).

13 Dalgaard P, *Freshness, Quality and Safety in Seafoods*. Teagasc, The National Food Centre, Dublin (2000).

14 Hong H, Regenstein JM and Luo Y, The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: a review. *Crit Rev Food Sci Nutr* **57**:1787–1798 (2017).

15 Maqsood S, Benjakul S and Kamal-Eldin A, Haemoglobin-mediated lipid oxidation in the fish muscle: a review. *Trends Food Sci Technol* **28**:33–43 (2012).

16 Hematyar N, Masilko J, Mraz J and Sampels S, Nutritional quality, oxidation, and sensory parameters in fillets of common carp (*Cyprinus carpio* L.) influenced by frozen storage (−20 °C). *J Food Process Preserv* **42**:e13589 (2018).

17 Erikson U and Misimi E, Atlantic salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis, and ice storage. *J Food Sci* **73**:C50–C59 (2008).

18 Li D, Jia S, Zhang L, Wang Z, Pan J, Zhu B *et al.*, Effect of using a high voltage electrostatic field on microbial communities, degradation of adenosine triphosphate, and water loss when thawing lightly-salted, frozen common carp (*Cyprinus carpio*). *J Food Eng* **212**: 226–233 (2017).

19 Pan J, Shen H and Luo Y, Cryoprotective effects of trehalose on grass carp (Ctenopharyngodon idellus) surimi during frozen storage. *J Food Process Preserv* **34**:715–727 (2010).

20 Sampels S, Asu M and Vogt G, Berry marinades enhance oxidative stability of herring fillets. *J Agric Food Chem* **58**:12230–12237 (2010).

21 Hao R, Liu Y, Sun L, Xia L, Jia H, Li Q *et al.*, Sodium alginate coating with plant extract affected microbial communities, biogenic amine formation and quality properties of abalone (*Haliotis discus* hannai Ino) during chill storage. *LWT – Food Sci Technol* **81**:1–9 (2017).

22 Hong H, Luo Y, Zhou Z, Bao Y, Lu H and Shen H, Effects of different freezing treatments on the biogenic amine and quality changes of bighead carp (*Aristichthys nobilis*) heads during ice storage. *Food Chem* **138**:1476–1482 (2013).

23 Lu H, Hong H, Luo Y and Desk S, The seasonal fatty acids composition in different tissues of farmed common carp (*Cyprinus carpio*). *SDRP J Food Sci Technol* **1**:11–19 (2016).

24 Hegde MV, Zanwar AA and Adekar SP, Nutrition, life, disease, and death, in *Omega-3 Fatty Acids: Keys to Nutritional Health*, ed. by Hegde MV, Zanwar AA and Adekar SP. Springer, Cham, pp. 1–10 (2016).

25 Zhang WG, Lonergan SM, Gardner MA and Huff-Lonergan E, Contribution of postmortem changes of integrin, desmin and μ-calpain to variation in water holding capacity of pork. *Meat Sci* **74**:578–585 (2006).

26 Zhang L, Li Q, Lyu J, Kong C, Song S and Luo Y, The impact of stunning methods on stress conditions and quality of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4°C during 72h postmortem. *Food Chem* **216**:130–137 (2017).

27 Qin N, Li D, Hong H, Zhang Y, Zhu B and Luo Y, Effects of different stunning methods on the flesh quality of grass carp (*Ctenopharyngodon idellus*) fillets stored at 4°C. *Food Chem* **201**:131–138 (2016).

28 Duran A, Erdemli U, Karakaya M and Tyilmaz M, Effects of slaughter methods on physical, biochemical and microbiological quality of rainbow trout *Oncorhynchus mykiss* and mirror carp *Cyprinus carpio* filleted in pre-, in- or post-rigor periods. *Fish Sci* **74**:1146–1156 (2008).

29 Bao Y, Zhu S, Luo Y and Shen H, Comparison of postmortem changes in blunt-snout bream (*Megalobrama amblycephala*) during short-term storage at chilled and partial freezing temperatures. *J Aquat Food Prod Technol* **24**:752–761 (2015).

30 Alasalvar C, Taylor KDA and Shahidi F, Comparative quality assessment of cultured and wild sea bream (*Sparus aurata*) stored in ice. *J Agric Food Chem* **50**:2039–2045 (2002).

31 Shiba T, Shiraki N, Furushita M and Maeda T, Free amino acid and ATP-related compounds in sterile Tiger Puffer fish (*Takifugu rubripes*) fillets stored at 4C. *J Food Process Preserv* **38**:791–797 (2014).

32 Hernández-Cázares AS, Aristoy M-C and Toldrá F, Nucleotides and their degradation products during processing of dry-cured ham, measured by HPLC and an enzyme sensor. *Meat Sci* **87**:125–129 (2011).

33 Hamada-Sato N, Usui K, Kobayashi T, Imada C and Watanabe E, Quality assurance of raw fish based on HACCP concept. *Food Control* **16**: 301–307 (2005).

34 Li D, Zhang L, Song S, Wang Z, Kong C and Luo Y, The role of microorganisms in the degradation of adenosine triphosphate (ATP) in chill-stored common carp (*Cyprinus carpio*) fillets. *Food Chem* **224**: 347–352 (2017).

35 Delbarre-Ladrat C, Chéret R, Taylor R and Verrez-Bagnis V, Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. *Crit Rev Food Sci Nutr* **46**: 409–421 (2006).

36 ICMSF I, *Microorganisms in Foods*. University of Toronto Press, Toronto (1986).

37 Shi C, Cui J, Yin X, Luo Y and Zhou Z, Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: effect on lipid and protein oxidation. *Food Control* **40**:134–139 (2014).

38 Guerrero P, O'Sullivan MG, Kerry JP and de la Caba K, Application of soy protein coatings and their effect on the quality and shelf-life stability of beef patties. *RSC Adv* **5**:8182–8189 (2015).

39 Martinez I, Wang PA, Slizyté R, Jorge A, Dahle SW, Cañas B *et al.*, Protein expression and enzymatic activities in normal and soft textured Atlantic salmon (*Salmo salar*) muscle. *Food Chem* **126**:140–148 (2011).

40 Cai L, Wu X, Li X, Zhong K, Li Y and Li J, Effects of different freezing treatments on physicochemical responses and microbial characteristics of Japanese sea bass (Lateolabrax japonicas) fillets during refrigerated storage. *LWT – Food Sci Technol* **59**:122–129 (2014).

41 Duun AS and Rustad T, Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at −1.4 and −3.6°C. *Food Chem* **106**:122–131 (2008).

42 Skipnes D, Johnsen SO, Skåra T, Sivertsvik M and Lekang O, Optimization of heat processing of farmed Atlantic cod (*Gadus morhua*) muscle with respect to cook loss, water holding capacity, color, and texture. *J Aquat Food Prod Technol* **20**:331–340 (2011).

CHAPTER 3

CRITICAL REVIEW ON THE USE OF ESSENTIAL OILS AGAINST SPOILAGE IN CHILLED STORED FISH: A QUANTITATIVE META-ANALYSES

Hao, R.Y., Roy, K., Pan, J., Shah, B. R., Mraz, J., 2021. Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses. Trends in Food Science & Technology 111, 175–190.

My share on this work was about 45%.

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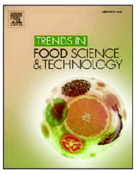

Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses

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ABSTRACT

Background: Presently, ~68.6 million tons of chilled-stored seafood are available globally for human consumption, worth ~129.5 billion €. At least ~13.7 million tons (worth 25.8 billion €) are probably spoilt each year, from post-catch till consumption. A growing interest in essential oils (EOs) as bio-preservatives in chilled-stored seafood is recently visible – prolific research during 2015–2020.

Scope and approach: Data from 180 scientific articles were reviewed and meta-analyzed. Our data-driven review aims to corroborate the promises of EOs in the chilled-stored seafood industry– where we stand and where to go (?).

Key findings and conclusions: Microbial load explain 60–90% of spoilage indicators' progression in chilled-stored fish flesh. Beyond TVC 5–7 log CFU g−1, spoilage progresses exponentially. We identified 6 EOs with extraordinary TVC reduction potential (>4.61 log CFU g−1 per % concentration) that can ensure compliance with EU safety standards for raw fish – citrus, mentha, origanum, thymus, zataria, and zingiberaceae (probably chamomile and star anise in future). Not all EOs can suppress all specific microbes, especially anaerobic H_2S producing bacteria. Only origanum, zingiberaceae, and thymus have complete-spectrum efficacy. Their right application method is essential (hurdle technology; active film-nanonemulsion; special packaging). 0.5–1% concentration of most EOs impart little interference on the natural odor of fresh fish. The rate of sensory score deterioration in EO treated fish flesh is ~2.5–5 times slower than normal refrigerated ones. Selected EOs at mild concentrations with the right application method can promote safety, sensory and shelf-life agendas of chilled-stored seafood. The guidelines, warnings, knowledge gaps, and research needs are discussed.

1. Introduction

Presently about 156 million tons of aquatic food products predominated by fish (generically termed as seafood) are used for human consumption (FAO, 2020). It contributes a first-sale value worth of ~294.9 billion € to the worldwide food industry (recalculated from FAO, 2020). The present estimated annual supply of seafood for the global population is about 20.5 kg per capita, with a majority of 44% of seafood being consumed as 'live, fresh or chilled' form. It is equivalent to ~68.6 million tons of chilled seafood globally, worth ~129.5 billion € (FAO, 2020). The high moisture, low amount of connective tissues, reactive endogenous enzymes, and enriched nutrients make fish susceptible to biochemical and microbial spoilage (Yu et al., 2020). Significant spoilage of fish flesh occurs every year at different production chain levels (post-harvest handling, processing, storage, and distribution), causing perceptible economic losses, product quality deterioration, and consumer safety concerns. For example, even in Europe with the least food losses documented for meat products, up to 20% of fish flesh is lost from 'post-catch' till 'consumption' (FAO, 2011, p. 37). Assuming this minimum 20% loss on a global scale (estimates above), it adds up to ~13.7 million tons (worth 25.8 billion €) of chilled-stored seafood lost annually, almost equal to total capture fisheries production by China (FAO 2020, p. 37).

Though frozen storage (−18 °C) is the most effective method of extending fish shelf-life, chilled storage is gaining popularity among consumers with the availability of cold-chains and fresh seafood concepts. Chilled-stored fish have the potential to maintain original flavor, texture, and freshness. Without the time-consuming thawing process involved, chilled products suffer no ice crystal damage and are

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Abbreviations

Adenosine diphosphate	(ADP)
Adenosine monophosphate	(AMP)
Air-package	(AP)
Adenosine triphosphate	(ATP)
Biogenic amine	(BA)
Colony forming unit	(CFU)
Dominant spoilage microorganism	(DSM)
Enterobacteriaceae	(ENT)
Essential oil	(EO)
H2S producing bacteria	(HSP)
Inosine	(HxR)
Hypoxanthine	(Hx)
Inosine monophosphate	(IMP)
Lactic acid bacteria	(LAB)
Modified atmosphere package	(MAP)
Pseudomonas	(PSE)
Specific spoilage organism	(SSO)
Trimethylamine nitrogen	(TMA)
Total volatile basic nitrogen	(TVBN)
Total viable counts	(TVC)
Volatile organic compound	(VOC)
Vacuum package	(VP)

convenient for cooking (Benjakul et al., 2003). Nevertheless, fish quality still deteriorates severely during chilled storage, leading to texture deterioration, off-odor, and shelf-life reduction. The rapid growth and metabolism of microorganisms naturally present or from contamination drive this process (Yu et al., 2020). Microbial spoilage can cause the decomposition of protein, results in the formation of volatile organic compounds (VOCs) with unpleasant off-flavors and the accumulation of deleterious substances, such as biogenic amines (BAs). It might also promote nucleotide degradation to form HxR and Hx. All of these give off-flavors, putrid odors, and bitterness to the flesh. Thus, controlling the microbial spoilage is a critical step in guaranteeing a high quality of chilled-stored fish flesh (Hao et al., 2020).

Fish is an excellent source of polyunsaturated fatty acids (mainly eicosapentaenoic acid and docosahexaenoic acid), easily digestible protein and amino acids, specific vitamins (e.g., vitamin B, D, tocopherols, and carotenoids), and many minerals (e.g., selenium, phosphorus, and calcium) (Tilami et al., 2018; Yin et al., 2016). Several chemical or synthetic preservatives have been used in chilled-stored fish to inactivate microorganisms responsible for spoilage. The most common and permitted synthetic preservatives in chilled stored seafoods are butylated hydroxytoluene (BHT), sodium benzoate, potassium sorbate, sodium acetate, sodium sulfite, and ethylenediamine tetra-acetic acid (EDTA). Their advantages include effectivity at lower concentrations and low to no interference with the original organoleptic properties (Olatunde & Benjakul, 2018; Hyldgaard et al., 2012). However, their use often brings side-effects on human health (supplementary Table S1), which is a significant disadvantage. Essential oils (EOs) application in food preservation has amplified in recent years due to the increasingly negative consumer perception of synthetic preservatives (Hassoun & Çoban, 2017; Hyldgaard et al., 2012). EOs are naturally derived aromatic liquids, including terpenoids, sesquiterpenes, and diterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones (Atarés et al., 2016; Moosavi-Nasab et al., 2019). They are regarded as safe additives. Many EOs exhibit strong antibacterial, antiviral, antifungal, and antioxidative properties, which enable their application in foods (Atarés et al., 2016; Shojaee-Aliabad et al., 2018). Their antimicrobial properties are related to the main bioactive compounds present in them (reviewed in Hyldgaard et al., 2012). The fundamental problem with any EO is that they are 'desirably effective' at higher concentrations than synthetic preservatives. Such higher concentration negatively interferes with the original organoleptic property of the food itself, despite suppressing spoilage. Some EOs are even weak against specific spoilage microbes (Hyldgaard et al., 2012; Olatunde & Benjakul, 2018).

There has been a growing interest in using EOs as bio-preservatives for chilled-stored seafood or fish flesh per se. A trend analysis on research concerning the usage of EOs in chilled-stored fish flesh shows rapidly growing popularity in the last half-decade (2015–2020). Before the year 2007, there was no footprint of this research (further discussed below). Not many, but few classical reviews on the preservative effects of EOs on fish flesh have accrued over time, e.g., Moosavi-Nasab et al. (2019), Hassoun and Çoban (2017), and Patel (2015). They have mostly compiled information and provided theoretical background on the chemical composition, antioxidant, and antimicrobial properties of EOs with few case examples on seafood. Hassoun and Çoban (2017) is perhaps the most comprehensive attempt to review EOs in the context of seafood so far. All those classical reviews have focused on compiling information, highlight successful case examples and textual findings from related research – somewhat 'qualitatively.' The main novelty of the present review is in its approach. We looked at the accrued data (information) objectively and attempted to generate applied information with 'quantitative' evidence. To the best of our knowledge, this is the first such metadata synthesis in the domain of EOs and seafood. Some novel aspects of this review, for example, are: (a) analyses on the sensory impact and degradation reaction in chilled-stored fish by spoilage microbes or essential oils independently; (b) intricacies of EO application (for effective shelf-life prolongation) and its negative interferences on 'original' organoleptic properties of fish (caused by EO treatment itself); (c) identification of top EOs and their application methods to safely comply with microbial hygiene limits (set internationally) for chilled-stored fish.

Through a data-driven approach, we attempted to unravel hidden knowledge and understanding of the manipulation of microbial properties by EOs. Data from an exhaustive list of scientific articles (n = 180) surrounding spoilage microbes and EOs in chilled-stored fish flesh were reviewed, studied, and meta-analyzed. The review aims to corroborate the promises of EOs in the chilled-stored seafood – where we stand and where to go (?). The purposes of the review or main research hypotheses were to: (a) list major genera of spoilage microorganisms in chilled-stored fish; (b) understand how spoilage progresses in chilled-stored fish with microbial load; (c) highlight recent trends in research and application of essential oils as bio-preservatives for chilled-stored fish; (d) review anti-microbial properties of EOs and physiological responses of spoilage microbes to EO exposure; (e) review various application methods for EO treatment of chilled-stored fish and their comparisons; (f) meta-analyze if any EO can be highly effective against all major spoilage microbes; (g) understand how the choice of application methods can boost or suppress the performance of top EOs (identified before); (h) simulate our identified top EOs and their application methods against microbial safety limits officially imposed in the EU, USA or Oceania for chilled-stored fish products; and, (i) search key knowledge gaps and research needs. The managerial implication of this review is to guide proper and effective usage of EOs in the chilled-stored seafood sector (both for industry and research), with the possible replacement of synthetic preservatives.

2. Methods of literature review and metadata analyses

2.1. Microbial load and associated spoilage indicators data in chilled-stored fish flesh

Using Web of Science, Scopus, ScienceDirect, and Google Scholar online databases targeted published data were collected and compiled. Keywords such as 'fish' or 'fillet' and 'chilled storage' and 'microbial enumeration' or '16s rRNA gene analysis' and/or 'volatile organic compound' and/or 'biogenic amine' were used in various combinations to get matches. Altogether 41 peer-reviewed and published articles in English (from 2001 to 2020) were collected (Appendix 2 in supplementary text). A checklist of common or dominant spoilage microorganisms in chilled-stored fish was created. As spoilage indicators, three established spoilage parameters were selected due to the completeness of their data – (a) hypoxanthine (Hx); (b) trimethylamine (TMA); (c) total volatile basic nitrogen (TVBN). As a proxy of microbial population or load, total viable counts (TVC) were collected in pairs to such spoilage indicators. The data subjects for further analyses had to qualify the chilled storage temperature conditions (0–6 °C) and somewhat packed (either AP, MAP, or VP). Taking TVC as an independent variable and the spoilage indicators as response variables (Hx, TMA, and TVBN), generalized additive models (GAMs) were generated to quantify the degree and nature of the relationship between microbial load and spoilage indicators in chilled-stored fish, using 'mgcv' package in RStudio v1.2 (Wood, 2017). Based on the visual trend of the GAM curve, the cut-offs were identified beyond which spoilage aggravate markedly.

2.2. Collection of data concerning essential oils (EOs) application on chilled-stored fish flesh

The keywords such as 'fish' or 'fillet' and 'quality' and 'chilled storage' and 'essential oil' and 'microbiological' and/or 'antimicrobial' and/or 'microbial' were used in various combinations to get matches from the Web of Science, Scopus, ScienceDirect, and Google Scholar. Altogether, 140 peer-reviewed articles in English, published from 2003 till 2020, fulfilled our search criteria. Based on research outputs encountered in this genre, multi-dimension research trend analyses were conducted (year wise published output, candidate species for EO application, type of EOs, application methods of EOs, packaging types, etc.). All articles had to fulfill 'non-frozen' fish flesh criteria elaborated above (i.e., chilled-stored temperature conditions and somewhat packed). Information on microorganisms such as Lactic acid bacteria (LAB), Pseudomonas (PSE), H2S producing bacteria (HSP), Enterobacteriaceae (ENT), and others were collected. Data on total viable counts (TVC), total volatile basic nitrogen (TVBN), trimethylamine nitrogen (TMA), inosine (HxR), hypoxanthine (Hx), and BAs were focused. All raw data sources ($n = 140$) used in the meta-analyses and presented in the main document can be found as 'Appendix 1' (see supplementary text). Since the review is critical, sources prior to the year 2000 were excluded. The oldest reference is from 2003.

2.3. Meta-analysis of anti-microbial efficacy of individual EOs and application method interferences

From the available literature, raw data in 380 groups (91 groups as control + 289 groups as EO-applied) were compiled. From the raw grouped (by experiment, EO species, application type) and paired data (non-EO/control and EO-applied/treatment), parameters like general microbial load (overall TVC) and four major specific spoilage microorganisms group (TVC of Pseudomonas, Enterobacteriaceae, H2S producing bacteria and lactic acid bacteria) were extracted. To avoid any confusion, TVC of specific groups Pseudomonas, Enterobacteriaceae, H2S producing bacteria, and lactic acid bacteria were denoted as PSE, ENT, HSP, and LAB, respectively. From the paired data, the TVC in the treatment group (i.e., EO-applied) was subtracted from the control group (without EO) to calculate reduction. For example, TVC reduction = TVCtreatment – TVCcontrol; at fixed time point and temperature conditions. The TVC reduction was then divided by the concentration of EO used (in %) to arrive at 'TVC reduction potential' (expressed as log CFU g−1 per % concentration). The same calculations were repeated for PSE, ENT, HSP, and LAB (expressed in log CFU g−1) for each EO.

Firstly, irrespective of EO application methods, the TVC reduction potential of all the EOs was pooled. A Shapiro-Wilk's normality test was done to check whether the data is skewed. In the present case, the data seemed to be positively skewed (skewness 2.59), highly peaked (kurtosis 7.81), and not normally distributed ($p < 0.01$), indicating many low 'TVC reduction potential' values dominated in our dataset. The mean (TVC reduction potential 4.15 log CFU g−1) and median (2.12 log CFU g−1) differed to such a degree that either of them could not be assumed as an accurate measure of central tendency (or a representative). Therefore, to obtain representative values, the interquartile range (IR) of TVC reduction potential of pooled EOs was calculated. In the IR, the upper value (i.e., third quartile or 75th percentile) proved to be the closest (and slightly higher) match with the mean. The 75th percentile was then identified as a benchmark for being 'above average.' Any EO whose TVC reduction potential IR, in any way, surpassed this 75th percentile benchmark was flagged as an EO having 'extraordinary (≈above average)' TVC reduction potential. Any outlier (extreme upper values only) was removed from the dataset to prevent any overestimation bias. However, the articles from which the outliers originated were traced. The reasons behind this were separately investigated individually. The abovementioned exercise was also repeated for PSE, ENT, HSP, and LAB reduction potentials for each of the EOs.

Secondly, EOs flagged as having extraordinary TVC reduction potential were marked as 'Top EOs.' Those top EOs were 'specially traced' down-the-chain (i.e., subsequent graphical models for specific spoilage microbes, PSE → ENT → HSP → LAB), if they demonstrate any extraordinary reduction potential(s) for PSE, ENT, HSP, and LAB too, respectively. Besides, those EOs with high reduction potentials of specific microbes but sub-average TVC reduction potential were noted as 'species-specific EOs.' Third, the top EOs were coded as 0 (low/average efficacy) or 1 (extraordinary efficacy) against specific spoilage microorganism categories like PSE, ENT, HSP, and LAB. A heatmap was generated to test their broad-spectrum antimicrobial efficacy and identify any EO(s) that could single-handedly 'close the loop' under diverse spoilage microbes. Fourth, the TVC reduction potential of the top EOs was compared across their different application methods. It was done to identify any application method(s), be it EO-specific or not, that could guarantee the highest anti-microbial efficacy of the top EOs for chilled-stored fish flesh.

Lastly, top broad-spectrum EOs and their best application methods were screened. In terms of application method, seven primary application methods of top EOs were encountered: normal; normal + special packaging; film; film + special packaging; emulsion; emulsion + film; and hurdle. Here, film means 'active film'; emulsion indicates 'nanoemulsion'; normal = bulk EO; special packaging = either modified atmosphere packaging (MAP) or vacuum packaging (VP); and hurdle = hurdle technology. The scheme of meta-analyses is illustrated in supplementary Fig S1. All graphical models (qplot, heatmap, and facet wrap plots) were done using ggplot2 package in RStudio v1.2 (Wickham, 2016).

2.4. Meta-analysis of interferences caused by EO application on sensory properties of fish flesh

Based on limited available information in this regard (only 32 out of 140 reviewed articles), we investigated the EO application's influence at three time points (in days). They include – (a) sensory score right at the start (time point t0); (b) time point where control fish flesh (without EO) sensory scores became unacceptable (tnon-EO), and; (c) time point where a sensory score of EO-applied flesh became unacceptable (tEO).

The authors have used different sensory score scales. We normalized such scales by converting them on a percentage scale, i.e., 0% → 100%. When sensory scores dropped below the 60% mark, they were deemed 'unacceptable.' Here, by 'sensory score,' we imply 'odor values' of raw flesh since odor values were most frequently assessed within the limited data pool. Other sensory scores like the color (after EO application) and taste (after EO application and cooking) were scanty to non-existent. Intuitive exploratory analysis of the available data was conducted.

Whether EO application itself deteriorates sensory score at t0 was analyzed by calculating percentage difference of treatment group (=EO applied) sensory score from control group (=fresh flesh, without EO) sensory score. The results obtained were corrected and normalized for the EO concentration interferences, and all results were expressed 'per unit concentration' of EO. Likewise, the percentage difference in sensory score of treatment flesh at tnon-EO, per unit EO concentration was determined. For calculating the rate of sensory quality deterioration (with or without EO treatment), the percentage differences were divided by the number of days taken to reach from 'fresh' to 'unacceptable' status. This daily rate measured how much sensory score deterioration in fish flesh was delayed by EO application per unit of their concentration. Outliers were identified and excluded (if percentage differences exceeded 100% or absolute differences exceeded scale extremes, zero differences were also excluded to prevent statistical biasedness). The interquartile range was derived from the calculated values using the summary function in RStudio, and the coefficient of variance was manually calculated (=standard deviation × 100 ÷ mean).

3. Results of literature review and metadata analyses

3.1. *Major genera of spoilage microorganisms associated with chilled-stored fish flesh*

The initial microorganisms in fish consist of their endogenous microbiota and exogenous microbes from the environment (fishing, transportation, and processing). Numerous studies have reported that fish's initial microorganisms are characterized by vast diversity and low relative abundance (Jääskeläinen et al., 2019; Parlapani et al., 2015). Not all initial microorganisms can survive and grow to a great extent during storage. Only a fraction of them could survive under the specific processing and storage condition and rapidly grow into dominance (Parlapani et al., 2015). Dominant spoilage microorganism (DSM) is decided by endogenous microbiota, processing parameters (marinating, antibacterial agents, etc.), and storage conditions (temperature and atmosphere). Among DSM, only a few ones can produce large amounts of off-odor and metabolites. Those microorganisms are called specific spoilage organisms (SSO) (Gram et al., 2002). SSO is usually identified by analyzing targeted metabolites. However, Therefore, in most cases, SSO and DSM are considered synonyms.

The common microbes involved in chill-stored fish are summarized in Table S2. Some case examples of DSMs/SSOs (Pseudomonas spp. > Shewanella spp. > Aeromonas spp. > Lactic acid bacteria > others) in different fish species (fillets; chilled stored) and packaging conditions are highlighted in the supplementary text. Results suggest – (a) all microbes, except Aeromonas spp., seem indiscriminative to cause spoilage irrespective of fish habitat origin (freshwater/saltwater); (b) Aeromonas spp. was almost exclusively subjected to studies on freshwater fishes only; (c) Aeromonas spp. or Pseudomonas spp. exhibit interspecies inhibition while causing spoilage – one of the species becomes prevalent with time; (d) Shewanella spp. with H_2S producing capability dominate the spoilage under anaerobic conditions, especially in packaging like MAP or VP; (e) Lactic acid bacteria are common spoilage microorganism in chill-stored fish under MAP or VP too; (f) Enterobacteriaceae are usually observed in fish caught from the contaminated aquatic area and can cause spoilage even under MAP packaging condition.

3.2. *Microbial load and spoilage indicators in chilled-stored fish flesh*

Metabolites such as inosine (HxR), hypoxanthine (Hx), trimethylamine (TMA), ammonia, biogenic amines (BA), and volatile organic compounds (VOCs) can be formed through microbial activities during chilled storage. Microbial activity in fish flesh is commonly determined by microbial load or total viable counts (TVC). A detailed account of all these spoilage parameters and their connection with microorganisms is provided in the supplementary text. The putrid-smelling compounds like VOCs or BAs and their association with spoilage bacteria are reviewed in Tables S3 and S4. These obnoxious compounds have a negative sensory impact and related to consumer safety issues; further discussed in sections 3.5.5, 3.8, and 3.9. Accumulation of these metabolites through a synergistic effect of 'autolysis' and 'microbial decomposition' leads to spoilage. Our point of interest was to determine the contribution of microbial spoilage behind spoilage. If any significantly high relationship between TVC and spoilage indicators exists, it will make sense to have a retrospective evaluation of EOs (each) for their 'microbial load reduction potential.' In return, it would tell us how far the EOs can help in controlling the spoilage and even identify top EOs that have superior efficacy compared to others.

Our meta-analyses reinforce that fish flesh is highly susceptible to microbial spoilage. The generalized additive model (GAM) revealed that the predictor variable (=TVC) could significantly ($p < 0.01$) explain the majority of the deviance (63–93%) in the GAM function of the response variables (=Hx, TMA, and TVBN; Fig. 1A–C). It implies that microbial load is a statistically key driver of spoilage progression (tracked by indicators) in fish flesh, over other spoilage influences like autolytic protein degradation, lipid oxidation, etc. The nature of the relationship was highly positive and significant (Adj. R^2 +0.633 to +0.938; $p < 0.01$). With increasing microbial load (TVC), all three assessed spoilage indicators (i.e., Hx, TMA, and TVBN) increases. The narrow prediction band tightly wrapped around the GAM curves (indicated by greyish shade, Fig. 1A–C) indicates high confidence in the models' projections. From the model projections, few cut-offs (or thresholds) were evident beyond which the synthesis of Hx, TMA, or TVBN is significantly aggravated, resulting in marked deterioration of the product. The GAM between TVC-Hx revealed a highly significant and positive relationship in chilled-stored fish (Adj. R^2 0.938; $p < 0.01$) with a cut-off point at >7 log CFU g^{-1} TVC (Fig. 1A). The model between TVC-TMA also revealed a highly significant and positive relationship (Adj. R^2 0.641, $p < 0.01$) with a cut-off point at >5 log CFU g^{-1} TVC (Fig. 1B). Like TMA, an almost similar relationship (Adj. R^2 0.633, $p < 0.01$) between TVC and TVNB was observed with a cut-off point at >5–6 log CFU g^{-1} TVC (Fig. 1C). It is apparent that between 5 and 7 log CFU g^{-1} TVC, the spoilage indicators markedly deteriorate, and it marks the onset of rapid spoilage. At this point, the EOs must intervene and suppress the TVC values below such critical thresholds (presented below). Therefore, our focus to suppress microbial load in raw fish as the 'key' to protect both organoleptic and safety properties is justified by these findings. Below we systematically unfold how EO(s) can serve as that key.

3.3. *Trends in EOs research and application methods in chilled-stored fish flesh*

Presently there are approximately 300 commercial EOs in the market (Falleh et al., 2020). Many EOs, such as basil, cinnamon, citrus, clove, ginger, laurel, lemon, thyme, and oregano, are approved as "generally recognized as safe" (GRAS) food additives by the Food and Administration (www.fda.gov). The European Food Safety Authority (EFSA) (www.efsa.europa.eu) and the Chinese Food Additives & Ingredients Association (CFAA) (www.cfaa.cn) recommends many EOs as safe food additives including their maximum permissible concentrations (Donsì & Ferrari, 2016).

A trend analysis on research concerning the usage of EOs in chilled-stored fish flesh shows rapidly growing popularity in the last half-decade

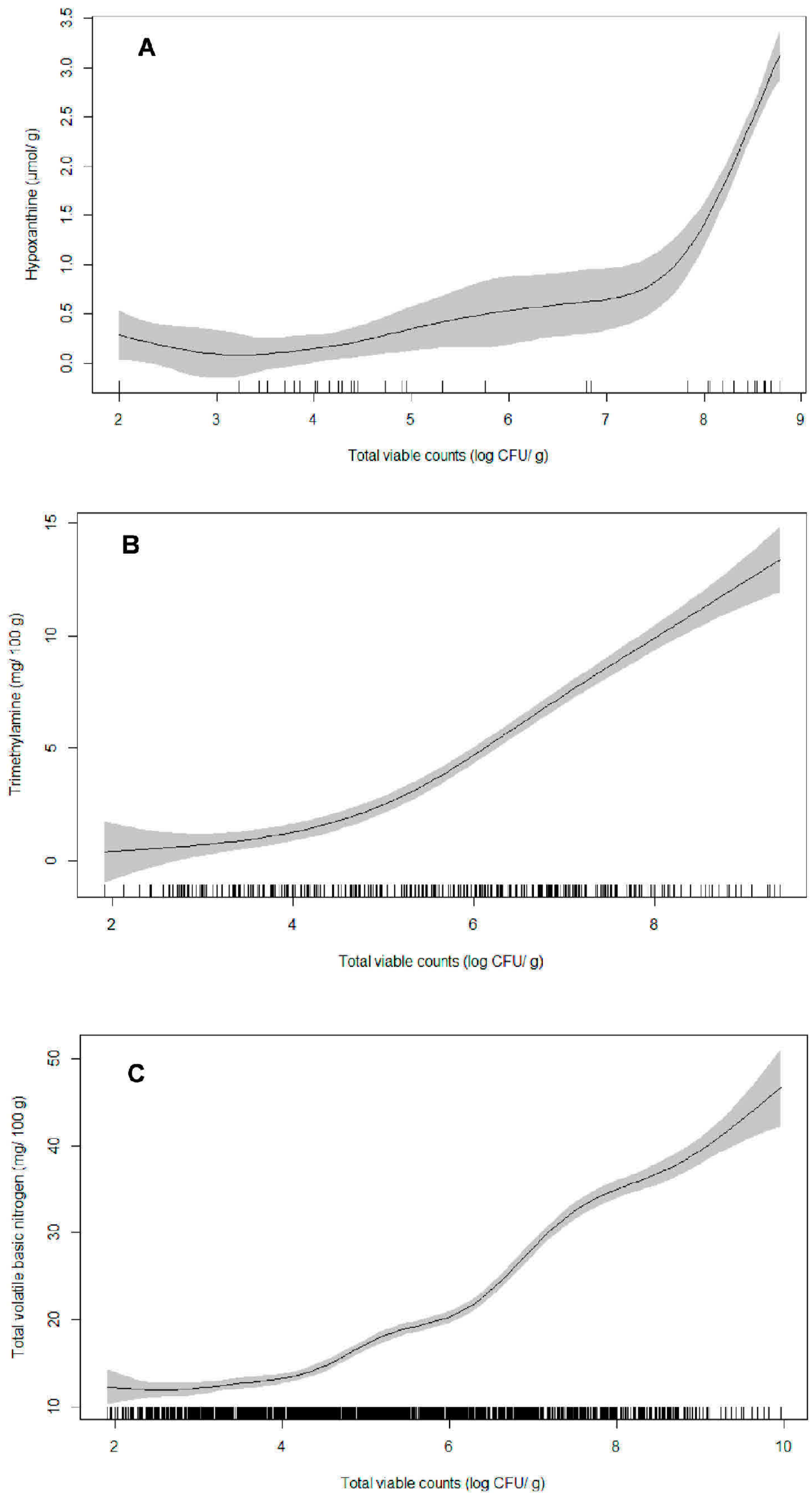

Fig. 1. (A–C): Generalized additive models on microbial load (TVC) and spoilage indicators (A = Hx; B = TMA; C = TVBN) in chilled-stored fish flesh. Microbial load showed a significantly high positive relationship with either of the spoilage indicators (Adj. R2 0.63–0.93; $p < 0.01$). Between 5 and 7 log CFU g−1 TVC, the spoilage indicators markedly deteriorate, and it marks the onset of rapid spoilage in chilled-stored fish flesh (logic axes = microbial load → spoilage indicators; see introduction).

(2015–2020; Fig S2A). On average, 18 published articles have accrued per annum during 2015–2020. In the previous half-decade (2009–2014), only ~5 published articles accrued per annum. Before the year 2007, there was no significant footprint of this research. It implies within one decade (2009–2020), there has been a 3–4 folds increase in attention towards EOs as bio-preservatives for non-frozen, chilled-stored fish flesh. Our collected articles covered 35 fish species (Fig S2B), e.g., rainbow trout, grass carp, sea bass, silver carp, tilapia, bream, etc. Rainbow trout was the most prevalent candidate for EOs application research, followed by grass carp, seabass, and sea bream (Fig S2B). From 140 articles reviewed, we encountered approximately 34 EOs in chill-stored fish (Fig S2C). The most popular ones were thymus, origanum, clove, cinnamon, mentha, and rosemary EO, altogether occupying at least 60% of reviewed studies. Lately, the EOs from Zataria multiflora boiss, citrus, bay laurel, ginger, and sage are also gaining much popularity.

EOs have been applied using various methods, i.e., original state/ bulk EO, EO emulsion, coupled with other preservative methods (e.g., active film, packaging, additives, and pre-treatment known as ‘hurdle technology’) (Fig S2B). The major EO application methods in chill-stored fish (alone or in combination) are highlighted in Fig S2D. In terms of recent popularity, ‘nanoemulsion of EO + active film’ followed by ‘bulk EO + active film’ → ‘bulk EO alone’ (with different packaging systems) have been the major application methods. Fig S2E summarizes further breakdown of these categories illustrated in Fig S2D. For example – the active film with EO nanoemulsion can be inedible or edible. Bulk EO may be applied directly through immersion, spray, pipetting, or evaporation. Special packaging requirements (after EO application) could be either modified atmosphere packaging or vacuumed.

3.4. Background on EO’s anti-microbial properties relevant to chilled-stored fish flesh

EOs are aromatic substances obtained from plant materials such as flowers, buds, leaves, stems, bark, and seeds (Hassoun & Çoban, 2017). Typically, EOs are a complex mixture of hundreds of individual compounds and characterized by two or three principal components at high concentrations (20–70%) (Van Haute et al., 2016), which could be: (i) terpene compounds (e.g., p-cymene, terpinene, limonene); (ii) terpenoids (subdivided into alcohols, esters, aldehydes, ketones, ethers, and phenols); (iii) phenylpropanoids (subdivided into phenols, aldehyde, alcohol and methoxy derivatives) (Jayasena et al., 2013). These bioactive compounds have antimicrobial properties (Hassoun & Çoban, 2017; Hyldgaard et al., 2012). The dominant bioactive compounds of different EOs are reviewed in Table S5. Also reviewed in Hyldgaard et al. (2012) and Patel (2015).

EO’s antibacterial mechanism has not been fully understood, which might be attributed to more than one mechanism (Falleh et al., 2020). Lipophilicity, the principal character of EOs, enable EOs to penetrate cytoplasm easily and disturb the phospholipid bilayer of inner membrane and mitochondria, leading to the instability of cellular structure and increasing cellular permeability (Fig. 2A–a, b) (Hassoun & Çoban, 2017; Shojaee-Aliabad et al., 2018, pp. 191–216). As a result, the leakage of ions (K+, Na+, Mg2+) and cytoplasmic constituents (e.g., DNA and RNA) (Fig. 2A–c, d) occurs (Hassoun & Çoban, 2017; Prakash et al., 2018). These are mainly caused by lipophilic hydrocarbons, such as terpenes and phenolics from EOs. Lipophilic hydrocarbons in EOs could also distort the lipid-protein interaction in a bacterial cell and interfere with ATPases necessary for producing ATP (Fig. 2A–e, f) (Mei et al., 2019). Moreover, phenolics in EOs could disrupt the proton motive force, electron flow, and cytoplasmic coagulation (Fig. 2A–g, h) (Shojaee-Aliabad et al., 2018, pp. 191–216). All these changes could inhibit the activity of bacteria. It can prevent active compounds in EOs from reaching the inner membrane. Thus, Gram-negative bacteria might be more resistant to EOs than Gram-positive bacteria (Hassoun & Çoban, 2017). However, hydrophobic compounds of EOs could pass through this barrier through porin on the outer membrane (Fig. 2A–i) (Nazzaro et al., 2013). It provides the necessary access for the EO to invade Gram-negative bacteria. In general, the physiological response exhibited by bacteria on exposure to EO bioactive compounds are: (a) depolarized and permeabilized membrane, damaged cell wall; (b) damaged cell structure, shape, and integrity leading to cell lysis; (c) leakage of potassium, ATP, and other cellular contents like membrane vesicles; (d) coagulation of cytoplasmic material; (e) reduced intracellular pH; (f) inhibition of enzymes like histidine decarboxylase and also respiration (from Hyldgaard et al., 2012).

The effect of EOs on the microbial community and spoilage indicators in chill-stored fish are summarized in Table 1. The highlights are: (a) the inhibition of microbial population varies among EO species; (b) it is possible to inhibit microorganism using lower doses of EO with hurdle-based application systems; (c) large fluctuations in an EO’s inhibitory effects on microbes can occur even at the same dose (could be explained by different fish species, EO categories, and EO application methods); (d) microbial community structure on chilled-stored fish is modified by EO (suppress one group and advantage on other groups; see supplementary text for examples); (e) formation of nucleotide degradation products (formation of Hx, HxR), spoilage indicators like TVBN, TMA, biogenic amines (like histamine, putrescine, cadaverine) and even some volatile organic molecules are suppressed by the EOs (see supplementary text for examples). These observations confirm our meta-data derived relationship scores (i.e. 63-93% of deviance explained) presented in section 3.2 between the TVC and selected spoilage indicators.

3.5. Application methods of EOs on chilled-stored fish flesh and their comparison

3.5.1. Bulk EOs application

Applying bulk EOs directly on chill-stored fish is the most convenient way (Fig. 2B). In Fig. S2D, altogether, 78 cases were found using bulk EO on chill-stored fish. Immersion (31 out of 78 cases) was a primary style for EO’s direct application in fish, allowing EO to thoroughly and evenly adsorbed onto the fish surface (Fig. 2B). EO can also be applied via spraying (9/78 cases) or pipette dropping method followed by massaging (25/78 cases) (Fig. 2B and S2E). These methods usually have similar preservative effects on chilled-stored fish. For example - rainbow trout immersed with 3% thyme EO (Tokur et al., 2016) showed a similar change of TVC and other quality parameters with the counterpart received pipette-dropped 3.5% thyme EO (Meral et al., 2019). EOs can also be applied in an untouched manner by adding them into an absorbent pad (Kilinc et al., 2016), filter paper (Cai et al., 2015), or other vapor-forming apparatus (Navarro-Segura, Ros-Chumillas, Martínez-Hernández, & López-Gómez, 2020), and then sealed with fish in a container for several hours to evaporate (Fig. 2B). EO vapor could reach fish surface thoroughly and uniformly, which might improve its inhibitory effect on microorganisms. Navarro-Segura et al. (2020) found the oregano EO vapor method fared better in sea bream than the EO application method in the conventional touch way. However, EO vapor is not widely applied in chill-stored fish, and only 4 cases were found in our collected data.

