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THE EFFECT OF DIPPING TREATMENT ON PRESERVATION OF FISH (MACKEREL) USING CHITOSAN, SORBATE AND ACETIC ACID

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ABSTRACT

This project focused on evaluating the effect of chitosan, sorbate and acetic acid treatment on preservation of fish (mackerel) in ice. Mackerel samples were soaked in chitosan solution (0.4%), sorbate solution (2%), acetic acid (0.1%), and then stored in 0℃ ice for 20 days. As contrast, mackerel without any treatment were stored at the same condition. The pH, Thiobarbituric Acid (TBA), Total Volatile Basic Nitrogen (TVB-N) and Total Viable Count (TVC) were determined in 0, 5, 10, 15, 20 day of storage. The results of this study showed that rate of microbiological growth in mackerel, which were treated with chitosan, sorbate and acetic acid was considerably slower than in mackerel without treatment. Also, the inhibitory effect of chitosan, sorbate and acetic acid against bacteria strengthened with increase of chitosan, sorbate and acetic acid concentration and treatment time. Especially, chitosan and sorbate treatment can be used to prolong quality of fish.

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1 INTRODUCTION

The production of chitosan has not a long history in DPR of Korea, but is high in relation to its exploitation and research work. In recent years, chitosan has been considered good for human health, which has inspired researches on chitosan to understand its specifics. The main reasons for this recent research in chitosan are:

- It is a good medicine for the people`s health.
- It is not harmful in food processing.
- The processing method is simple and inexpensive.
- It is relatively easy to get the raw material.
- Its price is high on the international market.

At present the actual production of the chitosan in DPR of Korea is unknown, but many institutes and experimental factories, including the ministry of public health, are making the products with chitosan, especially medicines, and exporting successfully to other countries such as China.

The food processing with chitosan has begun with processing result at experimental level. The problem is to reduce the cost of the chitosan production. Now researching is being done on new production methods of chitosan from the shells of oyster and scallops. Freshness is a major contribution to the quality of seafood, which is a very perishable product. From the moment the seafood is caught, the deterioration process starts and its quality for use as a food product is affected. Changes occur in composition and structure caused by biochemical, physical, enzymatic and bacterial reactions, negatively affecting the sensory quality of the product (Magnusson and Martinsdóttir 1995). The edible films can improve shelf life and food quality with good and selective barriers to moisture transfer, oxygen uptake, lipid oxidation, losses of volatiles aromas and flavours (Kester and Fennema 1986), better visual aspect, and reduction of the microbiologic contamination (Nisperos-Carriedo 1994).

Most of the chemical compounds found in spoiling seafood are volatile compounds produced by bacteria. These include trimethylamine, volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine and other low molecular weight compounds (Huss 1995). On live and newly caught fish, the microorganisms are found on the skin, gills and in the intestines. The total number of organisms varies enormously depending on the environment and on the fish species. Fish caught in very cold, clean waters carry lower numbers compared to fish caught in warm waters, which have slightly higher counts.

The flesh of a healthy live or newly caught fish is sterile. When a fish dies, the bacteria are allowed to proliferate at the beginning on the skin and during storage, they eventually invade the flesh *(*Huss 1995). Chilling and freezing is an excellent process for preserving the quality of fish. At low temperature, growth of bacteria is retarded, but never completely stopped. Gram *et al.* (1987) studied total viable count (TVC) and H_2S-producing bacteria on whole cod and vacuum packed cod fillets. After 10 days of storage at 0˚C, the total viable count (TVC) was 6×10^6~10^8cfu/g and the number of H_2S-producing bacteria varied from 5×10^6 to 8×10^7cfu/g. Magnusson and Martinsdottir (1995) reported total viable count (TVC) of 10^6~10^7cfu/g for fresh cod fillets stored in ice at 0~1˚C. The preceding reports showed that the number of bacteria existing in fish had increased slowly even during ice storage and quality of fish had fallen resulting of increased volatile compounds and protein decomposition substance.

Thus, it is very important to remove bacteria existing in fish before storage. Chitin and chitosan, a natural antibacterial substance, has been widely used in food processing industry and its antibacterial rate is more than 90 %.

The aim of this study was to research shelf life and freshness of fish by treating it with chitosan, sorbate and acetic acid solution. The quality development of mackerel treated with chitosan, sorbate, and acetic acid was monitored. Using chitosan may be considered a natural way of sterilisation in seafood products and to ensure the safety for the consumer. The goal of the study was to explore the possibility to use natural substances such as chitosan to preserve food to ensure the safety of the consumers. The specific objectives were to:

- Compare the effectives of the chitosan to other chemicals in prohibiting bacterial growth.
- Compare prohibit bacterial growth in mackerel using different substances.

Many new processing methods are used, but there are problems with the cost and safety. But, the processing method with chitosan is very effective and simple in safety of seafood.

2 LITERATURE REVIEW

2.1 Structure and properties of Chitosan, Sorbate and Acetic acid

2.1.1 Structure and properties of Chitosan

Chitosan is a natural polymer, derived from chitin, material that participates in the composition of exoskeleton of shellfish, such as shrimps, lobsters and crabs and insects, such as ants and beetles (Majeti and Kumar 2000). Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acety1 D-glucosamine. It has a number of commercial and possible biomedical uses (Sandford and Hutchings 1987; Sandford 1989; Kean *et al.* 2005) (Figure 1).

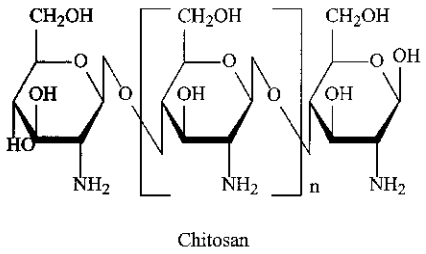


Figure 1: Chemical structure of chitosan.

The processing of crustacean shells mainly involves the removal of proteins and the dissolution of calcium carbonate that is present in crab shells in high concentrations. The resulting chitin is deacetylated in 40% sodium hydroxide at 120˚C for 1-3h. This treatment produces 70% deacetylated chitosan. Chitosan is a weak base and is insoluble in water and organic solvent. However, it is soluble in dilute aqueous acidic solution (pH<6.5), which can convert glucosamine units into soluble from R-NH_3 (Kumar *et al.* 2004). It gets precipitated in alkaline solution or with polyanions and forms gel at lower pH. To produce 1 kg of 70% deacetylated

chitosan from shrimp shells 6.3 kg of HCL and 1.8 kg of NaOH was required in addition to nitrogen, processed water (0.5t), cooling water (0.9t). Important items in estimating the production cost include transportation, which varies depending on labour and location. The worldwide price of chitosan is ca. US $ 7.5/10 g (Kumar 1999).