Many disadvantages of using bulk EO solely pose limitations to its application. The high volatility, high sensitivity to environmental conditions, and low stability could lower its antibacterial properties. The direct application usually requires a high EO dose to ensure a good preservative effect, bringing strong unpleasant odors to fish and might result in sensory rejection. Thus, other preservative methods are often combined to overcome these deficiencies. We encountered 60 cases integrating bulk EO with other methods (Fig. S2D). Among them, our meta-analyses revealed that bulk EO + MAP or VP packaging ensures reasonably good antimicrobial efficacy of EOs, even sometimes comparable to active film or nanoemulsion based delivery systems (further

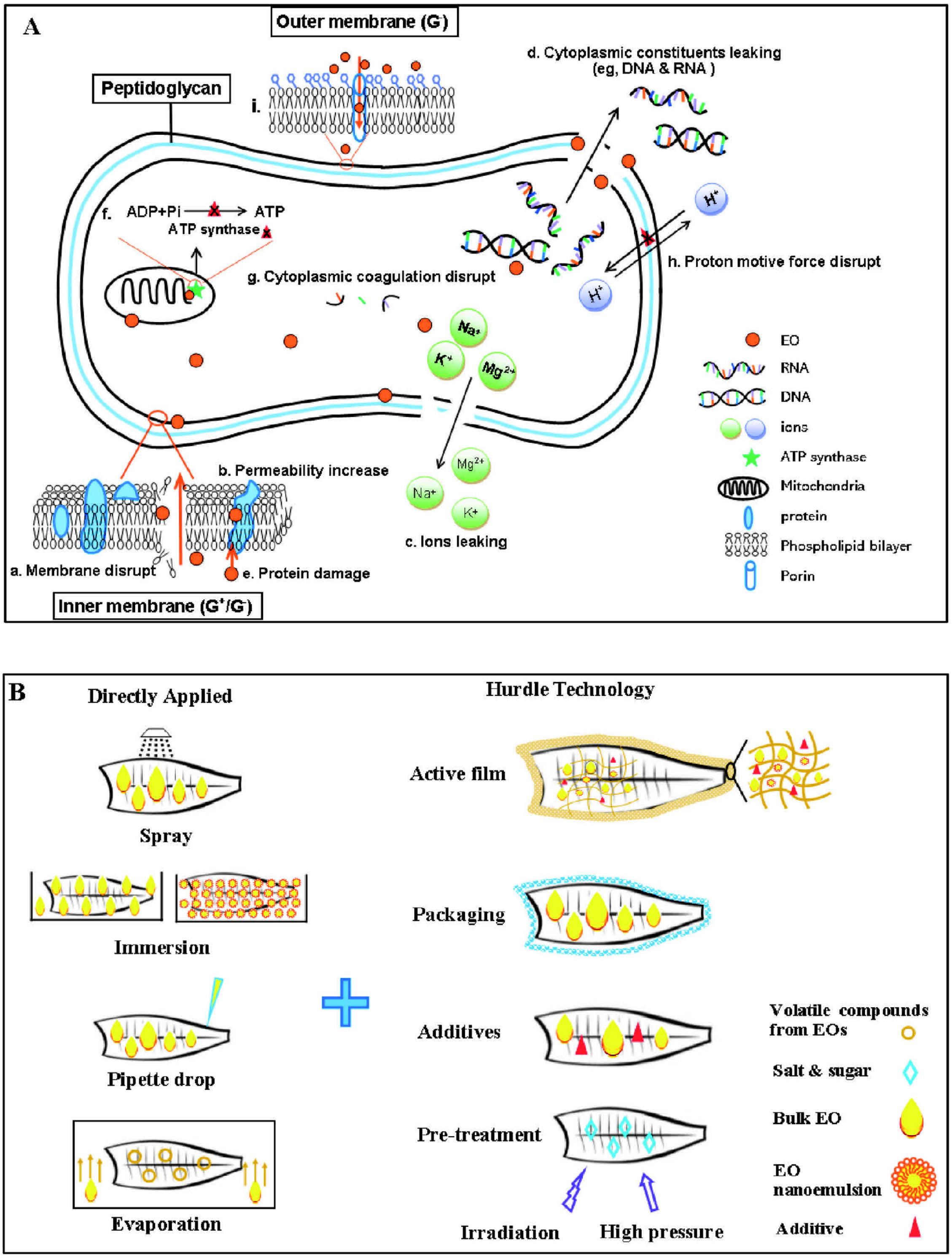

Fig. 2. (A and B): Mechanisms of the antibacterial function of EOs (A) and application methods (B) in chill-stored fish. Abbreviations: MAP - modified atmosphere package; VP - vacuum package; Pre-T – pre-treatment; HPP - high-pressure processing.

Table 1
Effects of essential oils (EOs) on microbial community and relevant quality parameters of chill-stored fish.

Fish	EOs	Preservation condition	Microbial composition		BAs changes (mg/kg)	Nucleotide degradation (μmol/g)	Other parameters	Reference
			Control	Groups				
Grass carp	Clove 0.5%	Nanoemulsion, 2% chitosan AP, 4 °C	Shewanellaceae spp. 35.4% Pseudomonadaceae spp. 26.1% Flavobacteriaceae spp. 21.5%	Lachnospiraceae spp. 15.6% Lactobacillaceae spp. 12.4% Ruminococcaceae spp. 10.2% Moraxellaceae spp. 6.0%		Hx 2.1↓ HxR 2.5↓	TVBN 6.5 mg/100g ↓ TMA 3.4mgN/100g↓	Yu, Regenstein, et al. (2018)
Grass carp	Oregano 0.1%	Nanoemulsion, AP, 4 °C	Aeromonas spp. 48.76%, Pseudomonas spp. 23.85% Shewanella spp. 9.64%	Aeromonas spp. 19.1% Pseudomonas spp. 65.0% Shewanella spp. 1.7%	PUT 2.6↓	Hx 1.2↓	TVBN 10.5 mg/100g↓	Huang et al. (2018)
Grass carp	Thyme 0.1%	Nanoemulsion, AP, 4 °C		Aeromonas spp. 38.2%, Pseudomonas spp. 33.1% Shewanella spp. 9.17%	PUT 1.2↓ CAD 0.4↓	Hx 0.7↓	TVBN 12.5 mg/100g↓	Huang et al. (2018)
Grass carp	Star anise 0.1%	Nanoemulsion, AP, 4 °C		Aeromonas spp. 36.5% Pseudomonas spp. 28.8% Shewanella spp. 7.28%	PUT 2.8↓ CAD 0.6↓	Hx 0.5↓	TVBN 13.5 mg/100g↓	Huang et al. (2018)
Grass carp	Cinnamon bark 0.1%	Nanoemulsion, AP, 4 °C	Pseudomonas spp. 65.4% Aeromonas spp. 19.7% Shewanella spp. 7.6%	Pseudomonas spp. 92.0% Aeromonas spp. 0.1% Shewanella spp. 1.40%	PUT 3.2↓ CAD 0.7↓	Hx 2.1↓	TVBN 18 mg/100g↓	Huang et al. (2017)
Common carp	Cinnamon 0.1%	Nanoemulsion, AP, 4 °C	Aeromonas spp. >90%	Lactococcus spp. >90%	PUT 9↓ CAD 6.5↓		TVBN 30.2%↓	Zhang, Li, et al. (2017)
Grass carp	Clove 0.1%	Nanoemulsion, 2% chitosan, AP, 4 °C				Hx 1.99↓ HxR 2.3↓	TMA 3 mg N/100g↓	Yu, Xu, et al. (2018)
Grass carp	Clove 0.5%	Nanoemulsion, 2% chitosan AP, 4 °C				Hx 1.97↓ HxR 2.5↓	TMA 3.5 mg N/100g↓	Yu, Xu, et al. (2018)
Grass carp	Clove 1%	Nanoemulsion, 2% chitosan AP, 4 °C				Hx 1.91↓ HxR 2.8↓	TMA 3.8 mg N/100g↓	Yu, Xu, et al. (2018)
Sea bass	Thyme 0.05%	AP, 0–2 °C				Hx 0.08↓	TVBN 3.6 mg/100g↓	Harpaz et al. (2003)
Sea bass	Oregano 0.05%	AP, 0–2 °C				Hx 0.06↓	TVBN 3.2 mg/100g↓	Harpaz et al. (2003)
Common carp	Oregano 112 ± 13 mg/fillet	VP, UV treated, 3.5 °C			PUT 20↓ CAD 38↓			Křížek et al. (2018)
Common carp	Thyme 112 ± 13 mg/fillet	VP, UV treated, 3.5 °C			PUT 19↓ CAD 37↓			Křížek et al. (2018)
Red drum	Clove 0.4%	AP, 4 °C			PUT 11↓ CAD 9↓ HIM 2↓			Cai et al. (2015)
Red drum	Cumin 0.4%	AP, 4 °C			PUT 11↓ CAD 9↓ HIM 12↓			Cai et al. (2015)
Red drum	Spearmint 0.4%	AP, 4 °C			PUT 12↓ CAD 10↓ HIM 12↓			Cai et al. (2015)
Rainbow trout	Citrus (grapefruit peel) 4%	Nanoemulsion, AP, 4 °C			PUT 32↓ CAD 18↓ HIM 0.4↓			Kosker (2020)
Rainbow trout	Citrus (lemon peel) 4%	Nanoemulsion, AP, 4 °C			PUT 31↓ CAD 12↓ HIM 2.4↓			Kosker (2020)
Rainbow trout	Citrus (mandarin peel) 4%	Nanoemulsion, AP, 4 °C			PUT 31↓ CAD 16↓ HIM 0.7↓			Kosker (2020)
Rainbow trout	Citrus (orange peel) 4%	Nanoemulsion, AP, 4 ± 2 °C			PUT 24↓ CAD 13↓ HIM 2.4↓			Kosker (2020)

Note: AP, air package; VP, vacuum package; MAP, modified air package; BA, biogenic amine; HIM, Histamine; PUT, putrescine; CAD, cadaverine; HXR, Inosine; Hx, Hypoxanthine; TVBN, total volatile basic nitrogen; TMA, trimethylamine nitrogen; ↓, decrease.

described below).

3.5.2. *EO in nanoemulsion*

EO nanoemulsion is an oil-in-water delivery system in colloidal dispersions (Fig. 2B). It is formed by combining two non-mixable phases like EO and water, which are stabilized by a food-grade surfactant (e.g., polysorbates, sugar ester) with a droplet size between 20 and 200 nm (Donsì et al., 2016). Studies suggest that EO nanoemulsions are more effective in preserving chill-stored fish. For example - in chill-stored rainbow trout treated with 4% sage EO nanoemulsion (Ozogul et al., 2017), the microbial inhibitory effect was doubled compared to 4% sage EO applied in bulk method (Çoban et al., 2016). Most fish treated with EO nanoemulsion exhibited a more distinct reduction in TVBN and Enterobacteriaceae than the fish treated with bulk EO alone. Noticeably, EO nanoemulsion provides the possibility of using a lower EO dose to achieve a similar antibacterial effect compared to the same EO applied conventionally. For example - Khanzadi et al. (2020) reported that nanoemulsions of 0.25% Zataria multiflora boiss EO had the same preservative effect as 1% of the same bulk EO in chill-stored rainbow trout. As our collected data showed (Fig. S2D), EO nanoemulsions are mostly coupled with active film (66/82 cases) and various packaging (15/82 cases) to preserve chill-stored fish, followed by additives (8/82 cases) and pre-treatment (4/82 cases). From about 82 cases applying EO nanoemulsions (Fig S3D), 12 cases successfully used EO nanoemulsions solely. Therefore, a significant effort is necessary in this line over the coming years.

EO nanoemulsion exhibits several advantages over conventional methods. It could stabilize (protect) EO, enables sustained release of EO active ingredients, evenly distribute to the fish's surface quite fast and enhance the passive cellular uptake by bacteria. Altogether this could improve EO's antimicrobial activity, reduce the application dose and minimize the sensory effects of EO on fish (section 3.9). Albeit the perceived advantages, our metadata-based observations hint nanoemulsion based delivery systems 'alone' cannot yield high antimicrobial efficacy all the time. Nanoemulsion EO incorporated into active films perform much better than EO-nanoemulsion alone. Nanoemulsion EO often performed lower than other application methods like bulk EO + MAP/VP, active film, or hurdle systems (further demonstrated below).

3.5.3. *EO in active film*

Another strategy to apply EOs is to incorporate them into active films to wrap chill-stored fish (Fig. 2B). In this case, active compounds in EO can be gradually released to perform their long-time preservative function and minimize adverse organoleptic effects on fish (Echeverría et al., 2018). We found 102 cases using film-EO combination for preserving chill-stored fish (Fig. S2D). Among them, 3 cases used inedible film prepared from polyethylene (LDPE) (Abedi et al., 2016; Dong et al., 2019), polypropylene (PP) (Dong et al., 2019), and ethylene-vinyl alcohol copolymer (EVOH) (Yang et al., 2016). In contrast, the other 99 cases used edible film. Chitosan film (35 cases) is the most studied edible film integrating EOs for fish preservation, followed by films from gelatin (10 cases), alginate (9 cases), carboxymethyl cellulose (7 cases), and whey protein isolate (6 cases). EO nanoemulsion can also be incorporated into film (66 cases). Our metadata analyses suggest active film incorporating EO nanoemulsions show much better antimicrobial efficacy than normal film containing bulk EO. Also, film-EO system was better than the sole bulk-EO application (further demonstrated below).

3.5.4. *Hurdle technology*

Combining two or more preservative technologies to establish a series of barriers to limit the proliferation of target microorganisms is called 'hurdle technology' (Fig. 2B). Due to the synergistic effect, EOs in a hurdle system often exhibit significant inhibitory effects on spoilage microorganisms than their sole application. It also enables a low dose of EO. Among our reviewed studies, EOs were commonly applied through such a hurdle system (130/160 cases) for chill-stored fish (Fig. S2D). Film-EO is the most common hurdle system (discussed above). Another standard hurdle system is combining packaging, mainly vacuum or modified atmosphere packaging, with EOs (Fig. 2B). VP or MAP could create an atmosphere that is not conducive for microorganisms to propagate, strengthening the preservative effect of EOs. We encountered 47 cases using the packaging-EO hurdle system. The Packaging-EO hurdle system usually shows a more substantial perseverative effect than sole EO in chill-stored fish, especially for LAB, PSE, HSP, and ENT. Some additives could be used with EOs better to protect the fish quality (Fig. 2B). We found 17 cases where eleven additives were applied with EO to create various additive-EO hurdle systems (Fig. S2D). We encountered a higher reduction in TVC by additive-EO hurdle systems than by sole EO. It is noticed that some pre-treatments, e.g., marinating (Van Haute et al., 2016), high hydrostatic pressure (HHP) (Gómez-Estaca et al., 2018), γ-irradiation (Abdeldaiem et al., 2018) and UV irradiation (Křížek et al., 2018) have been given to chill-stored fish before EO exposure (Fig. 2B). Pre-treatments could reduce the initial microbial load in fish, thereby improving the antibacterial effect of EO. We encountered only ten such cases in the collected studies. Such pretreatment-EO hurdle systems showed a lower microbial population, especially for LAB, PSE, and HSP, during chilled storage. Our metadata suggests hurdle technology, in general (with pre-treatment, additives, film and special packaging in various combinations), are good and reliable application methods for EO (further demonstrated below).

3.5.5. *Role of essential oils in the hurdle systems with pre-treatments*

Under chilled-storage conditions (0–6 °C), raw fish flesh without any pre-treatment or EO application whatsoever reaches unacceptable microbiological load (TVC limit = 7 log CFU g^{-1}; section 3.8) in 5–9 days (interquartile range, IR). With some pre-treatments like non-EO coatings and normal or special packaging, such shelf life could be extended by +1 to +6 days. As soon as EOs are combined with such pre-treatments (i.e., hurdle system), raw fish can be stored up to 11–20 days (IR) under chilled storage conditions. Such a shelf-life of 2–3 weeks of raw fish, with acceptable microbiological quality, is crucial for retailers and consumers. Although non-EO coatings (e.g., salt, sodium tripolyphosphate, casein, gelatin, zein, chitosan, alginate, methylcellulose, tuber starch, carrageenan, Persian gum, pectin, quinoa, nisin, lactoperoxidase) and special packaging (e.g., vacuum, modified atmosphere, ultraviolet irradiation) prolong the shelf life of raw fish, further prolongation is made possible by adding EOs to the equation. The essential role of EOs, precisely the bioactive compounds of the EOs (Table S5), in a hurdle system is demonstrated in Fig. 3.

3.6. *Microbial load reduction potential of EOs in chilled-stored fish flesh*

3.6.1. *General microbial load (TVC)*

The interquartile range (IR) of TVC reduction potential of EOs in chilled-stored fish flesh, irrespective of EO species and application method, was 0.87–4.61 log CFU g^{-1} per % concentration. Therefore, quite a high variability in EO's antimicrobial efficacy is apparent overall, on an average, 60% coefficient of variance. It implies not all EOs and/or application methods can yield promising results. The choice of EO species itself is an important variable that must be considered. Out of the 22 EOs we screened, only six qualified as top EOs (citrus, mentha, origanum, thymus, zataria, and zingiberaceae) having extraordinary TVC reduction potential (>4.61 log CFU g^{-1} per % concentration). Other EOs were somewhat comparable to each other. Seven EOs, i.e., basil, black pepper, chamomile, eucalyptus, satureja, and star anise, were identified as data deficient. They should be studied more. Even among the top EOs, high variability in anti-microbial efficacy was apparent, particularly in origanum followed by mentha and zingiberaceae (Fig. 4).

Few outliers were also encountered. Outliers had TVC reduction potential >30 log CFU g^{-1} per % concentration and/or PSE/ENT/HSE/LAB reduction potential >30 log CFU g^{-1} per % concentration. The outliers were mainly restricted within the EOs rosemary (more frequently), origanum, thymus, bay laurel, and cinnamon. They were excluded from the analyses and dealt with individually. Any common factor(s) which might be responsible was qualitatively investigated and presented in the next section.

3.6.2. *Pseudomonas (PSE)*

The IR of PSE reduction potential of EOs in chilled-stored fish flesh was 0.74–6.9 log CFU g^{-1} per % concentration; again, showing high

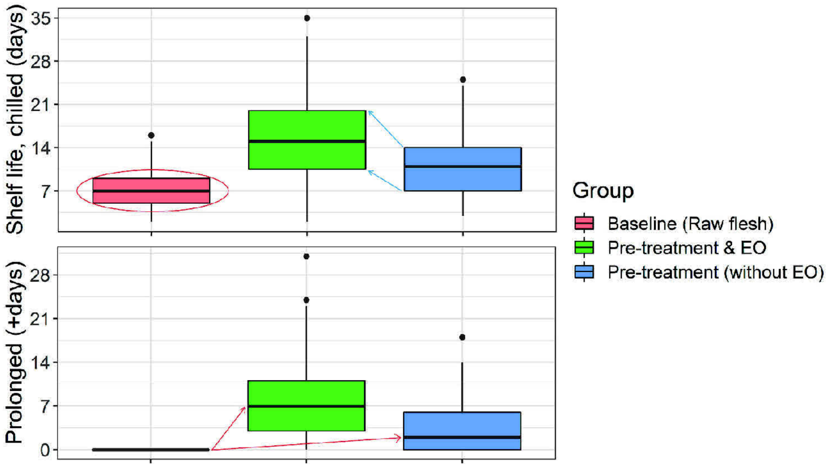

Fig. 3. Chilled-stored shelf life (days; top panel) and prolongation of shelf life (+days; bottom panel) of raw fish by pre-treatments alone or pre-treatment combined with essential oil application (hurdle system). Shelf life = day of breaching unacceptable TVC limit (7 log CFU g−1) under chilled storage conditions. The arrows highlighting improvement of quartile boundaries/mean indicate EO's presence in pre-treatments is advantageous. The red circle indicates the raw fish without any treatment(s) whatsoever. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

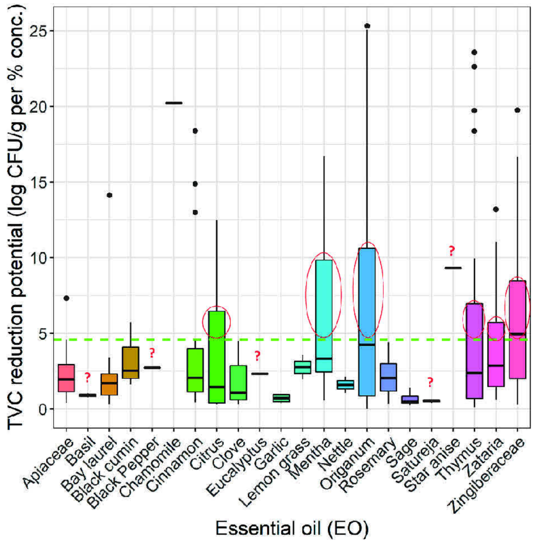

Fig. 4. General microbial load (TVC) reduction potential of different essential oils (EO) in chilled-stored fish flesh. Further breakdown against specific spoilage microbes is illustrated in Fig. 6. Note: red circles indicate top EOs performing above the extraordinary benchmark. The extraordinary benchmark is indicated by a green dashed line (values are given in sections 3.6.1 to 3.6.5). Question marks indicate data deficiency. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

variability (discussed above). Out of the 16 EOs with available data, only 5 EOs (cinnamon, mentha, origanum, rosemary, and zingiberaceae) showed extraordinary PSE reduction potential (>6.9 log CFU g−1 per % concentration). Origanum and rosemary had the most variable efficacy. Basil, chamomile, satureja, and star anise were data deficient (Fig. 5 and S3A). In terms of physiological response, EO can damage the macromolecules in Pseudomonas spp. cell membranes, causing loss of membrane permeability, efflux of K+, and respiratory activity inhibition leading to cell death. Moreover, coagulated cytoplasmic material and leaked intracellular material in the surrounding environment hinting rupture of cells on EO exposure (Bouhdid et al., 2010; Huang et al., 2019). Furthermore, the Pseudomonas spp. cell shape may change to coccoid form on EO exposure (Leja et al., 2019).

3.6.3. *Enterobacteriaceae (ENT)*

The IR of ENT reduction potential by EOs was 0.7–4.63 log CFU g−1 per % concentration, with high variability. Out of 16 EOs, only 5 EOs (apiaceae, mentha, origanum, thymus, and zataria) had extraordinary ENT reduction potential (>4.63 log CFU g−1 per % concentration). Origanum and mentha were the most variable ones. Basil, black pepper, garlic, lemongrass, sage, and satureja were data deficient (Fig. 5 and S3B). EO can damage the Escherichia coli cell membrane, increase cell membrane permeability, inhibit efflux pump and respiratory activity (de Sousa Guedes & de Souza, 2018). E. coli cells can become deformed, pitted, or shriveled. Leakage of cytoplasmic materials such as DNA materials, proteins, potassium ions, phosphate ions, and ATP can occur (Zhang, Ye, et al., 2017). EO can inhibit the endoenzymes like ATPase, ALP, topoisomerases, bioenergetic pathways like HMP pathway of E. coli, and DNA metabolism (Cui et al., 2015, 2018).

3.6.4. *H2S (hydrogen sulfide) producing bacteria (HSE)*

The HSE reduction potential by EOs had an IR of 0.76–6.91 log CFU g−1 per % concentration, much like the reduction potential of Pseudomonas spp. Out of the 15 EOs, only 3 EOs (origanum, thymus, and zingiberaceae) exhibited extraordinary ENT reduction potential (>6.91 log CFU g−1 per % concentration). Bothe origanum and thymus show high variabilities in their efficacy. Most of the EOs were data-deficient, namely apiaceae, basil, cinnamon, lemongrass, citrus, garlic, satureja, and star anise (Fig. 5 and S3C). Shewanella putrefaciens cells treated with EO lost their continuous structures, with unsmooth surface and almost no intracellular protoplasm. Moreover, EO damaged the macromolecules in S. putrefaciens cell membranes and specific membrane proteins (Huang et al., 2019). The cell membrane destruction decreased intracellular ATP through leakage (Lyu et al., 2018).

3.6.5. *Lactic acid-producing bacteria (LAB)*

With comparatively lowest variability, the LAB reduction potential of EOs had an IR of 0.6–3.82 log CFU g−1 per % concentration. Out of 18 EOs, only 5 EOs (bay laurel, origanum, thymus, zataria, and zingiberaceae) exhibited extraordinary LAB reduction potential (>3.82 log CFU g−1 per % concentration). Only origanum had the most variable efficacy. Basil, black pepper, chamomile, garlic, and lemongrass were the data deficient EOs (Fig. 5 and S3D). EO increased the membrane permeability and caused disruptive effects on the integrity of Lactobacillus spp. cells. Increased release of essential cell constituents, such as sugars and proteins, were recorded on exposure to EO (Ambrosio et al., 2020; Ziaee et al., 2018).

3.6.6. *Top EOs with complete broad-spectrum efficacy*

The top 6 EOs showing extraordinary TVC reduction potential (≈effect on general microbes) were screened against specific microbes (PSE, ENT, HSP, and LAB) too. Although all top EOs can have extraordinary TVC reduction potential but still may be weak against some specific microbe(s) (Fig. 6). Any EO which can be extraordinarily effective against all the major spoilage microbes in chilled stored fish may be a boon for the industry. In terms of complete or broad-spectrum efficacy, only 3 out of 6 top EOs qualified, i.e., origanum → zingiberaceae → thymus. Please note that zingiberaceae lacks data for the ENT category but based on existing observations, we extrapolated it to have complete-spectrum efficacy. It should be noted that the antimicrobial properties of these oils are related to the main bioactive compounds present in them. Interestingly, the top EOs shared few bioactive compounds in common: thymol, carvacrol, and γ-Terpinene (Table S5). The dominant bioactive compounds present in the reviewed EOs and their demonstrated antimicrobial efficacy is presented in Table S5.

Zataria and mentha were not extraordinarily effective against PSE and HSP, respectively (Fig. 6). The most limited choice of high efficacy EOs seems to be against HSP. Thus, more options need to be explored against HSP (Fig. 6 and S3D). Despite extraordinarily high TVC reduction potential, citrus could not demonstrate high effectivity against PSE, HSP, and LAB (Fig. 5D). Some EOs were also species-specific. For example – cinnamon and rosemary seem highly effective against PSE. Apiaceae and bay laurel seems highly effective against ENT and LAB, respectively. However, they had sub-average TVC reduction potential. We conclude only origanum, zingiberaceae, and thymus are the top broad-spectrum EOs having the potential to single-handedly 'close-the-loop' against diverse spoilage microorganisms in chilled-stored fish (Fig. 6). Origanum demonstrated the highest variability in performance

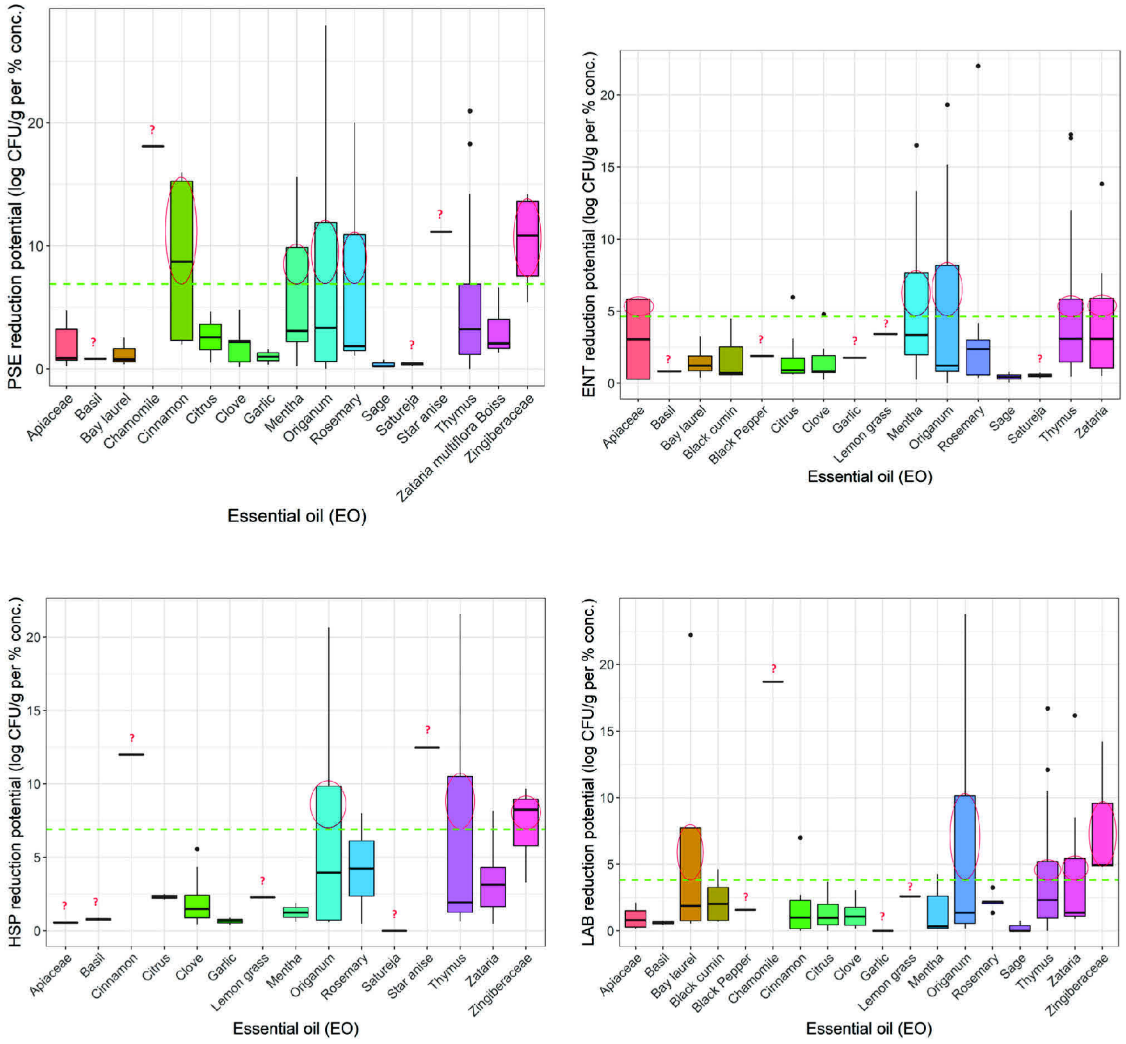

Fig. 5. Comparative account of essential oils (EO) antimicrobial efficacy against specific spoilage microbes in chilled-stored fish flesh. PSE= Pseudomonas spp.; ENT = Enterobacteriaceae spp.; HSP= H2S or sulfur producing bacteria reduction potential; E = lactic acid-producing bacteria. Note: red circles indicate top EOs performing above the extraordinary benchmark. The extraordinary benchmark is indicated by a green dashed line (values are given in sections 3.6.1 to 3.6.5). Question marks indicate data deficiency. High definition individual graphs can be found in the supplementary Fig S2A-D. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

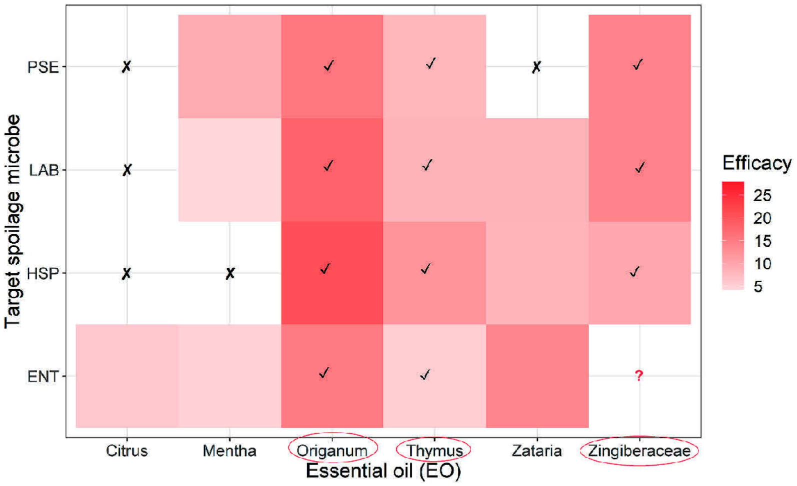

Fig. 6. Heatmap of the anti-microbial efficacy of top 6 EOs with extraordinary TVC reduction potential against specific spoilage microbes. Efficacy = microbial load reduction potential (log CFU g−1 per % concentration); ✓ = high specific efficacy across all microbe categories; ✗ = average to low efficacy against specific microbes. Red circles indicate top EOs with complete or almost-complete spectrum efficacy. Question mark indicate data unavailability. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Figs. 4 and 5). Hence, the application method selected for origanum EO could be a decisive factor in guaranteeing its highest performance (presented below).

3.7. Application method interferences on top EO's performances

Out of 7 primary application methods screened, four application methods seem to be associated with comparatively better TVC reduction potential per unit concentration (of the 6 top EOs traced). They are: (a) hurdle system – suitable for origanum, thymus, and zataria; (b) bulk EO + MAP/VP – good for origanum, thymus, and zataria; (c) active film – suitable for citrus and zingiberaceae, and; (d) nanoemulsion + active film – suitable for citrus and mentha (Fig. 7). Not all application methods have been equally explored for all the top EOs (e.g., citrus, mentha, zingiberaceae, and zataria); data-deficiency exists. Especially zingiberaceae should be explored more with different application methods (like already done for origanum). Modern delivery systems like nanoemulsions of EO might not always prove efficient (mostly alone); it is probably better when incorporated with an active film. Even the conventional EO application (i.e., bulk EO) can deliver high anti-microbial efficacy if combined with special packaging (Fig. 7).

After an investigation of the outliers (i.e., too high TVC/PSE/ENT/HSE/LAB reduction potentials), few common factors were noted: (a) chitosan coating of the fish flesh (as an additive), even by gelatin; (b) pre-treatment of meat by salting; (c) modified atmosphere packaging (purged by gaseous N2); (d) dissolving the EO in a cryoprotectant like

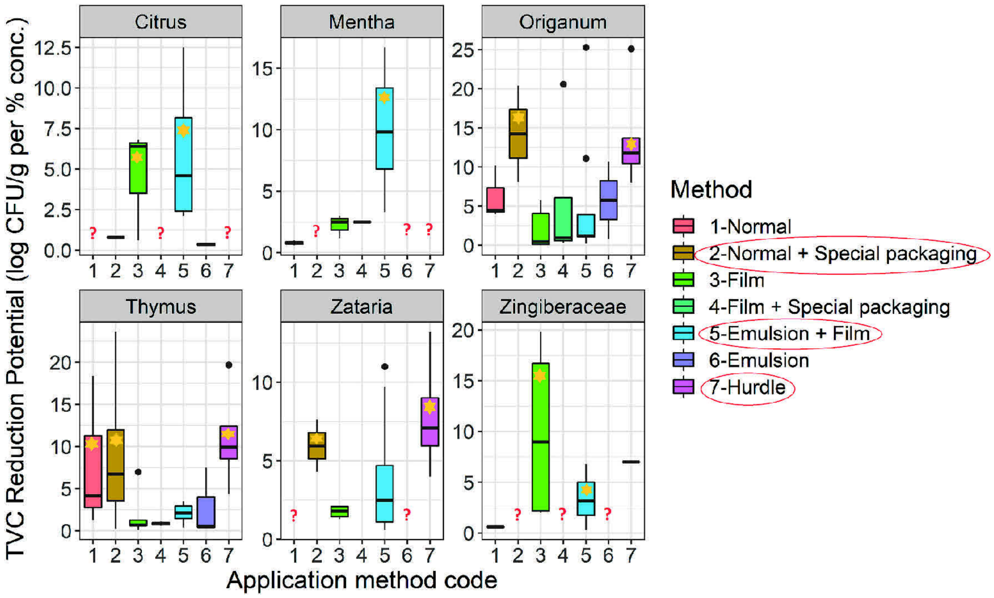

Fig. 7. Application method-specific microbial load reduction (TVC; log CFU g−1) by top EOs per unit of their concentration. Stars highlight 'good' application methods of the EOs associated with comparably better anti-microbial efficacy. Red circles indicate best performing application methods. Question marks imply insufficient data. Special packaging indicates vacuum or modified atmosphere packaging. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dimethyl sulfoxide (DMSO) and emulsifying it. With these additional hurdle technology components, we suspect even the performance of some EOs with average to sub-average TVC reduction potential (e.g., rosemary, bay laurel, cinnamon; see previous section) could be boosted.

Not all the EOs could be applied in the same way to obtain the highest efficacy. To some extent, the individual nature and characteristics of the EO(s) seem to play a role. For example – the TVC reduction potential in origanum (main volatile organic compound = carvacrol), thymus (thymol), or zingiberaceae (zingiberene) by 'nanoemulsion + active film' method was always below 5 log CFU g^{-1} per % concentration. However, in citrus (limonene), mentha (menthol), and zataria (linalool), the TVC reduction potential by 'nanoemulsion + active film' could surpass >5 log CFU g^{-1} per % concentration. Revisiting this trend from chemistry, the flash point of carvacrol, thymol, and zingiberene is above 100 °C (102–107 °C). Whereas, the flash point of limonene, menthol, and linalool were below 100 °C (48–93 °C) (www.guidechem.com/dictionary/en/). Whether such molecular traits have implications on an EO's anti-microbial efficacy when applied through different application methods needs to be researched in the future.

3.8. Metadata validation in the context of the international regulations

The present European Commission Regulation (EC) No. 2073/2005 sets legal microbiological criteria 'only' for histamine in fish and fishery products (http://data.europa.eu/eli/reg/2005/2073/2020-03-08). As previously pointed out (section 3.2. and Table S4), histamine formation is closely linked with spoilage microbes (more specifically by Enterobacteriaceae spp.; Table S4). Closely following commission regulation (EC No. 2073/2005), the countries have broadened and set their legislations regarding the acceptable quality of fish flesh or products. For example – a recent revision by the Food Safety Authority of Ireland (FSAI) has set 'borderline of quality' specifying permissible limits of microbial load (TVC or APC) in raw fish flesh at 6–7 log CFU g^{-1} (FSAI, 2019); same specifications exist in United Kingdom (HPA 2009). In North America (e.g., USA, Canada following ICMSF 1986) or Oceania (e.g., Australia, New Zealand following FSANZ, 2018) too, raw fish flesh having microbial load (TVC or APC) ≥6–7 log CFU g^{-1} is considered unacceptable. Therefore, internationally TVC below 6 log CFU g^{-1} in chilled-stored fish may be deemed satisfactory. These limits fall well in agreement with our GAM derived thresholds (aggravated spoilage at ≥5–7 log CFU g^{-1}; section 3.2).

Let us simulate an example. Assume at some time point (under chilled-stored conditions) some fish flesh has a microbial load >75% close (= TVC ≥4.5 log CFU g^{-1}) to the borderline (= TVC 6 log CFU g^{-1}). It could have been easily avoided by using EOs like origanum or zingiberaceae, or thymus. Per unit concentration, these EOs have the potential to reduce TVC by > 4.61 log CFU g^{-1} when applied by the right method (sections 3.6.1 and 3.7). If a mild 0.5% concentration origanum or thymus EO is applied with Bulk EO + VP/MAP packaging, or hurdle system method, the TVC may be reduced by at least >2.3 log CFU g^{-1}. Additionally, they are highly effective against histamine synthesizing microbes like Enterobacteriaceae spp. (section 3.6.4). Assuming an initial TVC load of ≥4.5 log CFU g^{-1}, the final TVC load would be ≤ 2.2 log CFU g^{-1}. This is only ≥37% close to (or ~60% far from) the borderline, ensuring acceptable quality and suppressing histamine formation. Therefore, our metadata derived recommendations (sections 3.6 and 3.7) are much relevant in the context of international regulation on microbiological safety of fishery products.

3.9. Impact of EO on sensory properties of fish flesh

The sensory properties of any meat or meat-like product (here, fish flesh) matter most for the end customers. Their perception and measurement are highly circumstantial and qualitative, respectively. It can be independent of spoilage (if EO itself interferes in a fresh fish) or intertwined with spoilage (if spoilage imparts a foul smell and taste). We could only assess the sense of odor in EO-treated fish versus untreated fresh fish from our present metadata due to sufficient data available on this aspect. Other important sensory properties like color or taste could not be assessed due to scattered to insufficient data. Usually, fish flesh without EO achieves an unacceptable odor score in 5–9 days (=tnon-EO). At tnon-EO, the EO treated flesh still has a better odor than control (IR +1.3 to +3.2 odor score per % EO concentration (than control)). Hence, reaching an unacceptable odor score in EO treated flesh is effectively delayed by +2 to +8 days, per % concentration of EO (=tEO). The daily sensory score deterioration rate is also faster in untreated fish flesh (IR −30% to −68.8%). Such deterioration rate (decrease in sensory score per day) in EO treated flesh is ~2.5–5 times slower (IR −6% to −28.5%, per % EO concentration). The LOESS (locally weighted scatterplot smoothing) model simulated on available data (Fig. 8) shows EO treatment effectively prolonging the shelf life of raw fish flesh ($p < 0.05$) with acceptable odor properties.

From the marketing perspective, any EO treatment must not significantly alter the original sensory properties of fresh fish. Right on EO application (=t0), the difference in odor values of EO treated flesh was IR −0.5% to +0.15% per % EO concentration than untreated fresh fish (=control). Extreme variability exists (CV >1000%) in these differences. We attributed them to some specific EOs, their threshold concentrations, or interactions with particular fish species. For example – EOs like star anise or citrus when used on carp or rainbow trout respectively, or rosemary when used at ≥1% concentrations, caused 22–25% deterioration in odor properties than control. Whereas oregano EO at ≥1% concentration improved the odor score of European eel flesh by +17 to +22% than control. In general, our data implies 0.5–1% concentration of most EOs impart little interference on odor properties of fresh fish since the percentage change could be negligible (close to 0%). Data on EO specific impact on sensory properties right on the application are usually scanty (Fig. 9). Based on limited data, it is evident that some EOs might have a detrimental impact on the odor properties of fresh fish right on application (e.g., citrus, star anise, thyme). Caution must be exercised for them. Simultaneously, some EOs like origanum could even boost the odor properties of fresh fish (Fig. 9). Despite any small drop in odor properties caused by EO treatment initially, such little disadvantage may be transformed to perceptible advantage over the chilled storage duration. However, relying only on odor values might be misleading in the absence of other sensory parameters like color and taste. For example – even if the odor values are acceptable, EO application must not interfere with the aftertaste of cooked fish or color (e.g., rosemary's flavor in cooked fish or greenish tinge in raw flesh at higher concentrations; Linhartova et al., 2019). Such information is much needed for the future.

3.10. Key knowledge gaps and research needs

From a research perspective – data deficiencies of some less-explored, probably non-conventional EOs in the chilled-stored seafood sector should be addressed soon. For example, data on basil, black pepper, chamomile, eucalyptus, garlic, sage, satureja, and star anise EOs are required. Based on hints from limited data, two EOs, namely chamomile and star anise, may prove to be 'wild card' entries among the top EOs category. The anti-microbial efficacy of most EOs against sulfur-producing bacteria is still mostly unknown. They are perhaps the most difficult microbes to suppress since they can thrive even in nitrogen-purged or vacuum-based packaging. Especially, zingiberaceae EO should be assessed against H2S producing bacteria in chilled-stored fish flesh. It would close this EO's loop against all the major spoilage microbes. The interferences of different application methods on the anti-microbial efficacy of some top-performing EOs are still unknown. For example - hurdle or bulk EO type application methods integrated with special packaging (MAP or VP) are yet to be tested in zingiberaceae, mentha, and citrus.