Most of the naturally occurring polysaccharides e.g. cellulose, dextran, pectin, alginic acid, agar, agarose, carragenans are neutral or acidic in nature, whereas chitin and chitosan are the examples of highly basic polysaccharides. Their unique properties include solubility behaviour, polyoxysalt formation, of ability to form films, chelate metal ions and optical structural characteristics (Austin *et al.* 1981).

Like cellulose, it naturally functions as a structural polysaccharide, but differs from cellulose in the properties (Muzzarelli 1977). Chitin is highly hydrophobic and is insoluble in water and most organic solvents but chitosan, the deacetylated product of chitin is soluble in very dilute acids like acetic acid and formic acid.

2.1.2 Structure and properties of sorbate

Sorbate appears as white, free flowing, extruded pellets or spherical beads with a mild and characteristic odor. It is very soluble in water, and slightly soluble in alcohol. Sorbate is generally regarded as safe and effective for what purposes and has approximately the same toxicity as table salt (Deuel *et al.*1954).

Potassium sorbate has the molecular formula $C_6H_{702}K$ although the formula $(CH_3)(CH)_4COOK$ shows its structure more clearly (Figure 2). The first carbon atom on one end has three hydrogen atoms. The next four carbon atoms have one hydrogen atom, a single bond with one of the adjoining carbon atoms and a double bond with the other adjoining carbon atom. The carbon atom on the other end of the potassium sorbate molecule has a double bond with an oxygen atom and a single bond with the remaining oxygen atom. This oxygen atom also shares an ionic bond with the potassium (Windholz *et al*. 1976).

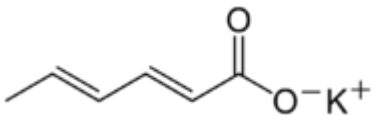


Figure 2: Chemical structure of sorbate**.**

Potassium sorbate is a salt of sorbic acid and is prepared by reacting sorbic acid with potassium hydroxide. It is a white or yellowish crystalline powder or granule, potassium sorbate is soluble in water. Once dissolved in water, it produces sorbic acid. It is effective as a preservative up to a pH of 6.5. Its effectiveness is reduced as pH is lowered. It is inexpensive to produce, safe and easy to use as an ingredient in foods and other products requiring antimicrobial activity. Preservative efficacy is increased with increasing temperature, and increasing concentration of potassium sorbate (Lusher *et al.* 1984). The efficacy of potassium sorbate is also increased when used in combination with other antimicrobial preservatives or glycols since synergistic effects occur. Potassium sorbate has a molar mass of 150.22 grams per mole and a density of 1.363 g per cubic centimetre. It decomposes at 270 degrees Celsius. It has a solubility of 58.2 % in water at 20˚C. Potassium sorbate is also soluble in ethanol and propylene glycol, and slightly soluble in acetone (Branen *et al.*, 1983).

2.1.2 Structure and properties of Acetic acid

Acetic acid is one of the most common organic acids and has been known for quite a long time in the form of vinegar. It is also present free in a number of fruit juices. In the combined state it occurs in many oils and essential oils. Acetic acid, also known as ethanoic acid, is an organic chemical compound, giving vinegar its sour taste and pungent smell. Pure, water-free acetic acid (glacial acetic acid) is a colourless liquid that absorbs water from the environment (hygroscopy), and freezes below 16.7 A ℃ (62 *A*°F) to a colourless crystalline solid. A molecule of acetic acid contains two carbon, four hydrogen and two oxygen atoms, which is often written as CH_3COOH to reflect its actual molecular structure (Figure 3). Acetic acid has the empirical formula CH_2O (Akeroyd 1993).

Figure 3: Chemical structure of acetic acid.

The hydrogen (H) atom in the carboxyl group (a COOH) in carboxylic acetic acids can be given off as an H+ ion (proton), giving them their acidic character. Acetic acid is a weak, effectively monoprotic acid in aqueous solution, with a PKa value of 4.8. The crystal structure of acetic acid shows that the molecules pair up into dimmers connected by hydrogen bonds (Figure 4) (Jones and Templeton 1958).

Figure 4: Chemical properties of acetic acid.

Acetic acid is corrosive to many metals including iron, magnesium, and zinc, forming hydrogen gas and metal salts called acetates. Metal acetates can also be prepared from acetic acid and an appropriate base, as in the popular "baking soda + vinegar" reaction.

$$Mg(s) + 2CH_3C00H\ (aq) \rightarrow (CH_3COO)_2\ Mg(aq) + H_2(g).$$

$$NaHCO_3(s) + CH_3COOH\ (aq) \rightarrow CH_3COONa(aq) + CO_2(g) + H_2O(l).$$

Acetic acid undergoes the typical chemical reactions of a carboxylic acid, notably the formation of ethanol by reduction, and formation of derivatives such as acetyl chloride via nucleophilic acyl substitution. Acetates when heated with arsenic trioxide from cacodyls oxide, which can be detected by its malodorous vapours. The acetyl group, derived from acetic acid, is fundamental to the biochemistry of virtually all froms of life. Acetic acid is produced and excreted by acetic acid bacteria, notably the acetobacter genus and clostridium acetobutylicum (Buckingham 1996). Acetic acid is produced both synthetically and by bacterial fermentation. Total worldwide production of virgin acetic acid is estimated at 5 Mt/a (million tonnes per year) (Yoneda *et al.* 2001).

2.2 Utilization of Chitosan, Sorbate and Acetic acid

2.2.1 Utilization of Chitosan

Chitosan a waste product of the shellfish industry has been shown to be non-toxic (Arai *et al.* 1986) and safe (Ando *et al.*1968). Chitosan is easily derived from chitin by N-deacetylation and appears to be more useful than chitin because it has both hydroxyl and amino groups that can be modified easily. This polymer has been the object of studies for several decades, and recent review articles outline much of the broad ranging research on this polymer to date (Tang *et al.* 2002). Chitin and its deacetylated product, chitosan are high-molecular-weight biopolymers and are recognized as versatile, environmentally friendly raw materials. There are many applications for these chitinous materials including use in agriculture, food processing, fruit, medicine, cosmetics, and biotechnology (Ghaouth *et al*. 1992, Zhang and Quantick 1998, Bautista-Banos *et al*. 2004, Du *et al*. 1997, Capdeville *et al*. 2002, Liu *et al*. 2007, Meng *et al.* 2008, Sun *et al*. 2008). Because chitosan has various practical properties such as microbial resistance, nontoxicity, biodegradability and metal ion adsorption, many investigators have concentrated on applying chitosan to a wide variety of textiles (Howgate 1998).