Frequent and high variabilities in the anti-microbial efficacy of any

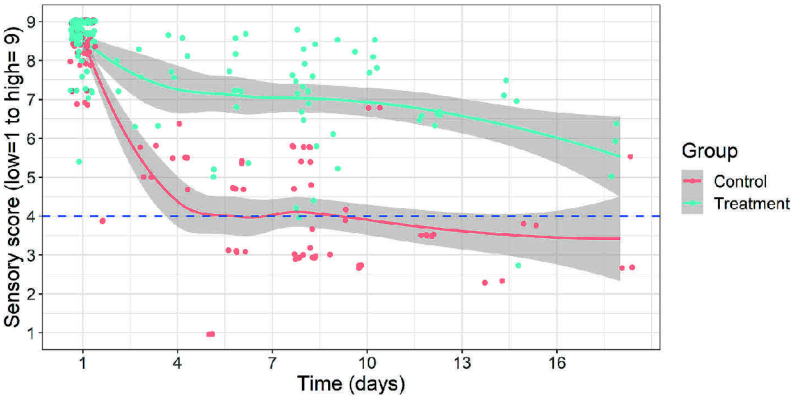

Fig. 8. Change of sensory properties with time in non-EO applied (control) versus EO-applied fish flesh (treatment) under chilled-storage conditions. The horizontal blue line shows the threshold of generally unacceptable odor score (below 4). EO application visibly prolongs the shelf-life of raw fish-flesh with an acceptable sensory score up to 2–2.5 weeks at least, under chilled-storage conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

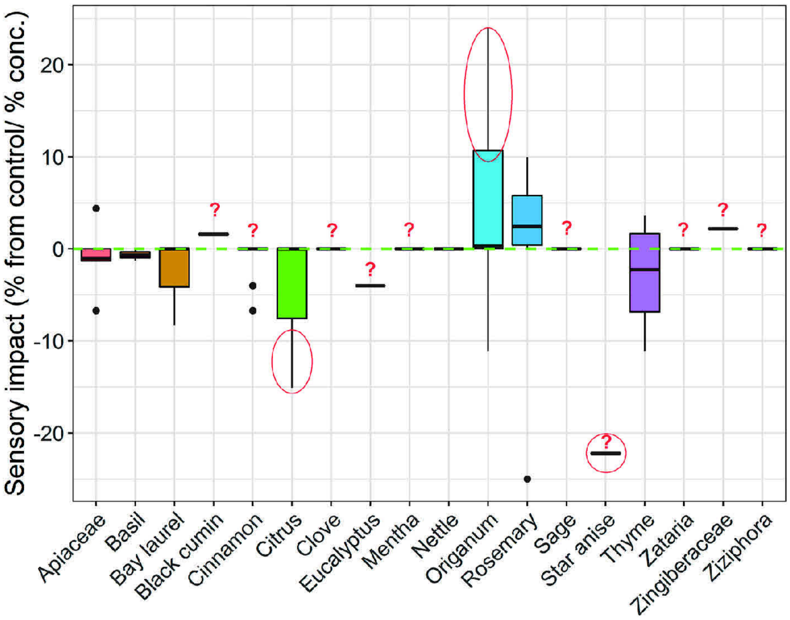

Fig. 9. Sensory impact of specific EOs right on application (at t0) relative to fresh fish flesh (control; horizontal green line). Positive values indicate EOs amplifying the original sensory properties of fresh fish on application. Negative values indicate detrimental sensory impact of EOs on application. Red circles indicate EOs with possibly high impact on the sensory property at higher concentrations. Question mark indicate data deficiency. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

EO cast doubt on its adoption over a large scale. In this regard, future efforts should be focused on reducing the variability in the performance of EOs; particularly, origanum. It may be accomplished by optimizing the 'already good' application methods (hurdle system; bulk EO + modified atmosphere or vacuum packaging) with or without performance boosters (additives like chitosan coating, salt pre-treatment, dissolving EO in cryoprotectants, N2 purged packaging). Existing evidence suggest nanoemulsion-based delivery systems integrated with active films perform better, much in contrast to EO nanoemulsions sprayed directly on fish flesh. It needs to be further validated. New ways of designing experiments could also be explored, e.g., flash-point based application methods for specific EOs.

Content of different bioactive compounds in the plants (and EOs derived from them) may be influenced by different factors such as plant varieties (strains), soil, agro-climatic conditions, altitude, the process of drying, etc. For example – altitude factor in apiaceae (Sanli and Karadogan, 2017), location × drying method interactions in mentha (Teles et al., 2013), or region-specific variations eugenol content in clove oil (Amelia et al., 2017). These may likely influence the antimicrobial efficacy of EOs too. Jordanian origanum EO suppressed E. coli much better than origanum from Saudi Arabia, despite having comparable carvacrol content (Khan et al., 2019). There is a knowledge gap in this regard as most studies do not report the composition, country, and technology of origin of the studied EOs. Therefore, the relation between origin and effect cannot be evaluated now.

From an industry perspective – there have been some limited attempts to isolate the active ingredient of EOs (Hyldgaard et al., 2012) and apply those isolates to increase anti-microbial efficacy. For example – rosemary extract 'Inolens 4', at low concentrations (≤0.5%), have been successfully tested to reduce microbial load and spoilage of fish fillets under refrigerated conditions (Linhartova et al., 2019; Sternisa et al., 2020). Although they allow lower treatment concentrations (below 1%) to successfully inhibit microbial spoilage, they can still bring undesirable interferences with the product's sensory qualities (also essential for the customers). For example, at concentrations >0.5%, 'Inolens 4' progressively interferes with fresh fish's original sensory attributes despite inhibiting microbes (Linhartova et al., 2019). One of the drawbacks we realized in our reviewed article pool was the missing evaluations of sensory properties. Only 32 out of 142 reviewed articles (22% of the studies) incorporated any sensory evaluation in their experiment – that too with varying sensory scores scale(s). Future researchers should consider including the impact of EO treatments on sensory properties of fish flesh, keeping a standard scale (1–9, with <4 as unacceptable), if possible. Advanced sensory tests should be conducted considering a broad-spectrum of sensory parameters – odor, color, and texture (in both raw and cooked meat) and taste, aftertaste (in cooked meat). For commercial applications, many people (100–200) should be considered in the sensory test panel rather than an internal evaluation with limited people (10–30). Fish product and EO species-specific life cycle assessment (LCA; precisely economic LCAs) studies are also necessary. Information on the economic feasibility of top EOs in combination with their right application methods against different retail price-range of chilled-stored products should be generated. The possibility of reducing the concentration of EOs without compromising anti-microbial efficacy but significantly minimizing sensory quality interferences should be intuitively explored.

4. Conclusion

The microbial load is a significant spoilage driver in chilled stored seafood, even under chilled temperature and different packaging conditions. EOs provide a useful natural tool to suppress microbial load and spoilage under chilled-stored conditions. Out of the new spectrum of EOs used, only a limited assortment of EOs have high anti-microbial and broad-spectrum efficacy. The high efficacy is further ensured by the selection of the right application method(s). Based on present microbiological safety limits adopted internationally for raw fish flesh, selected EOs with the right application method can assure satisfactory quality even at mild concentrations. A judicious EO treatment can effectively prolong shelf life without compromising the products' original sensory properties. The present article provides those good management practices of EO's application in chilled stored seafood. Critical knowledge gaps and research needs to advance this field are highlighted.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2021.02.054.

References

Abdeldaiem, M. H., Mohammad, H. G., & Ramadan, M. F. (2018). Improving the quality of silver carp fish fillets by gamma irradiation and coatings containing rosemary oil. *Journal of Aquatic Food Product Technology, 27*(5), 568–579. https://doi.org/10.1080/10498850.2018.1461157

Abedi, E., Naseri, M., Ghanbarian, G. A., & Vazirzadeh, A. (2016). Coverage of polyethylene film with essential oils of thyme (Thymus daenensis celak) and savory (Satureja bachtiarica bunge) for lipid oxidation control in rainbow trout (Oncorhynchus mykiss) fillets during short-term storage in the refrigerator. *Journal of Food Processing and Preservation, 40*(3), 483–491. https://doi.org/10.1111/jfpp.12627

Ambrosio, C. M. S., Contreras-Castillo, C. J., & Da Gloria, E. M. (2020). In vitro mechanism of antibacterial action of a citrus essential oil on an enterotoxigenic Escherichia coli and Lactobacillus rhamnosus. *Journal of Applied Microbiology, 129*, 541–553.

Amelia, B., Saepudin, E., Cahyana, A. H., Rahayu, D. U., Sulistyoningrum, A. S., & Haib, J. (2017). GC-MS analysis of clove (Syzygium aromaticum) bud essential oil from Java and Manado. In *, 1862. AIP Conference Proceedings*. AIP Publishing LLC, Article 030082, 1.

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology, 48*, 51–62.

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2003). Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. *Food Research International, 36*(8), 787–795. https://doi.org/10.1016/S0963-9969(03)00073-5

Bouhdid, S., Abrini, J., Amensour, M., Zhiri, A., Espuny, M. J., & Manresa, A. (2010). Functional and ultrastructural changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Cinnamomum verum essential oil. *Journal of Applied Microbiology, 109*, 1139–1149.

Cai, L., Cao, A., Li, Y., Song, Z., Leng, L., & Li, J. (2015). The effects of essential oil treatment on the biogenic amines inhibition and quality preservation of red drum (Sciaenops ocellatus) fillets. *Food Control, 56*, 1–8. https://doi.org/10.1016/j.foodcont.2015.03.009

Çoban, O. E., Patir, B., Özpolat, E., & Kuzgun, N. K. (2016). Improving the quality of fresh rainbow trout by sage essential oil and packaging treatments. *Journal of Food Safety, 36*(3), 299–307. https://doi.org/10.1111/jfs.12242

Cui, H., Bai, M., Sun, Y., Abdel-Samie, M. A.-S., & Lin, L. (2018). Antibacterial activity and mechanism of Chuzhou chrysanthemum essential oil. *Journal of Functional Foods, 48*, 159–166.

Cui, H., Zhang, X., Zhou, H., Zhao, C., & Lin, L. (2015). Antimicrobial activity and mechanisms of Salvia sclarea essential oil. *Botanical Studies, 56*, 16.

Dong, Z., Luo, C., Guo, Y., Ahmed, I., Pavase, T. R., Lv, L., et al. (2019). Characterization of new active packaging based on PP/LDPE composite films containing attapulgite loaded with Allium sativum essence oil and its application for large yellow croaker (Pseudosciaena crocea) fillets. *Food Packaging and Shelf Life, 20*, 100320. https://doi.org/10.1016/j.fpsl.2019.100320

Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. *Journal of Biotechnology, 233*, 106–120. https://doi.org/10.1016/j.jbiotec.2016.07.005

Echeverría, I., López-Caballero, M. E., Gómez-Guillén, M. C., Mauri, A. N., & Montero, M. P. (2018). Active nanocomposite films based on soy proteins-montmorillonite- clove essential oil for the preservation of refrigerated bluefin tuna (Thunnus thynnus) fillets. *International Journal of Food Microbiology, 266*, 142–149. https://doi.org/10.1016/j.ijfoodmicro.2017.10.003

Falleh, H., Ben Jemaa, M., Saada, M., & Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. *Food Chemistry, 330*, 127268. https://doi.org/10.1016/j.foodchem.2020.127268

FAO, Food and Agriculture Organization. (2011). *Global food losses and food waste - extent causes and prevention Global food losses and food waste - extent causes and prevention*. Rome: Italy.

FAO, Food and Agriculture Organization. (2020). *The state of world fisheries and aquaculture 2020*. https://doi.org/10.4060/ca9229en. Sustainability in action. Rome.

FSAI, Food Safety Authority of Ireland. (2019). *Guidance note No. 3 guidelines for the interpretation of results of microbiological testing of ready-to-eat foods placed on the market (revision 3)*. Dublin: Food Safety Authority of Ireland, ISBN 0-9539183-5-1.

FSANZ. (2018). *Compendium of microbiological criteria for food (january 2018)* (p. 51p). Food Standards Australia New Zealand.

Gómez-Estaca, J., López-Caballero, M. E., Martínez-Bartolomé, M.Á., de Lacey, A. M. L., Gómez-Guillen, M. C., & Montero, M. P. (2018). The effect of the combined use of high pressure treatment and antimicrobial edible film on the quality of salmon carpaccio. *International Journal of Food Microbiology, 283*, 28–36. https://doi.org/10.1016/j.ijfoodmicro.2018.06.015

Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology, 13*(3), 262–266. https://doi.org/10.1016/s0958-1669(02)00309-9

Hao, R., Pan, J., Tilami, S. K., Shah, B. R., & Mráz, J. (2021). Post-mortem quality changes of common carp (Cyprinus carpio) during chilled storage from two culture systems. *Journal of the Science of Food and Agriculture, 101*(1), 91–100.

Harpaz, S., Glatman, L., Drabkin, V., & Gelman, A. (2003). Effects of herbal essential oils used to extend the shelf life of freshwater-reared asian sea bass fish (Lates calcarifer). *Journal of Food Protection, 66*(3), 410–417. https://doi.org/10.4315/0362-028x-66.3.410

Hassoun, A., & Çoban, Ö. E. (2017). Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. *Trends in Food Science & Technology, 68*, 26–36.

HPA, Health Protection Agency. (2009). *Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market*. London: Health Protection Agency.

Huang, Z., Jia, S., Zhang, L., Liu, X., & Luo, Y. (2019). Inhibitory effects and membrane damage caused to fish spoilage bacteria by cinnamon bark (Cinnamomum tamala) oil. *Lebensmittel-Wissenschaft und -Technologie, 112*, 108195.

Huang, Z., Liu, X., Jia, S., & Luo, Y. (2017). Antimicrobial effects of cinnamon bark oil on microbial composition and quality of grass carp (Ctenopharyngodon idellus) fillets during chilled storage. *Food Control, 82*, 316–324.

Huang, Z., Liu, X., Jia, S., Zhang, L., & Luo, Y. (2018). The effect of essential oils on microbial composition and quality of grass carp (Ctenopharyngodon idellus) fillets during chilled storage. *International Journal of Food Microbiology, 266*, 52–59. https://doi.org/10.1016/j.ijfoodmicro.2017.11.003

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology, 3*, 12.

ICMSF, International Commission on Microbiological Specifications for Foods. (1986). Microorganisms in foods 2. Sampling for microbiological analysis: Principles and specific applications. In *International commission on microbiological specifications for foods* (2nd ed.) Netherlands.

Jääskeläinen, E., Jakobsen, L. M. A., Hultman, J., Eggers, N., Bertram, H. C., & Björkroth, J. (2019). Metabolomics and bacterial diversity of packaged yellowfin tuna (Thunnus albacares) and salmon (Salmo salar) show fish species-specific spoilage development during chilled storage. *International Journal of Food Microbiology, 293*, 44–52. https://doi.org/10.1016/j.ijfoodmicro.2018.12.021

Jayasena, D. D., & Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology, 34*(2), 96–108. https://doi.org/10.1016/j.tifs.2013.09.002

Khan, M., Khan, S. T., Khan, M., Mousa, A. A., Mahmood, A., & Alkhathlan, H. Z. (2019). Chemical diversity in leaf and stem essential oils of Origanum vulgare L. and their effects on microbicidal activities. *AMB Express, 9*(1), 176.

Khanzadi, S., Keykhosravy, K., Hashemi, M., & Azizzadeh, M. (2020). Alginate coarse/nanoemulsions containing Zataria multiflora Boiss essential oil as edible coatings and the impact on microbial quality of trout fillet. *Aquaculture Research, 51*(3), 873–881. https://doi.org/10.1111/are.14418

Kilinc, B., & Altas, S. (2016). Effect of absorbent pads containing black seed or rosemary oils on the shelf life of sardine [Sardina pilchardus (Walbaum, 1792)] fillets. *Journal of Applied Ichthyology, 32*(3), 552–558. https://doi.org/10.1111/jai.13044

Kosker, A. R. (2020). The effects of nanoemulsions based on citrus essential oils on the formation of biogenic amines in trout fillets stored at 4 ± 2°C. *Journal of Food Safety, 40*(1), Article e12762. https://doi.org/10.1111/jfs.12762

Křížek, M., Dadáková, E., Vácha, F., Pelikánová, T., & Matějková, K. (2018). The effects of two essential oil and UV-light irradiation treatments on the formation of biogenic amines in vacuum packed fillets of carp (Cyprinus carpio). *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 95*, 268–273. https://doi.org/10.1016/j.lwt.2018.04.097

Leja, K., Szudera-Kończal, K., Świtała, E., Juzwa, W., Kowalczewski, P.Ł., & Czaczyk, K. (2019). The influence of selected plant essential oils on morphological and physiological characteristics in Pseudomonas orientalis. *Foods, 8*, 277.

Linhartová, Z., Lunda, R., Dvořák, P., Bárta, J., Bártová, V., Kadlec, J., et al. (2019). Influence of rosemary extract (Rosmarinus officinalis) Inolens to extend the shelf life of vacuum-packed rainbow trout (Oncorhynchus mykiss) fillets stored under refrigerated conditions. *Aquaculture International, 27*(3), 833–847.

Lyu, F., Hong, Y.-l., Cai, J.-h., Wei, Q.-q., Zhou, X., Ding, Y.-t., Liu, Z.-f., & Liu, L. (2018). Antimicrobial effect and mechanism of cinnamon oil and gamma radiation on Shewanella putrefaciens. *Journal of Food Science & Technology, 55*, 3353–3361.

Mei, J., Ma, X., & Xie, J. (2019). Review on natural preservatives for extending fish shelf life. *Foods, 8*(10), 490.

Meral, R., Ceylan, Z., & Kose, S. (2019). Limitation of microbial spoilage of rainbow trout fillets using characterized thyme oil antibacterial nanoemulsions. *Journal of Food Safety, 39*(4), Article e12644. https://doi.org/10.1111/jfs.12644

Moosavi-Nasab, M., Mirzapour-Kouhdasht, A., & Oliyaei, N. (2019). *Application of essential oils for shelf-life extension of seafood products Essential Oils-Oils of Nature*. IntechOpen.

Navarro-Segura, L., Ros-Chumillas, M., Martínez-Hernández, G. B., & López-Gómez, A. (2020). A new advanced packaging system for extending the shelf life of refrigerated farmed fish fillets. *Journal of the Science of Food and Agriculture, 100*, 4601–4611, 10.1002/jsfa.10520.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals, 6*(12), 1451–1474. https://doi.org/10.3390/ph6121451

Olatunde, O. O., & Benjakul, S. (2018). Natural preservatives for extending the shelf-life of seafood: A revisit. *Comprehensive Reviews in Food Science and Food Safety, 17*(6), 1595–1612.

Ozogul, Y., Yuvka, İ., Ucar, Y., Durmus, M., Kösker, A. R., Öz, M., & Ozogul, F. (2017). Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (Oncorhynchus mykiss) fillets during ice storage. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 75*, 677–684. https://doi.org/10.1016/j.lwt.2016.10.009

Parlapani, F. F., Haroutounian, S. A., Nychas, G.-J. E., & Boziaris, I. S. (2015). Microbiological spoilage and volatiles production of gutted European sea bass stored under air and commercial modified atmosphere package at 2 °C. *Food Microbiology, 50*, 44–53. https://doi.org/10.1016/j.fm.2015.03.006

Patel, S. (2015). Plant essential oils and allied volatile fractions as multifunctional additives in meat and fish-based food products: A review. *Food Additives & Contaminants: Part A, 32*(7), 1049–1064. https://doi.org/10.1080/19440049.2015.1040081

Prakash, B., Kujur, A., Yadav, A., Kumar, A., Singh, P. P., & Dubey, N. K. (2018). Nanoencapsulation: An efficient technology to boost the antimicrobial potential of plant essential oils in food system. *Food Control, 89*, 1–11. https://doi.org/10.1016/j.foodcont.2018.01.018

Şanli, A., & Karadoğan, T. (2017). Geographical impact on essential oil composition of endemic Kundmannia anatolica Hub.-Mor.(Apiaceae). *African Journal of Traditional, Complementary and Alternative Medicines, 14*(1), 131–137.

Shojaee-Aliabad, S., Hosseini, S. M., & Mirmoghtadaie, L. (2018). *Antimicrobial activity of essential oil essential oils in food processing: Chemistry, safety and applications*. USA: John Wiley & Sons Ltd.

de Sousa Guedes, J. P., & de Souza, E. L. (2018). Investigation of damage to Escherichia coli, Listeria monocytogenes and Salmonella Enteritidis exposed to Mentha arvensis L. and M. piperita L. essential oils in pineapple and mango juice by flow cytometry. *Food Microbiology, 76*, 564–571.

Sternisa, M., Purgatorio, C., Paparella, A., Mraz, J., & Smole Možina, S. (2020). Combination of rosemary extract and buffered vinegar inhibits Pseudomonas and Shewanella growth in common carp (Cyprinus carpio). *Journal of the Science of Food and Agriculture, 100*(5), 2305–2312.

Teles, S., Pereira, J. A., Santos, C. H., Menezes, R. V., Malheiro, R., Lucchese, A. M., & Silva, F. (2013). Effect of geographical origin on the essential oil content and composition of fresh and dried Mentha× villosa Hudson leaves. *Industrial Crops and Products, 46*, 1–7.

Tilami, S. K., Sampels, S., Zajíc, T., Krejsa, J., Másílko, J., & Mráz, J. (2018). Nutritional value of several commercially important river fish species from the Czech Republic. *PeerJ, 6*, Article e5729.

Tokur, B. K., Sert, F., Aksun, E. T., & Özoğul, F. (2016). The effect of whey protein isolate coating enriched with thyme essential oils on trout quality at refrigerated storage (4 ± 2°C). *Journal of Aquatic Food Product Technology, 25*(4), 585–596. https://doi.org/10.1080/10498850.2014.896063

Van Haute, S., Raes, K., Van Der Meeren, P., & Sampers, I. (2016). The effect of cinnamon, oregano and thyme essential oils in marinade on the microbial shelf life of fish and meat products. *Food Control, 68*, 30–39.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.

Wood, S. N. (2017). *Generalized additive models- an introduction with R* (2 ed.). Chapman and Hall/CRC Press.

Yang, H., Wang, J., Yang, F., Chen, M., Zhou, D., & Li, L. (2016). Active packaging films from ethylene vinyl alcohol copolymer and clove essential oil as shelf life extenders for grass carp slice. *Packaging Technology and Science, 29*(7), 383–396. https://doi.org/10.1002/pts.2215

Yin, X., Luo, Y., Fan, H., Feng, L., & Shen, H. (2016). Effect of freeze-chilled treatment on flavor of grass carp (ctenopharyngodon idellus) fillets and soups during short-term storage. *Journal of Aquatic Food Product Technology, 25*(5), 777–787. https://doi.org/10.1080/10498850.2014.987862

Yu, D., Regenstein, J. M., Zang, J., Jiang, Q., Xia, W., & Xu, Y. (2018a). Inhibition of microbial spoilage of grass carp (Ctenopharyngodon idellus) fillets with a chitosan-based coating during refrigerated storage. *International Journal of Food Microbiology, 285*, 61–68. https://doi.org/10.1016/j.ijfoodmicro.2018.07.010

Yu, D., Wu, L., Regenstein, J. M., Jiang, Q., Yang, F., Xu, Y., & Xia, W. (2020). Recent advances in quality retention of non-frozen fish and fishery products: A review. *Critical Reviews in Food Science and Nutrition, 60*(10), 1747–1759. https://doi.org/10.1080/10408398.2019.1596067

Yu, D., Xu, Y., Regenstein, J. M., Xia, W., Yang, F., Jiang, Q., & Wang, B. (2018b). The effects of edible chitosan-based coatings on flavor quality of raw grass carp (Ctenopharyngodon idellus) fillets during refrigerated storage. *Food Chemistry, 242*, 412–420. https://doi.org/10.1016/j.foodchem.2017.09.037

Zhang, Y., Li, D., Lv, J., Li, Q., Kong, C., & Luo, Y. (2017b). Effect of cinnamon essential oil on bacterial diversity and shelf-life in vacuum-packaged common carp (Cyprinus carpio) during refrigerated storage. *International Journal of Food Microbiology, 249*, 1–8.

Zhang, J., Ye, K.-P., Zhang, X., Pan, D.-D., Sun, Y.-Y., & Cao, J.-X. (2017). Antibacterial activity and mechanism of action of black pepper essential oil on meat-borne Escherichia coli. *Frontiers in Microbiology, 7*.

Ziaee, E., Razmjooei, M., Shad, E., & Eskandari, M. H. (2018). Antibacterial mechanisms of Zataria multiflora Boiss. essential oil against Lactobacillus curvatus. *Lebensmittel-Wissenschaft und -Technologie, 87*, 406–412.

CHAPTER 4

DEVELOPMENT OF ESSENTIAL OIL-EMULSION BASED COATING AND ITS PRESERVATIVE EFFECTS ON COMMON CARP

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Development of essential oil-emulsion based coating and its preservative effects on common carp

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Storage quality

ABSTRACT

Fish fillets are susceptible to microbial spoilage during chilled storage. Green consumerism promotes the development of biodegradable, recyclable and environmentally friendly food packaging. The study selected three essential oils (EOs), i.e. thyme, oregano and pimento EO, with good antibacterial activity, to develop an EO-emulsion based alginate coating. A stable alginate coating with good antibacterial effects could be prepared under optimized conditions of homogenization (18,000 rpm, 3 min) and EO/Tween-80 ratio (20:1, v/v). When loaded with 1% EO-emulsion, the coating showed good antibacterial effects with a high-value sensory assessment. No effect was found on the coating properties under different temperatures (4 and 20 °C) and pH conditions (6, 7 and 8). Alginate coatings with 1% thyme, oregano, pimento EO-emulsion were applied for chill-stored carp fillets. All coatings with EO-emulsion delayed pH change, decreased total volatile basic nitrogen and total viable count and inhibited *Pseudomonas* sp., H_2S-producing bacteria and Enterobacteriaceae.

1. Introduction

Common carp *(Cyprinus carpio)* had the fourth highest production (4189.5 thousand tons) among the major species in world aquaculture (FAO, 2020, p. 244). It is the most important fish species produced and consumed in the Czech Republic. The carp has a high nutritional value, such as high-quality protein, healthy unsaturated fatty acids. However, its high nutrition, high moisture content and low connective tissue content make carp perishable, leading to severe quality deterioration. Microorganisms are one of the main causes of fish quality deterioration during chilled storage. Various chemical preservatives along with packaging have been used for chill-stored fish to inhibit the proliferation of spoilage microorganisms. Nowadays, the concept of human consumption is experiencing a so-called 'green consumerism', where more natural, minimally processed products are preferred (as well as) potentially biodegradable, recyclable and environmentally friendly food packaging, but synthetic food additives are unexpected (Falleh, Ben Jemaa, Saada, & Ksouri, 2020; Shahidi & Hossain, 2020). Therefore, it is necessary to develop an effective and environmentally friendly preservation system for fish to extend shelf life and maintain quality.

Essential oils (EOs) are of plant origin and many of them are approved as 'generally recognized as safe' (GRAS) food additives by the Food and Drug Administration (FDA) in the USA (Code of federal regulations (CFR), 2019) and by the European Food Safety Authority (EFSA) (Donsì & Ferrari, 2016). Many EOs exhibit antioxidative and antibacterial activities due to their organic compounds such as terpenes, terpenoids, aromatics (phenylpropanoids) (Hassoun & Çoban, 2017; Wang, Wang, Li, & Luo, 2018). For example, oregano and thyme EOs were found to be effective in preserving carp (Huang, Liu, Jia, Zhang, & Luo, 2018; Wu et al., 2014). However, our previous study indicated that the antibacterial activities of EOs against specific spoilage organisms (SSOs) might differ. Few EOs showed a broad-spectrum of antibacterial activities (Hao, Roy, Pan, Shah, & Mráz, 2021). Thus, a targeted selection of EOs with the most effective antibacterial activities for chill stored fish needs to be performed.

Sodium alginate is extracted from brown seaweed and is approved by the FDA as a GRAS food additive (FDA, 2019). Recently, coating food materials with alginate for controlling microbial growth and reducing oxidation has become a new preservative approach. In addition, alginate can serve as an emulsifier to enhance the stability of EO, inhibit volatilization and minimise the organoleptic effects of EO (Wang et al., 2018). As a promising delivery system for EO, the emulsion can improve the antimicrobial and antioxidant stability of EOs and their functionalities, as well as the organoleptic properties of food (Donsì & Ferrari,

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2016). It is suggested that emulsion can increase the solubility, dispensability and deliverability of EO in water-based foods and modulate the release of the antimicrobial organic compound to reach the sites where microorganisms proliferate, thus improving the biological activity of EO (Prakash, Baskaran, Paramasivam, & Vadivel, 2018). Recently, many studies have focused on using EO or EO enriched coating as a preservative method for fish. However, only a few studies have applied EO-emulsion into the coating for fish preservation. Moreover, the organoleptic acceptability of fish fillets preserved with EO has not been adequately investigated in previous studies (Hao et al., 2021), as this is an important aspect from a marketing perspective.

To address these gaps above, the study firstly made a purposive selection of the EOs with the most effective antibacterial activity for chill stored fish. Secondly, considering the potential negative organoleptic effects of EOs on fish materials, a delivery system EO-emulsion was developed to be used instead of bulk to form the antibacterial coating. The EOs identified in the first part were used to optimize the preparation process. The aim was to develop a stable EO-emulsion based alginate coating system to provide an effective green strategy for chill-stored preservation of carp fillets. The physical and antibacterial properties were tested to optimize the preparation of coatings with EO-emulsion. In order to verify the preservative activity of the coatings, a chilled storage test of common carp fillets was performed, which included: monitoring of carp freshness, monitoring of microbial contamination and sensory evaluation.

2. Materials and methods

2.1. EOs selection

Twelve commercial food-grade EOs, i.e., thyme, pimento, oregano, ginger, rosemary, lime, sage, basil, garlic, lemon, clove, and cinnamon, obtained by steam distillation, were provided by Kalsec Europe Ltd. Mildenhall, UK. According to recent studies, they are considered to have effective antibacterial activities (Baptista, Horita, & Sant'Ana, 2020; Falleh et al., 2020; Hassoun & Çoban, 2017).

2.1.1. Bacteria strains and growth conditions

Two fish spoilage bacteria, *Pseudomonas fragi* ŽM648 and *Shewanella putrefaciens* ŽM654, and two pathogenic bacteria found in fish meat, *Listeria monocytogenes* ŽM58 and *Escherichia coli* ŽM370, were used to evaluate the antibacterial activity of the EOs. All strains were provided by the Laboratory for Food Microbiology at the Department of Food Science, Biotechnical Faculty, University of Ljubljana, Slovenia. Strains were stored at −80 °C and revitalized on tryptic soy agar (TSA, Biolife, Milan, Italy). For assays, strains were diluted in tryptic soy broth (TSB, Biolife, Milan, Italy) to 10^5–10^6 colony forming units (CFU)/mL. *L. monocytogenes* and *E. coli* were incubated at 37 °C, while *P. fragi* and *S. putrefaciens* were incubated at 30 °C.

2.1.2. Analysis of antibacterial activity of EOs and EO-emulsions

First, the antibacterial activity of 12 EO solutions was evaluated, and then the antibacterial activity of EO-emulsions of three of the most effective EOs (thyme, pimento, oregano) was further analyzed. For the preparation of EO solutions, the EOs were dissolved in TSB medium containing 2% dimethyl sulfoxide (DMSO), which does not affect the growth of the tested bacteria at this concentration (Ziaee, Razmjooei, Shad, & Eskandari, 2018). For the preparation of EO-emulsions, EOs were homogenized with Tween-80 (Sigma Aldrich, St. Louis, USA) using T18 Ultra-Turrax mixer (IKA, Staufen, Germany) at different ratios, homogenization speed and times) (see Table 1 for abbreviations of the different parameters.

Table 1
Abbreviation of emulsion systems.

Abbreviation	5E-101	10E-101	20E-101	20E-103	20E-181	20E-183
Ratio (EO: Tween 80) (v/v)	5:1	10:1	20:1	20:1	20:1	20:1
Homogenization speed (rpm)	10,000	10,000	10,000	10,000	18,000	18,000
Homogenization time (min)	1	1	1	3	1	3

The antibacterial activities of the EO solutions and emulsions were analyzed using the broth microdilution test according to Javidi, Hosseini, and Rezaei (2016) and Sterniša, Bucar, Kunert, and Smole Možina (2020) with modifications. Two-fold serial dilutions of EO solutions and emulsions were performed in 96-well microtiter plates (Nunc Thermo-Fisher, Waltham, US) with final concentrations of EOs in the range of 0.03125–2% (v/v) in a final volume of 100 μL. After overnight incubation, the minimal inhibitory concentration (MIC) was determined by adding 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT, Sigma Aldrich, St. Louis, USA). The MIC was the lowest concentration at which no bacterial growth was detected as reduction of INT to red formazan. Suspensions from wells in which no color change was observed were sub-cultured to TSA and incubated overnight. The minimal bactericidal concentration (MBC) was determined as the lowest concentration at which no visible growth of bacteria was detected. All measurements were repeated in duplicate.

2.1.3. Antibacterial effect of coatings on carp fillets

The antibacterial activity of coatings loaded EO-emulsion (thyme, pimento, oregano) at different EO concentrations of 0.5%, 1.0%, 1.5% on carp fillets were investigated to select the effective EO concentration. The coating solution and coating processing were prepared according to Cai, Cao, Bai, and Li (2015). Thyme, oregano and pimento EO-emulsions were prepared as described in section 2.1.2 and added to 2% (w/v) sodium alginate solution to achieve concentrations of 0.5%, 1% and 1.5% (v/v) of EO. Glycerol (final concentration 10% v/v, Sigma Aldrich, St. Louis, USA) was added as a plasticizer. The mixture was homogenized at 8000 rpm for 1 min using a digital mixer T18 Ultra-Turrax (IKA, Staufen, Germany). The coating solution was degassed and cooled to room temperature before being applied to fish meat. Common carp (obtained from ponds in Vodňany, Czech Republic) fillets were immersed in the above coating solution for 1 min, air-dried for 1 min, immersed in 2% $CaCl_2$ for 1 min to gelatinize, and then air-dried for 25 min (processing in icebox), The coated carp fillets were packed in polyethene bags and stored at 4 ± 1 °C. Uncoated carp fillets were used as control.

According to our previous study (Hao, Pan, Khalili Tilami, Shah, & Mráz, 2020), chill-stored common carp fillets were spoiled on day 6. Thus, samples were taken for total viable count (TVC) analysis on day 0 and day 6 of chill storage. The TVC analysis was performed according to Joukar, Hosseini, Moosavi-Nasab, Mesbahi, and Behzadnia (2017). Enumeration of microbial communities was recorded as log CFU/g muscle. Three random samples were collected at each time point for analysis.

2.1.4. Sensory assessment of EO

The sensory assessment of EO-emulsions (thyme, oregano, pimento) was tested by evaluating raw and cooked fish muscle cubes coated with alginate coating with 1% or 1.5% EO-emulsion. All tested fish muscle cubes were served in two ways, i.e. with or without coating (coating was removed before serving for raw fish cubes and before cooking for cooked fish cubes). A well-trained panel of 8 members evaluated the organoleptic properties of the samples. Sensory questionnaires measured intensity on a 9-point hedonic scale (weak to strong) for the following properties color, odor, texture, taste and acceptability. In this sense, 1, 4, 5, and 9 determine extreme dislike, mild dislike, neither like nor dislike (neutral), and extreme like, respectively (Mailgaad, Civille, & Carr, 1999; Meral et al., 2019). Color, texture, odor, and acceptability were evaluated for sensory assessment of raw carp fillets. Moreover, taste was evaluated for sensory assessment of cooked carp fillets.

2.2. *Optimize the effect of EO-emulsion systems on coating characterization based on pimento EO*

2.2.1. *Coating preparation*

Pimento EO was used to optimize the conditions for the preparation of EO-emulsion and the effect of EO-emulsion systems on coating characterization. Six pimento EO-emulsions (1% v/v) were prepared with different EO/Tween-80 ratios, homogenizing speeds, and times conditions (Table 1). The coating solution was prepared as described in section 2.1.3. The coating solutions (3 g) were cast into 40 mm diameter glass Petri dishes (P-Lab, Praha, Czech Republic) and cross-linked with 2% $CaCl_2$ (Sigma Aldrich, St. Louis, USA).

2.2.2. *Color properties and thickness analysis*

The color properties of the coatings were determined using a spectrophotometer colorimeter (Minolta CM-600d, Tokyo, Japan). The values of L*, a* and b* were recorded. The total color difference (ΔE) was calculated using the following formula: $\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$. L_0*, a_0*, and b_0* are the color parameters related to the standard indicator. The thickness of the coatings was measured using a CoatingTest-Master (Umarex Gmbh & Co. KG, Arnsberg, Germany). Each analysis was performed at six points.

2.2.3. *Coating solubility analysis*

According to Haghighi et al. (2019), the effects of pH and temperature on coating solubility were tested with modifications. The initial dry matter content of the coating was determined by drying in an oven at 105 ± 2 °C (W_a) to a constant weight. The coatings were immersed in buffers with pH 2, 4, 6, 7 and 8 at 4 °C or pH 7 at 25 °C. After 24 h, the coatings were dripped and dried to a constant weight at 105 ± 2 °C (W_b). The coating solubility (%) was calculated as $(W_a\text{-}W_b)/W_a*100$.

2.2.4. *Optic microscope observation*

All EO-emulsions and EO-emulsion based coatings were observed with an optical-light microscope (Olympus BX53, Shinjuku, Japan) equipped with a digital camera (Olympus U-tv 0.63x C, Shinjuku, Japan). A drop of pimento EO-emulsion or a small piece of the coating was placed on a microscope slide and observed at 60 × magnification. Images were taken by the Olympus cellSens software. In addition, the coatings with pimento EO-emulsions of 20E-183 and 10E-101 were stored at 4 °C for 30 days. The EO release status of the coating was analyzed by observation with an optical microscope on days 0, 7, 14 and 30 of storage.

2.2.5. *Scanning electron microscopy (SEM) analysis*

The coating sample (with pimento EO-emulsions of 20E-183 and 10E-101) for SEM observation was prepared according to the method of Fabra, Falcó, Randazzo, Sánchez, and López-Rubio (2018) with modifications. The coating was frozen in liquid nitrogen and transferred to an ALTO 2500 high vacuum preparation chamber (Gatan, Pleasanton, California). The sample was fractured at −140 °C and sublimated at −95 °C for 3 min and then coated with a 3 nm thick platinum layer. The microstructure of the sample was observed with a JSM-7401F SEM (JEOL, Tokyo, Japan). Two images (one from the surface and one from the cross-section) were obtained using the secondary electron signal at an accelerating voltage of 1 kV using Gentle Beam high mode.

2.3. *Preservative effects of coating on chill-stored common carp fillets*

2.3.1. *Sample preparation*

Thyme, oregano, and pimento EO-emulsions at 1% (v/v) in 20E-183 system were prepared as described in section 2.1.1. and mixed into the coating solution as described in section 2.1.3. Four-year-old marketable size common carp (weight: 2.65 ± 0.43 kg; n = 15) were obtained from ponds in Vodňany, Czech Republic, in November 2019. Fish were transported alive to the laboratory at the Institute of Aquaculture and Protection of Waters in České Budějovice and killed by blows to the head followed by bleeding in accordance with the ethical standard and legislation of the Czech Republic. Three fillets (~$7 \times 9 \times 1.5$ cm^3) were obtained from each side of the common carp (without the tail part). A total of 90 fillets were obtained and randomly divided into five groups, which were assigned the following treatment: CK-, control, untreated; CK+, EO-free-coating; P, 1% pimento EO-emulsion-coating; T, 1% thyme EO-emulsion-coating; O, 1% oregano EO-emulsion-coating. The carp fillets were coated and packed as described in section 2.1.3 and stored at 4 ± 1 °C for 10 days. Samples were collected every 2 days for analysis. At each time point, three random samples were taken for analysis.

2.3.2. *pH and total volatile basic nitrogen (TVBN) analysis*

The pH value of carp fillets was measured using a 206 digital pH meter (Testo AG, Lenzkirch, Germany) by inserting a probe 8 mm deep into the muscle. TVBN was measured using Conway's dish microdiffusion method according to Chuesiang, Sanguandeekul, and Siripatrawan (2020) with modifications. Five grams of the minced sample was stirred in 50 mL of deionized water for 30 min, and then the mixture was filtered. The evaporation from the filtrate was produced under the catalysis of saturated K_2CO_3 and absorbed by a boric acid solution (20 g/L) with methyl red-methine blue indicator (2 g/L) during a 2 h reaction at 37 °C in Conway's dish. The TVBN value was calculated by the titration volume of a 0.01M HCl standard solution.

2.3.3. *Microbiological parameters*

The enumeration of microorganisms was performed according to Joukar et al. (2017) with modifications. Homogenates with bacteria were prepared as described in 2.1.3. Serial decimal dilution method was used for microbial analysis. TVC was measured using plate count agar incubated at 30 °C for 72 h. Enterobacteriaceae (ENT) were enumerated in violet red bile glucose agar (Condalab, Madrid, Spain) incubated at 30 °C for 24 h. *Pseudomonas* sp. (PSE) were determined on *Pseudomonas* selective CFC agar (Condalab, Madrid, Spain) incubated at 20 °C for 48 h H_2S-producing bacteria (HSP) were evaluated on iron agar medium (Condalab, Madrid, Spain) incubated at 20 °C for 4 days. Microbial community counts were recorded as log CFU/g muscle.

2.3.4. *Sensory evaluation*

The odor and acceptability of the raw fish fillet samples were evaluated according to the sensory questionnaires inform section 2.1.4. Six samples were taken randomly, served with the coating, and evaluated by a well-trained panel of 3 members at each time point.

2.4. *Statistical analysis*

Data were expressed as mean ± standard deviation. Results were analyzed by one-way analysis of variance (ANOVA) and Duncan's test using Statistical Package for Social Science 16.0 (SPSS Inc, Chicago, USA). When $p < 0.05$, the difference was considered significant.

3. Results and discussion

3.1. *EOs selection*

3.1.1. *Antibacterial activity of EOs and EO-emulsions*

Twelve commercial EOs known from previous reports for their positive effects on food preservation (Baptista et al., 2020; Falleh et al., 2020; Hassoun & Çoban, 2017) were selected to evaluate their potential antibacterial function. *L. monocytogenes* is one of the most dangerous foodborne pathogens in aquatic products (Baptista et al., 2020). In

Table 2
Antibacterial activity of twelve essential oils (EOs) determined by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) as percentage (v/v).

EO	*L. monocytogenes* ŽM 58		*E. coli*ŽM 370		*P. fragi*ŽM 648		*S. putrefaciens*ŽM 654	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Thyme	0.25	0.25	0.25	0.25	0.125	0.25	0.0625	0.0625
Pimento	1	1	0.5	0.5	0.5	0.5	0.25	0.25
Oregano	0.25	0.25	0.125	0.125	0.5	1	0.03125	0.0625
Ginger	>2	>2	>2	>2	>2	>2	2	>2
Rosemary	>2	>2	2	2	2	>2	2	2
Lime	2	>2	>2	>2	>2	>2	1	1
Sage	1	2	>2	>2	>2	>2	0.5	0.5
Basil	2	2	1	1	>2	>2	1	1
Garlic	1	1	>2	>2	2	2	0.25	1
Lemon	0.5	1	>2	>2	1	>2	0.5	0.5
Clove	1	2	0.5	0.5	0.5	1	0.125	0.25
Cinnamon	2	>2	0.5	0.5	0.5	0.5	0.25	0.25

Table 2, thyme and oregano EO had the lowest MIC and MBC (0.25% v/v) for *L. monocytogenes*, followed by pimento, lemon and garlic EO, whose MIC and MBC were less than or equal to 1%. E. *coli* is considered as an indicator of the hygiene status of fish fillets (Socaciu, Semeniuc, & Vodnar, 2018). The lowest MIC and MBC (0.125%) was found in oregano EO, followed by thyme EO (0.25%). Pimento, clove and cinnamon EO showed the same MIC and MBC (0.5%). *P. fragi* and *S. putrefaciens* are common dominant spoilage microorganisms in chill-stored freshwater fish (Socaciu et al., 2018). Thyme EO showed the lowest *P. fragi* MIC (0.125%) and MBC (0.25%). The antibacterial activity of thyme, oregano and pimento EO on *Pseudomonas* measured by broth microdilution assay showed that thyme EO had higher inhibitory activity than oregano and pimento EO (Girova et al., 2010). The MIC and MBC of oregano and thyme EO on *S. putrefaciens* were <0.0625%, while those of pimento, clove and cinnamon EO were 0.25% or below. The results indicated that thyme EO had the most effective bacteriostatic and bactericidal activity, followed by pimento and oregano EO. It was suggested that these three EOs be used as EO-emulsions for further antibacterial testing.

In Table 3, all three EO-emulsions against four bacterial strains showed lower MIC and MBC than EOs applied as EO-bulks, with the exception of thyme EO-emulsion against *P. fragi*. This suggests that the emulsion system could amplify the antibacterial functions of EO, which was also previously reported (Donsì & Ferrari, 2016). The EO-emulsion system could promote the interaction of EO with microbial cell membranes by increasing the surface area and passive transport across the outer cell membrane (Moghimi, Ghaderi, Rafati, Aliahmadi, & McClements, 2016). Meral et al. (2019) also reported that MIC and MBC values of nisin emulsion were lower than those of nisin. Various pimento EO-emulsions were prepared to investigate the effects of homogenization conditions and EO/Tween-80 ratio on the antibacterial activity of EO-emulsions. Table 3 shows that the six pimento EO-emulsions did not have different antibacterial activities against P. fragi and S. putrefaciens. 5E-101 showed the highest antimicrobial activity against *L. monocytogenes* and *E. coli* (0.0625%). This suggests that a high concentration of Tween-80 could enhance the antibacterial activity of EO-emulsions. This could be due to the fact that the high emulsifier content (Tween-80) reduced the droplets of EO-emulsion and provided a large surface area for EO, which further increased the antibacterial activity of EO-emulsions. To achieve a reliable preservation effect for fish fillets, namely inhibition of most bacterial species, the maximum MIC of all tested bacteria should be equal to the minimum recommended application concentration (MRAC). It was found that the EOs of thyme, oregano and pimento had the same MRAC value (0.25%) in the 5E-101 system, as the choke point was the MIC value of *P. fragi*. The same MRAC value (0.25%) was observed in the pimento EO-emulsions (5E-101, 10E-101 and 20E-183), which was lower than the other systems (0.5%). This suggests that increasing the homogenization speed and time could positively affect the antibacterial activity of the EO-emulsions. This could be due to the fact that the droplet size of EO-emulsion was reduced at high homogenization speed and time. Consumers could better accept low-dose food additives. Thus, it was recommended to increase the homogenization speed and time for the formation of EO-emulsion using physical methods to achieve a similar antibacterial effect with lower emulsifier content (Tween-80). EO-emulsion system 20E-183 was found to be most suitable for use for alginate coating.

3.1.2. *Antibacterial activity of coating on carp fillets*

In Table 4, alginate coating with 1% or 1.5% thyme, oregano and pimento EO-emulsions showed significant ($p < 0.05$) reduction in TVC in carp fillets. Jouki, Yazdi, Mortazavi, Koocheki, and Khazaei (2014), Oguzhan Yildiz (2017) and Tokur, Sert, Aksun, and Özoğul (2016) also reported a reduction in TVC in rainbow trout fillets treated with quince seed mucilage coating with 1% oregano EO, chitosan coating with 1%

Table 3
Antibacterial activity of essential oil (EO) emulsion systems determined as minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) as a percentage of EO (v/v). For abbreviations see Table 1.

EO	EmulsionSystem	*L. monocytogenes*ŽM 58		*E. coli*ŽM 370		*P. fragi*ŽM 648		*S. putrefaciens*ŽM 654	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Thyme	5E-101	0.125	0.125	0.0625	0.0625	0.25	1.0	0.0156	0.0156
Oregano	5E-101	0.125	0.125	0.0625	0.0625	0.25	0.25	0.0156	0.0312
Pimento	5E-101	0.0625	0.0625	0.0625	0.0625	0.25	0.5	0.0312	0.0312
Pimento	5E-101	0.0625	0.0625	0.0625	0.0625	0.25	0.5	0.0312	0.0312
	10E-101	0.125	0.125	0.125	0.125	0.25	0.5	0.0312	0.0312
	20E-101	0.25	0.25	0.5	0.5	0.25	0.5	0.0312	0.0312
	20E-103	0.25	0.25	0.5	0.5	0.25	0.5	0.0312	0.0312
	20E-181	0.125	0.125	0.5	0.5	0.25	0.5	0.0312	0.0312
	20E-183	0.125	0.125	0.125	0.125	0.25	0.5	0.0312	0.0312

Table 4
Total viable count (log CFU/g) of common carp fillets treated without coating (CK-) and with alginate coating incorporating 0.5%, 1.0%, and 1.5% EO emulsions during chilled storage.

Groups		Storage time (day)	
EOs concentration (%)	EOs name	0	6
–	–	3.22 ± 0.16 aA	7.35 ± 0.34 aB
0.5%	Thyme	3.11 ± 0.19 aA	6.76 ± 0.34 aB
0.5%	Oregano	3.10 ± 0.14 aA	6.81 ± 0.37 aB
0.5%	Pimento	3.06 ± 0.29 aA	6.73 ± 0.32 aB
1%	Thyme	2.26 ± 0.24bA	4.27 ± 0.34bcB
1%	Oregano	2.36 ± 0.10bA	4.46 ± 0.42bB
1%	Pimento	2.26 ± 0.24bA	4.13 ± 0.38bcB
1.5%	Thyme	2.10 ± 0.17bA	3.59 ± 0.35 cB
1.5%	Oregano	2.20 ± 0.17bA	3.49 ± 0.25 cB
1.5%	Pimento	2.16 ± 0.28bA	3.56 ± 0.39 cB

Lowercase letters in the same column indicate significant levels ($p < 0.05$), uppercase letters in the same row indicate significant levels ($p < 0.05$).

thyme EO, and whey protein isolate coating with 3–7% thyme EO, respectively. This suggested that a high content of EO-emulsion could reduce the initial bacterial load in the fish. A relatively high content of EO could enhance the destruction of the bacterial outer wall during the coating process and increase the amount of EO entering the bacterial cytoplasm, which could lead to the leakage of bacterial intracellular material and the termination of DNA synthesis, resulting in the death of bacteria and a decrease in TVC (Hassoun & Çoban, 2017).

On day 6, the TVC of carp fillets without coating was above 7 log CFU/g muscle, which is above the acceptable level (ICMSF, 1986). The TVC of carp fillets treated with a coating with 0.5% EO-emulsion was close to the limit, implying that 0.5% EO-emulsion did not achieve the goal of extending the microbial shelf life of carp fillets. However, the TVC of carp fillets treated with coatings containing 1% or 1.5% thyme, oregano and pimento EO-emulsion were below 4.5 log CFU/g muscle on day 6, significantly lower than that of the control ($p < 0.05$).

The high concentration of EO, applied along with the coating, showed strong antibacterial activity. A similar result was found in hake fillets coated with whey protein isolate loaded with 1% or 3% thyme and oregano EO (Carrión-Granda, Fernández-Pan, Rovira, & Maté, 2018). The emulsion at a high concentration of EO, applied to the carp fillets, showed a clear function in inhibiting spoilage bacteria. However, this does not mean that the higher the concentration, the better it is for practical application. Economic cost and customer preference should also be considered.

3.1.3. *Sensory assessment*

For all sensory parameters, a score of less than 4 was considered unacceptable to customers. In Figure A.1, the color and texture of raw (Fig. A.1 A) and cooked fish (Fig. A.1 B) were not significantly affected by coating with EO-emulsions and serving methods, i.e. with coating or with the coating removed. It could be that the alginate coating was thin and transparent so that its effects on color and texture were not noticeable to the consumer.

For odor and acceptability, the CK + group showed a slightly lower score than CK- regardless of serving styles for raw fish cubes (Fig. 1 A) and cooked cubes (Fig. 1 B), but no significant ($p > 0.05$) differences were found. The coating loaded 1% pimento, thyme and oregano EO-emulsion showed a decrease i ($p < 0.05$) in odor and acceptability scores in raw fish cubes (Fig. 1 A) and odor, acceptability and taste scores in cooked fish cubes (Fig. 1 B) served with coating. However, these negative effects on odor, acceptability and taste of raw (Fig. 1 A) and cooked fish cubes (Fig. 1 B) could be eliminated by removing the coating before serving. Coating with 1.5% pimento, thyme and oregano EO-emulsion showed remarkable negative effects on odor and acceptability in raw fish (Fig. 1 A) and odor, acceptability and taste in cooked fish (Fig. 1 B), regardless of whether the fish cubes were served with coating or the coating was removed.

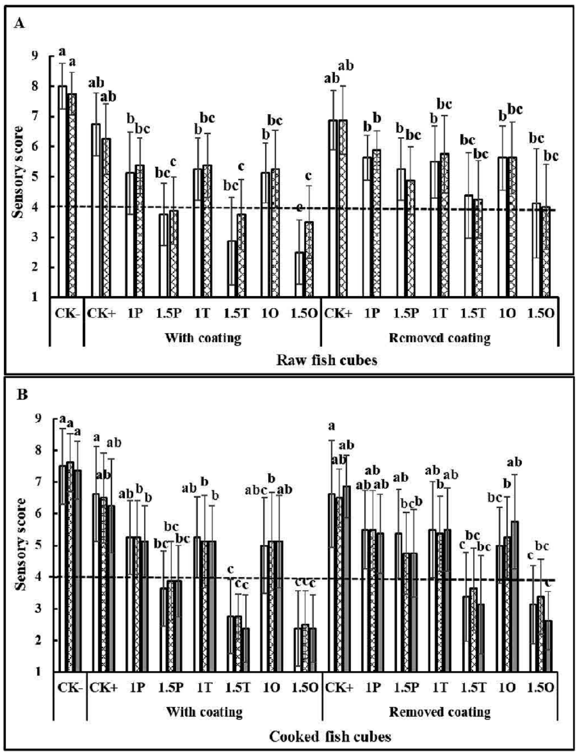

Fig. 1. Sensory test (☐ odor, ☒ acceptability, and ■ taste) on raw fish cubes (A) and cooked fish cubes (B) served with coating or removed coating (0 day). CK-, fillet without coating treatment; CK+, fillet coated with only alginate. Others are fillet coated with alginate including different EO emulsions. 1 and 1.5 denotes 1 and 1.5% EO-emulsion. P, pimento EO; T, thyme EO; O, oregano EO. All EO emulsions were prepared as 20E-183 emulsion system. Letters (a, b, c) indicate significant different levels ($p < 0.05$). Error bars represent the standard deviation of the mean (n = 8). The horizontal lines represent the maximum customers' acceptable level in each parameter.

The application of EO as an antibacterial agent could have negative sensory effects on the food. This has been frequently observed in fish, e. g., rainbow trout fillets coated with chitosan loaded 1% lemon verbena EO (Rezaeifar, Mehdizadeh, Mojaddar Langroodi, & Rezaei, 2020) and sea bass treated with 1.5% bay laurel EO (Öztürk Kerimoğlu, KavuŞAn, & SerdaroĞLu, 2020). Our results indicated that emulsion with a relatively low EO concentration (e.g., no higher than 1% EO) embedded in a removable alginate coating could effectively reduce the negative sensory influence of EO. However, a removable alginate coating could not eliminate the negative influence of EO on the sensory characteristics of carp fillets when EO was applied at a high concentration, such as the 1.5% EO in our study. A high concentration EO-emulsion could increase the penetration and adherence of the active ingredients of EO on the surface of carp fillets and achieve a strong antibacterial effect. Nevertheless, more odorous EO remain on the fish surface, resulting in low sensory value. Together with the results of the antibacterial trials on carp fillets, it was proposed to incorporate an emulsion of 1% EO into the alginate coating to preserve the carp fillets.

3.2. *Coating characterization*

3.2.1. *Color, thickness and solubility of coatings*

As shown in Table 5, the color parameters and thickness of all

Table 5
Thickness and color properties of alginate coating without EO (CK) and incorporating 1% pimento EO emulsions. For abbreviations see Table 1.

	Thickness (mm)	Color			
		L*	a*	b *	ΔE
CK	0.0607 ± 0.0055a	89.91 ± 1.17a	−0.51 ± 0.01a	6.87 ± 0.14a	2.95 ± 0.54a
5E-101	0.0612 ± 0.0051a	89.56 ± 0.45a	−0.54 ± 0.03 ab	6.59 ± 0.15a	2.68 ± 0.29a
10E-101	0.0607 ± 0.0058a	89.86 ± 0.30a	−0.54 ± 0.03 ab	6.63 ± 0.22a	2.56 ± 0.26a
20E-101	0.0599 ± 0.0058a	90.16 ± 0.36a	−0.55 ± 0.02b	6.65 ± 0.22a	2.47 ± 0.24a
20E-103	0.0606 ± 0.0066a	90.02 ± 0.47a	−0.54 ± 0.02 ab	6.70 ± 0.15a	2.57 ± 0.25a
20E-181	0.0613 ± 0.0041a	89.79 ± 0.26a	−0.56 ± 0.03b	6.75 ± 0.11a	2.69 ± 0.15a
20E-183	0.0597 ± 0.0062a	89.67 ± 0.59a	−0.53 ± 0.03 ab	6.83 ± 0.26a	2.86 ± 0.30a

Letters a, b in the same line indicate the significantly different levels ($p < 0.05$).

coatings containing 1% EO-emulsion were similar, indicating that the various EO-emulsions did not affect the color and thickness of the coating. Only 20E-101 and 20E-181 were observed to have a slightly higher a* value than CK. The difference could be attributed to the reflection effects of the EO-emulsion droplets buried in the alginate coating. Our results differed from the study on an alginate coating loaded with oregano EO, in which it was reported that the incorporation of EO into the coating resulted in an increase in thickness (Benavides, Villalobos-Carvajal, & Reyes, 2012).

The solubility (Fig. A.2) of all coatings (≈6.13%) was found similar at pH 6–8, and temperatures of 4 and 25 °C, which denoted that the emulsion had no additional effect on coating stability and alginate coatings with EO-emulsions were stable under general conditions of fish processing, transport, and preservation. Chuang et al. (2017) reported that the water solubility of the alginate film did not change at pH 3–11. Higher solubility of the coatings was observed at pH 2 than at pH 6–8 was observed. This suggests that alginate coatings with EO-emulsion are unstable under extremely acidic conditions, which could be due to the change in the properties of alginate under the acidic matrix.

3.2.2. *Optical microscope observation*

Pimento EO-emulsions prepared under different conditions (Fig. 2 A), and alginate coatings containing these EO-emulsions (Fig. 2 B) were observed under the optical microscope. All EO-emulsions and alginate coatings showed regular and uniform droplets, indicating that all these EO-emulsions were homogeneously dispersed in the alginate matrix, which could positively affect EO stability in the final coating. Purwanti et al. (2018) also reported that alginate coating solution loaded with clove EO-emulsion prepared at different homogenization speeds exhibited uniform droplets.

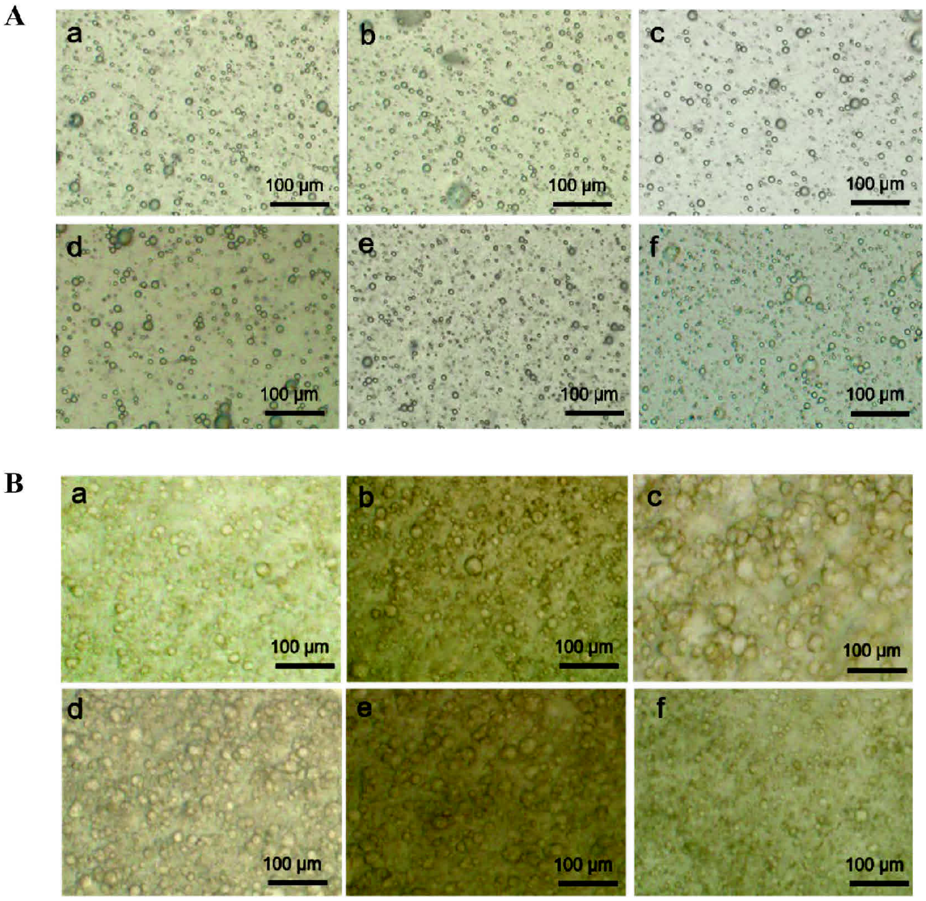

Fig. 2. Optical microscopy images of 1% pimento EO emulsion systems (A) and alginate coating incorporating these EO emulsions (B). a, 5E-101; b,10E-101; c, 20E-101; d, 20E-181; e, 20E-103; f, 20E-183. For abbreviations see Table S1.

The stability of alginate coatings containing EO-emulsions was evaluated during chilled storage by observation using optical microscopy. The stability of two EO-emulsions, 10E-101 (Fig. A.3 A) and 20E-183 (Fig. A.3 B), with the same antibacterial activity (MIC and MBC) was investigated. EO-emulsion droplets in coatings were small and uniform until day 7. After 14 days, EO-emulsion droplets turned slightly larger and less uniform, and this tendency is more pronounced on day 30, suggesting the unstable state of EO-emulsions after a long time. Since most chill-stored fish have a shelf life of about 7–10 days, the results denote that the alginate coating with EO-emulsion could be stable enough to release EO gradually from the coating on carp fillets.

3.2.3. *Microstructure*

The coating's microstructure or internal morphological structures depend on the interactions between the coating components that affect the final physical, optical, mechanical, and barrier properties. The microstructure of the coatings with emulsions 10E-101 and 20E-183 is shown in Fig. 3. Both coatings showed heterogeneous surfaces with many pores, which could be due to the evaporation of the embedded EO-emulsion droplets during the drying process. Remarkable pores were also observed in alginate film, including cinnamon EO-emulsions in a study by Frank, Garcia, Shin, and Kim (2018). However, the surface of coating 20E-183 was smoother and had fewer pores. The cross-section of the two coatings showed discontinuities and heterogeneous structure, implying the presence of EO-emulsion droplets. Nevertheless, the cross-section of coating 20E-183 was less discontinuous. The above results imply that coating 20E-183 could embed EO-emulsions of smaller size than coating 10E-101, which was more stable during drying and formed a more compact, sponge-like structure.

3.3. *Storage trial of common carp fillets*

3.3.1. *pH value*

In Fig. 4A, the pH of CK- and CK + decreased from 6.88 to 6.94 to 6.31 and 6.42, respectively, within 2 days and then returned to 7.21 and 7.14, respectively, on day 10. However, the pH of the pimento, oregano and thyme EO (P, O and T) groups decreased to the lowest values of 6.32, 6.25 and 6.38 on day 4 and increased to 6.64, 6.61 and 6.70 on day 10, respectively. The initial pH decrease could be due to the breakdown of ATP, creatine phosphate and glycogen, while the following increase could be caused by bacterial metabolites, protein degradation by endogenous or microbial enzymes and the formation of volatile basic components and amine (Atrea, Papavergou, Amvrosiadis, & Savvaidis, 2009). It is noticeable that the pH of CK- was the highest from the 4th day of storage. The alginate coating could create an anaerobic atmosphere that promotes anaerobic respiration in the carp fillets and generates lactic acid that lowers the pH. The growth of lactic acid bacteria could be another way of lowering pH (Hao et al., 2017). The lowest pH in groups P, O and T was observed on day 4. It remained lower than the control during the rest of the storage, indicating that alginate coating with EO-emulsion could delay the transition point of pH. The above results suggest that an alginate coating could reduce microorganisms and inhibit the activity of endogenous enzymes, which would extend the shelf life of chill-stored fish. Pimento, oregano and thyme EO-emulsion could elevate this capability.

3.3.2. *Total volatile basic nitrogen (TVBN)*

TVBN is closely related to spoilage microorganisms in fish and is often used as a quality index to evaluate the microbial shelf life of fish. In

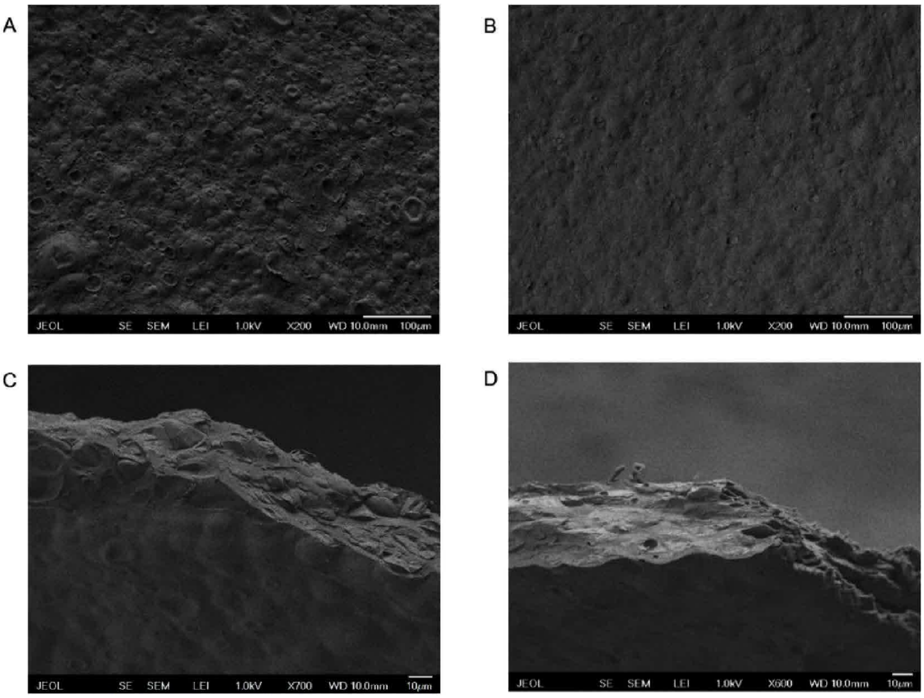

Fig. 3. Scanning electron microscopy (SEM) observation on microstructure of alginate coating incorporating EO emulsions. A, surface, 10E-101; B, surface, 20E-183; C, cross-section, 10E-101; D, cross-section, 20E-183. For abbreviations of emulsion systems see Table 1.

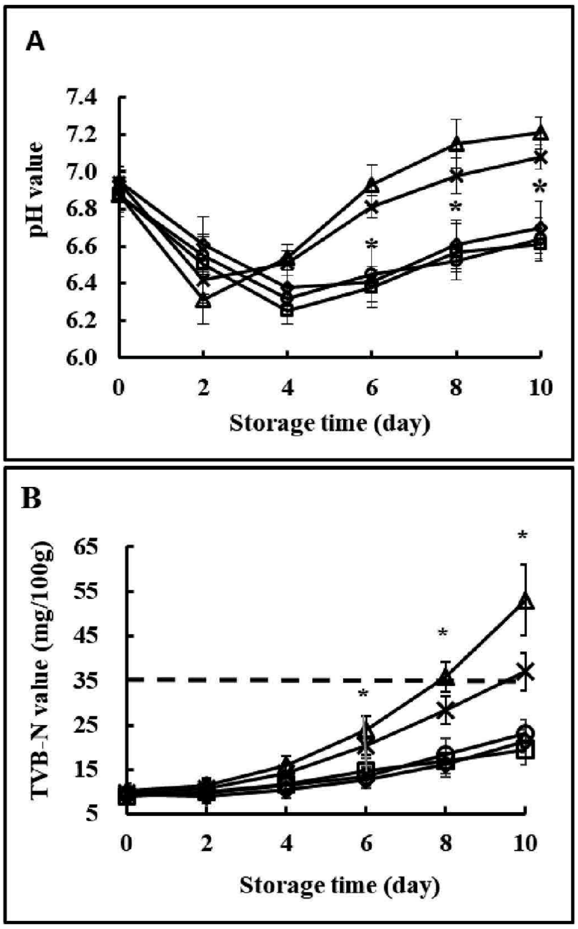

Fig. 4. pH value (A) and total volatile basic nitrogen (TVBN, B) of common carp fillets during chilled storage. CK-, △ fillets without coating; CK+, × fillets with EO-free-coating; P, ○ fillet coated with 1% pimento EO-emulsion; T, ⋄ fillet coated with 1% thyme EO-emulsion; O, □ fillet coated with 1% oregano EO-emulsion. Error bars represent the standard deviation of the mean (n = 6). The horizontal line in B represents the maximum limit of 25 mg/100g. * denotes significant differences between fillets coated with EO-emulsion (P, O and T) and two control groups (CK- and CK+), no significant difference was observed among three coated groups (P, O, and T).

Fig. 4B, the TVBN value of CK- and CK + at day 8 was 35.83 and 28.45 mg/100 g, which is above the limit of 25 mg/100 g for aquatic food (Giménez, Roncalés, & Beltrán, 2002), indicating severe bacterial spoilage and deterioration of carp fillets. After the 6th day, significantly lower TVBN ($p < 0.05$) was observed in the carp fillets coated with EO than in the CK- and CK + groups. Meanwhile, TVBN of the P, O and T groups remained <25 mg/100g until the end of chilled storage. A similar reduction in TVBN was observed by Wu et al. (2014) in grass carp fillets treated with a gelatin-chitosan coating containing 4% oregano EO. The TVBN of carp fillets treated with a coating containing 1% pimento, thyme and oregano EO-emulsion showed no differences throughout the storage period on the same day, suggesting their preservative capability for carp fillets.

3.3.3. *Microbial enumeration*

In Fig. 5 A, the initial TVC in CK- and CK+ was 2.91 and 2.86 log CFU/g muscle, respectively. P, T and O showed slightly lower initial TVC of 2.10–2.21 log CFU/g muscle, indicating that the EO-emulsion could somehow reduce the initial amount of microorganisms. The initial TVC reduction of 0.56 log CFU/g muscle was observed in grass carp (*Ctenopharyngodon idellus*) fillets treated for 30 min in 0.1% (v/v) cinnamon bark EO-emulsion (Huang, Liu, Jia, & Luo, 2017). Asian sea bass fillets soaked in a solution of 1% (v/v) cinnamon (*Cinnamomum zeylanicum*) EO for 30 min showed an initial TVC reduction of 0.84 log CFU/g (Chuesiang et al., 2020). Furthermore, a reduction in initial TVC of 1.01 log CFU/g was reported by (Yu, Xu, Jiang, & Xia, 2017) in grass carp (*Ctenopharyngodon idellus*) fillets immersed in 1% (v/v) lemongrass EO for 5 min. Significant differences ($p < 0.05$) in TVC between P, T, O and CK- CK+ were observed from day 4. The TVC of CK- and CK + exceeded the maximum acceptable level for freshwater fish (7.0 log CFU/g muscle) on day 6 and day 8, while the EO-coating groups came close to it on day 10. This demonstrates that the alginate coating loaded 1% pimento, thyme or oregano EO-emulsion effectively inhibited the growth of bacteria in the carp fillets during chilled storage. No differences in TVC were observed between P, O and T throughout the storage period. It is suggested that alginate coating with 1% pimento, thyme or oregano EO-emulsion could extend the chemical shelf life of chill-stored common carp fillets by 2–4 days.

Typical specific spoilage organisms (SSOs), including PSE (Fig. 5 B), HSP (Fig. 5 C), and ENT (Fig. 5 D), were analyzed to better understand the effects of coating and EO-emulsions on microorganisms in chilled carp fillets. The PSE and HSP counts of CK- and CK+ were initially 2.3–2.5 log CFU/g muscle and increased to about 8 log CFU/g muscle at day 10. However, the P, O and T groups had PSE count of 5.76–6.15 log CFU/g muscle and HSP count of 5.81–6.19 log CFU/g muscle at day 10. The differences ($p < 0.05$) of PSE and HSP between P, O and T and CK-, CK+ were significant from day 6. Jouki et al. (2014) also reported significant inhibition of PSE and HSP in rainbow trout by oregano and thyme EO loaded coating. ENT count is an important criterion for assessing the hygiene status of chill-stored foods. Initially, it was 2.00–2.21 log CFU/g muscle which increased to 7.24 and 7.06 log CFU/g muscle on day 10 in CK- and CK + respectively. ENT count increased more slowly (8.7–23.4%) than the other microbial flora in the early storage period (0–6 days). ENT levels of P, O and T were significantly lower than those of CK- and CK + after day 8, indicating that coating with the three EO-emulsions slowed the growth of ENT in carp fillets.

3.3.4. *Sensory evaluation*

The changes in sensory properties of the raw carp fillets throughout the storage period are illustrated in Figure A.4. A-B. Acceptable fish fillets for human consumption received a sensory score of at least 4. The control samples (CK- and CK + groups) were unacceptable in the odor and acceptability after day 6. In contrast, the EO coating treated samples (P, O and T groups) remained within the permissible range until day 10. Significant differences were found between the control groups (CK- and CK + groups) and the treated groups (P, O and T groups). In the first days, the control groups showed better sensory scores than the treated groups, suggesting that the treatment with EOs slightly negatively affected the odor and acceptability of the fillets. It is noticeable that although the sensory scores of the treated groups were not as high as

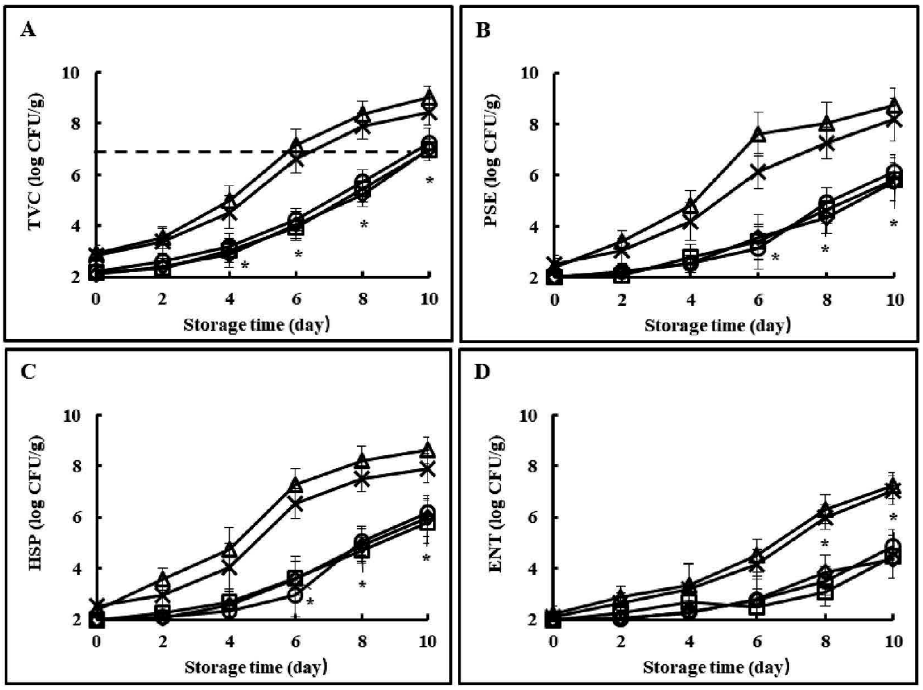

Fig. 5. Microbial communities of common carp fillets during chilled storage. (A) TVC, total viable count; (B) PSE, *Pseudomonas* sp.; (C) HSP, H_2S-producing bacteria; (D) ENT, Enterobacteriaceae. CK-, △ fillets without coating; CK+, × fillets with EO-free-coating; P, ⊖ fillet coated with 1% pimento EO-emulsion; T, ◇ fillet coated with 1% thyme EO-emulsion; O, ⊟ fillet coated with 1% oregano EO-emulsion. Error bars represent the standard deviation of the mean (n = 6). The horizontal line in A represents the maximum limit TVC (7.0 log CFU/g). * denotes significant differences between fillets coated with EO-emulsion (P, O and T) and two control groups (CK- and CK+), no significant difference was observed among three coated groups (P, O, and T).

those of the control groups, they were still well above the permissible range, which did not influence the choice of customers. Together with the TVBN and TVC values determined above, the alginate coating loaded with 1% pimento, thyme, or oregano EO-emulsion could extend the shelf life of chill-stored common carp fillets by 2–4 days.

4. Conclusion

Thyme, oregano and pimento EOs have good antibacterial activity and their EO-emulsion form showed stronger antimicrobial activity than their EO-bulk. The antibacterial activity of the EO-emulsions was affected by the homogenization speed and time and the amount of emulsifier (Tween-80) used to form the EO-emulsions. No effect was found on the stability of alginate coating loaded different EO-emulsions at pH 6–8 and temperature 4–25 °C. Coatings loaded 1% EO-emulsions were acceptable by customers and slightly more welcomed when the coating was removed before consumption. Alginate coating loaded with 1% EO-emulsions was proven to maintain the quality and extend the shelf life of chill-stored common carp fillets by 2–4 days.

CRediT authorship contribution statement

Ruoyi Hao: Experiment design, data analysis, figures preparation, writing – original draft, preparation, reviewing manuscript. **Bakht Ramin Shah:** Some experiment design, some experimental analysis, revising manuscript. **Meta Sterniša:** Revising manuscript, some methodology, some experimental analysis. **Sonja Smole Možina:** Some methodology, revising manuscript. **Jan Mráz:** Some experiment design, revising manuscript.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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Appendix

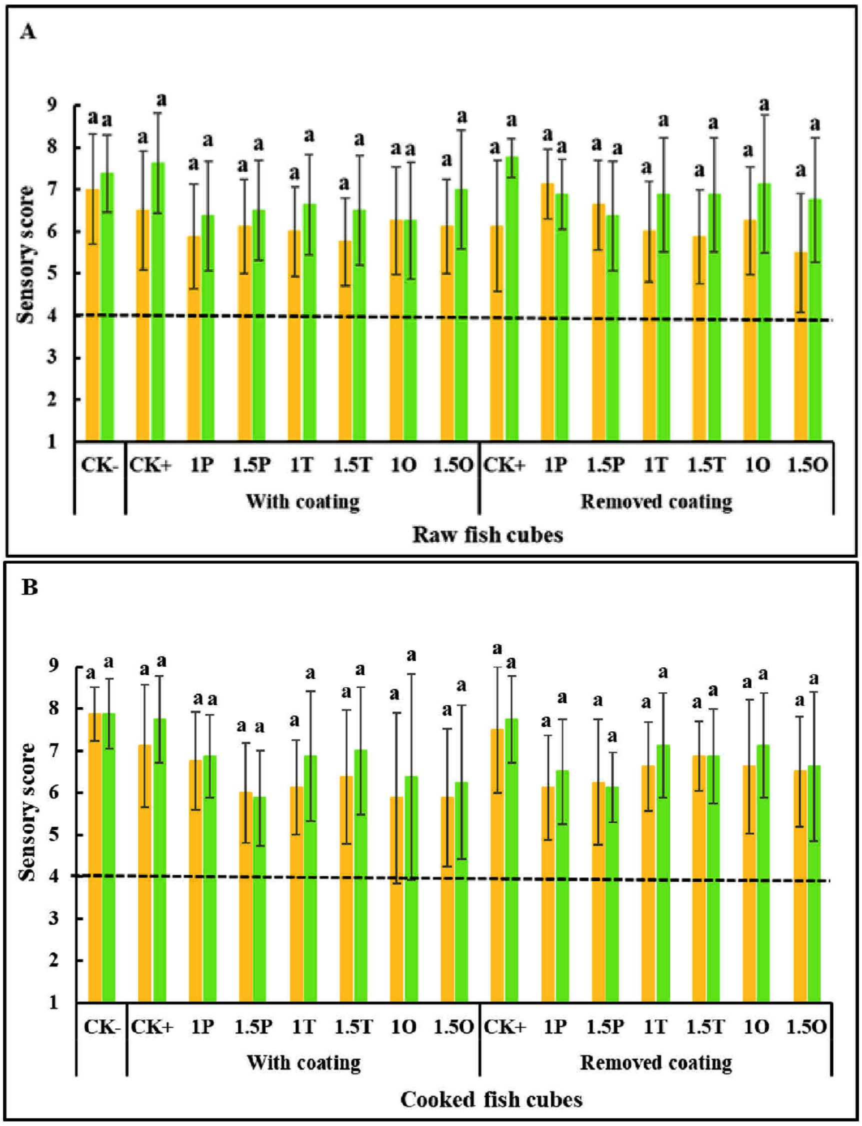

Figure A.1. Sensory test (🟧 color, and 🟩 texture) on raw fish cubes (A) and cooked fish cubes (B) served with coating or removed coating (0 day). CK-, fillet without coating treatment; CK+, fillet coated with only alginate. Others are fillet coated with alginate including different EO emulsions. 1 and 1.5 denotes 1 and 1.5% EO-emulsion. P, pimento EO; T, thyme EO; O, oregano EO. All EO emulsions were prepared as 20E-183 emulsion system. Letters (a, b, c) indicate significant different levels ($p < 0.05$). Error bars represent the standard deviation of the mean (n = 8). The horizontal lines represent the maximum customers' acceptable level in each parameter.

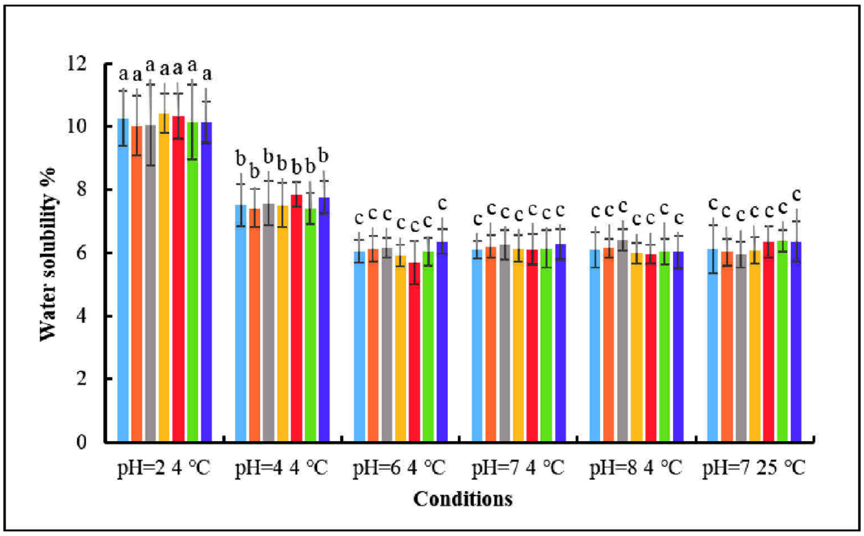

Figure A.2. Water solubility of alginate coatings without EO (🟦 CK) and with different EO emulsions (🟧 5E-101; ■ 10E-101; 🟨 20E-101; 🟥 20E-181; 🟩 20E-103; 🟪 20E-183) in different pH buffer and temperatures. Error bars represent the standard deviation of the mean (n = 6). Letters (a, b, c) denote significant different levels ($p < 0.05$) of coatings. For abbreviations see Table S1

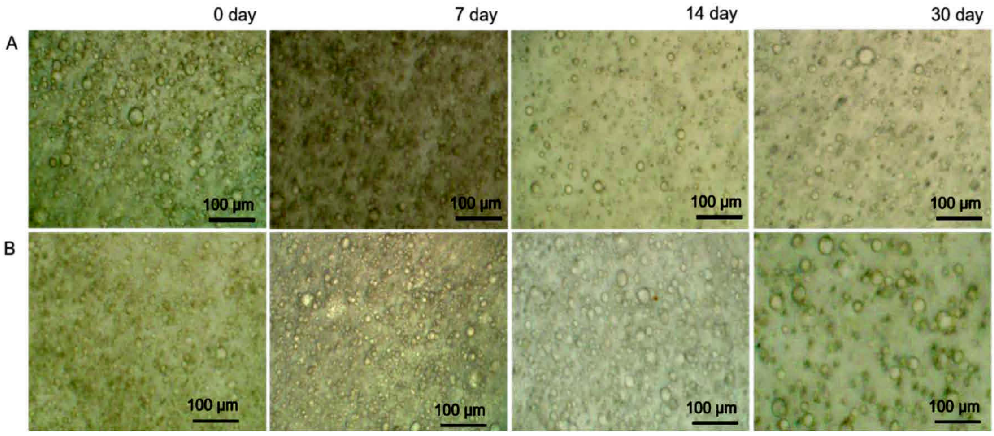

Figure A.3. Optical microscopy images of alginate coating loaded 1% pimento EO emulsion prepared as 10E-101 (A) and 20E-183 (B) during 4 °C storage for 30 days. For abbreviations see Table 1.