Chitosan were developed for controlled drug release, removal of heavy metal ion from waste water, such as Hg (II), UO_2 (II), Cd(II), Zn(II), Cu(II) and Ni(II) ions, and were also applied to immobilized biological agents, such as yeast cell, E. coli, protease, lipoprotein lipase and bovine serum albumin. Recently macroporous chitosan scaffolds were explored as a material used for tissue engineering. Due to its wide application in chemical, biochemical and biomedical fields of researches, chitosan has become an important biomaterial.

Chitosan is inexpensive, biodegradable, and nontoxic for mammals. This makes it suitable for use as additive in the food industry (Koide 1998, Shahidi *et al.* 1999), as a hydrating agent in cosmetics, and more recently as a pharmaceutical agent in biomedicine (Dodane and Vilivalam 1998; Illum 2003; Khor and Lim 2003). It has been patented as a lipid binding food additive (Furda 1984) and demonstrated its emulsion properties and dye binding capacity (Knorr 1982, Knorr 1983). The antimicrobial activity of chitosan against different groups of microorganisms has received considerable attention in recent years (Rabea *et al.* 2003).

Chitosan, however, shows its antibacterial activity only in an acidic medium, which is usually ascribed to the poor solubility of chitosan at high pH (Wang 1992). These reported antimicrobial activities might be the effect of dissolved chitosan in acidic media such as acetic acid (Devlieghere *et al*. 2004) and hydrochloric acid (Chung *et al*. 2003). Chitosan is a biocompatible polymer reported to exhibit a great variety of useful biological properties such as anticholesteremic and ionsequestering actions. Antibacterial and antifungal activities of chitosan have been shown to inhibit growth of a wide variety of bacteria and fungi have high killing rate and low toxicity toward mammalian cells (Tsai *et al.* 1999).

In the preservation of fruits, it has been used as a coating and antifungal agent, resulting in increased quality and storability of fresh strawberries (Ghaouth *et al.* 1992). The utilization of chitosan for the development of the preservative qualities of meat has been presented by Darmadji *et al.* (1992). They examined the inhibitory effect of chitosan against some spoilage bacteria in beef. The result indicated that chitosan inhibited Micrococci, Staphylococci, Pseudomonades and Coliforms and this effect increased with increase of chitosan content and incubation time. The antimicrobial activity of chitosan will depend on several factors such as the kind of chitosan (deacetylation degree, molecular weight) used, the pH of the medium, the

temperature and the presence of several food components (Papineau *et al*. 1991, Sudarshan *et al*. 1992). In China, shrimps were treated with 0.0075~0.01% chitosan solution and stored for 20 days, showing antibacterial reaction against several microorganisms and in 0.1% concentration almost bacteria were inhibited (Wang 2002).

2.2.2 Utilization of Sorbate

Potassium sorbate is an antimicrobial preservative, with antibacterial and antifungal properties used in pharmaceuticals, foods, enteral preparations and cosmetics (Smolinske 1992). Potassium sorbate use in food increased rapidly following its discovery. However, there are few references that potassium sorbate has been used as a seed treatment or for any other crop uses in either organic or conventional agriculture (Dorko 1997). Potassium sorbate is effective against yeasts, molds, and select bacteria, and is widely used at 0.025 to 0.10% levels in cheeses, dips, yogurt, sour cream, bread, cakes, pies and fillings, baking mixes, dough, icings, fudges, toppings, beverages, margarine, salads, fermented and acidified vegetables, olives, fruit products, dressings, smoked and salted fish, confections and mayonnaise (Anonymous 1961; Moline *et al.* 1963). Sorbic acid is widely used in the food industry as a preservative because it is harmless to animals while being an effective inhibitor of fungal growth in acidic environments (Anonymous 1961; Deuel *et al.* 1954). Sorbic acid and other unsaturated aliphatic mono-carboxylic acids and their salts were discovered to be effective at inhibiting the growth of microorganisms between the late-1930s and mid-1940 (Deuel *et al*. 1954). More serious is the limitation imposed by pH on the activity of sorbic acid (Bell *et al.* 1959; Nomoto *et al.* 1995, Juven 1976). When used at the pH levels of most mildly acidic food products (pH 5.5-6.0), Sorbates are the most effective preservatives against a wider spectrum of food spoilage microorganisms than benzoates or propionates. Sorbate efficacy increases with greater acidity. Above pH 4.0, Sorbates are more effective than sodium benzoate and sodium or calcium propionate. At pH 2.5 to 3.0 sorbate are still somewhat more effective than sodium benzoate as yeast and mold inhibitor and more than twice as potent as propionates. Sorbates are at their optimum effectiveness used below pH 6.0. Its function is ineffective at pH 7.0 and above.

2.2.3 Utilization of Acetic acid

Preserving seafood with vinegar (acetic acid) is one of the easiest food-preservation techniques know (Khanna *et al*. 2001). Acetic acid is one of the world`s most important intermediate chemicals, and is used in the manufacture of vinyl acetate monomer, purified terephthalic acid, acetic anhydride, monochloroactic acid, and acetate esters. Polyvinyl acetate and copolymers of vinyl acetate monomer are used in the manufacture of paints, adhesives, paper coatings, textile treatments and plastics. Solutions of lactic and acetic acid are commonly used by the slaughter industry as antimicrobial interventions to reduce the microbial load on freshly slaughtered carcasses (Berry and Cutter 2000). Acetic acid is used to produce purified terephthalic acid, which is a key intermediate for a range of applications, including polyester fibres, bottles for water and soft drinks, photographic film and magnetic tapes. Another important use for acetic acid is in the production of acetic anhydride. Acetic anhydride has a wide range of applications, the predominant one being the production of cellulose acetate. Cellulose acetate is used to produce textile fibres and cigarette filter tow. Other applications for acetic anhydride are plastics, agricultural chemicals and pharmaceuticals. Carboxymethly cellulose is used in a variety of applications including foods, pharmaceuticals, cosmetics and textiles. Monochloroacetic acid is also used to produce herbicides for agriculture. Acetic acid is used to produce a broad range of acetate esters; the most important of which are ethyl acetate,

n-butyl acetate and isopropyl acetate. These solvents find applications in coatings, inks, adhesives and cosmetics. It is used as a solvent for gums, resins, volatile oils, and other organic compounds (Yoneda *et al.* 2001).