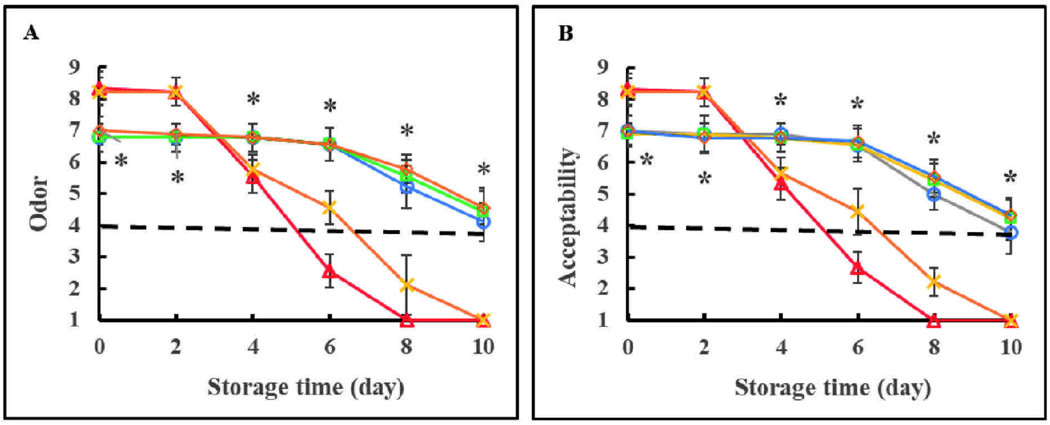

Figure A.4. Odor (A) and acceptability (B) score of common carp fillets during chilled storage. CK-, △ fillets without coating; CK+, × fillets with EO-free-coating; P, ○ fillet coated with 1% pimento EO-emulsion; T, ◇ fillet coated with 1% thyme EO-emulsion; O, □ fillet coated with 1% oregano EO-emulsion. Error bars represent the standard deviation of the mean (n = 9). The horizontal lines represent the maximum customers' acceptable level in each parameter. * denotes significant differences between fillets coated with EO-emulsion (P, O and T) and two control groups (CK- and CK+), no significant difference was observed among three coated groups (P, O, and T).

References

Atrea, I., Papavergou, A., Amvrosiadis, I., & Savvaidis, I. N. (2009). Combined effect of vacuum-packaging and oregano essential oil on the shelf-life of Mediterranean octopus (Octopus vulgaris) from the Aegean Sea stored at 4°C. *Food Microbiology, 26*(2), 166–172. https://doi.org/10.1016/j.fm.2008.10.005

Baptista, R. C., Horita, C. N., & Sant'Ana, A. S. (2020). Natural products with preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood: A review. *Food Research International, 127*, Article 108762. https://doi.org/10.1016/j.foodres.2019.108762

Benavides, S., Villalobos-Carvajal, R., & Reyes, J. E. (2012). Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. *Journal of Food Engineering, 110*(2), 232–239. https://doi.org/10.1016/j.jfoodeng.2011.05.023

Cai, L., Cao, A., Bai, F., & Li, J. (2015). Effect of ε-polylysine in combination with alginate coating treatment on physicochemical and microbial characteristics of Japanese sea bass (Lateolabrax japonicas) during refrigerated storage. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 62*(2), 1053–1059. https://doi.org/10.1016/j.lwt.2015.02.002

Carrión-Granda, X., Fernández-Pan, I., Rovira, J., & Maté, J. I. (2018). Effect of antimicrobial edible coatings and modified atmosphere packaging on the microbiological quality of cold stored hake (Merluccius merluccius) fillets. *Journal of Food Quality*, 1–12. https://doi.org/10.1155/2018/6194906, 2018.

Chuang, J.-J., Huang, Y.-Y., Lo, S.-H., Hsu, T.-F., Huang, W.-Y., Huang, S.-L., et al. (2017). Effects of pH on the shape of alginate particles and its release behavior. *International Journal of Polymer Science, 2017.* , Article 3902704. https://doi.org/10.1155/2017/3902704

Chuesiang, P., Sanguandeekul, R., & Siripatrawan, U. (2020). Phase inversion temperature-fabricated cinnamon oil nanoemulsion as a natural preservative for prolonging shelf-life of chilled Asian seabass (Lates calcarifer) fillets. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology.* , Article 109122. https://doi.org/10.1016/j.lwt.2020.109122

Code of federal regulations (CFR). (2019). *Title 21: Food and drugs. Chapter I - food and drug administration, department of health and human services, subchapter B - food for human consumption (continued), Part 182- substances generally recognized as safe (GRAS), subpart A - general provisions, subpart 182.20 - essential oils, oleoresins (solvent-free), and natural extractives (including distillates)*.

Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. *Journal of Biotechnology, 233*, 106–120. https://doi.org/10.1016/j.jbiotec.2016.07.005

Fabra, M. J., Falcó, I., Randazzo, W., Sánchez, G., & López-Rubio, A. (2018). Antiviral and antioxidant properties of active alginate edible films containing phenolic extracts. *Food Hydrocolloids, 81*, 96–103. https://doi.org/10.1016/j.foodhyd.2018.02.026

Falleh, H., Ben Jemaa, M., Saada, M., & Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. *Food Chemistry, 330*, Article 127268. https://doi.org/10.1016/j.foodchem.2020.127268

FAO. (2020). *FAO yearbook. Fishery and aquaculture statistics FAO yearbook. Fishery and aquaculture statistics.* Roma, Italy: Food and Agriculture Organization.

Frank, K., Garcia, C. V., Shin, G. H., & Kim, J. T. (2018). Alginate biocomposite films incorporated with cinnamon essential oil nanoemulsions: Physical, mechanical, and antibacterial properties; SEM; activity of CEO-NEs and biocomposite films. *International Journal of Polymer Science, 2018, 8.* https://doi.org/10.1155/2018/1519407

Giménez, B., Roncalés, P., & Beltrán, J. A. (2002). *Modified atmosphere packaging of filleted rainbow trout, 82*(10), 1154–1159. https://doi.org/10.1002/jsfa.1136

Girova, T., Gochev, V., Jirovetz, L., Buchbauer, G., Schmidt, E., & Stoyanova, A. (2010). Antimicrobial activity of essential oils from spices against psychrotrophic food spoilage microorganisms. *Biotechnology & Biotechnological Equipment, 24*(sup1), 547–552. https://doi.org/10.1080/13102818.2010.10817895

Haghighi, H., Biard, S., Bigi, F., De Leo, R., Bedin, E., Pfeifer, F., … Pulvirenti, A. (2019). Comprehensive characterization of active chitosan-gelatin blend films enriched with different essential oils. *Food Hydrocolloids, 95*, 33–42. https://doi.org/10.1016/j.foodhyd.2019.04.019

Hao, R. Y., Liu, Y., Sun, L. M., Xia, L. N., Jia, H., Li, Q., et al. (2017). Sodium alginate coating with plant extract affected microbial communities, biogenic amine formation and quality properties of abalone (Haliotis discus hannai Ino) during chill storage. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 81*, 1–9. https://doi.org/10.1016/j.lwt.2017.03.031

Hao, R. Y., Pan, J. F., Khalili Tilami, S., Shah, B. R., & Mráz, J. (2020). Post-mortem quality changes of common carp (Cyprinus carpio) during chilled storage from two culture systems. *Journal of the Science of Food and Agriculture, 101*(n/a), 91–100. https://doi.org/10.1016/10.1002/jsfa.10618

Hao, R. Y., Roy, K., Pan, J. F., Shah, B. R., & Mráz, J. (2021). Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses.

Trends in Food Science & Technology, 111, 175–190. https://doi.org/10.1016/j.tifs.2021.02.054

Hassoun, A., & Çoban, Ö. E. (2017). Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. *Trends in Food Science & Technology, 68*, 26–36. https://doi.org/10.1016/j.tifs.2017.07.016

Huang, Z., Liu, X., Jia, S., & Luo, Y. (2017). Antimicrobial effects of cinnamon bark oil on microbial composition and quality of grass carp (Ctenopharyngodon idellus) fillets during chilled storage. *Food Control, 82*, 316–324. https://doi.org/10.1016/j.foodcont.2017.07.017

Huang, Z., Liu, X. C., Jia, S. L., Zhang, L. T., & Luo, Y. K. (2018). The effect of essential oils on microbial composition and quality of grass carp (Ctenopharyngodon idellus) fillets during chilled storage. *International Journal of Food Microbiology, 266*, 52–59. https://doi.org/10.1016/j.ijfoodmicro.2017.11.003

ICMSF. (1986). *Microorganisms in foods* (Vol. 2). Toronto: University of Toronto Press.

Javidi, Z., Hosseini, S. F., & Rezaei, M. (2016). Development of flexible bactericidal films based on poly(lactic acid) and essential oil and its effectiveness to reduce microbial growth of refrigerated rainbow trout. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 72*, 251–260. https://doi.org/10.1016/j.lwt.2016.04.052

Joukar, F., Hosseini, S. M. H., Moosavi-Nasab, M., Mesbahi, G. R., & Behzadnia, A. (2017). Effect of Farsi gum-based antimicrobial adhesive coatings on the refrigeration shelf life of rainbow trout fillets. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 80*, 1–9. https://doi.org/10.1016/j.lwt.2017.01.074

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

Mailgaad, M., Civille, G. V., & Carr, B. T. (1999). *Sensory evaluation techniques*. Boca Raton, Florida: CRS Press.

Meral, R., Alav, A., Karakas, C., Dertli, E., Yilmaz, M. T., & Ceylan, Z. (2019). Effect of electrospun nisin and curcumin loaded nanomats on the microbial quality, hardness and sensory characteristics of rainbow trout fillet. *Lebensmittel-Wissenschaft und -Technologie, 113*, Article 108292. https://doi.org/10.1016/j.lwt.2019.108292

Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & McClements, D. J. (2016). Superior antibacterial activity of nanoemulsion of Thymus daenensis essential oil against E. coli. *Food Chemistry, 194*, 410–415. https://doi.org/10.1016/j.foodchem.2015.07.139

Oguzhan Yildiz, P. (2017). The effects of chitosan coatings enriched with thyme oil on the quality of rainbow trout. *Journal of Food Measurement and Characterization, 11* (3), 1398–1405. https://doi.org/10.1007/s11694-017-9518-1

Öztürk Kerimoğlu, B., KavuŞAn, H. S., & SerdaroĞLu, M. (2020). The impacts of laurel (Laurus nobilis) and basil (Ocimum basilicum) essential oils on oxidative stability and freshness of sous-vide sea bass fillets [Article] *Turkish Journal of Veterinary and Animal Sciences*, (1), 101–109. https://doi.org/10.3906/vet-1908-25.

Prakash, A., Baskaran, R., Paramasivam, N., & Vadivel, V. (2018). Essential oil based nanoemulsions to improve the microbial quality of minimally processed fruits and vegetables: A review. *Food Research International, 111*, 509–523. https://doi.org/10.1016/j.foodres.2018.05.066

Purwanti, N., Zehn, A. S., Pusfitasari, E. D., Khalid, N., Febrianto, E. Y., Mardjan, S. S., … Kobayashi, I. (2018). Emulsion stability of clove oil in chitosan and sodium alginate matrix. *International Journal of Food Properties, 21*(1), 566–581. https://doi.org/10.1080/10942912.2018.1454946

Rezaeifar, M., Mehdizadeh, T., Mojaddar Langroodi, A., & Rezaei, F. (2020). Effect of chitosan edible coating enriched with lemon verbena extract and essential oil on the shelf life of vacuum rainbow trout (Oncorhynchus mykiss). *Journal of Food Safety, 40* (3), Article e12781. https://doi.org/10.1111/jfs.12781

Shahidi, F., & Hossain, A. (2020). Preservation of aquatic food using edible films and coatings containing essential oils: A review. *Critical Reviews in Food Science and Nutrition*, 1–40. https://doi.org/10.1080/10408398.2020.1812048

Socaciu, M.-I., Semeniuc, C., & Vodnar, D. (2018). Edible films and coatings for fresh fish packaging: Focus on quality changes and shelf-life extension. *Coatings, 8*(10), 366. https://doi.org/10.3390/coatings8100366

Sterniša, M., Bucar, F., Kunert, O., & Smole Možina, S. (2020). Targeting fish spoilers Pseudomonas and Shewanella with oregano and nettle extracts. *International Journal of Food Microbiology, 328*, Article 108664. https://doi.org/10.1016/j.ijfoodmicro.2020.108664

Tokur, B. K., Sert, F., Aksun, E. T., & Özoğul, F. (2016). The effect of whey protein isolate coating enriched with thyme essential oils on trout quality at refrigerated storage (4 ± 2°C). *Journal of Aquatic Food Product Technology, 25*(4), 585–596. https://doi.org/10.1080/10498850.2014.896063

Wang, H., Wang, H. Y., Li, D. P., & Luo, Y. K. (2018). Effect of chitosan and garlic essential oil on microbiological and biochemical changes that affect quality in grass carp (ctenopharyngodon idellus) fillets during storage at 4°C. *Journal of Aquatic Food Product Technology, 27*(1), 80–90. https://doi.org/10.1080/10498850.2017.1403525

Wu, J. L., Ge, S. Y., Liu, H., Wang, S., Chen, S. F., Wang, J. H., … Zhang, Q. Q. (2014). Properties and antimicrobial activity of silver carp (Hypophthalmichthys molitrix) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. *Food Packaging and Shelf Life, 2*(1), 7–16. https://doi.org/10.1016/j.fpsl.2014.04.004

Yu, D., Xu, Y., Jiang, Q., & Xia, W. (2017). Effects of chitosan coating combined with essential oils on quality and antioxidant enzyme activities of grass carp (Ctenopharyngodon idellus) fillets stored at 4 °C. *International Journal of Food Science and Technology, 52*(2), 404–412. https://doi.org/10.1111/ijfs.13295

Ziaee, E., Razmjooei, M., Shad, E., & Eskandari, M. H. (2018). Antibacterial mechanisms of Zataria multiflora Boiss. essential oil against Lactobacillus curvatus. *Lebensmittel-Wissenschaft und -Technologie, 87*, 406–412. https://doi.org/10.1016/j.lwt.2017.08.089

CHAPTER 5

DOSE AFFECTED THE ROLE OF GALLIC ACID ON MEDIATING GELLING PROPERTIES OF OXIDATIVELY STRESSED JAPANESE SEERFISH MYOFIBRILLAR PROTEIN

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Dose affected the role of gallic acid on mediating gelling properties of oxidatively stressed Japanese seerfish myofibrillar protein

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2,4,6-Trinitrobenzenesulfonic acid (PubChem CID: 11045)
5,5′-Dithiobis-(2-nitrobenzoic acid) (PubChem CID: 6254)
β-mercaptoethanol (PubChem CID: 1567)
Phenylmethylsulfonyl fluoride (PubChem CID: 4784)
N-ethylmaleimide (PubChem CID: 4362)

ABSTRACT

The study investigated effects of gallic acid (GA, 0, 1, 5, 25 and 125 μmol/g) on properties of oxidatively stressed Japanese seerfish myofibrillar protein (MFP). Results showed that GA alleviated carbonyls formation and protected free amine. 5 μmol/g GA stabilized sulphydryls and secondary structure while 125 μmol/g GA enabled great loss of sulphydryls and reduced α-helix structure. Analysis of tryptophan fluorescence and surface hydrophobicity indicated that GA induced the unfolding of MFP structure but not in a dose-response fashion. Polymers were formed along with marked attenuation of myosin heavy chain in MFP with 125 μmol/g GA, and its particle size was the largest. Compared with purely oxidized MFP, MFP with 125 μmol/g GA showed a radical peak with narrower peak width but higher intensity. Results imply that high dose GA formed thiol-quinone adducts, enhancing polymerization. It also formed stable protein-bound phenoxyl radicals, inhibiting protein oxidation. Compared with non-oxidized group, storage modulus of MFP with 5 μmol/g GA increased sharply but that of MFP with 125 μmol/g GA decreased distinctly. The study suggests the role of GA on MFP depends much on its dose. Low dose GA could be used for improving fish MFP gelling property.

1. Introduction

Phenolic compounds are secondary metabolites commonly existing in herbs and fruits. As natural antioxidants, they show good radical-scavenging capabilities and beneficial influences on human health such as attenuate inflammation, inhibit tumor growth, promote cardiovascular functions, etc. (Maqsood, Benjakul, Abushelaibi, & Alam, 2014; Shahidi & Zhong, 2010). The advantages in availability, safety and health enable the incorporation of them into food for enhancing food stability and quality a good strategy. Numerous studies have approved the effective roles of phenolic compounds on retarding lipid oxidation (Brewer, 2011; Cao, Ai, True, & Xiong, 2018; Jiang & Xiong, 2016). However, their effects on protein oxidation are not consistent, could be anti- or pro-oxidative both, resulting in variable protein properties (Cao, True, Chen, & Xiong, 2016; Ganhão, Morcuende, & Estévez, 2010; Sabeena Farvin, Grejsen, & Jacobsen, 2012; Shi, Cui, Yin, Luo, & Zhou, 2014).

In fact, phenolic compounds could interact with proteins in both covalent and non-covalent style to modify protein structure, side-chain groups and pattern, leading to the improvement or deterioration of protein functionalities, depend on phenolics category, concentration and food matrix. Balange and Benjakul (2009b), Balange and Benjakul (2009a) reported that optimum levels of oxidized ferulic acid (0.2%), tannic acid (0.05%) and caffeic acid (0.15%) could induce conformational changes in myofibrillar protein (MFP) from bigeye sanpper and mackerel, enhancing cross-linking through amino groups or disulphide

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bond to form stronger surimi gel. Nevertheless, non-oxidized phenolics did not show this effect. Jia, Wang, Shao, Liu, and Kong (2017) found 10 μmol/g catechin led to increased MFP gel strength while 50–200 μmol/g catechin caused severe deterioration of gelation. Some studies claimed that low and moderate content of phenolics might induce the unfolding of MFP to improve MFP gel properties, however, high dose of phenolics could lead to excessive aggregation, preventing the development of fine gel structure (Cao & Xiong, 2015; Feng et al., 2017; Jongberg, Tørngren, Gunvig, Skibsted, & Lund, 2013). But mechanism behind this dose selection effect is not fully understood. Therefore, phenolics impacts on protein are highly contingent and the underlying mechanisms need further elucidations.

Gallic acid (GA) is a water-soluble phenolic compound with strong anti-oxidative (Abdelwahed et al., 2007) and pharmacological actions (Karimi-Khouzani, Heidarian, & Amini, 2017; Precupas, Leonties, Neacsu, Sandu, & Popa, 2019). It has been showed to play dual role (anti- and pro-oxidant) in mediating pork MFP gelation (Cao et al., 2016). But no report of its application in fish gel food is available to our best knowledge. Japanese seerfish (JS) is a common economic fish species in China. Its muscle is usually used for producing gel food. However, its MFP showed poor gelation capability and is susceptible to oxidation due to abundant oxidation initiators such as H_2O_2, hemoglobin and lipids. This study investigated dose effect of GA on structure and gelling potential of JS MFP exposed to imitated Fenton system. Free radical intensity in samples was analyzed by electron spin resonance (ESR) for better understanding of GA role. Results would provide theory support for manipulating oxidation and improving JS MFP gelling properties by GA.

2. Materials and methods

2.1. Materials

Analytical grade GA was purchased from Sangon Biotech Co. Ltd., Shanghai, China. Japanese seerfish (*Scomberomours niphonius*) (350–500 g) were fished in oceanic area of Changxing Island, Dalian, China in October. The fish were immersed into liquid nitrogen to be frozen, transferred into lab at National Engineering Research Center for Seafood in 12 h and stored at −80 °C.

2.2. Preparation of MFP sample

2.2.1. MFP extraction

Frozen mackerels were partially thawed to let filleting dorsal muscle. MFP was extracted according to the method of Park, Xiong, and Alderton (2007) with modifications. Dorsal muscle was homogenized with isolation buffer (0.1 M NaCl-0.02 mM Tris-Maleic acid, pH = 6.8, 1:4, g:mL), centrifuged (10,000 g, 10 min) and the supernatant was discarded. The above procedure was repeated once. Obtained precipitates were re-suspended and filtered with gauze to remove insoluble tissue. The filtered was centrifuged and pellets were collected as MFP. For MFP solution used for experiment, MFP pellets were dissolved in 0.6 M Tris-NaCl and protein concentration was measured using a Biuret assay kit (Beijing Biolab Co. Ltd., Beijing, China).

2.2.2. Oxidation with GA

MFP solution (40 mg/ml) was prepared and treated with GA in Fenton oxidation system according to Cao and Xiong (2015). Different levels of GA (0, 1, 5, 25 and 125 μM/g protein) were added into MFP solutions within Fenton oxidation system (10 μM $FeCl_3$, 100 μM ascorbic acid, 1 mM H_2O_2). These systems were incubated at 4 °C for 12 h and oxidation was terminated by adding 1 mM trolox. Samples were labeled as: non-oxidized sample, NOX; samples oxidized with 0, 1, 5, 25 and 125 μmol/g protein GA, OX+0, OX+1, OX+5, OX+25 and OX+125.

2.3. Changes in amino acid side-chain groups

Carbonyl content was measured using 2,4-dinitrophenylhydrazine (DNPH) method as Li, Xiong, and Chen (2012) with modification. The sample was reacted with 10 mM DNPH and terminated with 20% TCA. The precipitates were washed using ethanol/ethyl acetate and dissolved in 6 M guanidine hydrochloride. After 15 min incubation at 37 °C, absorbance at 370 nm was recorded. Carbonyl content was calculated using a molar extinction coefficient 22 000 L M^{-1}·cm^{-1}.

Total sulphydryl (SH) content was determined by 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) method as Liu, Xiong, and Butterfield (2000) with modifications. Sample was reacted with DTNB (10 mM) at room temperature for 15 min. Absorbance at 412 nm was recorded. Molar extinction coefficient 13,600 L M^{-1}·cm^{-1} was used for calculating SH content.

Free amine content was measured by 2,4,6-trinitrobenzenesulfonic acid (TNBS) method as Adler-Nissen (1979) with modifications. Sample was reacted with 0.01%TNBS at 50 °C for 30 min. Reaction was stopped with 0.1 M Na_2SO_3. Absorbance at 420 nm was recorded. Free amine content was calculated from a standard curve produced with L-leucine.

2.4. Changes in structure

Secondary structure of MFP was analyzed using a J-1500 circular dichroism (CD) meter (JASCO Co. Ltd., Tokyo, Japan). MFP was diluted to 0.2 mg/mL and scanned from 200 to 260 nm within a 0.1 cm quartz cell.

Tertiary structure information of MFP was examined by analyzing tryptophan fluorescence and surface hydrophobicity. Tryptophan fluorescence of MFP (0.4 mg/ml) was measured using a fluorescence spectrophotometer (F-2700, Hitachi Co. Ltd., Tokyo, Japan). Excitation wavelength was set as 283 nm and emission spectra were 300–400 nm. Surface hydrophobicity was measured using bromophenol blue (BPB)-binding method as Chelh, Gatellier, and Santé-Lhoutellier (2006). Sample was incubated with 1% BPB at room temperature for 10 min and centrifuged. Absorbance at 595 nm of supernatant (diluted × 100) was recorded. Surface hydrophobicity calculated as formula:

$$\text{BPB boud } (\mu g) = 20\ \mu g \times (A_{CO} - A_{sample})/A_{CO}$$

2.5. Particle size distribution

Diluted MFP (1 mg/mL) was used for particle size distribution analysis using a Zetasizer 3000HSA (Malvern Malvern Panalytical Ltd., UK). Excitation wavelength was 633 nm and scattered light intensity detector angle was 173°.

2.6. MFP cross-linking

Cross-linking was analyzed using SDS−PAGE electrophoresis. MFP was diluted with loading buffer with or without β-mercaptoethanol (βME, 10%). N-ethylmaleimide (NEM, 1 mM) was added into sample without βME for avoiding thermal cross-linking. 5% stacking gel and 12% resolving gel were used for protein separation. 20 μg MFP was loaded. Electrophoresis was done at 30 mA current for 1 h using a Mini Unit AE-8135 (ATTO Corp., Tokyo, Japan). Gel was stained with Coomassie brilliant blue and destained with methanol and acetic acid.

To expose cross-linking location, MFP was hydrolyzed by chymotrypsin as E:S = 1:500 at 25 °C for 1 h and terminated with 0.5 mM PMSF. The hydrolyzed sample was analyzed by above method.

2.7. Rheological properties

Rheological properties of MFP (40 mg/ml) were analyzed using a Discovery HR-1 rheometer (TA Instrument, New Castle, UK). Sample was loaded into 1 mm gap between two parallel plates (upper plate, 40 mm in diameter). Temperature ramp test was performed as: frequency 0.2 Hz, stress 0.6 Pa, temperature 20–80 °C, heating rate 1 °C/min. Storage modulus (G') was recorded.

2.8. Protein radical intensity

Radical intensity in sample was analyzed by ESR. Lyophilized MFP powder was filled into a fused quartz ESR tube until the height of filling was 3 cm. Tube with sample was placed into an ESR spectrometer A200 (BRUKER Corporation, Karisruhe, Germany) and analyzed as following condition: microwave power, 4 mV; center field, 3460 Gauss; sweep width, 200 Gauss; sweep time, 491.52 s; modulation width, 2 Gauss; modulation amplitude, 1 Gauss; time constant, 5242.88 ms.

2.9. Statistical analysis

Data were presented as mean ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA). Comparison of means was done with Duncan's multiple range tests using SPSS 19.0 (SPSS Inc, Chicago, IL, USA). Difference was significant if $p < 0.05$.

3. Results and discussion

3.1. Changes in amino acid side-chain groups

3.1.1. Carbonyls

Amino acid side-chain groups are prone to forming carbonyl derivatives during oxidation. Carbonyl content in NOX was 0.43 µmol/g protein. It showed a net increase of 1.26 µmol/g after oxidation (Fig. 1A). Compared with OX + 0, addition of 1, 5, 25, 125 µmol/g GA lowered carbonyl content by 54%, 66%, 32%, 56%, respectively. GA could act as ·OH scavenger through GA autoxidation to form phenoxyl radicals that can neutralizes ·OH (Vijayalakshmi, Adinarayana, & Rao, 2010). As metal ion chelator, it also plays a key role on hampering ferrous ions that could initiate oxidative modification toward amino acid side-chain groups and form carbonyls (Stadtman, 2006). Low dose GA (1, 5 µmol/g) most likely inhibit oxidation through above mechanisms. Additionally, high dose (125 µmol/g) GA might protect against carbonyl formation by forming stable protein-bound phenoxyl radicals that could decelerate oxidation (Jongberg et al., 2013). This was approved by the radical assessment using ESR in our study (see section 3.7). It is noticed that the inhibition of oxidation by GA is not a dose-dependent fashion. Though the overall effect was anti-oxidative, middle dose (25 µmol/g) GA might promote H_2O_2 production, resulting in higher carbonyls. Similar pro-oxidant effect of GA was reported by Utrera and Estévez (2013) and Yen, Duh, and Tsai (2002). Thus, anti-oxidative or pro-oxidative effects of GA on MFP depended much on its concentration and matrix.

3.1.2. Sulphydryls

SH in myosin are susceptible to ·OH and often oxidized into disulfide bonds, resulting in polymerization. Decline of SH content by 14.6% and 18.3% were observed in OX + 0 and OX + 1 (Fig. 1B). 5 µmol/g GA preserved SH content at 96%, but 25 µmol/g GA did not show protective effect. It is speculated that low dose GA could act as anti-oxidant to protect SH while middle dose GA might have a combined effect of anti-oxidant and pro-oxidant which might not so effective on SH protection. Compared with NOX, SH content of OX + 125 was reduced by 47.8%. Heavy loss of SH with high dose phenolics was also observed in pork MFP oxidatively stressed with chlorogenic acid (CA) (Cao & Xiong, 2015) and catechin (Jia et al., 2017). Phenolic compounds can be oxidized into quinone, which could react with protein SH to form thiol-quinone adducts by Michael addition, resulting in its distinct loss (Cao

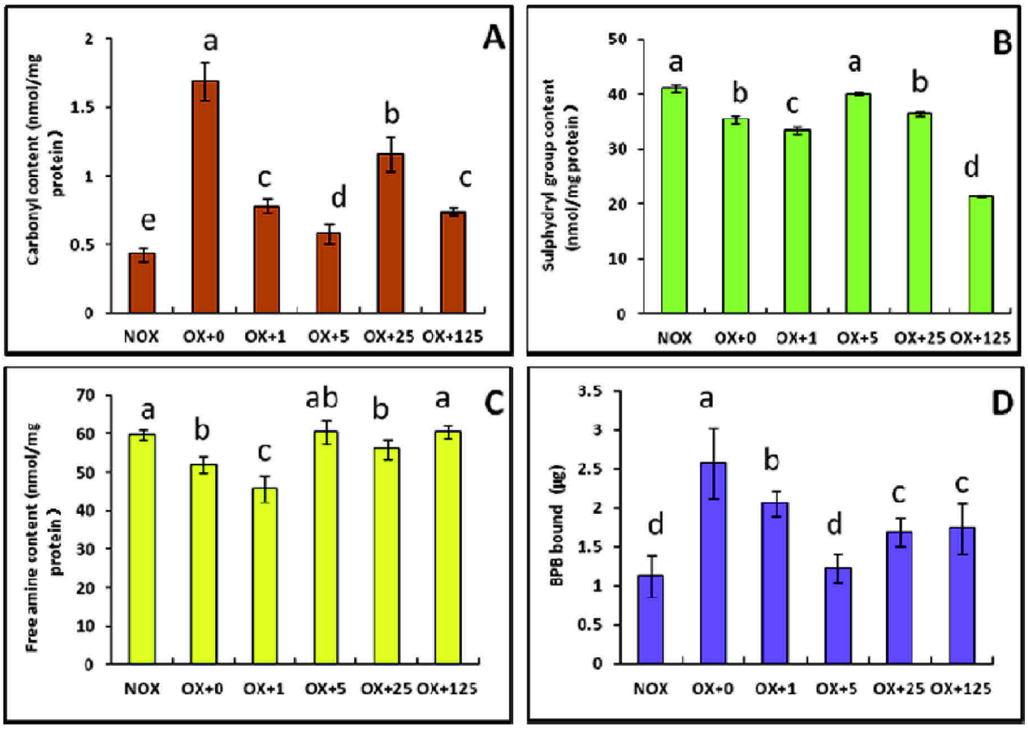

Fig. 1. Physiochemical properties of myofibrillar protein of Japanese seerfish oxidatively stressed with different levels (0, 1, 5, 25 and 125 µmol/g protein) of gallic acid. NOX, non-oxidized; OX, oxidized with gallic acid. The lowercase letters denote significant differences between groups ($p < 0.05$).

& Xiong, 2015; Jongberg et al., 2011, 2013). GA might also promote SH loss through this mechanism. Since the carbonyl content of OX + 125 was low, it is not likely that SH groups were largely oxidized into disulfide bonds but mainly formed thiol-quinone adducts. More discussions are given in section 3.4.

3.1.3. Free amines

NH_2 group could react with carbonyl derivatives, leading to the decrease of free amine content (Levine et al., 1990). In Fig. 1C, free amine content in OX + 0 and OX + 1 decreased by 13% and 23% compared with NOX, coincided with their increased carbonyl content. Different from Cao and Xiong (2015) who reported that CA hardly inhibited the ·OH-induced free amine loss, free amine of MFP with GA was well protected. This could be attributed to the smaller size and more phenol hydroxyl groups of GA than CA, which provides higher anti-oxidative activity, especially for low and middle dose of GA-adding groups. Stable protein-bound phenoxyl radicals formed at high dose of GA might also alleviate oxidation and maintain more free amines. It is proposed that covalent adduction of quinone formed by phenolics to free amines could lead to their decrease, which was observed in whey protein, soy protein (Kroll, uuml, Rawel, & Rohn, 2003) and pork MFP (Cao et al., 2016; Cao & Xiong, 2015). Our results indicate that high dose GA did not show this phenolic-initiated free amine loss, indicating the pathway for GA to form amine-quinone adducts might not occur.

3.2. Changes in secondary structure

Protein oxidation could lead to change in secondary structure. In Fig. 2, peaks representing α-helix structure were observed at 210 and 223 nm in CD spectrum of NOX. Nevertheless, intensity of them sharply declined in OX + 0 and OX + 1, suggesting the great loss of α-helix conformation caused by oxidation, in agreement with previous findings by Sun, Zhou, Sun, and Zhao (2013). OX + 5 showed similar altitude of two peaks with NOX, which could be attributed to the anti-oxidative role of GA. However, attenuated peak intensity was observed in OX + 25 and OX + 125, but both were higher than that in NOX. It is possible that GA interacted with MFP directly or indirectly as quinone, leading to the loss of α-helix structure. Above results indicate that low level GA could stabilize the secondary structure of MFP, but middle and high dose of GA might disrupt its initial structure. Cao and Xiong (2015) also found decreased intensity of α-helix-representing peak in oxidatively stressed MFP with 30–150 μmol/g CA, which was even lower than that of oxidized group without CA. α-helix conformation is mainly maintained by hydrogen bonds between C=O and –NH. The presence of multiple hydroxyl groups in CA could disturb the hydrogen bonds. Since GA contains less hydroxyl groups than CA, thus, the intervention to protein structure by GA might not be pronounced as CA. Thus, modification of protein secondary structure depends on the structure and dose of phenolic compound.

3.3. Changes in tertiary structure

3.3.1. Surface hydrophobicity

Protein bound BPB content is often applied for estimating surface hydrophobicity which can represent protein structure unfolding (Chelh et al., 2006). Compared with NOX, BPB content of OX + 0 increased by 128%, and that of GA-adding groups, other than OX + 5, increased as well (Fig. 1D). Oxidation could induce unfolding of protein structure to expose more hydrophobic area, leading to an increase BPB binding content. 1 μmol/g GA was not effective on inhibiting oxidation, thus BPB content increased sharply. However, 5 μmol/g GA showed a good anti-oxidative role. The MFP structure might not heavily affected by oxidation, showing a stable BPB content. As aforementioned, 25 μmol/g GA exerted a net effect of pro-oxidant. As a result, MFP might suffer fair oxidation and expose more hydrophobic amino acids. It is reported that high dose of phenolics could promote protein unfolding and increase surface hydrophobicity (Cao & Xiong, 2015; Jia et al., 2017). Increased BPB content in OX + 125 is consistent with the conclusion, but different from the decreased surface hydrophobicity in pork MFP oxidatively stressed with epigallocatechin-3-gallate (Cao et al., 2018). Thus, category and dose of phenolic compounds decide the role of them on protein.

3.3.2. Tryptophan fluorescence

Protein tryptophan fluorescence is often used for indicating protein conformational change (Papadopoulou, Green, & Frazier, 2005). In Fig. 3, tryptophan fluorescence intensity of OX + 0 was much higher than that of NOX, suggesting MFP unfolding after oxidation, coincided with increased surface hydrophobicity. Tryptophan fluorescence intensity decreased with increasing GA content, exhibiting a typical dose-response style, and a weak red shift of λm from 333 to 336 nm was observed in OX + 125. Similar red shift was also observed in EGCG and CA interaction with pork MFP (Cao et al., 2018). Results imply that tryptophan residues were brought into a more hydrophilic environment by GA binding. The quenching constant K_{sv} and quenching rate constant K_q of GA calculated by Stern-Volmer equation were

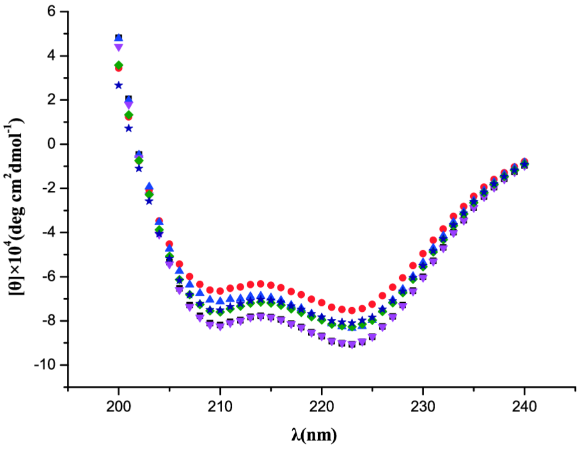

Fig. 2. Circular dichroism spectroscopy of (0.2 mg/mL) myofibrillar protein of Japanese seerfish oxidatively stressed with different levels (0, 1, 5, 25 and 125 μmol/g protein) of gallic acid. ■ NOX, ● OX + 0, ▲ OX + 1, ▼ OX + 5, ◆ OX + 25, ★ OX + 125.

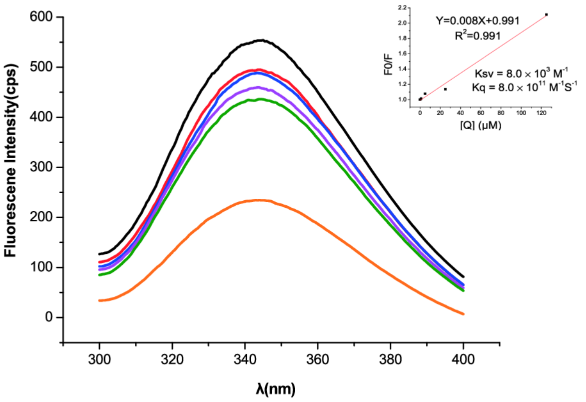

Fig. 3. Tryptophan fluorescence of (0.4 mg/mL) myofibrillar protein of Japanese seerfish oxidatively stressed with different levels (0, 1, 5, 25 and 125 μmol/g protein) of gallic acid (GA). ▬ NOX, ▬ OX + 0, ▬ OX + 1, ▬ OX + 5, ▬ OX + 25, ▬ OX + 125.

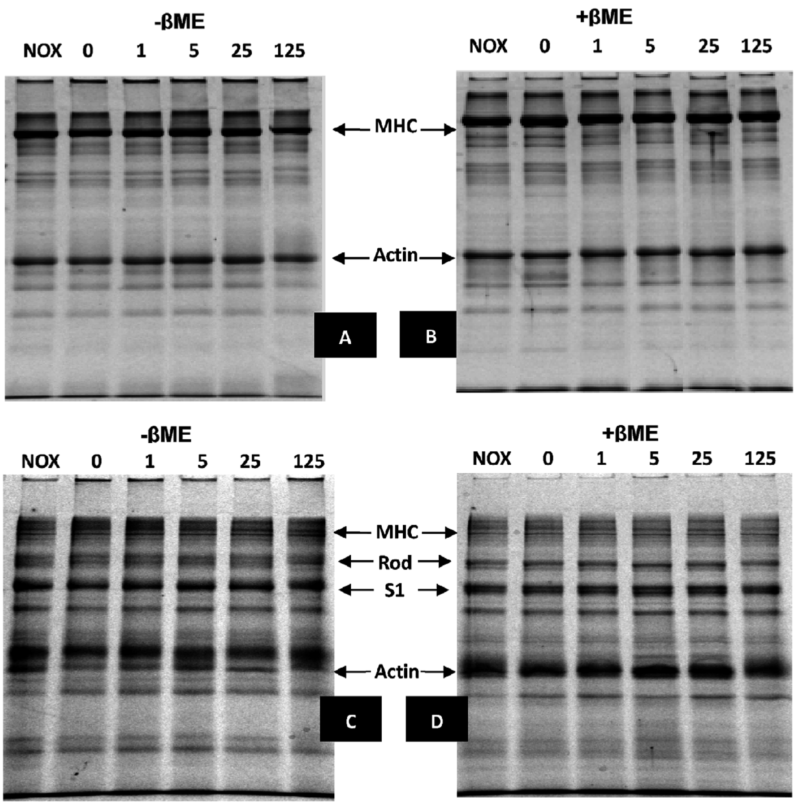

Fig. 4. SDS–PAGE patterns of myofibrillar protein of Japanese seerfish oxidatively stressed in the absence of gallic acid at different levels (0, 1, 5, 25 and 125 μmol/g protein) in different styles: A and B, integrate JSMP; C and D, digested by chymotrypsin into MHC, Actin, Rod, S1 style. Samples were prepared in the presence (+βME) or absence (-βME) of β-mercaptoethanol. NOX, non-oxidized; MHC, myosin heavy chain.

$8.0 \times 10^3\ M^{-1}$ and $8.0 \times 10^{11}\ M^{-1}\ S^{-1}$, similar with those ($6.9 \times 10^3\ M^{-1}$ and $6.9 \times 10^{11}\ M^{-1}\ S^{-1}$) reported by Cao et al. (2016). Since Kq was much larger than the maximum diffusion collision quenching rate constant of various quenchers with biopolymer ($2.0 \times 10^{10}\ M^{-1}\ S^{-1}$) (Ware, 1962), the fluorescence quenching by GA should be a static style. Interestingly, both tryptophan fluorescence intensity and surface hydrophobicity of OX+25 and OX+125 was lower than that of OX+0, indicating the decreased tryptophan fluorescence intensity was mainly attributed to the binding of GA with MFP which brought polar hydrol groups but not only the unfolding of protein structure.

3.4. Cross-linkings in MFP

In Fig. 4A, all groups showed polymers on the top stacking gel compared with NOX, and MHC band in OX+0, OX+1 and OX+125 was attenuated markedly while that of OX+5 and OX+25 remained strong. In reducing condition (Fig. 4B), these polymers largely disappeared accompanied with recovery of most MHC bands. It is well established that protein cross-linking could be formed via disulfide and non-disulfide bonds during oxidation (Youling L. Xiong, 2000), which is evidenced by decreased sulfhydryls and free amine content in OX+0 and OX+1. As aforementioned, low dose GA could inhibit oxidation, thus, MHC might be protected. For the heavy loss of MHC in OX+125, it is speculated that high dose GA could form quinone to promote MFP cross-linking. Jongberg et al. (2013) and Cao and Xiong (2015) also observed enhanced MFP polymerization along with distinct MHC loss in pork sausage and MFP with high dose green tea extract or CA. A proposed reaction mechanism of GA with JS MFP is showed in Fig. 5. It is deduced that GA quinone is formed by ·OH attack firstly and then it reacts with protein SH to form thiol-quinone adducts. These thiol-quinone adducts might be further oxidized into a new quinone that could react with protein SH again to form GA-mediated protein polymer which could be reduced by βME. Different from stable actin observed in MFP with CA (Cao & Xiong, 2015), actin in OX+0 and OX+125 faded fairly, indicating its susceptibility to oxidation and involvement in interaction with GA. Samples were hydrolyzed into Rod and S1 by chymotrypsin to investigate the cross-linking location. In Fig. 4C, polymers were showed on the top of stacking gel in OX+0, OX+1 and OX+125, but those in NOX, OX+5 and OX+25 were not remarkable, indicating severe cross-linking of MFP in OX+0, OX+1 and OX+125 occurred, forming polymers possessing compact structure resistant to chymotrypsin. Sharp declined intensity of Rod and S1 was also observed in OX+125, suggesting their participation in the interaction with GA.