2.3 Atlantic Mackerel

2.3.1 Mackerel catches

The mackerel has been a consistently popular fish throughout European history. Atlantic mackerel is found in the North Atlantic Ocean. In the eastern Atlantic, they range from the southern Baltic Sea and lceland to northern Africa including Mediterranean and Black Seas. The mackerel fisheries took off in the mid 60s and in the mid 70s the catch reached 1.1million tons. There are important fisheries of *Scomber scombrus* in Northwest and Northeast Atlantic, but the world catch was declined to about 580000 t (Figure 5) in 2007. In 1981 (FAO 1983) Atlantic mackerel was mainly caught with purse seines, sometimes together with sardines. Surface catches are best when the summer thermo cline is not deeper than 15 to 20 meters so as to prevent the mackerel from escaping into deeper water. Other types of gear used include trolling lines, gillnets, traps, beach seines, and midwinter trawls. The countries with the largest catches were UK and Norway. This species is traded fresh, frozen, smoked and canned (Collette and Nauen 1983).

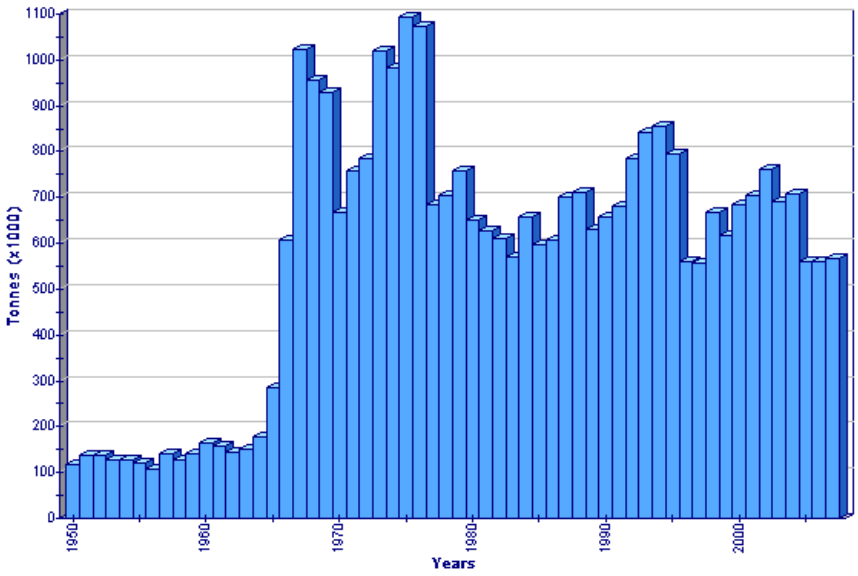


Figure 5: Global Capture production for *Scomber scombrus* (FAO Fishery Statistics).

2.3.2 Varieties of mackerel

Atlantic mackerel (also called Boston mackerel) is often used in sashimi. Spanish mackerel has only a small percentage of red meat and a milder taste than other types of mackerel. King mackerel (also called kingfish or cavalla) has a firm texture and distinct taste. Cero mackerel (also called Cerro or painted mackerel), caught in waters along the coast of Florida, has leaner flesh and more delicate flavour than most varieties. Pacific mackerel (also called American, blue, or chub) is an oily fish with an assertive flavour. Pacific jack mackerel (also called horse mackerel) is often canned (Collette *et al.*, 1983).

2.3.3 Characteristics and the use of mackerel

The Atlantic mackerel (*Scomber scombrus*) is a schooling fish found on both sides of the North Atlantic Ocean. The Atlantic mackerel is by far the most common of the ten species of the family that are caught in the North Atlantic. It travels in big shoals migrating towards the coast to feed on small fish and prawns during the summer (Jenkins *et al.* 1985). Mackerel is abundant in cold and temperate shelf areas. They overwinter in deeper waters but move closer to shore in the spring when water temperatures range between 11℃ and 14℃. Male and female Atlantic mackerel grow at about the same rate, reaching a maximum age of about 20 years and a maximum fork length of about 47 cm. Most Atlantic mackerel are sexually mature by the age of three years. The fish spawns in May-June the eggs are released into the sea in great numbers, up to 90,000 per spawning. The eggs are between 1 and 1.4 mm in size and are planktonic. Hatching occurs after 2-6 days. The juvenile fish stay offshore for about 2 years until they are sexually mature. At this time they join the great shoals of mackerel that form during spawning time. Mackerel is an excellent source of omega-3 fatty acids, selenium, and vitamin B_{12}. The polyunsaturated fatty acids are believed to be beneficial to general health (reduction of high blood pressure) and possible prevention of many diseases such as coronary heart disease, rheumatoid arthritis and possibly some cancers (CSIRO 2003). Table 1-3 shows nutritional values and information about on calories, minerals and vitamins for mackerel (USDA 2006).

Table 1: Nutrition and Calories Mackerel.

Water content (grams per 100 g) 53.27
Calorie content of food (Kcals per 100 g/3.5 oz) 262
Protein content (grams per 100 g) 23.85
Fat content (lipids) (grams per 100 g) 17.81
Ash content (grams per 100 g) 1.53

Table 2: Minerals Nutrition in Atlantic Mackerel.

Calcium content (mg per 100 g) 15	Sodium content (mg per 100 g) 83
Lron content (mg per 100 g) 1.57	Zine content (mg per 100 g) 0.94
Magnesium content (mg per 100 g) 97	Copper content (mg per 100 g) 0.094
Phosphorus content (mg per 100 g) 278	Manganese content (mg per 100 g) 0.02
Potassium content (mg per 100 g) 401	Selenium content (µ per 100 g) 51.6

Table 3: Vitamins Nutrition in Mackerel.

Vitamin C (Ascorbic acid) content (mg per 100 g) 0.4	Food Folate content (µg per 100 g) 2
Thiamin content (vitamin B-1) (mg per 100 g) 0.159	Folate content (DFE µg per 100 g) 2
Riboflavin content (vitamin B-12) (mg per 100 g) 0.412	Vitamin B-12 content (µg per 100 g) 19
Niacin content (vitamin B-3) (mg per 100 g) 6.85	Vitamin A content (µg per 100 g) 180
Pantothenic Acid content (vitamin B-3) (mg per 100 g) 6.85	Vitamin A content (lnt. Units,lU,per 100 g) 54
Vitamin B-3 content (mg per 100 g) 0.46	Vitamin E (alpha-tocopherol) content (µg per 100 g) N/A
Folate content (µg per 100 g) 2	Rentionl content (µg per 100 g) 54

3 MATERIALS AND METHODS

3.1 Material

3.1.1 Raw material

Atlantic mackerel was caught in North-west of Iceland in August 2009 and wept frozen until start of the project. The freshness of mackerel was good. The samples of Mackerel, chitosan, sorbate and aectic acid were kindly provided by MATIS (Iceland). Chitosan is insoluble in water but soluble in a very dilute acid, thus in this experiment chitosan was dissolved in 0.1% acetic acid solution.

3.1.2 Chitosan, Sorbate and Acetic acid preparation

Chitosan solution was prepared by dissolving 20 g of chitosan in 4973.8 ml of distilled water with 6.25 g of acetic acid, then heating with constant agitation for 24h. At the same above condition, sorbate solution was planned by dissolving 100 g of sorbate in 4900 ml of distilled water, and then heating with constant agitation for 24 h. Acetic acid solution was also made by dissolving 6.25 g of acetic acid in 4993.8 ml of distilled water.

3.1.3 Sampling

A total of 72 mackerels were used for experiment the average weight per one was 390 g. All the mackerel were marked with different colours plastic tags and divided into four groups. The mackerels that were kept in at 0℃, were put in B group (mixture of 0.4% chitosan solution, 0.1% acetic acid solution and water) for one minute, then taken out of solution and kept in at 0℃ again. The same methods are following to C group (mixture of 2% sorbate solution and water) and D group (mixture of 0.1% acetic acid and water).

3.2 Experimental design

The experiment was set up to test different treatments on the growth of bacteria in mackerel. The treatments were 4 groups (A, B, C and D) (Figure 6). A group was prepared with the raw material, B group was mixture of the chitosan and acetic acid, C group was sorbate and D group was treated with acetic acid.

3.2.1 Trial

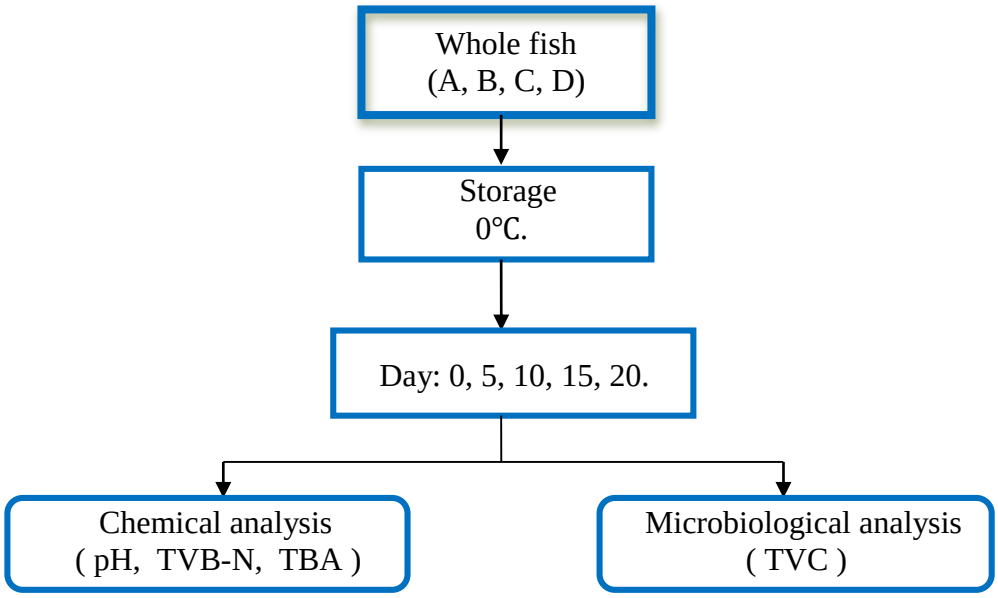
A group: Un-gutted whole mackerels, without any treatment put in fresh water for one minute then stored in 0℃ ice.

B group: Un-gutted whole mackerels, is soaked in mixture of 0.4% chitosan solution, 0.1% acetic acid solution and water for one minute, put them out and then stored in 0℃ ice.

C group: Un-gutted whole mackerels, is soaked in mixture of 2% sorbate solution and water for one minute, put them out and then stored in 0℃ ice.

D group: Un-gut whole mackerels, is soaked in mixture of 0.1% acetic acid and water for one minute, and then stored in 0℃ ice.

On 0^{th}, 5^{th}, 10^{th}, 15^{th}, 20^{th} day after storage, then pH, Total Viable Count (TVC), Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric acid (TBA) of select samples from each group are measured and determined.



NO	Group	Chitosan(0.4%)+Acetic acid (0.1%)	Sorbate (2%)	Acetic acid (0.1%)
1.	A	–	–	–
2.	B	+	–	+
3.	C	–	+	–
4.	D	–	–	+

Figure 6: Flow chart for trials.

3.3 Method

3.3.1 Protein content (Kjeldahl method)

2 g of the minced fish fillet was weighed and transferred into Kjeldahl method digesting flask with a catalyst (2 tablets) and 17.5 ml H_2SO_4 and heated for 3 hours at 420 ℃. Then, the solution was cooled and measured in auto distillation unit (ISO1979).

3.3.2 Fat content (soxtec method)

After drying 5g of minced fish was weighed and transferred into a paper filter (extraction thimble) and put in a tin calumet match in the soxtec system. Fat was extracted with petroleum at 60℃ for 82 minutes (AOSC 1997).

3.3.3 pH measurements

The pH was measured using a calomel electrode (SE 104) pH meter (Knick-Portamess 913(X) pH meter, Germany, Berlin). Glass calomel electrode was dipped into minced fish flesh at room temperature.