3.5. Particle size distribution

In Fig. 6, particle size distribution of OX+0 showed a new peak at 2223 nm, though a main peak around 710 nm similar to that of NOX was observed. Oxidation could induce protein unfolding and promote the interaction of protein molecules to form aggregations, resulting in large particle size. This is confirmed by increased surface hydrophobicity and decreased sulfhydryl content as well as new formed polymers. With GA addition, main peak of particle size transferred to 920–1170 nm in OX+1, OX+5 and OX+25 while that of OX+125 sharply increased to approximate 1300 nm. GA could interact with protein molecules to induce aggregation, which is evidenced by the decreased sulfhydryl content and formed polymers. The much larger particle size of OX+125 should be mainly attributed to the polymerization mediated by large amount of GA thiol-quinine adduct as showed in Fig. 5. Particle size distribution of protein closely correlates with the interactions of protein with protein and protein with other

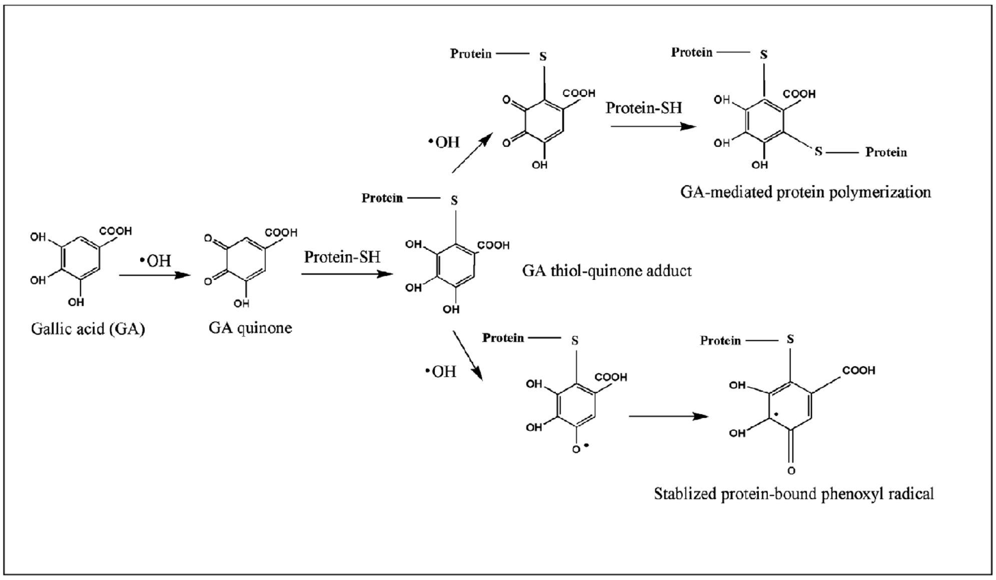

Fig. 5. A proposed pathway of gallic acid quinone reaction with Japanese seerfish myofibrillar protein based o the study of Jongberg et al. (2011) and Jongberg et al. (2013).

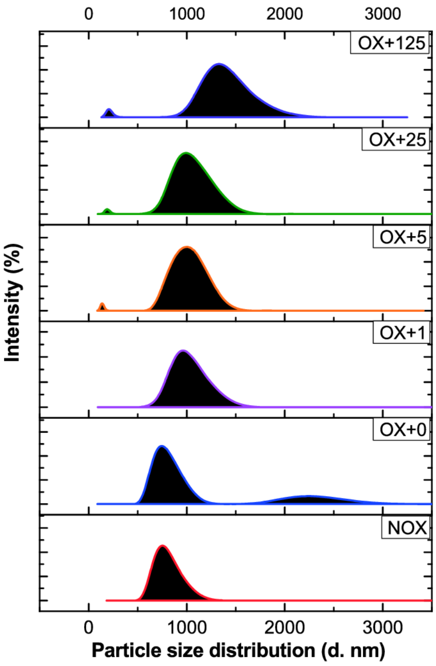

Fig. 6. Particle size distribution of myofibrillar protein of Japanese seerfish oxidatively stressed in the presence of gallic acid at different levels (0, 1, 5, 25 and 125 μmol/g protein).

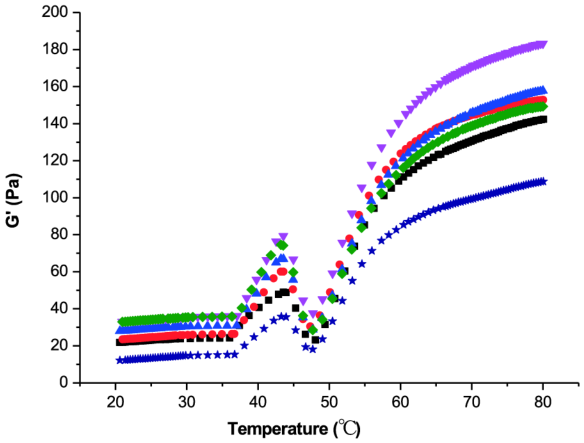

Fig. 7. Storage modulus (G′) of myofibrillar protein of Japanese seerfish oxidatively stressed in the presence of gallic acid at different levels (0, 1, 5, 25 and 125 μmol/g protein) during thermal gelation. ■ NOX, ● OX + 0, ▲ OX + 1, ▼ OX + 5, ◆ OX + 25, ★ OX + 125.

components in matrix. Above results suggest that GA could interact with MFP via covalent and non-covalent way to promote its aggregation, which would affect final gelling properties.

3.6. Rheological properties

Compared with NOX, G′ of OX + 0, OX + 1 and OX + 25 increased slightly while that of OX + 5 climbed distinctly (Fig. 7). It is known that mild oxidation could induce the unfolding of protein, facilitating the interaction of protein molecules during heating to form fine gel structure (Youling L. Xiong, Blanchard, Ooizumi, & Ma, 2010). Increased surface hydrophobicity together with decreased tryptophan fluorescence intensity was found in OX + 0 and OX + 1, and the decrease of sulfhydryl and free amine content in them was not distinct. These denote that mild oxidation induce protein structure change without scarifying functional groups, therefore, improved gelling properties. 5 μmol/g GA preserved the highest level of sulfhydryls and free amine and induced the unfolding of protein. Together with possible quinone-protein interaction, it thus greatly improved rheological properties of MFP. However, 20 μmol/g GA showed subtle pro-oxidative role. It might also form excessive aggregations induced by thiol-quinone adduct which could hamper protein interaction for forming fine gel

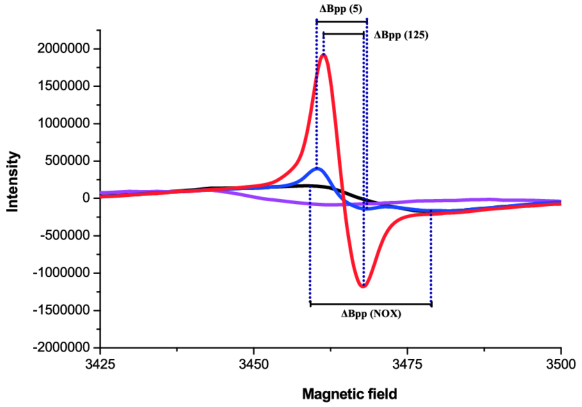

Fig. 8. ESR spectra of myofibrillar protein of Japanese seerfish (freeze dried) oxidatively stressed with the presence of gallic acid (0, 5, 125 μmol/g protein). ΔB_{PP} indicates peak to peak width of radical signal. ▬ NOX, ▬ OX + 0, ▬ OX + 5, ▬ OX + 125.

network. Thus, G′ value did not elevate largely. In contrast, 125 μmol/g GA greatly lowered G' value. High dose GA might lead to excessive aggregation through thiol-quinine adduct pathway, which is unfavorable for forming an ordered gel structure. The superabundant GA could also shield reactive groups and obstruct their participation in gel formation (Cao & Xiong, 2015). Similarly, high dose CA (150 μmol/g) and catechin (50–200 μmol/g) were found to impair pork MFP gelation (Cao & Xiong, 2015; Jia et al., 2017). These results imply that low dose phenolic compounds such as CA and GA can improve MFP gel properties, but high dose of them might impair MFP gel potential. Thus, surimi product industry should avoid using high dose phenolic compounds so as to enable gel property improvement with lower cost.

3.7. Protein radical formation

Radicals, as intermediate products or precursors of oxidative modification, directly relate with oxidation progress in matrix. The radical intensity of JS MFP was determined by ESR spectroscopy to clarify the role of GA at 5 and 125 μmol/g. Fig. 8 shows that OX + 125 had much higher radical intensity than OX + 0 and NOX, indicating heavy accumulation of radicals in it. In most case, increased radical intensity signifies increased oxidative instability, but the increased radical intensity in two GA-adding groups could be discussed. Jongberg et al. (2013) considered that phenolics could donate hydrogen atoms to radicals and be oxidized into phenoxyl radicals themselves. GA might generate protein-bound phenoxyl radicals through this way (see Fig. 5). Since phenoxyl radicals have low reactivity, their formation could inhibit further protein oxidation which is evidenced by low carbonyl content in OX + 125. Interestingly, the peak to peak width (ΔB_{PP}) of radical signal in OX + 125 was much narrower than that in NOX, OX + 0, but similar with it in OX + 5. This suggests that the accumulated radicals in OX + 125 and OX + 5 were different in nature from the counterparts in NOX and OX + 0, supporting the hypothesis of forming protein-bound phenoxyl radicals. More investigations on this stable radical need to be continued for better understanding its role in oxidation process.

4. Conclusions

Low dose (5 μmol/g) of GA could induce partial unfolding of protein structure while not cause server loss of functional groups. Meanwhile, thiol-quinone adducts formed by GA and MFP could enhance the cross-linking of MFP. Thus, low dose of GA could be applied for improving gelation properties of oxidatively stressed JS MFP. However, high dose of GA (125 μmol/g) might form stable protein-bound phenoxyl radical to alleviate oxidation progress, but lead to excessive polymerization of MFP. This would cause great loss of sulphydryls and block reactive functional groups that are supposed to participate in gelation. Therefore, high dose of GA might inhibit gelling properties of oxidatively stressed JS MFP. Fish gel food industry can apply low dose GA to obtain improved gel properties.

Author contribution statement

Jinfeng Pan: Experiment design, data analysis, reviewing manuscript.
Hongliang Lian: Original draft preparation, most experiment.
Hui Jia: Partial experiment, partial data analysis.
Ruoyi Hao: Partial data analysis, figures preparation.
Yujie Wang: Partial experiment analysis.
Huapeng Ju: Partial experiment analysis.
Shengjie Li: Some methodology.
Xiuping Dong: Conceptualization.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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References

Abdelwahed, A., Bouhlel, I., Skandrani, I., Valenti, K., Kadri, M., Guiraud, P., et al. (2007). Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from Pistacia lentiscus. Confirmation by microarray expression profiling. *Chemico-Biological Interactions, 165*(1), 1–13. https://doi.org/10.1016/j.cbi.2006.10.003.

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry, 27*(6), 1256–1262. https://doi.org/10.1021/jf60226a042.

Balange, A. K., & Benjakul, S. (2009a). Effect of oxidised phenolic compounds on the gel property of mackerel (Rastrelliger kanagurta) surimi. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 42*(6), 1059–1064. https://doi.org/10.1016/j.lwt.2009.01.013.

Balange, A. K., & Benjakul, S. (2009b). Enhancement of gel strength of bigeye snapper (Priacanthus tayenus) surimi using oxidised phenolic compounds. *Food Chemistry, 113*(1), 61–70. https://doi.org/10.1016/j.foodchem.2008.07.039.

Brewer, M. S. (2011). Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety, 10*(4), 221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x.

Cao, Y., Ai, N., True, A. D., & Xiong, Y. L. (2018). Effects of (−)-epigallocatechin-3-gallate incorporation on the physicochemical and oxidative stability of myofibrillar protein–soybean oil emulsions. *Food Chemistry, 245*(Supplement C), 439–445. https://doi.org/10.1016/j.foodchem.2017.10.111.

Cao, Y., True, A. D., Chen, J., & Xiong, Y. L. (2016). Dual role (anti- and pro-oxidant) of gallic acid in mediating myofibrillar protein gelation and gel in vitro digestion. *Journal of Agricultural and Food Chemistry, 64*(15), 3054–3061. https://doi.org/10.1021/acs.jafc.6b00314.

Cao, Y., & Xiong, Y. L. (2015). Chlorogenic acid-mediated gel formation of oxidatively stressed myofibrillar protein. *Food Chemistry, 180*, 235–243. https://doi.org/10.1016/j.foodchem.2015.02.036.

Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. *Meat Science, 74*(4), 681–683. https://doi.org/10.1016/j.meatsci.2006.05.019.

Feng, X., Chen, L., Lei, N., Wang, S., Xu, X., Zhou, G., et al. (2017). Emulsifying properties of oxidatively stressed myofibrillar protein emulsion gels prepared with (−)-Epigallocatechin-3-gallate and NaCl. *Journal of Agricultural and Food Chemistry, 65*(13), 2816–2826. https://doi.org/10.1021/acs.jafc.6b05517.

Ganhão, R., Morcuende, D., & Estévez, M. (2010). Tryptophan depletion and formation of α-aminoadipic and γ-glutamic semialdehydes in porcine burger patties with added phenolic-rich fruit extracts. *Journal of Agricultural and Food Chemistry, 58*(6), 3541–3548. https://doi.org/10.1021/jf903356m.

Jiang, J., & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science, 120*, 107–117. https://doi.org/10.1016/j.meatsci.2016.04.005.

Jia, N., Wang, L., Shao, J., Liu, D., & Kong, B. (2017). Changes in the structural and gel properties of pork myofibrillar protein induced by catechin modification. *Meat Science, 127*, 45–50. https://doi.org/10.1016/j.meatsci.2017.01.004.

Jongberg, S., Gislason, N. E., Lund, M. N., Skibsted, L. H., & Waterhouse, A. L. (2011). Thiol–quinone adduct formation in myofibrillar proteins detected by LC-MS. *Journal of Agricultural and Food Chemistry, 59*(13), 6900–6905. https://doi.org/10.1021/jf200965s.

Jongberg, S., Tørngren, M. A., Gunvig, A., Skibsted, L. H., & Lund, M. N. (2013). Effect of green tea or rosemary extract on protein oxidation in Bologna type sausages prepared from oxidatively stressed pork. *Meat Science, 93*(3), 538–546. https://doi.org/10.1016/j.meatsci.2012.11.005.

Karimi-Khouzani, O., Heidarian, E., & Amini, S. A. (2017). Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. *Pharmacological Reports : PR, 69*(4), 830–835. https://doi.org/10.1016/j.pharep.2017.03.011.

Kroll, J., uuml, rgen, Rawel, H. M., & Rohn, S. (2003). Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. *Food Science and Technology Research, 9*(3), 205–218. https://doi.org/10.3136/fstr.9.205.

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A.-G., et al. (1990). *Determination of carbonyl content in oxidatively modified proteins Methods in Enzymology, Vol. 186*, Academic Press464–478.

Liu, G., Xiong, Y. L., & Butterfield, D. A. (2000). Chemical, physical, and gel-forming properties of oxidized myofibrils and whey- and soy-protein isolates. *Journal of Food Science, 65*(5), 811–818. https://doi.org/10.1111/j.1365-2621.2000.tb13592.x.

Li, C., Xiong, Y. L., & Chen, J. (2012). Oxidation-induced unfolding facilitates myosin cross-linking in myofibrillar protein by microbial transglutaminase. *Journal of Agricultural and Food Chemistry, 60*(32), 8020–8027. https://doi.org/10.1021/jf302150h.

Maqsood, S., Benjakul, S., Abushelaibi, A., & Alam, A. (2014). Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in Seafood: A detailed review. *Comprehensive Reviews in Food Science and Food Safety, 13*(6), 1125–1140. https://doi.org/10.1111/1541-4337.12106.

Papadopoulou, A., Green, R. J., & Frazier, R. A. (2005). Interaction of flavonoids with bovine serum Albumin: A fluorescence quenching study. *Journal of Agricultural and Food Chemistry, 53*(1), 158–163. https://doi.org/10.1021/jf048693g.

Park, D., Xiong, Y. L., & Alderton, A. L. (2007). Concentration effects of hydroxyl radical oxidizing systems on biochemical properties of porcine muscle myofibrillar protein. *Food Chemistry, 101*(3), 1239–1246. https://doi.org/10.1016/j.foodchem.2006.03.028.

Precupas, A., Leonties, A. R., Neacsu, A., Sandu, R., & Popa, V. T. (2019). Gallic acid influence on bovine serum albumin thermal stability. *New Journal of Chemistry, 43*(9), 3891–3898. https://doi.org/10.1039/C9NJ00115H.

Sabeena Farvin, K. H., Grejsen, H. D., & Jacobsen, C. (2012). Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (Trachurus trachurus): Effect on lipid and protein oxidation. *Food Chemistry, 131*(3), 843–851. https://doi.org/10.1016/j.foodchem.2011.09.056.

Shahidi, F., & Zhong, Y. (2010). Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science and Technology, 112*(9), 930–940. https://doi.org/10.1002/ejlt.201000044.

Shi, C., Cui, J., Yin, X., Luo, Y., & Zhou, Z. (2014). Grape seed and clove bud extracts as natural antioxidants in silver carp (Hypophthalmichthys molitrix) fillets during chilled storage: Effect on lipid and protein oxidation. *Food Control, 40*, 134–139. https://doi.org/10.1016/j.foodcont.2013.12.001.

Stadtman, E. R. (2006). Protein oxidation and aging. [Article]. *Free Radical Research, 40*(12), 1250–1258. https://doi.org/10.1080/10715760600918142.

Sun, W., Zhou, F., Sun, D.-W., & Zhao, M. (2013). Effect of oxidation on the emulsifying properties of myofibrillar proteins. *Food and Bioprocess Technology, 6*(7), 1703–1712. https://doi.org/10.1007/s11947-012-0823-8.

Utrera, M., & Estévez, M. (2013). Impact of trolox, quercetin, genistein and gallic acid on the oxidative damage to myofibrillar proteins: The carbonylation pathway. *Food Chemistry, 141*(4), 4000–4009. https://doi.org/10.1016/j.foodchem.2013.06.107.

Vijayalakshmi, G., Adinarayana, M., & Rao, P. J. (2010). Kinetics and mechanisms of oxidation of some antioxidants with photochemically generated tert-butoxyl radicals. *Indian Journal of Biochemistry & Biophysics, 47*(5), 292–297.

Ware, W. R. (1962). Oxygen quenching of fluorescence in solution: An experimental study of the diffusion process. *Journal of Physical Chemistry, 66*(3), 316–320. https://doi.org/10.1021/j100809a020.

Xiong, Y. L. (2000). Protein oxidation and implication for muscle food quality. In E. Decker, C. Faustman, & C. J. Lopez-Bote (Eds.). *Antioxidants in muscle foods* (pp. 85–111). Chichester: John Wiley & Sons.

Xiong, Y. L., Blanchard, S. P., Ooizumi, T., & Ma, Y. (2010). Hydroxyl radical and ferryl-generating systems promote gel network formation of myofibrillar protein. *Journal of Food Science, 75*(2), C215–C221. https://doi.org/10.1111/j.1750-3841.2009.01511.x.

Yen, G.-C., Duh, P.-D., & Tsai, H.-L. (2002). Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chemistry, 79*(3), 307–313. https://doi.org/10.1016/s0308-8146(02)00145-0.

CHAPTER 6

ULTRASOUND TREATMENT MODIFIED THE FUNCTIONAL MODE OF GALLIC ACID ON PROPERTIES OF FISH MYOFIBRILLAR PROTEIN

Pan, J.F., Lian, H.L., Jia, H., Li, S.J., Hao, R.Y., Wang, Y.J., Zhang, X.N., Dong, X.P., 2020. Ultrasound treatment modified the functional mode of gallic acid on properties of fish myofibrillar protein. Food Chemistry 320, 126637.

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Ultrasound treatment modified the functional mode of gallic acid on properties of fish myofibrillar protein

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Bromophenol blue (PubChem CID: 8272)
2,4,6-Trinitrobenzenesulfonic acid (PubChem CID: 11045)
5,5′-Dithiobis-(2-nitrobenzoic acid) (PubChem CID: 6254)
β-Mercaptoethanol (PubChem CID: 1567)
Sodium dodecyl sulfate (PubChem CID: 3423265)
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ABSTRACT

Effects of 0, 1, 5, 25 and 125 μmol/g gallic acid (GA) without or with ultrasound treatment (20 kHz, 400 w, 5 min) (NU or U groups) on properties of Japanese seerfish myofibrillar protein (MP) were studied. After sonication, tryptophan fluorescence decreased while surface hydrophobicity, free amine and SH content (not U125) and solubility increased. After heating, NU125 showed the heaviest polymers among NU groups, but U5 exhibited the strongest while U125 showed the weakest polymers in U groups. Storage modulus (G′) of NU groups showed a dose-dependent style, but for U groups, U5 had the highest G′ while U125 had the lowest G′. Mass analysis confirmed the formation of Cys-GA-Cys and Lys-GA-Lys polymers in U125. Thus, ultrasound promoted structural unfolding and reactive groups exposure, producing GA quinone by triggering OH·. These together led to the G′ improvement by low dose GA but deterioration by high does GA.

1. Introduction

Phenolic compounds are widely existing in fruits and herbs. They show good antioxidative function and positive role on human health (Maqsood, Benjakul, Abushelaibi, & Alam, 2014; Shahidi & Zhong, 2010), thus, are commonly incorporated into food matrix for improving food quality and stability. Phenolic compounds are able to modify gelling properties of proteins, but their effects are contingent, depending on phenolics category, dose and food matrix. For example, Balange and Benjakul (2009a, 2009b) reported that oxidized ferulic acid, tannic acid and caffeic acid enhanced cross-linking of myofibrillar protein (MP) from bigeye snapper and mackerel, forming good surimi gel, but the non-oxidized counterparts did not exert the gel-improving effect. Cao and Xiong (2015) found chlorogenic acid (CA) increased storage modulus (G′) of pork MP in a dose-dependent style, but under oxidative stressing, low dose CA strengthened G′ while high dose CA lowered G′. Therefore, how to properly use phenolic compounds to maximize their positive effects on rheological properties of protein is of interest.

Gallic acid (GA) is a water-soluble phenolic compound with strong

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radical scavenging and metal ion chelating capability (Abdelwahed et al., 2007; Vijayalakshmi, Adinarayana, & Rao, 2010). It exhibits pharmacological actions including anti-inflammatory (Karimi-Khouzani, Heidarian, & Amini, 2017), cardioprotective (Jin et al., 2018), anticarcinogenic (Chia, Rajbanshi, Calhoun, & Chiu, 2010) properties, etc. Cao, True, Chen, and Xiong (2016) reported that low dose (6 μmol/g) GA increased G′ of oxidatively stressed pork MP by nearly 50% while high dose (150 μmol/g) GA decreased G′. It is proposed that GA might form quinone–NH_2 or –SH adducts to enhance G′ by promoting MP cross-linking. These imply it is possible to obtain good MP gelling properties by reducing dose of phenolic compound with the aid of other treatments.

As an efficient and environment-friendly technology, ultrasound is promising to be applied for improving food quality (Chemat, Zill & Khan, 2011). Ultrasound can cause cavitation bubbles and microstreaming currents, resulting in high temperature, high pressure, high shear energy wave and turbulence, which might modify structural and functional properties of proteins (Soria & Villamiel, 2010). Studies showed that ultrasound exposed reactive groups, reduced particle size, improved solubility, emulsifying and rheological properties of soy protein (Lin, Lu, Hsieh, & Kuo, 2016) and millet protein (Nazari, Mohammadifar, Shojaee-Aliabadi, Feizollahi, & Mirmoghtadaie, 2018). It also enhanced the gel-strengthen role of transglutaminase on proteins (Qin et al., 2016; Qin et al., 2017). It is hypothesized that ultrasound can induce protein structure changes and promote protein-phenolics interactions. Further, ultrasonic cavitation can generate highly reactive OH· from water molecules ($H_2O \rightarrow H^{\cdot} + OH^{\cdot}$) (Gülseren, Güzey, Bruce, & Weiss, 2007), by which phenolic compounds can be oxidized into quinone, an intermediate crucial for quinone–NH_2 or –SH adducts formation, to promote protein cross-linking. Thus, ultrasound might influence the function of phenolic compounds on mediating gelling properties through protein structure modification and quinone adducts formation.

Japanese seerfish (JS) is a low economic value fish species in China. Its muscle is commonly used or used as ingredient for producing gel food, thin paste or pottage. Its MP has weak gelling properties. This study aimed to expose the role and mechanism of GA on mediating gel properties of JS MP with the aid of ultrasound. Physicochemical, structural and rheological properties of MP added with different levels of GA receiving ultrasound treatment were investigated. It would provide theoretical support toward proper utilization of GA and ultrasound technology for improving fish MP rheological properties.

2. Materials and methods

2.1. Materials

Japanese seerfish (*Scomberomours niphonius*) (350–500 g) were fished in oceanic area of Changxing Island, Dalian, China in 2018, October. The fish were immediately killed by a blow on the head and then immersed into liquid nitrogen to be frozen. The fish were transferred into lab at National Engineering Research Center for Seafood in 12 h at −40 °C and stored at −80 °C. GA was purchased from Sangon Biotech Co. Ltd., Shanghai, China.

2.2. MP extraction

MP was extracted by the method of Park, Xiong, and Alderton (2006) with modifications. Dorsal muscle were taken and homogenized with isolation buffer, 0.1 M NaCl–0.02 mM Tris-Maleic acid (pH = 6.85, 1:4 = g:mL) using a IKA T25 homogenizer running at 16,000 rpm. The mixed was centrifuged at 10,000 × *g* and 4 °C for 10 min and the supernatant was discarded. The procedures were repeated once. The precipitates were re-suspended in isolation buffer and filtered with gauze to remove connective tissues. The sample was centrifuged again, and the pellets were collected. MP pellets were dissolved in 0.6 M NaCl-0.02 mM Tris-Maleic acid (pH = 6.85) for experiments use. Protein content was measured using a Biuret assay kit (Beijing Biolab Co. Ltd., Beijing, China).

2.3. MP exposed to GA with or without ultrasound treatment

MP (40 mg/mL) added with various content of GA was prepared. Approximate 100 mL sample was placed in a 200 mL beaker. For sample received ultrasound treatment, MP in the beaker was treated with an ultrasonic processor (Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) of 20 kHz equipped with a 2.0 cm diameter titanium probe at 400 W for 10 min. The probe was immersed 1 cm below liquid surface. Sonication was performed by a pulse duration with 2 s on and 2 s off. Sample was kept cool (< 10 °C) by ice water during this period. After sonication, sample was transferred into a 4 °C fridge and magnetically stirred for 2 h. Sample without ultrasound treatment was magnetically stirred at 4 °C for 2 h. Samples added with GA of 0, 1, 5, 25, 125 μmol/g protein received ultrasound treatment were labeled as: U0, U1, U5, U25, U125, while the counterparts received no ultrasound treatment were labeled as: NU0, NU1, NU5, NU25, NU125.

2.4. Changes in physical properties

2.4.1. Particle size

Particle size distribution was determined using a Zetasizer 3000HSA (Malvern Panalytical Ltd., UK). Excitation wavelength was 633 nm and scattered light intensity detector angle was 173°. Sample was diluted into 1 mg/mL for analysis.

2.4.2. Solubility

Solubility was studied as Li, Xiong, and Chen (2013). Briefly, sample after GA exposure with or without ultrasound MP solution (2 mg/ml) was centrifuged at 5000 × *g* for 15 min at 4 °C. Protein content of supernatant and original suspension was determined by a Biuret assay kit (Beijing Biolab Co. Ltd., Beijing, China). Solubility was expressed as:

$$\text{Solubility}\,(\%) = \frac{\text{supernatant protein content}}{\text{Initial protein content}} \times 100\%$$

2.4.3. Turbidity

Protein turbidity of MP solution (1 mg/ml) was measured as absorbance at 660 nm using a UV-5200 spectrophotometer (Yuanxi Instrument Co., Shanghai, China). Turbidity was expressed as the absorbance value.

2.5. Changes in amino acid side-chain groups

2.5.1. SH content

Total SH content (TSH) was measured using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) method as Liu, Xiong, and Butterfield (2000) with modifications. Reaction buffer (8.6 mM Tris-0.09 M Glycine-4 mM EDTA-8 M Urea, pH = 8.0) with 10 mM DTNB MP was added into MP sample (2 mg/ml) and incubated at room temperature for 15 min. Absorbance at 412 nm was recorded. Molar extinction coefficient 13,600 $L{\cdot}M^{-1}{\cdot}cm^{-1}$ was used for calculating SH content. Reactive SH content (RSH) was determined using the same reaction buffer in the absence of urea.

2.5.2. Free amine content

Free amine content (FAC) was measured by 2,4,6-trinitrobenzenesulfonic acid (TNBS) method. Sample was reacted with 0.01% TNBS at 50 °C for 30 min. Reaction was stopped with 0.1 M Na_2SO_3. Absorbance at 420 nm was recorded. FAC was calculated from a standard curve produced with L-leucine.

2.6. Changes in structure

Tertiary structure status of MP was evaluated by measuring tryptophan fluorescence (TF) and surface hydrophobicity. TF of MP (0.4 mg/mL) was measured using a fluorescence spectrophotometer (F-2700, Hitachi Co. Ltd., Tokyo, Japan). Excitation wavelength was set as 283 nm and emission spectra were 300–400 nm. Surface hydrophobicity was determined using bromophenol blue (BPB)-binding method as Chelh, Gatellier, and Santé-Lhoutellier (2006). MP sample (2 mg/mL) was incubated with 200 µL BPB (10 mg/mL) at room temperature for 10 min and centrifuged at 2000 g for 15 min. Absorbance at 595 nm of supernatant (diluted × 100) was recorded. Surface hydrophobicity was expressed as BPB bound content, calculated as below:

$$\text{BPB bound}(\mu\text{g}) = 20\mu\ \text{g}\times (\text{A}_{\text{CO}} - -\text{A}_{\text{sample}})/\text{A}_{\text{CO}}$$

where Aco is the absorbance of supernatant from blank without MP but only buffer; Asample is the absorbance of supernatant from MP sample; 20 µg is the total BPB in each sample.

2.7. Protein cross-linking

Protein cross-linking before and after thermal treatment was investigated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as Li et al. (2013) with modifications. Sample was diluted using loading buffer (0.125 M Tris-4% SDS-20% Glycerol, pH 6.85) with or without 10% β-mercaptoethanol (βME). *N*-ethylmaleimide (NEM, 1 mM) was added into sample without βME for avoiding thermal cross-linking. Twenty microgram protein was loaded onto a running gel composed of 5% stacking gel and 12% resolving gel and subjected to electrophoresis at 30 mA current for 1 h using a Mini Unit AE-8135 (ATTO Corp., Tokyo, Japan). Gel was stained with 0.5 g/L Coomassie blue R-250 in 45% alcohol-9% acetic acid and destained with 50% methanol-9% acetic acid.

2.8. Rheological properties

Rheological properties of MP were evaluated as G′ using a Discovery HR-1 rheometer (TA Instrument, New Castle, UK). Sample (40 mg/mL) was loaded onto 1 mm gap between two parallel plates (upper plate diameter = 40 mm). Temperature ramp test was performed as following condition: frequency, 0.2 Hz; stress, 0.6 Pa; temperature, 20–80 °C; heating rate, 1 °C/min.

2.9. Mass analysis of intermediate products by protein and GA

To confirm the formation of protein-S-GA-S-protein and protein-*N*-GA-*N*-protein polymers in U125, mass spectrometry analysis was performed. MP from U125 was precipitated using trichloroacetic acid and hydrolyzed with 6 M HCl at 110 °C for 20 h. Sample was dried with nitrogen gas, redissolved in water and analyzed using a triple quadrupole mass spectrometer (AB Sciex 5500 Qtrap System, America). MS/MS detection was operated in positive ionization mode. The data were obtained from multiple reaction monitoring (MRM) mode. Electrospray ionization (ESI) setting was as below: entrance potential 10 V, spray voltage 5500 V, ion source temperature 600 °C, source gas and auxiliary gas were 15 and 18 L/min. Gas source was 99% purity nitrogen.

2.10. Statistical analysis

Data were obtained from three independent trails using three batch of samples. Each trial was duplicated. Data were expressed as mean ± standard deviation (STD). One-way analysis of variance (ANOVA) was used for comparing means of U groups or NU groups with different content of GA. Means were compared with Duncan's multiple range tests using software SPSS 19.0 (SPSS Inc, Chicago, IL, USA). Student's *t* test was applied for analyzing differences between each two U and NU groups with the same dose GA. If $p < 0.05$, difference was defined as significant.

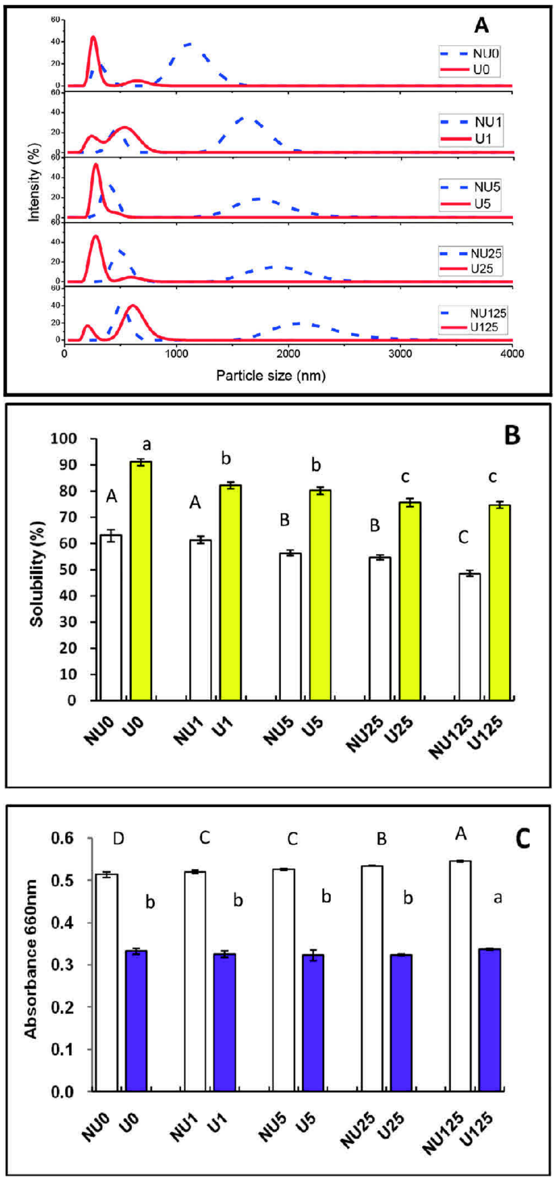

Fig. 1. Particle size (A), solubility (B) and turbidity (C) of Japanese seerfish myofibrillar protein (JSMP) exposed to 0, 1, 5, 25 and 125 µmol/g protein of gallic acid (GA) with or with no ultrasound treatment (U or NU). Uppercase letters denote significant difference between NU groups; lowercase letters denote significant difference between U groups.

3. Results and discussion

3.1. Physical changes

3.1.1. Particle size

In Fig. 1A, two peaks at 300 nm and 1100 nm were observed in particle size spectrum of NU0. As GA dose increased, they were shifted to larger size number, indicating that GA could promote MP aggregation. Phenolic compounds could interact with protein through covalent and non-covalent styles. Hydroxyl groups in phenolic compounds could form hydrogen bonds with –NH_2 or –OH in protein (Jia, Wang, Shao, Liu, & Kong, 2017). Meanwhile, phenolic compound oxidative derivative, quinone, could induce strong protein cross-linking (Cao & Xiong, 2015). Particle size of most U groups clustered in a range of 230–270 nm, much lower than that of their NU counterparts. Ultrasound often showed good effects on reducing particle size of proteins, such as duck liver protein, sunflower meal protein and millet protein (Malik, Sharma, & Saini, 2017; Nazari et al., 2018; Zou et al., 2017). It is considered that cavitation force, micro-streaming and turbulent force can exert strong agitation to dissociate protein aggregates, resulting in smaller particle size (Lu, Riyanto, & Weavers, 2002). Noticeably, U125 showed the main peak of particle size at 605 nm, confirming that the larger size of protein aggregates induced by high dose GA were more resistant to ultrasound.

3.1.2. *Solubility and turbidity*

In Fig. 1B, solubility of NU0 was 62%, and it decreased as GA level climbed. This could be attributed to the increased interaction between GA and MP, which caused aggregations and formed large size particles. Solubility of all U groups increased compared with their NU counterparts ($p < 0.05$). Protein in a natural state contains aggregates (Maity, Rasale, & Das, 2012). Ultrasonic cavitation could disrupt hydrogen bonds and hydrophobic interactions that maintain protein aggregates, forming smaller size aggregates with more surface area (Tang, Wang, Yang, & Li, 2009). This enables strong protein-water interactions and better solubility. Improved solubility by ultrasound also was observed in bean protein (Jiang et al., 2014) and millet protein (Nazari et al., 2018). Turbidity of NU125 was slightly higher than those of others, and all U groups showed much lower turbidity than their NU counterparts ($p < 0.05$) (Fig. 1C). This could be explained by their decreased particles size, which gave less light scattering. Changes in particle size, solubility and turbidity were in consistent with each other, which confirmed the distinct effect of ultrasound on dissociating MP aggregates.

3.2. *Amino acid side-chain group changes*

3.2.1. *SH content*

In Fig. 2A, TSH of NU0 was 56 nmol/mg protein. It showed no change in NU1 and NU5 but decrease in NU25 and NU125. Phenolic compounds could form their quinone derivatives by free radical attack, which would cross-link with protein-SH (Jongberg, Gislason, Lund, Skibsted, & Waterhouse, 2011). Since our samples were not treated under vacuum circumstance, GA might form some GA quinone-thiol adducts, consuming certain amount of SH. Thus, middle or high dose GA lowered TSH slightly. Compared with NU counterparts, TSH of U0, U1, U25 and U125 decreased by 17%, 7%, 10% and 30% while U5 showed no distinct change. Because ultrasound can produce OH·, it could facilitate the formation of protein-S-S-protein. Meanwhile, it is possible that OH· promoted the formation of GA quinone, which could interact with protein-SH to form GA quinone-thiol adduct, leading to polymerization and intensifying SH loss. The reduction of TSH in U0 could be attributed to the formation of S-S by radical oxidation. The two styles of polymerization were supported by the results of SDS-PAGE analysis see Section 3.4. Nevertheless, heavy loss of TSH in U125 could be due to the generation of large amount of GA quinone-thiol adducts. Further, low dose GA (1, 5 μmol/g) might mainly act as antioxidant to scavenge OH·, thus, alleviated SH loss.

In Fig. 2B, low dose GA (NU1 and NU5) did not bring distinct change to RSH, but slight decrease probably due to formation of GA quinone-thiol adduct was observed in NU25 and NU125. Compared with NU counterparts, RSH of U0, U1, U5 and U25 increased by 9%, 41%, 56% and 29% while it of U125 declined by 60%. It is known that ultrasound could reduce particle size of protein and exposed more interior SH onto molecule surface (Arzeni et al., 2012). Many studies reported increased RSH of protein by ultrasound (Arzeni et al., 2012; Malik et al., 2017). Since SH simultaneously suffered oxidation by OH·, RSH in U0 did not increase distinctly. RSH in U1, U5 and U25 might be protected by GA as antioxidant, thus it increased remarkably. In contrast, excessive GA in U125 might promote the interaction of protein-SH with GA quinone, consuming large amount of SH. Above results suggest that the role of ultrasound on SH in MP was affected by GA dose.

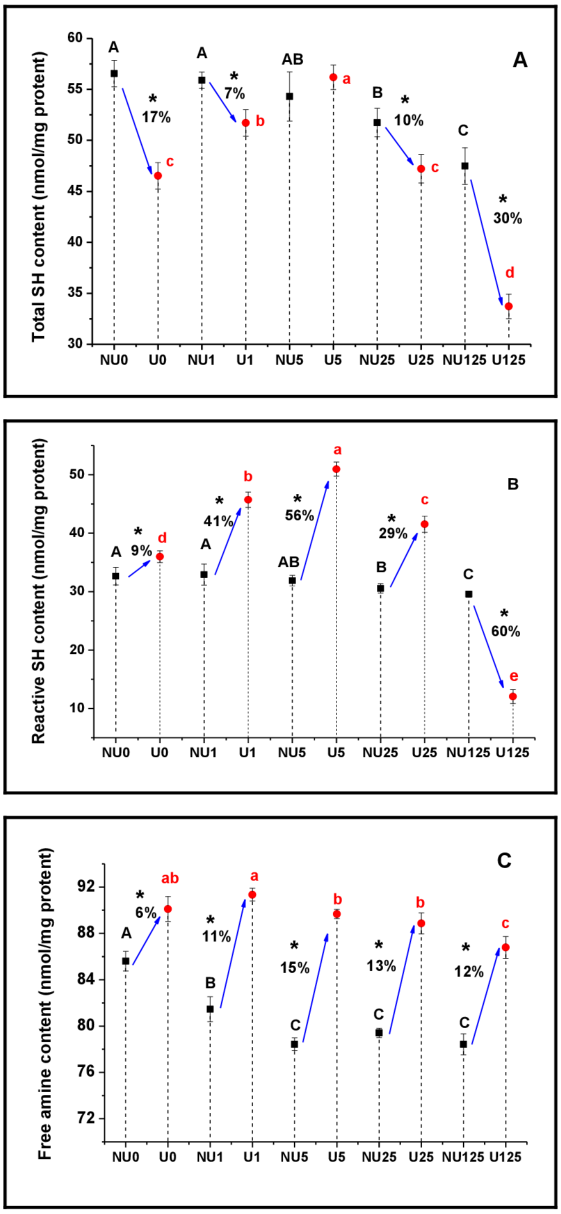

Fig. 2. Total SH (A), reactive SH (B) and free amine (C) content of JSMP exposed to 0, 1, 5, 25 and 125 μmol/g protein of GA with or with no ultrasound treatment. *Denotes significant decrease or increase between NU and U groups.

3.2.2. *Free amine content*

In Fig. 2C, FAC of NU groups decreased with increased GA content,

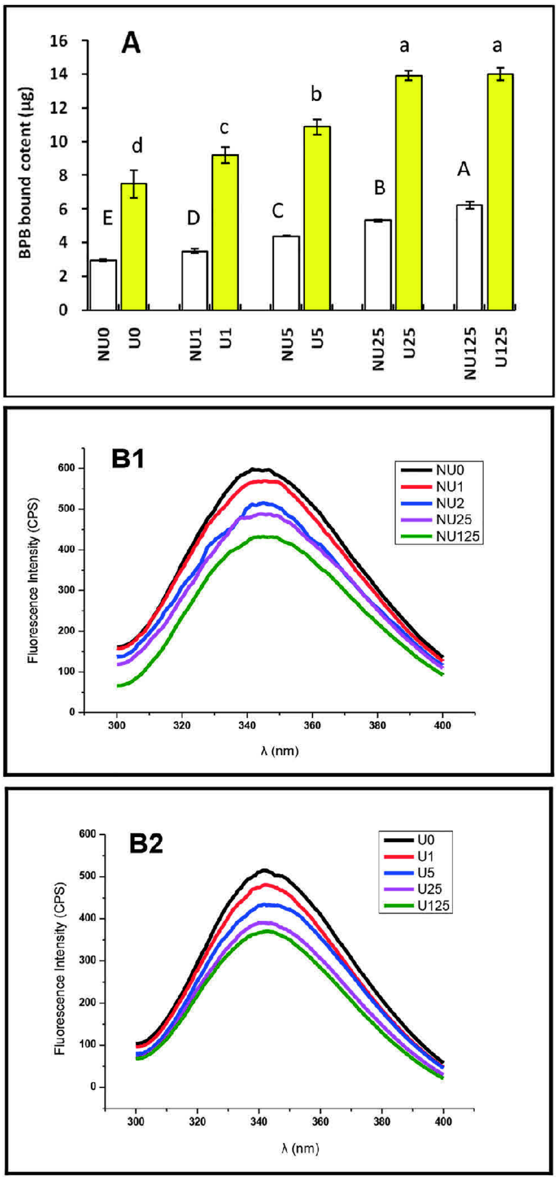

Fig. 3. Surface hydrophobicity (A) and fluorescence intensity (B1, B2) of JSMP exposed to 0, 1, 5, 25 and 125 μmol/g protein of GA with or with no ultrasound treatment. *Denotes significant decrease or increase between NU and U groups.

indicating GA or its derivatives might react with free amine groups in MP. After sonication, FAC of all U groups increased, suggesting that ultrasound could expose more free amine groups by inducing conformation change or particle size reduction. It is noticed that FAC of U5, U25 and U125 was slightly lower than that of U0, especially U125, indicating that GA might covalently interact with free amine groups. Cao and Xiong (2015) found decreased FAC in pork MP added with high dose CA and they considered that covalent adduction of oxidation-generated CA quinone to protein NH_2 occurred. Our results also suggest that ultrasound exposed more free amino groups and high dose GA could promote their cross-linking with GA quinone.

3.3. Structural changes

3.3.1. Surface hydrophobicity

BPB can bind to hydrophobic sites in protein to denote surface hydrophobicity of protein. For NU groups, BPB bound content increased from 2.96 to 6.25 μg as GA dose increased (Fig. 3A), implying increase of surface hydrophobicity by GA. Phenolic compounds are able to induce conformational change and unfolding of protein, exposing hydrophobic amino acids (Cao & Xiong, 2015; Jia et al., 2017). After sonication, BPB bound content of U0, U1, U5, U25 and U125 increased by 153%, 161%, 146%, 162% and 124% compared their NU counterparts respectively, indicating significant increase of surface hydrophobicity. Ultrasound could damage hydrogen bond, electrostatic interaction and hydration between protein molecules, allowing the hydrophobic groups previously buried in interior of molecules to be exposed (Higuera-Barraza et al., 2017). The involvement of GA might synergistically accelerate MP unfolding and expose more hydrophobic groups. This is supported by the increased BPB bound content with increasing GA content in U groups. However, U25 and U125 showed similar BPB bound content, indicating the synergistic effect of GA with ultrasound on surface hydrophobicity is fully achieved at 25 μmol/g. It has to be mentioned that ultrasound treatment could produce heat to increase temperature of targeted samples, which might also modify protein structure. Zhong and Xiong (2020) found that mung bean protein received ultrasound at 70 °C showed an accentuated exposure of inner hydrophobic groups compared with counterpart at 30 °C and 50 °C. Though the temperature fluctuation of MP during ultrasonication is limited (< 10 °C), since fish MP was susceptible to thermal denaturation, the changes in its tertiary structure of ultrasound-treated MP could be partially contributed by the temperature effect.

3.3.2. Tryptophan fluorescence

TF is sensitive to the polarity of tryptophan microenvironment, thus, it is a good monitor of conformational change of protein tertiary structure (Papadopoulou, Green, & Frazier, 2005). MP experienced enhanced TF loss with increasing GA dose (Fig. 3B1), suggesting structure unfolding by GA. GA might also interact with MP, bring tryptophan residues into a polar environment, leading to fluorescence quenching. GA-induced structural change could mainly due to non-covalent forces such as hydrophobic interactions between aromatic ring of GA and aromatic amino acid residues, hydrogen bonds between hydroxyl groups in GA and acceptors in MP (Guo & Xiong, 2019). Decreased TF was also observed in pork MP incubated with CA (Cao & Xiong, 2015), GA (Cao et al., 2016) and epigallocatechin gallate (EGCG) (Cao, Ai, True, & Xiong, 2018). All U groups showed lower TF intensity than their NU counterparts (Fig. 3B2), implying that ultrasound could promote molecular structure unfolding and destroy hydrophobic interactions, moving tryptophan residues to a more polar environment. Attenuated TF by ultrasound was also observed in duck liver protein (Zou et al., 2017). Changes in surface hydrophobicity, TF, RSH and FAC were consistent with each other, suggesting that ultrasound exposed protein structure.

3.4. Protein cross-linking

In Fig. 4A1, NU groups showed certain amounts of polymers on the top of stacking gel and MHC band of them was attenuated markedly. In reducing condition (+βME), most polymers disappeared while myosin heavy chain (MHC) recovered well. These indicate that some GA quinone induced by limited reactive oxygen might interact with protein-SH to form cross-linking, which is confirmed by the slight decrease of TSH (Fig. 2A). In Fig. 4B1, polymers on top of stacking gel in U groups turned heavier with the increase of GA dose while their MHC density was fading, especially for U125, indicating that ultrasound promoted the cross-linking by high dose GA. As aforementioned, ultrasound might provide OH· to propel the formation of GA quinone to enable further

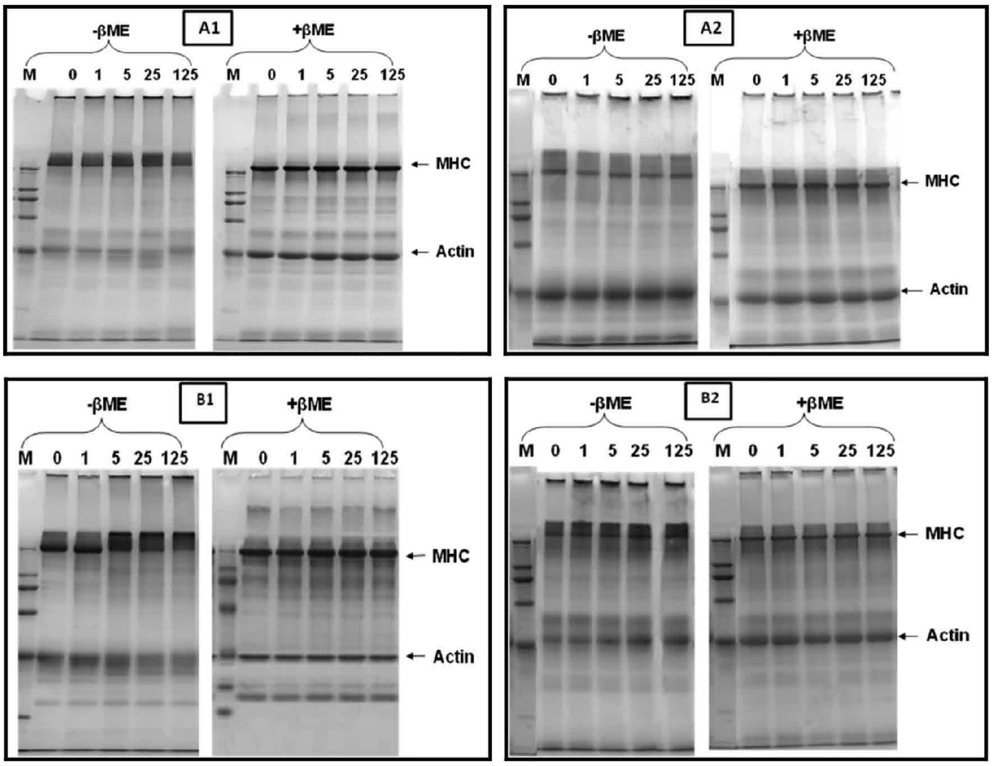

Fig. 4. Protein pattern by SDS-PAGE of JSMP exposed to 0, 1, 5, 25 and 125 μmol/g protein of GA with no (A1, A2) or with (B1, B2) ultrasound treatment before (A1, B1) and after (A2, B2) heating at 90 °C for 20 min. Samples were prepared in the presence (+βME) or absence (-βME) of β-mercaptoethanol. MHC, myosin heavy chain.

adducting with protein-SH. Thus, the higher GA dose, the more GA quinone-adducts formed, and the more polymers. Good recovery of MHC confirms that most polymers should be formed as protein-S-GA-S-protein but not protein-*N*-GA-*N*-protein, in agreement with sharp SH decrease but not distinct FAC reduction in U groups (Fig. 2C). Some studies reported that ultrasound degraded sunflower meal protein (Malik et al., 2017) and squid protein (Hu et al., 2014) to form low molecular weight fragments. Other studies reported ultrasound caused no changes to protein patterns of squid mantle protein (Higuera-Barraza et al., 2017) and fish gelatin (O'Sullivan, Murray, Flynn, & Norton, 2016). These inconsistent effects of ultrasound on protein pattern could be attributed to the different protein nature and intensity of applied ultrasound. The changes of JS MP in this study is the combined effect of GA and ultrasound.

MP of NU and U groups were heated to check their cross-linking potential. Fig. 4A2 showed that polymers on the top of stacking gel turned heavier as GA dose increased, indicating the cross-linking potential of NU groups complied a dose-dependent style. Nevertheless, U5 showed the highest intensity of polymers on the top of stacking gel among U groups (Fig. 4B2), denoting its highest gelling potential. Most polymers in U and NU groups disappeared when βME was added, implying these aggregations by heating should be mainly attributed to disulfide bond or protein-S-GA-S-protein.

3.5. Protein-S-GA-S-protein, protein-N-GA-N-protein polymers and proposed GA quinone pathway

Mass analysis was conducted to verify the existence of protein-S-GA-S-protein and protein-*N*-GA-*N*-protein polymers in U125. In Fig. 5A, a peak of 409.4 Da was observed in mass spectra. GA has a molecular weight (MW) of 170 Da, thus, it is deduced that two cysteine (121 Da) molecules could bind one GA molecule to form Cys-GA-Cys (409 Da). Meanwhile, the peak of 373.0 Da should be the dehydroxylated polymer deducting two hydroxyl groups. In Fig. 5B, a peak of 459.2 Da showed in mass spectra. Considering the MW of lysine (146 Da), it should be the signal of Lys-GA-Lys. Further, peak of 441 and 423 Da could be attributed to the dehydroxylation of Lys-GA-Lys by one and two hydroxyl groups. We did not quantify the Cys-GA-Cys and Lys-GA-Lys. Further study focusing on these products identification and quantification is of interest.

Above results provide direct evidence to the formation of GA-protein polymers through quinone-thiol and -amido adduct pathway (Fig. 5C). As proposed in Fig. 5C, GA quinone is formed by OH· attack firstly, and continues to react with -SH or -NH_2 of protein to produce GA quinone-thiol adduct or GA quinone-amino adduct. The two could further bind another protein molecule to form protein-S-GA-S-protein polymer or protein-*N*-GA-*N*-protein polymer. Noticeably, the decrease of SH content in U125 was more distinct than the decrease of FAC content, implying that the polymers in U125 should be mainly formed by thiol-quinone pathway.

3.6. Rheological properties

In Fig. 6A, final G′ value of NU125 was the highest (723 Pa), followed by NU25 (502 Pa), and NU1 and NU5 showed similar G′ value (361 and 410 Pa) that were slightly higher than G′ of NU0 (320 Pa). Results of GA were highly consistent with the results of polymerization showed in Fig. 4A2, where higher dose GA gave heavier polymerization after heating. Considering the decreased TSH content (Fig. 2A), GA should form some quinones to interact with MP to cause further

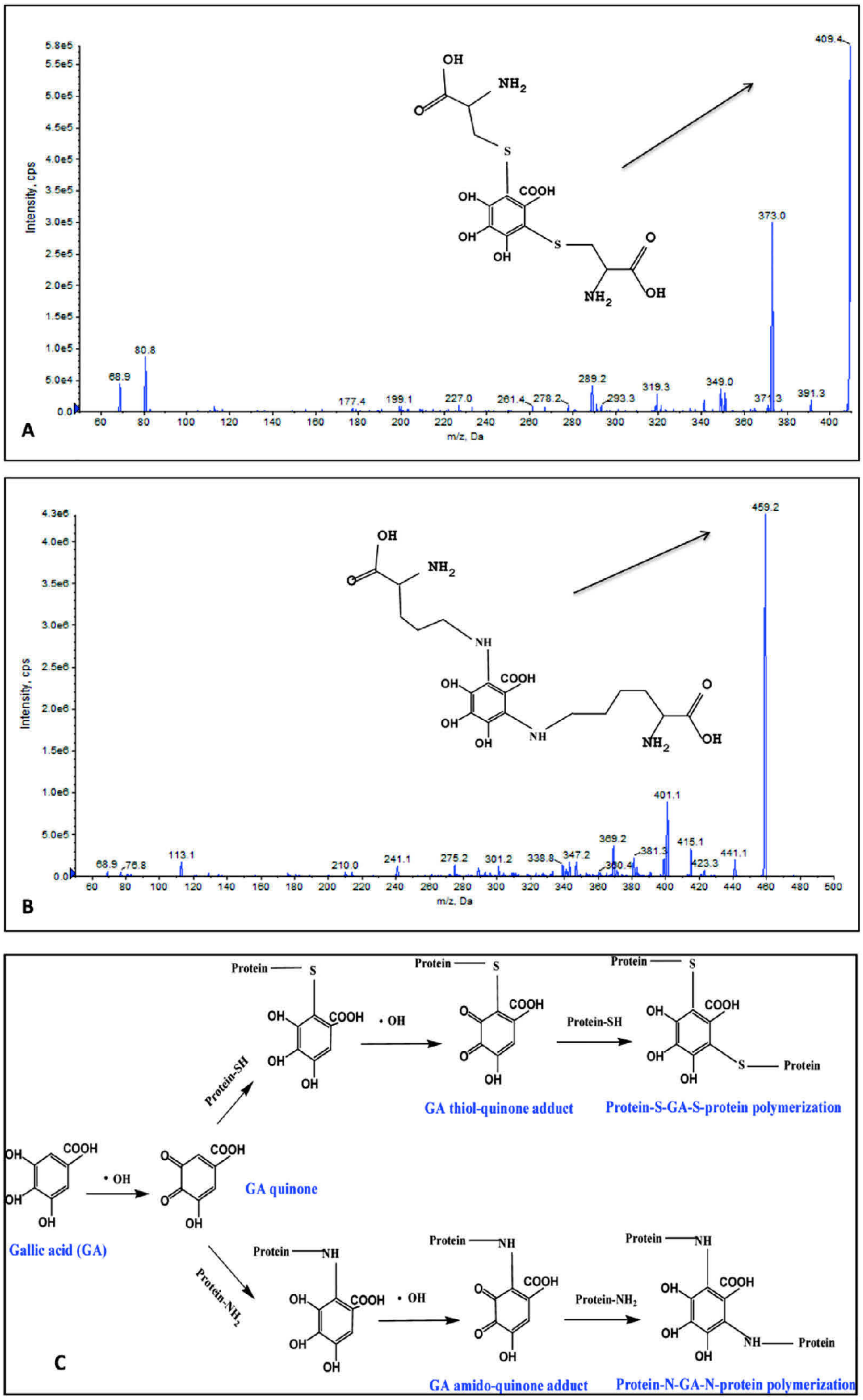

Fig. 5. Mass spectra of JSMP exposed to 125 μmol/g protein GA with ultrasound treatment (A, verification of Cys-GA-Cys; B, verification of Lys-GA-Lys) and proposed pathway of GA quinone reaction with JSMP based on Cao and Xiong (2015) (C). -S-GA-S- and -N-GA-N- are GA mediated protein polymers by reaction with SH or NH_2 group.

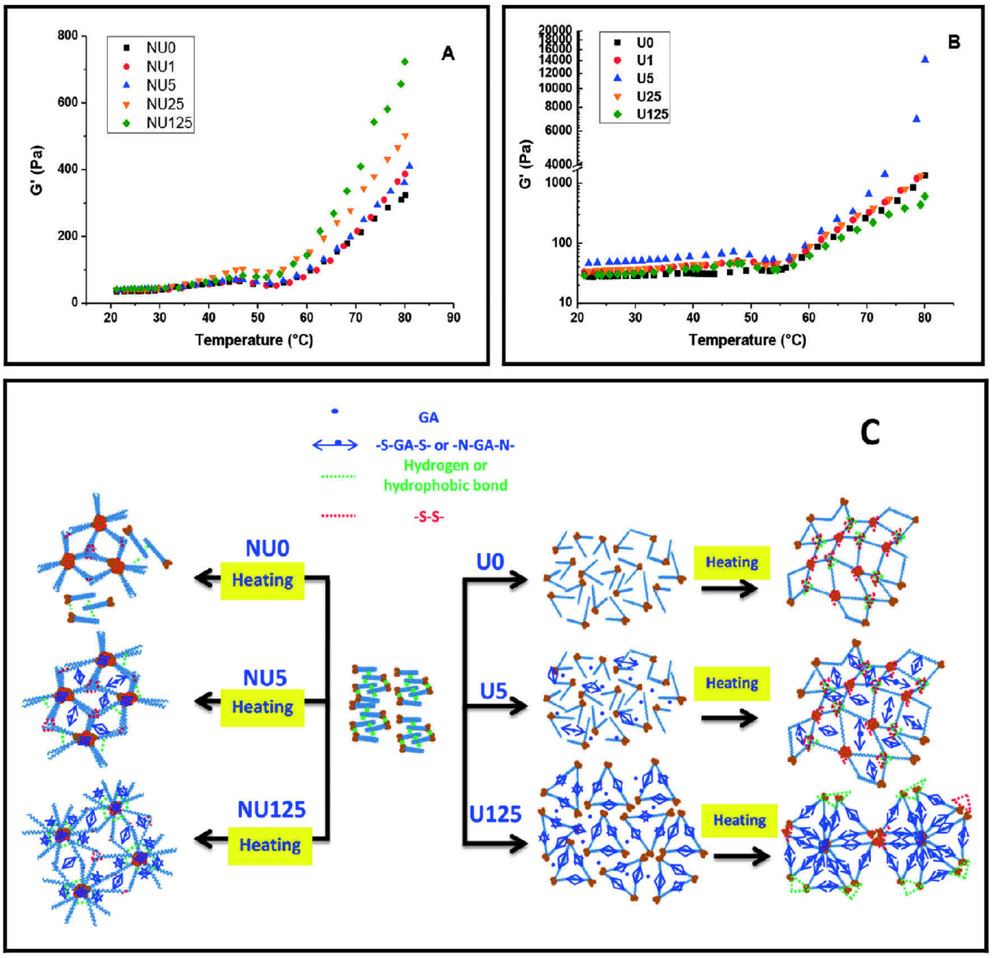

Fig. 6. Storage modulus (G') of JSMP exposed to 0, 1, 5, 25 and 125 µmol/g protein of GA with no (A) or with (B) ultrasound treatment and proposed mechanism of gelling mediations by high (125 µmol/g protein) and low (5 µmol/g protein) dose GA (C).

aggregations during heating and increase final G′. The conformational changes induced by GA (evidenced by increased TF and surface hydrophobicity in Fig. 3) might also facilitate cross-linking. Thus, GA role of mediating MP gelling properties without ultrasound was dose dependent. Similar effects of GA on G′ of porcine MP was reported by Guo and Xiong (2019).

In Fig. 6B, the highest G′ was observed in U5 (14137 Pa) while the lowest G′ was found in U125 (605 Pa), and G′ of U0, U1 and U25 was close to each other (1339, 1809 and 1990 Pa). Compared with NU0, U0 exhibited higher G′. It is established that ultrasound could unfold protein structure and expose more functional groups onto protein molecule surface, facilitating further cross-linking during heating and improving rheological properties. Zhao et al. (2014) and Li, Kang, Zou, Xu, and Zhou (2015) also reported improved gelling capacity of chicken MP due to enhanced hydrophobic interaction and disulfide bond formation by ultrasound. However, ultrasound might also bring OH· that might convert high dose GA (U125) into GA quinone to cause heavy cross-linking or aggregations prior to thermal treatment. These aggregations would shield partial reactive groups and hamper the further interactions between GA and protein or protein and protein due to steric hindrance. Therefore, U125 had a decreased gelling potential. In contrast, low dose GA (U5) could form limited GA quinone. Together with more exposed functional groups and smaller particles size, further cross-linking should be much easier to occur when heated (Fig. 4B2). Thus, gelling potential of U5 was greatly improved.

3.7. Mechanism of gelling mediations by GA with or without ultrasound

Compared with NU0, G′ of NU1, NU5, NU25 and NU125 increased by 11.8%, 26.9%, 55.4% and 124%, respectively while G′ of U1, U5, U25 and U125 increased by 458%, 4278%, 516% and 87.3%, respectively. Meanwhile, G′ of U0, U1, U5, U25 and U125 increased by 315%, 933%, 3348%, 296% and −16.3%, respectively compared with that of NU0, NU1, NU5, NU25 and NU12 (see supplementary Table 1). These indicate that ultrasound could promote MP interaction with GA, improving gelling potential. It modified the mediation role of GA on MP, leading to a deteriorated gelling potential with high dose GA while a highly improved gelling potential with low dose GA.

A proposed mechanism of gelling mediations by high and low dose GA with or without ultrasound was described in Fig. 6C. It is speculated that myosin of NU0 would interact with each other through limited amount of disulfide bonds and non-covalent bonds during heating. With the addition of GA, myosin was induced to unfold and expose more reactive groups, bringing myosin molecules close to each other. These enabled easy interactions of GA quinone with protein-SH and protein-SH with protein-SH, forming S-S or protein-S-GA-S-protein aggregations. Therefore, gelling potential was improved. Since structure unfolding was more thorough at high dose GA along with more GA quinone formation, NU125 showed a stronger linking structure than NU5. After sonication, myosin molecules unfolded greatly, exposed more SH and NH$_2$, leading to better cross-linking during heating. Due to GA quinone formed by OH· triggered by ultrasound, cross-linking might be strengthened as well. The two effects by ultrasound greatly improved U5 gelling potential. However, high dose GA in U125 might form excessive GA quinone, which caused heavy aggregations through protein-S-GA-S-protein or protein-*N*-GA-*N*-protein cross-linking prior to heating. These pre-aggregations severely inhibited further cross-linking due to huge steric hindrance and very limited SH, resulting in poor gel potential.

4. Conclusions

Ultrasound promoted structural unfolding and reactive groups exposure in JS MP. It might also form GA quinone by triggering OH·. Low dose GA with ultrasound greatly improved gel potential of MP due to further cross-linking by S-S and protein-S-GA-S-protein. High dose GA with ultrasound formed excessive GA quinone to cause severe protein aggregation, hampering further cross-linking during heating. Thus, ultrasound coupled low dose, but not high dose GA can be applied to improve gel properties of JS MP.

CRediT authorship contribution statement

Jinfeng Pan: Conceptualization, Funding acquisition, Methodology, Data curation, Supervision, Writing - original draft. **Hongliang Lian:** Investigation, Methodology, Software. **Hui Jia:** Investigation, Methodology. **Shengjie Li:** Methodology, Validation. **Ruoyi Hao:** Visualization, Writing - review & editing. **Yujie Wang:** Investigation. **Xuening Zhang:** Investigation. **Xiuping Dong:** Project administration, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126637.

References

Abdelwahed, A., Bouhlel, I., Skandrani, I., Valenti, K., Kadri, M., Guiraud, P., ... Chekir-Ghedira, L. (2007). Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from Pistacia lentiscus. Confirmation by microarray expression profiling. *Chemico-Biological Interactions, 165*(1), 1–13.

Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O. E., & Pilosof, A. M. R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering, 108*(3), 463–472.

Balange, A. K., & Benjakul, S. (2009a). Effect of oxidised phenolic compounds on the gel property of mackerel (Rastrelliger kanagurta) surimi. *LWT – Food Science and Technology, 42*(6), 1059–1064.

Balange, A. K., & Benjakul, S. (2009b). Enhancement of gel strength of bigeye snapper (Priacanthus tayenus) surimi using oxidised phenolic compounds. *Food Chemistry, 113*(1), 61–70.

Cao, Y., Ai, N., True, A. D., & Xiong, Y. L. (2018). Effects of (−)-epigallocatechin-3-gallate incorporation on the physicochemical and oxidative stability of myofibrillar protein–soybean oil emulsions. *Food Chemistry, 245*, 439–445.

Cao, Y., True, A. D., Chen, J., & Xiong, Y. L. (2016). Dual role (anti- and pro-oxidant) of gallic acid in mediating myofibrillar protein gelation and gel in vitro digestion. *Journal of Agriculture and Food Chemistry, 64*(15), 3054–3061.

Cao, Y., & Xiong, Y. L. (2015). Chlorogenic acid-mediated gel formation of oxidatively stressed myofibrillar protein. *Food Chemistry, 180*, 235–243.

Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. *Meat Science, 74*(4), 681–683.

Chemat, F., Zill, E. H., & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry, 18*(4), 813–835.

Chia, Y.-C., Rajbanshi, R., Calhoun, C., & Chiu, R. H. (2010). Anti-neoplastic effects of gallic acid, a major component of toona sinensis leaf extract, on oral squamous carcinoma cells. *Molecules, 15*(11), 8377–8389.

Gülseren, İ., Güzey, D., Bruce, B. D., & Weiss, J. (2007). Structural and functional changes in ultrasonicated bovine serum albumin solutions. *Ultrasonics Sonochemistry, 14*(2), 173–183.

Guo, A., & Xiong, Y. L. (2019). Glucose oxidase promotes gallic acid-myofibrillar protein interaction and thermal gelation. *Food Chemistry, 293*, 529–536.

Higuera-Barraza, O. A., Torres-Arreola, W., Ezquerra-Brauer, J. M., Cinco-Moroyoqui, F. J., Rodríguez Figueroa, J. C., & Marquez-Ríos, E. (2017). Effect of pulsed ultrasound on the physicochemical characteristics and emulsifying properties of squid (Dosidicus gigas) mantle proteins. *Ultrasonics Sonochemistry, 38*, 829–834.

Hu, Y., Yu, H., Dong, K., Yang, S., Ye, X., & Chen, S. (2014). Analysis of the tenderisation of jumbo squid (Dosidicus gigas) meat by ultrasonic treatment using response surface methodology. *Food Chemistry, 160*(10), 219–225.

Jia, N., Wang, L., Shao, J., Liu, D., & Kong, B. (2017). Changes in the structural and gel properties of pork myofibrillar protein induced by catechin modification. *Meat Science, 127*, 45–50.

Jiang, L., Jing, W., Yang, L., Wang, Z., Jing, L., Rui, W., ... Min, Z. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. *Food Research International, 62*(6), 595–601.

Jin, L., Sun, S., Ryu, Y., Piao, Z. H., Liu, B., Choi, S. Y., ... Jeong, M. H. (2018). Gallic acid improves cardiac dysfunction and fibrosis in pressure overload-induced heart failure. *Scientific Reports, 8*(1), 9302.

Jongberg, S., Gislason, N. E., Lund, M. N., Skibsted, L. H., & Waterhouse, A. L. (2011). Thiol-Quinone adduct formation in myofibrillar proteins detected by LC-MS. *Journal of Agriculture and Food Chemistry, 59*(13), 6900–6905.

Karimi-Khouzani, O., Heidarian, E., & Amini, S. A. (2017). Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. *Pharmacological Reports: PR, 69*(4), 830–835.

Li, C., Xiong, Y. L., & Chen, J. (2013). Protein oxidation at different salt concentrations affects the cross-linking and gelation of pork myofibrillar protein catalyzed by microbial transglutaminase. *Journal of Food Science, 78*(6), 823–831.

Li, K., Kang, Z.-L., Zou, Y.-F., Xu, X.-L., & Zhou, G.-H. (2015). Effect of ultrasound treatment on functional properties of reduced-salt chicken breast meat batter. *Journal of Food Science and Technology, 52*(5), 2622–2633.

Lin, H.-F., Lu, C.-P., Hsieh, J.-F., & Kuo, M.-I. (2016). Effect of ultrasonic treatment on the rheological property and microstructure of tofu made from different soybean cultivars. *Innovative Food Science & Emerging Technologies, 37*, 98–105.

Liu, G., Xiong, Y. L., & Butterfield, D. A. (2000). Chemical, physical, and gel-forming properties of oxidized myofibrils and whey- and soy-protein isolates. *Journal of Food Science, 65*(5), 811–818.

Lu, Y., Riyanto, N., & Weavers, L. K. (2002). Sonolysis of synthetic sediment particles: Particle characteristics affecting particle dissolution and size reduction. *Ultrasonics Sonochemistry, 9*(4), 181–188.

Maity, I., Rasale, D. B., & Das, A. K. (2012). Sonication induced peptide-appended bolaamphiphile hydrogels for in situ generation and catalytic activity of Pt nanoparticles. *Soft Matter, 8*(19), 5301–5308.

Malik, M. A., Sharma, H. K., & Saini, C. S. (2017). High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: Effect on physicochemical and functional properties. *Ultrasonics Sonochemistry, 39*, 511–519.

Maqsood, S., Benjakul, S., Abushelaibi, A., & Alam, A. (2014). Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: A detailed review. *Comprehensive Reviews in Food Science and Food Safety, 13*(6), 1125–1140.

Nazari, B., Mohammadifar, M. A., Shojaee-Aliabadi, S., Feizollahi, E., & Mirmoghtadaie, L. (2018). Effect of ultrasound treatments on functional properties and structure of millet protein concentrate. *Ultrasonics Sonochemistry, 41*, 382–388.

O'Sullivan, J., Murray, B., Flynn, C., & Norton, I. (2016). The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocolloids, 53*, 141–154.

Papadopoulou, A., Green, R. J., & Frazier, R. A. (2005). Interaction of flavonoids with bovine serum albumin: A fluorescence quenching study. *Journal of Agriculture and Food Chemistry, 53*(1), 158–163.

Park, D., Xiong, Y. L., & Alderton, A. L. (2006). Concentration effects of hydroxyl radical oxidizing systems on biochemical properties of porcine muscle myofibrillar protein. *Food Chemistry, 101*(3), 1239–1246.

Qin, X.-S., Luo, S.-Z., Cai, J., Zhong, X.-Y., Jiang, S.-T., Zhao, Y.-Y., & Zheng, Z. (2016). Transglutaminase-induced gelation properties of soy protein isolate and wheat gluten mixtures with high intensity ultrasonic pretreatment. *Ultrasonics Sonochemistry, 31*, 590–597.

Qin, X.-S., Sun, Q.-Q., Zhao, Y.-Y., Zhong, X.-Y., Mu, D.-D., Jiang, S.-T., ... Zheng, Z. (2017). Transglutaminase-set colloidal properties of wheat gluten with ultrasound pretreatments. *Ultrasonics Sonochemistry, 39*, 137–143.

Shahidi, F., & Zhong, Y. (2010). Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science and Technology, 112*(9), 930–940.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends in Food Science & Technology, 21*(7), 323–331.

Tang, C. H., Wang, X. Y., Yang, X. Q., & Li, L. (2009). Formation of soluble aggregates from insoluble commercial soy protein isolate by means of ultrasonic treatment and their gelling properties. *Journal of Food Engineering, 92*(4), 432–437.

Vijayalakshmi, G., Adinarayana, M., & Rao, P. J. (2010). Kinetics and mechanisms of oxidation of some antioxidants with photochemically generated tert-butoxyl radicals. *Indian Journal of Biochemistry & Biophysics, 47*(5), 292–297.

Zhao, Y.-Y., Wang, P., Zou, Y.-F., Li, K., Kang, Z.-L., Xu, X.-L., & Zhou, G.-H. (2014). Effect of pre-emulsification of plant lipid treated by pulsed ultrasound on the functional properties of chicken breast myofibrillar protein composite gel. *Food Research International, 58*, 98–104.

Zhong, Z., & Xiong, Y. L. (2020). Thermosonication-induced structural changes and solution properties of mung bean protein. *Ultrasonics Sonochemistry, 62*, 104908.

Zou, Y., Wang, L., Li, P., Cai, P., Zhang, M., Sun, Z., ... Wang, D. (2017). Effects of ultrasound assisted extraction on the physiochemical, structural and functional characteristics of duck liver protein isolate. *Process Biochemistry, 52*, 174–182.

CHAPTER 7

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

General discussion

Fish and fish products are rich in easily digestible animal protein and healthy n-3 polyunsaturated fatty acids. Thus, fish and fish products play an important role in the human diet. However, with high moisture and less connective tissue, fish muscle is perishable even under chilled storage. The key task is to maintain fish freshness and prolong the shelf life of chilled fish products. In order to fulfil this task, the fish muscle deterioration process and the typical freshness indicators changes should be well understood. Fish muscle deterioration and enzymatic autolysis occur mainly in the first few days of storage, while microbial spoilage usually occurs in the second stage. Enzymatic autolysis in fish muscle involves nucleotides, lipid and protein degradation, and they greatly affect the process of fish rigor mortis. Above deterioration processes and some common freshness indicators such as K value, TBARS value, salt-soluble protein content, etc., in chilled fish are discussed in sections 1, 2, and 3. Effective preservatives and preservation methods are necessary for maintaining fish freshness and extending the shelf-life of fish products. The 'green consumerism' mind makes foods with few synthetic additives but more natural ingredients popular. This novel consuming conception promotes the demand for developing friendly preservatives for fish. Antibacterial essential oils and alginate are generally considered safe. Their combination as film incorporating essential oil emulsion could develop a novel preservation strategy for chill-stored fish. This is discussed in section 4. Additionally, phenolic compounds commonly occur in plants, and they might be used as natural antioxidants or antibacterial agents for fish products. Wisely applying phenolic compounds could improve fish gel foods' properties and prolong their shelf-life, and these are discussed in section 5.

1. Fish autolysis

1.1. Fish rigor mortis

Rigor mortis of fish muscle is important for fish preservation. Its occurring time and lasting length greatly decide fish shelf-life. Fish rigor mortis depends on fish species, fish physiological status, slaughtering method, etc. However, very limited knowledge of the influences of the farming system and muscle nutrient profile on fish rigor mortis is available. In paper 1, the post-mortem quality changes of common carp raised by a patented and a traditional culture system were studied. It is found that the patented farming system could provide n-3 common carp (OCC) with a twice lower amount of total lipid and saturated fatty acid (SAFA) but four times higher n-3 PUFA, three-fold higher DHA+EPA and five times higher n-3/n-6 ratio compared with normal common carp (NCC) farmed by a traditional system. The study showed that the rigor mortis patterns of NCC and OCC were similar, and the change of pH value did not show any difference, either. Initial glycogen content was slightly higher in NCC. However, a similar metabolic rate of glycogen to form lactic acid was observed, with an inverse correlation between glycogen and lactic acid, which exhibited the same pattern with silver carp (Zhang et al., 2017) and grass carp (Qin et al., 2016). The result implies that the patented culture system and modified FA profile might have no distinct influence on the common carp rigor mortis process.

1.2. Fish nucleotides deterioration

The nucleic acid metabolism is a good proxy for checking biochemical changes in fish and could imply fish freshness. In paper 1, initial ATP content differed in NCC and OCC, but both declined to <0.1 μmol/g after 36 h and remained stable. The results suggest the rapid degradation of ATP, ADP, and AMP to IMP in OCC and NCC in 1 or 2 days, confirming the conclusion drawn by Alasalvar et al. (2002). The study found a distinct accumulation of IMP after 36 h, and the IMP level was slightly higher in OCC at 36 h. As an umami taste compound contributing to good fish flavor (Shiba et al., 2014), a higher IMP content implies the OCC flavor could be improved. Paper 1 calculated the K value to investigate the freshness of carp. According to Hong et al. (2017), NCC and OCC were very fresh before 36 h, but not fresh after 96 h, similar to that reported by Qin et al. (2016). No significant difference of K-value, Ki-value, and Hx-index between OCC and NCC was observed during the whole storage. The above results indicate that the patented farming system and modified FA profile have no remarkable impact on nucleotides deterioration of common carp during chilled storage.

1.3. Fish lipid and protein deterioration

In paper 1, lipid oxidation in NCC and OCC was evaluated by TBARS value. Though OCC is supposed to be more susceptible to lipid oxidation due to the enriched n-3 PUFA, the result showed no difference of TBARS between NCC and OCC, and only a slightly higher TBARS value in NCC was observed at 144 h. It is indicated that OCC might have similar or higher oxidative stability than NCC. Thus, the patented farming system and modified FA profile in fish muscle might not promote lipid oxidation in carp.

Protein is the main nutrient of fish muscle. Its degradation is crucial to fish freshness and texture. In paper 1, slightly higher SSP content in OCC than in NCC at the end of storage was observed. It indicates more integrated muscle protein in OCC than in NCC. Electrophoresis investigation showed that some fragments from the water-soluble protein of NCC exhibited higher intensity than the counterpart of OCC, suggesting more water-soluble protein loss. Meanwhile, the change in OCC was not as distinct as that in NCC, implying the alleviated hydrolysis of them in OCC. Coincidently, slightly higher hardness in OCC was observed at 144 h, while slightly lower drip loss (DL) and cooking loss (CL) were found in OCC. Proteolysis of the intracellular fish protein would cause texture deterioration of fish muscle, affecting liquid loss (Martinez et al., 2011; Duun and Rustad, 2008). Changes in hardness, DL, and CL in carp demonstrates that fish protein might be slightly more stable in OCC than in NCC. The above results indicate that the patented farming system might provide carp with a more stable lipid and protein than the traditional farming system.

2. Fish microbial spoilage

2.1. Fish common SSO

Fish spoilage is not contributed by all the microorganisms in fish. Only a few microbes defined as specific spoilage organisms (SSO) could survive and produce large amounts of off-odor and metabolites, resulting in spoilage and customer rejection (Jääskeläinen et al., 2019; Parlapani et al., 2015). By figuring out real target spoilage microorganisms, preservative methods could be applied more efficiently to maintain freshness and prolong the shelf-life of fish. In paper 2, *Pseudomonas* sp. (PSE), *Shewanella* sp. (HSP), Lactic acid bacteria (LAB), Enterobacteriaceae (ENT), and *Aeromonas* sp. are categorized as common SSOs in chill-stored fish according to the current peer-reviewed and published articles (42 in English, 2001–2020). The collected data from those 42 articles suggest that most above microbes have the opportunity to become SSOs in air package (AP), except LAB, which prevails in modified atmosphere package (MAP) and vacuum package (VP) (Jääskeläinen et al., 2019; Zhang et al., 2019). *PSE* and *Shewanella* sp. are the commonest SSOs in chilled fish, while *Shewanella* sp. have a higher chance to become SSOs in MAP or VP (Carrión-Granda et al., 2018; Zhang et al., 2016). Paper 1 exposed the interspecies inhibition in *Aeromonas* sp. (Liu et al., 2018b; Wang et al., 2014) and PSE (Jia et al., 2018; Li et al., 2018c). The results draw from these 42 articles suggest that *Aeromonas* sp. tend to be SSO only in freshwater fishes (Huang et al., 2018; Pan et al., 2018), and ENT is usually observed in fish from the contaminated aquatic area (Macé et al., 2013). These indicate that fish habitat could also greatly influence the occurrence of SSOs.

2.2. Microbial populations change

With the extension of storage time, more soluble nutrients are released via the degradation of lipid, protein, nucleotides, promoting microorganism reproduction. Thus, the population of spoilage microorganisms is often measured to investigate fish freshness (Gómez-Estaca et al., 2018; Jia et al., 2018). In Paper 1, the TVC of OCC and NCC during chilled storage were measured. Only slightly lower TVC in OCC (5.85 log CFU/g) than in NCC (6.47 log CFU/g) at the end of storage (144 h) was found. TVC of NCC increased slightly quicker than that of OCC after 96 h. This result might be due to a relative tightness muscle microstructure in OCC contributed by its higher salt-soluble protein (SSP) content and less distinct degradation of myosin heavy chain than traditional common carp. The results indicate patented farming system could provide a slightly more bacteria-resistant muscle.

In paper 3, the TVC of CK- (fillet without alginate coating) and CK+ (fillet with alginate coating) exceeded 7.0 log CFU/g muscle on day 6 and day 8, while the EO-coating groups came close to that on day 10. The results demonstrate that alginate coatings loaded 1% pimento (P), thyme (T) or oregano (O) EO-emulsions were effective in inhibiting spoilage bacteria in carp fillets during chilled storage, and they could extend the shelf-life of chill-stored common carp fillets by 2–4 days. The analysis of the SSOs population could provide a better understanding of the spoilage status of fish. In paper 3, the counts of typical SSOs, including PSE, HSP, and ENT were analyzed. It is found that PSE and HSP counts of CK- and CK+ increased to 8 log CFU/g muscle on day 10 while the P, O, and T groups had a PSE count of 5.76–6.15 log CFU/g muscle and an HSP count of 5.81–6.19 log CFU/g muscle on day 10. Jouki et al. (2014) also reported significant inhibition of PSE and HSP in rainbow trout by oregano and thyme EO loaded coating. It indicates the improved preservative effects of alginate coating with the three EO-emulsion. Additionally, ENT counts of P, O, and T were lower than those of CK- and CK+ after day 8, suggesting that coating with the EO-emulsions slowed down the proliferation of ENT in carp.