3.3.4 Total volatile basic nitrogen (TVB-N)

The method of Malle and Tao (1987) was used for total volatile bases (TVB-N) measurement. The TVB-N was determined by dissolving 100g of mackerel fish sample extract in 200 ml 7.5% aqueous trichloroacetic acid, filtering the mixture, and then mixing 25 ml of the extract in a distillation flask with 6 ml 10% NaOH. Into erlenmeyer flask put 10 ml of 4% boric acid (0.04ml of methyl red and bromocresol green indicator) and place under the condenser for the titration of ammonia. Distillate was titrated with 0.025N sulphuric acid solution. Complete neutralization is obtained when the color turned grey/pink on the addition of a further drop of sulphuric acid.

3.3.5 Total viable count (TVC)

20 g sample + 180 g dilutions buffer mixed in a stomacher 1 ml of 1/10 dilutions were transferred with pipettes to petri plates 10, 1 and 0.1 ml of 1/10 dilutions were transferred with pipettes to tubes with 10 ml of LST broth. Iron agar melted at 45 ℃ was poured on the plates and the content was mixed. After solidification the plates were covered with a thin layer of iron ager. Then the plates are incubated at 22 ℃ for 48 hours. The LST tubes were incubated at 37 ℃ for 48 hours. The values were the mean of the count of plants multiplied with the corresponding dilution factor (Gram *et al.* 1987).

3.3.6 Thiobarbituric acid (TBA)

Thiobarbituric reactive substances (TBARS) were determined by the extraction procedure described by Vyncke (1975) with a few modifications. The sample size was reduced to 15 g and homogenized with 30 ml of 7.5 % trichloroacetic acid solution containing 0.1 % of both propyl gallate and EDTA. The absorbance of samples and standards μg were measured at 530 nm. TBARS, expressed as µmol malondialdehyde per kilogram of sample (µmol MDA/kg), was calculated using malondialdehy-bis (diethyl acetate) as standard (Sorensen and Jorgensen 1996).

4 RESULTS

4.1 Assessment of raw material quality

The chemical and physical properties of the whole bled and gutted fish were analysed to assess the quality of the raw material. Samples for analysis were taken from muscle structure only. The water content of the raw material was 63.0 % at the beginning of the study, with the 6.11 pH, indicating a good quality. Total volatile basic nitrogen (TVB-N), Thiobarbituric acid (TBA) and Total viable count (TVC) concentration in the raw material was low (27.6 mg TVB-N/100 g sample, 50.3 µmol TBA/kg sample and log cfu 2.96 TVC/g), which indicates that fish was fresh (Table 4).

Table 4: Physical and chemical properties of the raw material.

Sample	pH	TVC (log cfu/g)	TVB-N (mg/100g)	TBA (µmol/kg)	Water (%)
Raw material (storage day 0)	6.11	2.96	27.6	50.3	63.0

4.2 The Inhibitory Effect in mixture of Chitosan (0.4%) with acetic acid (0.1%), sorbate (2%) and acetic acid (0.1%) against bacteria in mackerel fillet.

The value at day 0 in raw materials was 2.96, 5th day 3.67, 10th day 4.81, 15th day 6.14, 20th day 6.79 log CFU/g respectively. And value of 0 day in mixture of chitosan and acetic acid was 2.85, 5th day 3.16, 10th day 4.03, 15th day 5.16, 20th day 6.23 log CFU/g respectively. The value of 0 day in sorbate was 2.04, 5th day 3.58, 10th day 3.49, 15th day 5.22, 20th day 5.58 log CFU/g respectively. The value of 0 day in acetic acid was 2.75, 5th day 3.35, 10th day 4.24, 15th day 5.48, 20th day 6.62 log CFU/g respectively. The results showed that the effect of sorbate was best on 0 day, 10th day and 20th day among other groups. However, the effect of a mixture of chitosan with acetic acid was only best on 5th, 15th day among other groups. No treatment was least effective in inhibiting bacterial growth (Figure 7).

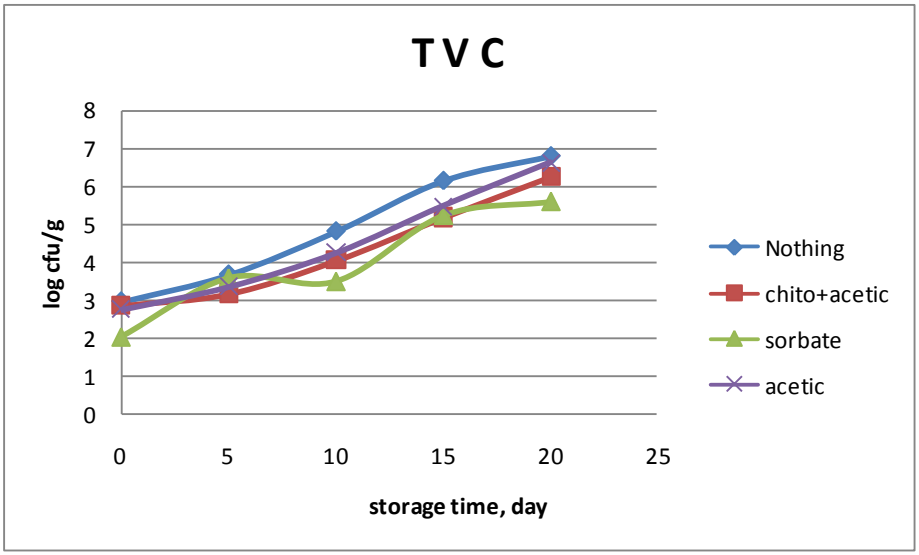


Figure 7: TVC of microbiological colonies in mackerel in each group during storage.

4.3 TVB-N changes in mackerel fillet mixture of chitosan (0.4%) with acetic acid (0.1%), sorbate (2%) and acetic acid (0.1%) treatment.

TVB-N in raw material was 27.6 mg/100 g before storage in ice (Table 4) and also as shown in Figure 8. TVB-N in contrast group steadily increased reaching 46.6 mg/100 g on 20th day of storage. The initial TVB-N in chitosan and acetic acid mixture was 24.8 mg/100g on 0 day and steadily increased to 41.3 mg/100 g on 20th day. TVB-N in sorbate was 22 mg/100 g on 0 day and steadily increased to 37.6 mg/100 g on 20th day. The initial TVB-N in acetic acid was 22.6 mg/100 g on 0 day and steadily increased to 37.2 mg/100 g on 20th day. The value of raw materials and flesh of mackerel dealt with mixture of chitosan and acetic acid decreased from

on 0 day to 5th day, after then increasing more and more. The value of sorbate and acetic acid increased from 0 to 20th day steadily.