2.3. Microorganism associated spoilage indicators

Besides the microbial population, some associated indicators might also evaluate microbial spoilage in fish. For example, Yu et al. (2018) report that TVBN was well correlated ($p<0.05$, R2=0.913–0.998) with TVC in chill-stored grass carp. Jouki, et al. (2014) found a close correlation between TMA and TVC in chill-stored rainbow trout. Using the generalized additive model (GAM) analysis, paper 2 revealed that microbial load is a key driver of spoilage progression in fish flesh over other spoilage influences, including autolytic protein degradation and lipid oxidation. Paper 2 found a highly significant and positive relationship between TVC and Hx, TVC and TMA, TVC and TVBN in chilled-stored fish when the TVC value exceeded 7 log CFU/g, 5 log CFU/g and 5–6 log CFU/g, respectively. These indicate that the three spoilage indicators markedly deteriorate when TVC is 5–7 log CFU/g, marking the onset of rapid spoilage. Thus, the three indicators could be analyzed to evaluate fish spoilage.

In paper 3, TVBN of common carp fillets with alginate coating and EO-emulsions treatment was evaluated during chilled storage. It was found that TVBN of CK- and CK+ were above the limit of 25 mg/100g for aquatic food (Ojagh et al., 2010) after 8 days, indicating the severe bacterial spoilage and deterioration of carp fillets. However, TVBN of the P, O, and T groups remained <25 mg/100g until day 10. These suggest the alginate coating loaded 1% pimento, thyme, or oregano EO-emulsions could improve the preservation quality and extend the shelf-life of chill-stored common carp fillets for 2–4 days. A similar reduction in TVBN was observed by Wu et al. (2014) in grass carp fillets treated with a gelatin-chitosan coating containing 4% oregano EO.

3. Fish freshness indicators and their limitations

The fish freshness and quality can be described by a variety of physical, chemical, and microbial properties, which can be assessed as indicators such as hardness, odor, Hx, HxR, K-value, TBARS, TMA, TVBN, biogenic amine (BA), TVC, etc. (Olafsdottir et al., 2004; Prabhakar et al., 2020). They are usually suggested with a recommended level to help to judge the freshness and acceptability of fish products.

3.1. Physical indicators of fish

In paper 1, various physical indicators were detected for obtaining a clear understating of carp freshness. Hardness is considered as a valuable quality attribute to evaluate fish fillets (Viji et al., 2019). Color properties (e.g. L* value reflects lightness) influence the acceptance of food by consumers. DL and CL denote water and water-soluble nutrient loss during storage or after cooking (Cai et al., 2014). No significant differences between NCC and OCC were found in hardness and springiness during storage, except a slightly lower hardness in NCC was observed at 144 h. Moreover, L* value, DL and CL showed no significant differences between NCC and OCC during storage. The above results of physical indicators indicated that the patent culture system does not lead to significant changes in common carp's physical qualities. Thus, it could be applied to produce carp with a high proportion of omega-3 fatty acid without compromising physical properties. However, considering the differences in fatty acid profile, it could be good to investigate fish's chemical and microbial properties to better understand the difference of quality between NCC and OCC.

In papers 5 and 6, the storage modulus (G') of the myofibrillar protein (MP) with the addition of gallic acid (GA) under oxidative stress or ultrasound treatment (U) were studied. G′ is the ability that the gel could store energy, and a higher G′ denotes the more solid-like property of the gel. G′ of MP increased sharply with a low dose of GA (5 μmol/g), indicating an improvement of the gelation properties of oxidatively stressed fish gel products. Similar effects of GA on G′ of porcine MP was reported by Guo and Xiong (2019). Moreover, U5 had the highest G′ while U125 had the lowest G. Those results indicated ultrasound coupled with low dose GA could improve the gel properties of fish products. Thus, low dose GA could be applied in fish gel products to improve their stability.

3.2. Chemical indicators of fish

In paper 1, a group of typical freshness parameters calculated from ATP-related compounds, e.g., K-value, Ki-value, and Hx-index, were investigated to reflect the spoilage of NCC and OCC. Based on limitations by Hong et al. (2017), OCC and NCC were not fresh at 120 h chilled storage, similar to that reported by Qin et al. (2016). Ki-value and Hx-index are also reported as alternative supplemental indicators for evaluating fish freshness since K-value is less effective than the other two parameters in some particular conditions (Hong et al., 2017). In our study, Ki-value and Hx-index showed similar change as K-value, exhibiting high correlation with each other (K-value versus Ki-value, r=0.980–0.984; K-value versus Hx-index, r=0.968–0.981, Pearsons two-tailed, $P<0.05$). Thus, they are all befitting for carp freshness evaluation. TBARS value is analyzed for assessing lipid oxidation in chill-stored common carp in paper 1. TBARS value increased from 0.075 and 0.095 mg/kg in NCC and OCC to 0.320 and 0.250 mg/kg after 144 h, suggesting lipid oxidation at that time. No difference in TBARS was found between NCC and OCC during storage. Only a slightly higher TBARS in NCC was observed at 144 h. Noticeably, the TBARS value suggested a freshness deterioration at 144 h. In comparison, K-value indicated a remarkable freshness decline at 120 h, indicating various chemical indicators also showed a slightly different freshness status of the carp.

3.3. Microbial indicators of fish

TVC is a widely used freshness indicator reflecting microbial contamination. The maximum acceptable microorganism level for raw freshwater fish is 7.0 log CFU/g (ICMSF, 1986). In paper 1, TVC of NCC increased to 6.47 log CFU/g, higher than that of OCC, 5.85 log CFU/g after 144 h, but both not exceeded the maximum acceptable level of microorganism for raw freshwater fish (7.0 log CFU/g). According to this, OCC and NCC are still acceptable after 144 hours under chilled storage. It is different from the conclusion drawn from K-value, which suggested the total freshness loss after 120 hours in OCC and NCC. Thus, to evaluate the freshness of fish products, only one freshness indicator is not enough. More indicators should be considered to gain a more comprehensive assessment of the freshness state of the aiming product. Noticeably, in paper 1, TBARS, TVC, CL, DL, and hardness in NCC and OCC showed no difference between the two groups before 96 h. However, higher TBARS, TVC, CL, and DL while lower hardness in NCC than in OCC was observed at 144 h. Thus, TBARS, TVC, CL, and DL might be more sensitive to show the difference between the two fish from different farming systems.

Freshness-related metabolites, such as HxR, Hx, TMA, ammonia, BAs, and VOCs, can be formed through microbial activities during chilled storage. There could be some relationships between TVC and these freshness indicators. In paper 2, metadata analysis based on peer-reviewed articles (n=140) studied the freshness of freshwater fish during storage by using a GAM. It revealed that TVC correlated with the Hx, TMA, and TVBN (R^2 0.63–0.93; $p<0.01$) in chilled-stored fish fillets when the TVC value exceeded 7 log CFU/g, 5 log CFU/g and 5–6 log CFU/g, respectively. It suggests it could be possible to measure just one or two of the four parameters to check the freshness of fish.

4. EOs for chill-stored fish preservation

4.1. EOs with antibacterial activity

Paper 2 summarized the effects of EOs application in fish. EOs are characterized by two or three principal components at high concentrations (20~70%) (Van Haute et al., 2016), which could be: (i) terpene compounds; (ii) terpenoids (subdivided into alcohols, esters, aldehydes, ketones, ethers, and phenols); (iii) phenylpropanoids (subdivided into phenols, aldehyde, alcohol and methoxy derivatives) (Jayasena and Jo, 2013). These compounds have antimicrobial properties. EOs' high lipophilicity enables EOs to penetrate the cytoplasm easily and disturb the phospholipid bilayer of the inner membrane and mitochondria, leading to the instability of cellular structure and increasing cellular permeability (Hassoun and Çoban, 2017; Shojaee-Aliabad et al., 2018). Lipophilic hydrocarbons in EOs could also distort the lipid-protein interaction in a bacterial cell and interfere with ATPases necessary for producing ATP (Mei et al., 2019). Moreover, phenolics in EOs could disrupt the proton motive force, electron flow, and cytoplasmic coagulation (Shojaee-Aliabad et al., 2018).

In paper 2, TVC or common SSOs in chill-stored fish as affected by various EOs were submitted for metadata analysis. It is found that citrus, mentha, origanum, thymus, zataria, and Zingiberaceae EOs have extraordinary TVC reduction potential. Cinnamon, mentha, origanum, rosemary, and Zingiberaceae EOs showed extraordinary PSE reduction potential while for HSP, there comes to be origanum, thymus, and Zingiberaceae EOs. Apiaceae, mentha, origanum, thymus, and zataria EOs had extraordinary ENT reduction potential, but only origanum, and zataria EOs exhibited extraordinary ENT reduction potential. Additionally, bay laurel, origanum, thymus, zataria, and Zingiberaceae EOs exhibited extraordinary LAB reduction potential. Another interesting question – is there any EO with a broad antibacterial spectrum, namely against the most common SSOs mentioned above? Paper 2 found that among the 6 top EOs with extraordinary TVC reduction potential, only 3 EOs, i.e., origanum, Zingiberaceae, and thymus EOs showed a full spectrum efficacy against all four SSOs (PSE, HSP, ENT, and LAB). That means origanum, Zingiberaceae, and thymus EOs have the capacity to inhibit diverse spoilage microorganisms in chilled-stored fish. These three EOs could have high value for the fish preservation industry.

Paper 3 also investigated the antibacterial activity of 12 commercial EOs on *L. monocytogenes, E. coli, P. fragi,* and *S. putrefaciens* by broth microdilution test. The results indicate that thyme, pimento, and oregano EOs had the most effective bacteriostatic and bactericidal functions, among which thyme EO exhibited the highest potential.

4.2. Application methods of EOs and their effects on EOs performance

Paper 2 discussed the methods for EOs application in chill-stored fish. View from 140 collected articles, EOs are often applied via bulk EO, EO-emulsion, applied alone, or via hurdle technology (e.g., combined with active film, packaging, additives, and pre-treatment). Seventy-eight cases were using bulk EOs for application in fish, including immersion (31 out of 78 cases), spraying (9/78 cases), pipette dropping, followed by massaging (25/78 cases), or EO vapor (4/78 cases). The disadvantages of using bulk EO are lowering its antibacterial properties, requiring a high dose of EO, bringing strong unpleasant odors to fish. Thus, other preservative methods are often combined to overcome these deficiencies.

According to the collected data in paper 2, EO-nanoemulsions are mostly coupled with active film (66/82 cases) and packaging (15/82 cases) to preserve chill-stored fish, followed by additives (8/82 cases) and pre-treatment (4/82 cases). Most fish treated with EO nanoemulsion exhibited a more distinct absolute reduction (calculated as the treatment group subtracted from the control at the fixed time point and temperature conditions) in TVBN and Enterobacteriaceae than those treated with bulk EO alone. On the other hand, paper 3 revealed the EO-emulsions from thyme, pimento, and oregano EOs against *L. monocytogenes, E. coli, P. fragi,* and *S. putrefaciens* by broth microdilution showed lower MIC and MBC compared to EOs applied alone, suggesting emulsion system could amplify the antibacterial functions of EO. Moreover, paper 3 found carp fillets treated with coatings containing 1% or 1.5% thyme, oregano, and pimento EO-emulsion showed TVC<4.5 log CFU/g muscle on day 6, significantly lower than that of the control, showing strong antibacterial activity. Paper 2 concluded that EO-nanoemulsion was more effective in preserving chill-stored fish. It summarized the advantages of EO-emulsion: improve EO’s antimicrobial activity, reduce the application dose and minimize the organoleptic effects of EO on fish.

Paper 2 found EOs were commonly applied through a hurdle system (130/160 cases) for chill-stored fish through metadata analysis. Film-EO is the commonest hurdle system, while combining packaging with EOs is another popular hurdle system. It is found that active film incorporating EO-nanoemulsion showed higher antimicrobial efficacy than normal film containing bulk EO. Also, the film-EO system was better than the sole bulk-EO application. Paper 2 indicated packaging-EO hurdle system usually showed a more substantial perseverative effect than sole EO in chill-stored fish, especially for controlling LAB, PSE, HSP, and Enterobacteriaceae. It has to be noticed that pre-treatments, e.g., marinating (Van Haute et al., 2016), high hydrostatic pressure (HHP) (Gómez-Estaca et al., 2018), -irradiation (Abdeldaiem et al., 2018) and UV irradiation (Křížek et al., 2018) could also improve the antibacterial effect of EOs. The metadata analysis suggests that hurdle technology, in general, with pre-treatment, additives, film, and special packaging in various combinations, are good and reliable application methods for EO.

4.3. EOs influences on fish sensory properties

EOs have a strong aroma, especially when used at a high concentration to achieve high efficiency against spoilage microbes. From the marketing perspective, any EO treatment (even if top-performing) must not significantly alter the sensory properties of fish flesh from the 'fresh-fish odor', or consumers will not accept it. Chitosan loaded 1% lemon verbena EO applied on rainbow trout fillets (Rezaeifar et al., 2020), 1% rosemary EO applied on mackerel fillets (Can et al., 2014), and 1.5% bay laurel EO applied on sea bass (Öztürk Kerimoğlu et al., 2020) were found to have side effects on their sensory properties. Metadata analysis in paper 2 indicated an insignificant effect on odor properties of fresh fish was observed by 0.5 to 1% concentration of most EOs, and citrus, star anise, and thyme EOs showed a detrimental impact on the odor properties of fresh fish, such as carp or rainbow trout (Farsanipour et al., 2020; Huang et al., 2018; Oguzhan Yildiz, 2017). Thus, caution must be exercised for them if higher concentrations are used.

In paper 3, coating loaded 1% pimento, thyme, and oregano EO-emulsions showed a decrease in the score of odor and acceptability in raw carp cubes and the score of odor and acceptability and taste in cooked carp cubes served with coating. Fortunately, these negative effects on the sensory properties of raw and cooked fish could be eliminated by removing the coating before serving. However, when 1.5% pimento, thyme, and oregano EO-emulsion were included, the negative influences on organoleptic properties of fish are remarkable, regardless of whether the fish was served with or with removed coating. Therefore, the paper suggested incorporating an emulsion of 1% EO into the alginate coating to preserve carp fillets.

5. Improvement of fish gel food stability by GA

5.1. Fish protein oxidation intervened by GA

GA is a water-soluble phenolic compound with strong anti-oxidative properties (Abdelwahed et al., 2007). It played a dual role (anti- and pro-oxidant) in mediating pork MP gelation (Cao et al., 2016). In paper 4, GA effect on the structure and gelling potential of Japanese seerfish (JS) MP exposed to imitated Fenton oxidation system (10 µM $FeCl_3$, 100 µM ascorbic acid, 1 mM H_2O_2) was studied. It was found that the addition of 5, 125 µmol/g GA alleviated carbonyl group formation significantly. 5 µmol/g GA preserved SH content at 96%, but 125 µmol/g GA led to heavy loss of SH, which was similar to pork MFP oxidatively stressed with chlorogenic acid (Cao and Xiong, 2015). OX+5 showed a similar altitude of two peaks representing α-helix structure at 210 and 223 nm in CD spectrum with NOX while attenuated peak intensity was observed in OX+125. Electron spin resonance analysis showed OX+125 had a much higher radical intensity than OX+0 and NOX, indicating a heavy accumulation of radicals of different nature in it. Results in paper 4 imply that GA in a low dose might play an anti-oxidative role, alleviate carbonyls formation, protect free amine, stabilize SH and secondary structure of the protein. Nevertheless, a high dose GA might play as a pro-oxidant to form more carbonyls and consume more SH to produce stable phenoxyl radicals.

In paper 5, the effects of GA without or with ultrasound treatment (NU or U groups) on properties of JS MP were studied. Total SH showed no change in U5 but a remarkable decrease in U125. Reactive SH increased in U5 but largely decreased in U125. Phenolic compounds could form their quinone derivatives to cross-link with protein SH (Jongberg et al., 2011). U5 treatment might expose more reactive SH (Malik et al., 2017), but it might also trigger radicals (Gülseren et al., 2007) to form GA quinone, which further cross-linked with MP. Reactive SH in U5 as an antioxidant. On the opposite, excessive GA in U125 might promote the interaction of protein-SH with GA quinone, consuming a large amount of SH. The above results suggest that the role of GA (anti- and pro-oxidant) in MP under ultrasound depends on its dose. Paper 5 identified two specific polymers Cys-GA-Cys and Lys-GA-Lys, by mass in U125, providing direct evidence of GA-protein polymers' formation through quinone-thiol and -amido adduct pathway.

5.2. Fish protein gel-forming properties intervened by GA

In paper 4, the storage modulus (G') of OX+5 climbed distinctly. It is known that mild oxidation could induce protein unfolding, facilitating the interaction of protein molecules during heating to form a fine gel structure (Xiong et al., 2010). 5 μmol/g GA might preserve a high level of SH and free amine and induce MP unfolding. Together with possible quinone-protein interaction, it greatly improved the rheological properties of MP. In contrast, 125 μmol/g GA might lead to excessive aggregation through the thiol-quinone adduct pathway, which is unfavorable for forming an ordered gel structure. The superabundant GA could also shield reactive groups and obstruct their participation in gel formation (Cao and Xiong, 2015). It can be concluded from paper 4 that a low dose of GA would improve gelation properties of oxidatively stressed JS MP. However, a high dose of GA might inhibit gelling properties of oxidatively stressed JS MP.

In paper 5, U5 showed the highest G' while U125 exhibited the lowest G'. Ultrasound could unfold protein structure and expose more functional groups onto protein molecule surface, facilitating further cross-linking during heating and improving rheological properties (Li et al., 2015). However, ultrasound might also bring OH· that might convert high dose GA (U125) into GA quinone to cause heavy cross-linking or aggregations before thermal treatment. These aggregations would shield partial reactive groups and hamper the further interactions between GA and protein or protein and protein due to steric hindrance, providing a decreased gelling potential.

Results in paper 4 and paper 5 suggest a low dose of GA could alleviate fish MP oxidation and enable high stability under both oxidative stress or ultrasound treatment, but a high dose of GA might promote protein oxidation, leading to unordered protein cross-linking. Low dose but not high dose GA can be applied to improve gel properties of JS MP.

4. Conclusions

This research filled the blank in the nutrient composition of Czech patented omega-3 carp; provided scientific data on the spoilage progress, freshness-related indicators, and shelf life of chill-stored omega-3 carp; supplemented the knowledge of manipulating spoilage microbe in chill-stored fish by EO-based preserving methods, which is lack of the consideration of sensory acceptability; established the strategy for using EO-emulsion coating to improve carp freshness; established smart strategy for controlling the oxidation of fish muscle protein to obtain good gel properties. It gives the below conclusions:

- Omega-3 carp from the patented culture system showed a slightly positive influence on carp muscle stability and slightly longer shelf-life than the carp from the traditional culture system in the Czech Republic. The patented system could be applied to produce carp with a high n-3 PUFA and n-3/n-6 ratio without compromising storage stability.
- Four SSOs, i.e., *Pseudomonas* sp., *Shewanella* sp., Lactic acid bacteria, and Enterobacteriaceae, are common spoilage microbe in chill-stored fishes. Six EOs, i.e., citrus, mentha, origanum, thymus, zataria, and Zingiberaceae EOs could inhibit TVC in fish. However, only origanum, Zingiberaceae, and thymus EOs showed complete-spectrum efficacy on the above four SSOs.
- To ensure a high preserving efficacy on the freshness and shelf-life of chill-stored fish, an appropriate combination of preservative methods, mainly hurdle technology, active film, nanoemulsion, and special packaging, is needed.
- Incorporation of an emulsion of 1% pimento, thyme, and oregano EO-emulsions into the alginate coating can properly preserve chill-stored common carp without causing unacceptable sensory properties.
- Protein oxidation can be well manipulated by an appropriate dose of gallic acid and ultrasound treatment to enable desirable protein properties modification for improving gel properties of fish products.

References

Abdeldaiem, M.H., Mohammad H.G., Ramadan M.F., 2018. Improving the quality of silver carp fish fillets by gamma irradiation and coatings containing rosemary oil. Journal of Aquatic Food Product Technology 27, 568–579.

Abdelwahed, A., Bouhlel I., Skandrani I., Valenti K., Kadri M., Guiraud P., Steiman R., Mariotte A.-M., Ghedira K., Laporte F., Dijoux-Franca M.-G., Chekir-Ghedira L., 2007. Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from *Pistacia lentiscus*. Confirmation by microarray expression profiling. Chem Biol Interact 165, 1–13.

Alasalvar, C., Taylor K.D.A., Shahidi F., 2002. Comparative quality assessment of cultured and wild sea bream (*Sparus Aurata*) stored in ice. Journal of Agricultural and Food Chemistry 50, 2039–2045.

Cai, L., Wu, X., Li, X., Zhong, K., Li, Y., & Li, J., 2014. Effects of different freezing treatments on physicochemical responses and microbial characteristics of Japanese sea bass (*Lateolabrax japonicas*) fillets during refrigerated storage. LWT – Food Science and Technology 59, 122–129.

Can, O.P., Sahin S., Yalçın H., 2014. Rosemary oil with decontamination of horse mackerel (*Trachurus trachurus l. 1758*) and detection of shelf life. Bulgarian Journal of Agricultural Science 20, 1054–1060.

Cao, Y., Xiong Y.L., 2015. Chlorogenic acid-mediated gel formation of oxidatively stressed myofibrillar protein. Food Chemistry 180, 235–243.

Cao, Y., True A.D., Chen J., Xiong Y.L., 2016. Dual role (anti- and pro-oxidant) of gallic acid in mediating myofibrillar protein gelation and gel in vitro digestion. Journal of Agricultural and Food Chemistry 64, 3054–3061.

Carrión-Granda, X., Fernández-Pan I., Rovira J., Maté J.I., 2018. Effect of antimicrobial edible coatings and modified atmosphere packaging on the microbiological quality of cold stored hake (*Merluccius merluccius*) fillets. Journal of Food Quality 2018, 1–12.

Duun, A.S., Rustad T., 2008. Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at -1.4 and -3.6 °C. Food Chemistry 106, 122–131.

Farsanipour, A., Khodanazary A., Hosseini S.M., 2020. Effect of chitosan-whey protein isolated coatings incorporated with tarragon Artemisia dracunculus essential oil on the quality of *Scomberoides commersonnianus* fillets at refrigerated condition. International Journal of Biological Macromolecules 155, 766–771.

Gómez-Estaca, J., López-Caballero M.E., Martínez-Bartolomé M.Á., de Lacey A.M.L., Gómez-Guillen M.C., Montero M.P., 2018. The effect of the combined use of high pressure treatment and antimicrobial edible film on the quality of salmon carpaccio. International Journal of Food Microbiology 283, 28–36.

Gülseren, İ., Güzey D., Bruce B.D., Weiss J., 2007. Structural and functional changes in ultrasonicated bovine serum albumin solutions. Ultrasonics Sonochemistry 14, 173–183.

Guo, A., & Xiong, Y. L., 2019. Glucose oxidase promotes gallic acid-myofibrillar protein interaction and thermal gelation. Food Chemistry 293, 529–536.

Hassoun, A., Çoban Ö.E., 2017. Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. Trends in Food Science & Technology 68, 26–36.

Hong, H., Regenstein J., Luo Y.K., 2017. The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: A review. Critical Reviews in Food Science and Nutrition 57, 1787–1798.

Huang, Z., Liu X.C., Jia S.L., Zhang L.T., Luo Y.K., 2018. The effect of essential oils on microbial composition and quality of grass carp (*Ctenopharyngodon idellus*) fillets during chilled storage. International Journal of Food Microbiology 266, 52–59.

ICMSF, 1986. Microorganisms in foods. University of Toronto Press Toronto, pp.

Jääskeläinen, E., Jakobsen L.M.A., Hultman J., Eggers N., Bertram H.C., Björkroth J., 2019. Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage. International Journal of Food Microbiology 293, 44–52.

Jayasena, D.D., Jo C., 2013. Essential oils as potential antimicrobial agents in meat and meat products: A review. Trends in Food Science & Technology 34, 96–108.

Jia, S.L., Liu X.C., Huang Z., Li Y., Zhang L.T., Luo Y.K., 2018. Effects of chitosan oligosaccharides on microbiota composition of silver carp (*Hypophthalmichthys molitrix*) determined by culture-dependent and independent methods during chilled storage. International Journal of Food Microbiology 268, 81–91.

J ongberg, S., Gislason N.E., Lund M.N., Skibsted L.H., Waterhouse A.L., 2011. Thiol–Quinone adduct formation in myofibrillar proteins detected by LC-MS. Journal of Agricultural and Food Chemistry 59, 6900–6905.

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.

Křížek, M., Dadáková E., Vácha F., Pelikánová T., Matějková K., 2018. The effects of two essential oil and UV-light irradiation treatments on the formation of biogenic amines in vacuum packed fillets of carp (*Cyprinus carpio*). LWT – Food Science and Technology 95, 268–273.

Li, K., Kang Z.-L., Zou Y.-F., Xu X.-L., Zhou G.-H., 2015. Effect of ultrasound treatment on functional properties of reduced-salt chicken breast meat batter. Journal of Food Science and Technology 52, 2622–2633.

Li, Y., Fang Y.D., Zhang J.B., Feng L.G., Lv Y.M., Luo Y.K., 2018. Changes in quality and microbial succession of lightly salted and sugar-salted blunt snout bream (*Megalobrama amblycephala*) fillets stored at 4 °C. Journal of Food Protection 81, 1293–1303.

Liu, X.C., Li D.P., Li K.F., Luo Y.K., 2018. Monitoring bacterial communities in ε-Polylysine-treated bighead carp (*Aristichthys nobilis*) fillets using culture-dependent and culture-independent techniques. Food Microbiology 76, 257–266.

Macé, S., Joffraud J.J., Cardinal M., Malcheva M., Cornet J., Lalanne V., Chevalier F., Sérot T., Pilet M.F., Dousset X., 2013. Evaluation of the spoilage potential of bacteria isolated from spoiled raw salmon (*Salmo salar*) fillets stored under modified atmosphere packaging. International Journal of Food Microbiology 160, 227–238.

Malik, M.A., Sharma H.K., Saini C.S., 2017. High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: Effect on physicochemical and functional properties. Ultrasonics Sonochemistry 39, 511–519.

Martinez, I., Wang P.A., Slizyté R., Jorge A., Dahle S.W., Cañas B., Yamashita M., Olsen R.L., Erikson U., 2011. Protein expression and enzymatic activities in normal and soft textured atlantic salmon (*Salmo salar*) muscle. Food Chemistry 126, 140–148.

Mei, J., Ma X., Xie J., 2019. Review on natural preservatives for extending fish shelf life. Foods 8, 490.

Oguzhan Yildiz, P., 2017. The effects of chitosan coatings enriched with thyme oil on the quality of rainbow trout. Journal of Food Measurement and Characterization 11, 1398–1405.

Ojagh, S.M., Rezaei M., Razavi S.H., Hosseini S.M.H., 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry 120, 193–198.

Olafsdottir, G., Nesvadba P., Di Natale C., Careche M., Oehlenschläger J., Tryggvadóttir S.a.V., Schubring R., Kroeger M., Heia K., Esaiassen M., Macagnano A., Jørgensen B.M., 2004. Multisensor for fish quality determination. Trends in Food Science & Technology 15, 86–93.

Öztürk Kerimoğlu, B., KavuŞAn H.S., SerdaroĞLu M., 2020. The impacts of laurel *(Laurus nobilis*) and basil (*Ocimum basilicum*) essential oils on oxidative stability and freshness of sous-vide sea bass fillets. Turkish Journal of Veterinary & Animal Sciences, 101–109.

Pan, Z.Q., Li L., Shen Z.H., Chen Y., Li M., 2018. Characterization of the microbiota in air- or vacuum-packed crisp grass carp (*Ctenopharyngodon idella c. Et v.*) fillets by 16s rRNA PCR–denaturing gradient gel electrophoresis and high-throughput sequencing. Journal of Food Protection 81, 1022–1029.

Parlapani, F.F., Haroutounian S.A., Nychas G.-J.E., Boziaris I.S., 2015. Microbiological spoilage and volatiles production of gutted European sea bass stored under air and commercial modified atmosphere package at 2 °C. Food Microbiology 50, 44–53.

Prabhakar, P.K., Vatsa S., Srivastav P.P., Pathak S.S., 2020. A comprehensive review on freshness of fish and assessment: Analytical methods and recent innovations. Food Research International 133, 109157.

Qin, N., Li D.P., Hong H., Zhang Y.M., Zhu B.W., Luo Y.K., 2016. Effects of different stunning methods on the flesh quality of grass carp (*Ctenopharyngodon idellus*) fillets stored at 4 °C. Food Chemistry 201, 131–138.

Rezaeifar, M., Mehdizadeh T., Mojaddar Langroodi A., Rezaei F., 2020. Effect of chitosan edible coating enriched with lemon verbena extract and essential oil on the shelf life of vacuum rainbow trout (*Oncorhynchus mykiss*). Journal of Food Safety 40, e12781.

Shiba, T., Shiraki N., Furushita M., Maeda T., 2014. Free amino acid and ATP-related compounds in sterile tiger puffer fish (*Takifugu rubripes*) fillets stored at 4 °C. Journal of Food Processing and Preservation 38, 791–797.

Shojaee-Aliabad, S., Hosseini S.M., Mirmoghtadaie L., 2018. Antimicrobial activity of essential oil, Essential Oils in Food Processing:Chemistry, Safety and Applications. John Wiley & Sons Ltd, USA, pp. 191–216.

Van Haute, S., Raes K., Van Der Meeren P., Sampers I., 2016. The effect of cinnamon, oregano and thyme essential oils in marinade on the microbial shelf life of fish and meat products. Food Control 68, 30–39.

Viji, P., Shanmuka Sai, K. S., Debbarma, J., Dhiju Das, P. H., Madhusudana Rao, B., & Ravishankar, C. N., 2019. Evaluation of physicochemical characteristics of microwave vacuum dried mackerel and inhibition of oxidation by essential oils. Journal of Food Science and Technology 56, 1890–1898.

Wang, H., Luo Y.K., Huang H.P., Xu Q., 2014. Microbial succession of grass carp (*Ctenopharyngodon idellus*) filets during storage at 4 °C and its contribution to biogenic amines' formation. International Journal of Food Microbiology 190, 66–71.

Wu, J.L., Ge S.Y., Liu H., Wang S., Chen S.F., Wang J.H., Li J.H., Zhang Q.Q., 2014. Properties and antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. Food Packaging and Shelf Life 2, 7–16.

Xiong, Y.L., Blanchard S.P., Ooizumi T., Ma Y., 2010. Hydroxyl radical and ferryl-generating systems promote gel network formation of myofibrillar protein. Journal of Food Science 75, C215–C221.

Yu, D.W., Regenstein J., Zang J.H., Jiang Q.X., Xia W.S., Xu Y.S., 2018. Inhibition of microbial spoilage of grass carp (*Ctenopharyngodon idellus*) fillets with a chitosan-based coating during refrigerated storage. International Journal of Food Microbiology 285, 61–68.

Zhang, C.L., Zhu S.Q., Wu H.H., Jatt A.N., Pan Y.R., Zeng M.Y., 2016. Quorum sensing involved in the spoilage process of the skin and flesh of vacuum-packaged farmed turbot (*Scophthalmus maximus*) stored at 4 °C Journal of Food Science 81, M2776–M2784.

Zhang, J.B., Li Y., Liu X.C., Lei Y.T., Regenstein J., Luo Y.K., 2019. Characterization of the microbial composition and quality of lightly salted grass carp (*Ctenopharyngodon idellus*) fillets with vacuum or modified atmosphere packaging. International Journal of Food Microbiology 293, 87–93.

Zhang, L.T., Li Q., Lyu J., Kong C.L., Song S.J., Luo Y.K., 2017. The impact of stunning methods on stress conditions and quality of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4 °C during 72h postmortem. Food Chemistry 216, 130–137.

English summary

Freshness and shelf-life of fish products

Ruoyi Hao

Fish and fish products play an important vital role in the human diet. However, fish suffer spoilage, such as deterioration of nucleotides, lipid and protein oxidation, and microbial growth, even though under chilled storage. Thus, maintaining freshness, improving quality, and prolong the shelf life of fish products is a crucial task. As the arising of 'green consumerism,' the methods with green materials (e.g., phenolic compounds, EOs, and active coating) against the spoilage or deterioration in a fish product receive much attention.

In chapter 2, our study supplemented the knowledge of nutrients patterns, deterioration progress, freshness loss, and shelf life of a patented "omega-3 carp". No significant differences in glycogen, lactic acid, and freshness indicator, i.e., Hx-index, K-value, Ki-value, were observed between traditional common carp and omega-3 carp during chilled storage. However, the protein pattern and lipid stability were found slightly better in omega-3 carp. TVC showed a lower value in the omega-3 carp group, indicating the shelf-life of omega-3 carp might be a few hours longer than traditional common carp. With these scientific bases, the patented Omega-3 common carp is recommended to be widely produced to gain a win-win stage in economic and healthy areas.

Chapter 3 and 4 provided a metadata analysis of the relationships among typical freshness indicators and several dominant spoilage microorganisms and the manipulation of spoilage microorganisms by EOs-based preserving method. The metadata analysis added more information in the chill-stored fish preservation area: a) *Pseudomonas* sp., *Shewanella* sp., Lactic acid bacteria, and Enterobacteriaceae are the most common SSOs in the chill-stored fillet; b) only origanum, Zingiberaceae, and thymus EOs exhibited a full-spectrum efficacy on all SSOs; c) properly select an application method (mainly hurdle technology, active film-emulsion, and special packaging) is very important to ensure a high efficacy of EO's antibacterial function; d) the sensory influence of EO-based preserving method on fish products needs to be considered. To fill the blanks found above, a sensory acceptable EOs-based antibacterial coating was developed for the preservation of chill stored fish fillets.

Chapters 5 and 6 established innovative strategies to control the fish muscle protein oxidation for good gel properties. Gallic acid in a lower dose could stabilize sulphydryls and the secondary structure of the fish myofibrillar protein. Meanwhile, ultrasound treatment could promote its structural unfolding and reactive group exposure and produce gallic acid quinone by triggering OH· from gallic acid. Thus, an appropriate combination of ultrasound treatment and gallic acid could offer a pleasant protein cross-linking, sharply increasing fish myofibrillar protein's storage modulus and improving fish gel food stability.

Czech summary

Čerstvost a trvanlivost rybích produktů

Ruoyi Hao

Ryby a rybí produkty jsou životně důležitou součástí lidského jídelníčku. Ačkoliv jsou ryby uchovávány v chladu, rychle podléhají zkáze, a to kvůli degradaci nukleotidů, oxidací bílkovin a lipidů a v neposlední řadě mikrobiální kontaminací. Velmi důležitým cílem je tedy udržení čerstvosti a prodloužení skladování těchto výrobků. S nástupem tzv. zeleného konzumerizmu je věnována velká pozornost metodám využívajícím přírodní látky (např. fenolové sloučeniny, esenciální oleje či aktivní obaly), jejichž aplikace brání snížení kvality či samotnému zkažení rybích produktů

Kapitola 2 této dizertace se věnuje doplnění informací o znalostech nutričních hodnot, procesu snížení kvality, ztrátě čerstvosti a trvanlivosti patentovaného "omega-3 kapra". Při porovnání omega-3 kapra s klasickým kaprem nebyly v průběhu skladování zjištěny žádné signifikantní rozdíly. A to z hlediska obsahu glykogenu, kyseliny mléčné, dále pak indikátorů čerstvosti jako H-index a hodnoty K a Ki. Avšak proteinový profil a stabilita lipidů dosahovaly u omega 3-kapra o něco příznivější hodnoty. TVC hodnota byla u omega-3 kapra nižší, což může znamenat, že skladovací životnost omega-3 kapra může být o několik hodin delší. Na základě těchto vědecky podložených zjištění se doporučuje produkce patentovaného omega-3 kapra v širším měřítku, čímž lze dosáhnou výhodné rovnováhy mezi ekonomickou i výživovou stránkou věci.

Kapitola 3 a 4 se věnuje analýze vztahů mezi typickými ukazateli čerstvosti a mikroorganizmy, které se považují za příčinu mikrobiální kontaminace (SSO). Dále se pak zabývá metodou konzervace ryb a rybích produktů na bázi esenciálních olejů (EO), které brání pomnožení SSO. Analýza metadat přinesla další informace v oblasti skladování ryb v chladu: a) nejběžnějšími SSO bakteriemi na filetu uloženém v chladu jsou *Pseudomonas* sp., *Shewanella* sp., bakterie mléčného kvašení a bakterie ze skupiny Enterobacteriaceae; b) pouze EO z oregana, zázvorovitých rostlin a tymiánu vykazovaly účinnost na celé spektrum SSO bakterií; c) pro vysokou účinnost antibakteriální funkce EO je velmi důležitá správná volba aplikační metody (především překážkové technologie, aktivní filmová emulze a speciální balení); d) je třeba také dbát na vliv EO konzervační metody na senzorické vlastnosti rybích produktů. Na základě těchto poznatků byl pro chlazené filety vyvinut senzoricky přijatelný antibakteriální film na bázi EO.

Kapitoly 5 a 6 stanovily inovativní strategie pro řízení oxidace proteinů rybího svalu za účelem dosažení dobrých gelových vlastností produktů typu surimi. Kyselina gallová v nízkých dávkách může stabilizovat sulphydryly a sekundární strukturu rybího myofibrilárního proteinu, zatímco ošetření ultrazvukem může podpořit rozvolnění jeho struktury a expozici reaktivních skupin. To vede k produkci chinonu kyseliny gallové aktivací OH• skupiny. Tedy vhodná kombinace ultrazvuku a kyseliny gallové by mohla nabídnout dostatečné provázání bílkovin, prudce zvýšit dobu uskladnění rybího myofibrilárního proteinu, a tak zlepšit stabilitu rybích produktů typu surimi.

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- CZ.02.1.01. /0.0 /0.0/16_025/0007370 Reproductive and genetic procedures for preserving fish biodiversity and aquaculture (2018–2021, responsible leader Prof. Martin Flajšhans)
- LM2018099 Large Research Infrastructures: CENAKVA – South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses (2019–2022, responsible leader: Prof. Otomar Linhart)
- GAJU (080/2019/Z) Using alginate coating coupled with essential oils to modulate microbial communities and improve quality of chill stored Common carp (*Cyprinus carpio*) fillets (2019–2020, responsible leader M.Sc. Ruoyi Hao)

List of publications

Peer-reviewed journals with IF

Hao, R.Y., Shah, B.R., Sterniša, M., Možina, S.S., Mraz, J., 2022. Development of essential oil-emulsion based coating and its preservative effects on common carp. LWT – Food Science and Technology 154, 112582. (IF 2020=4.952)

Hao, R.Y, Roy, K., Pan J.F., Shah, B. R., Mraz, J., 2021. Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses. Trends in Food Science & Technology 111, 175–190. (IF 2020=12.563)

Hao, R.Y., Pan, J.F., Khalili Tilami, S., Shah, B.R., Mráz, J., 2020. Post-mortem quality changes of common carp (*Cyprinus carpio*) during chilled storage from two culture systems. Journal of the Science of Food and Agriculture 101, 91–100. (IF 2020=3.638)

Pan, J.F., Lian, H.L., Jia, H., **Hao, R.Y.**, Wang, Y.J., Ju, H.P., Li, S.J., Dong, X.P., 2020. Dose affected the role of gallic acid on mediating gelling properties of oxidatively stressed Japanese seerfish myofibrillar protein. LWT – Food Science and Technology 118, 108849. (IF 2020=4.952)

Pan, J.F., Lian, H.L., Jia, H., Li, S.J., **Hao, R.Y.**, Wang, Y.J., Zhang, X.N., Dong, X.P., 2020. Ultrasound treatment modified the functional mode of gallic acid on properties of fish myofibrillar protein. Food Chemistry 320, 126637. (IF 2020=7.514)

Pan, J.F., Lian, H.L., Shang, M.J., Jin, W.G., **Hao, R.Y.**, Ning, Y., Zhang, X.N., Tang, Y., 2020. Physicochemical properties of Chinese giant salamander (*Andrias davidianus*) skin gelatin as affected by extraction temperature and in comparison with fish and bovine gelatin. Journal of Food Measurement and Characterization 14, 2656–2666. (IF 2020=2.431)

Hao R.Y., Liu Y., Sun L.M., Xia L.N., Jia H., Li Q., Pan J.F., 2017. Sodium alginate coating with plant extract affected microbial communities, biogenic amine formation and quality properties of abalone (*Haliotis discus hannai Ino*) during chill storage. LWT – Food Science and Technology 81, 1–9. (IF 2017=3.714)

Abstracts and conference proceedings

Hao, R.Y., Shah, B.R., Mráz, J., 2019. Optimization of alginate edible coatings incorporated with essential oil emulsion in physical and antibacterial properties. In: Book of abstracts "32nd international conference on Nanoscience, Nanotechnology and Nanoengineering 2019", 18–19 November 2019, Rome, Italy.

Training and supervision plan during study

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Research department	2018–2021 – Laboratory of Nutrition of FFPW
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Period	28th May 2018 until March 2022

Ph.D. courses	**Year**
Biostatistics	2019
Basic of scientific communication	2019
Pond aquaculture	2019
Applied hydrobiology	2020
Ichthyology and fish taxonomy	2020
English language	2020

Scientific seminars	**Year**
Seminar days of IAPW and FFPW	2019 2020 2021 2022

International conferences	**Year**
Hao, R.Y., Shah, B.R., Mráz, J., 2019. Optimization of alginate edible coatings incorporated with essential oil emulsion in physical and antibacterial properties. In: Book of abstracts "32nd international conference on Nanoscience, Nanotechnology and Nanoengineering 2019", 18–19 November 2019, Rome, Italy	2019

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Prof. Sonja Smole Možina, Biotechnical Faculty, Department of Food Science and Technology, University of Ljubljana, Ljubljana, Slovenia (half month, microbial analysis)	2019

Curriculum vitae

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2018 – present	Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic
2017–2014	M.Sc., in Food Resources and Comprehensive Utilization, School of Food Science and Technology, Dalian Polytechnic University, Dalian, China
2014-2010	B.Sc., in Food Science and Technology, Faculty of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China

PH.D. COURSES

Pond aquaculture, Applied hydrobiology, Basics of scientific communication, Biostatistics, Ichthyology and fish taxonomy, Czech language, English language

TRAINING

23/04–26/04 2019	Training – 'Hands-On Proteomics Workshop, 2D gel electrophoresis in combination with mass spectrometry', Vienna University of Technology, Vienna, Austria

RESEARCH STAY AND COLLABORATIONS

25/08–06/09 2019	Prof. Sonja Smole Možina, Biotechnical Faculty, Department of Food Science and Technology, University of Ljubljana, Ljubljana, Slovenia