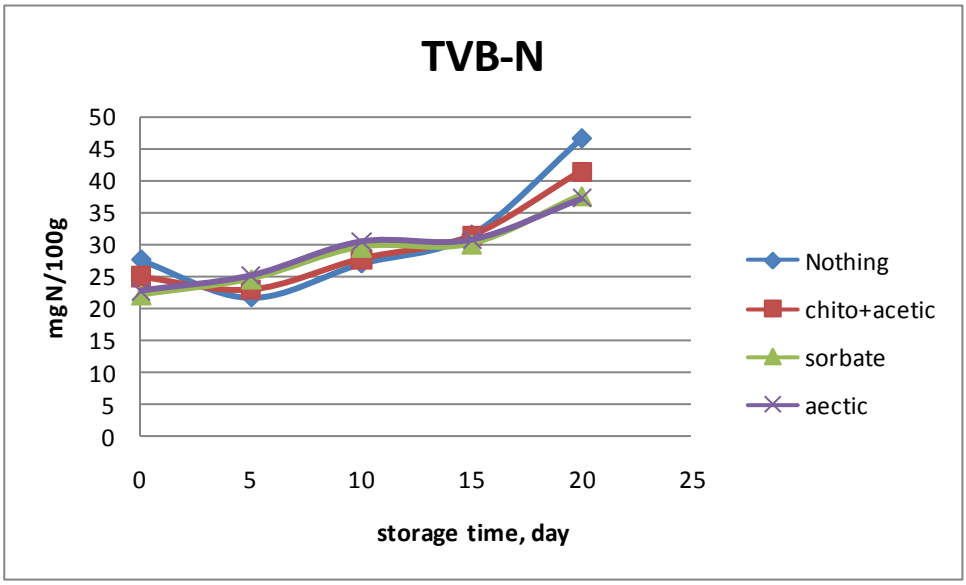


Figure 8: Changes in TVB-N in mackerel in each group during storage.

4.4 TBA Changes in mackerel fillet mixture of chitosan (0.4%) with acetic acid (0.1%), sorbate (2%) and acetic acid (0.1%) treatment.

The TBA value is a widely used indicator for the assessment of the degree of lipid oxidation. In the present study, the TBA value of fresh mackerel was 50.3µmol/kg on 0 day and 145µmol/kg on 20th day. The TBA value in mixture of chitosan and acetic acid was 33.3µmol/kg on 0 day and 130.8µmol/kg on 20th day and in sorbate was 36.4µmol/kg on 0 day and 122µmol/kg on 20th day, in acetic acid was 42.2µmol/kg on 0 day and 145.7µmol/kg on 20th day. The TBA value in mixture of chitosan and acetic acid rose increasingly from 0 day-10th day, after then decreased. The same applied to sorbate and acetic acid but the decline started on 15th day. But the value of sorbate and acetic acid increased from 0-15th day, and then it went down. TBA point out that treatment with chitosan and acetic acid gives lowest value of TBA but treatment with sorbate gives higher results. This may be something worth write to investigate further if chitosan is better to prevent oxidation than sorbate. We found the microbiological effect of sorbate and chitosan to be similar.

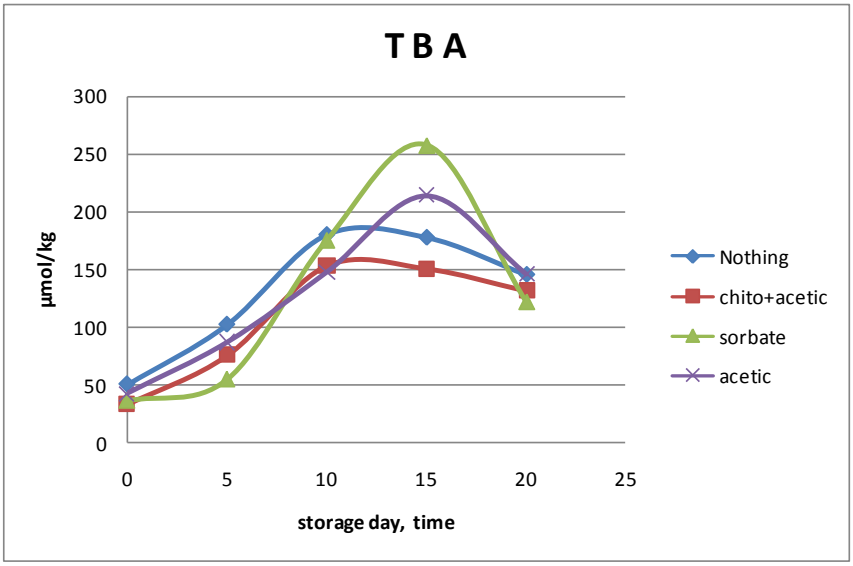


Figure 9: Changes in TBA in mackerel in each group during storage.

4.5 pH Changes in mackerel fillet mixture of chitosan (0.4%) with acetic acid (0.1%), sorbate (2%) and acetic acid (0.1%) treatment.

pH varied considerably from the initial value at day 0 to the 20 day storage period. pH of raw materials was 6.11 on 0 day and 6.06 on 20th day, in mixture of chitosan and acetic acid was 6.16 on 0day and 5.98 on 20th day , in sorbate was 6.06 on 0 day and 6.1 on 20th day and in acetic acid was 6.09 on 0 day and 6.23 on 20th day.

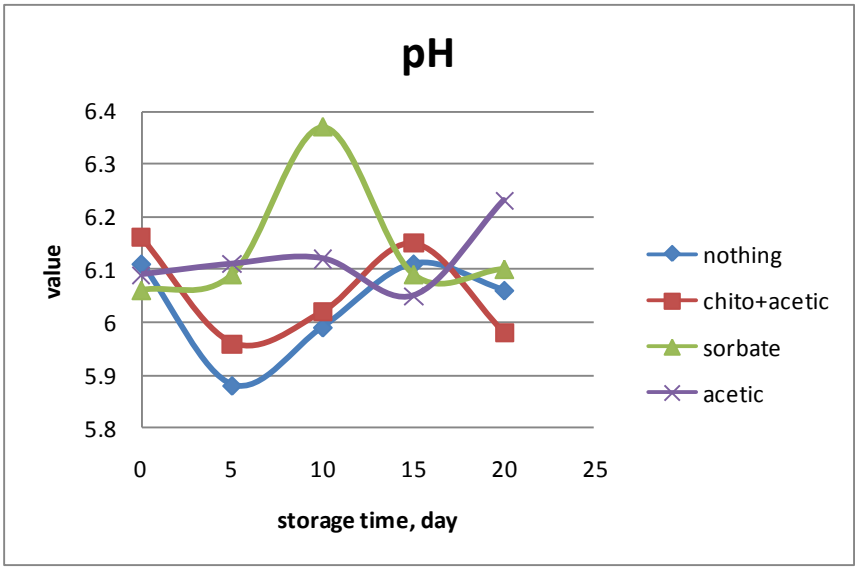


Figure 10: Changes in pH in mackerel in each group during storage.

5 DISCUSSION

The freshness of fresh or frozen fillets is normally determined by measuring bacteria content, e.g. with the Total viable count (TVC) and chemical components such as Total volatile base nitrogen (TVB-N) and Thiobarbituric acid (TBA) from the flesh. Guidelines, issued by MATIS (Icelandic Fisheries Laboratories) for fresh fish, determine good quality fish using total viable count TVC because chitosan, sorbate and acetic acid control the growth of the bacteria successfully over the raw materials. As the bacteria count goes above these safe limits, the quality decreases. Microorganisms isolated from seafood showed various degrees of sensitivity toward chitosan, sorbate and acetic acid. According to another document 0.5 % of chitosan and 1 % of sorbate can inhibit the activity of the bacteria for 14-day at 2 ˚C (Bautista-Banos *et al.* 2003). In this paper, Total viable count (TVC) of mackerel treated with chitosan, sorbate and acetic acid did not go above these safe limits at 20th day of storage in ice. But in contrast group without any treatment Total viable count (TVC) went above safe limit and the quality of mackerel decreased.

For mackerel stored at 0°C, Total volatile base nitrogen (TVB-N) measurements for all groups showed an increase towards the end of storage time. Freshness of mackerel in contrast group began to fall down after 5th day of storage and after then the value increased. Mackerel treated with sorbate and chitosan has comparably good quality. However, acetic acid didn't show a lot of effect on mackerel in controlling bacteria. The characteristics of chitosan that inhibits almost all bacteria may cause low Total volatile base nitrogen (TVB-N) levels of cod in 2st group. The mode of inhibition of chitosan on the growth of some bacteria might be due to the poly cationic nature of chitosan interferes with negatively charged of macro-molecules at the cell surface and interaction of chitosan with membranes or cell wall components resulting in increasing permeability of the membranes and leakage of cell materials from tissue (Young 1982).

The pH is an important intrinsic factor related to post-mortem changes of fish flesh. Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue and only small amounts of lactic acid are produced post-mortem (Gram and Huss 1987). pH of mackerel was within standard limit level in every treatment during storage. The value of thiobarbituric acid (TBA) was 0.1158µmol/kg, increased in mackerel dealt with mixture of chitosan and acetic acid and 0.1365µmol/kg in raw materials from 0-10th day and after then decreased. And the value of sorbate and acetic acid was 0.1959µmol/kg and 0.1625µmol/kg, increased from 0-15th day respectively and then decreased.

6 CONCLUSION

The results of the experiment can be summarised as follows:

- Chitosan, sorbate and acetic acid treatment may be used as a good method for preservation of fish quality. The level of inhibition of fungal growth is highly correlated with chitosan concentration. Recent studies have shown that chitosan treatment is effective in halting pathogen growth (Ben-Shalom *et al*. 2003; Liu *et al*. 2007; Meng *et al*. 2008; Xu *et al*.2006).
- Inhibitory effect against microorganism:
  During 0 ˚C ice storage for 20 days total viable count (TVC) in mackerel was as follows:
    - In raw material 20th day was 6.79 log cfu/g.
    - In mixture of chitosan and acetic acid was 6.23 log cfu/g.
    - In sorbate was 5.58 log cfu/g.
    - In acetic acid was 6.62 log cfu/g.

  As the results show, effect of sorbate was best on 0 day, 10th day and 20th day among other groups. However, the effect mixture of chitosan with acetic acid was only best on 5th, 15th day among other groups. That is, inhibitory effect of chitosan and sorbate against bacteria was higher than acetic acid, which was strong antiseptic substance.

- The optimum condition of mackerel freshness preservation by chitosan, sorbate and acetic acid treatment was as follows:

    - At pH 6.0 the antimicrobial activity of chitosan was significantly lower than at pH 4.0 (Devlieghere *et al.* 2004).
    - Chitosan shows its antibacterial activity only in an acidic medium, which is usually ascribed to the poor solubility of chitosan at high pH (Liu *et al.* 2004).
    - Also antimicrobial activities might be the effect of dissolved chitosan media such as acetic acid (Devlieghere *et al.* 2004).
    - More recent experiments and inventions claim that potassium sorbate is effective as a mold inhibitor sorbate with other synthetic fungicides used in tissue culture (Guri and Patel 1998).
    - The study demonstrated that the ability of inhibiting bacterial growth was more effective in sorbate of 2% and mixture of chitosan of 0.4% and acetic acid 0.1% than acetic acid 0.1%.
    - Also we find the microbiological effect of sorbate and chitosan similar.
    - In acetic acid of 0.1% inhibit effect didn’t show vivid differences.

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APPENDIX

Table 1: Physical and chemical properties of the raw material.

Sample	pH	TVC (log cfu/g)	TVB-N (mg/100g)	TBA (µmol/kg)	Water (%)
Raw material (storage day 0)	6.11	2.96	27.6	50.3	63.0

Table 2: Changes of pH in mackerel according to storage days in each group.

Group \ Day	0	5	10	15	20
Raw material	6.11	5.88	5.99	6.11	6.06
Chitosan(0.4%)+acetic acid(0.1%)	6.16	5.96	6.02	6.15	5.98
Sorbate (2%)	6.06	6.09	6.37	6.09	6.10
Acetic acid (0.1%)	6.09	6.11	6.12	6.05	6.23

Table 3: Changes of TVC in mackerel according to storage days in each group.

Group \ Day	0 (log cfu/g)	5 (log cfu/g)	10 (log cfu/g)	15 (log cfu/g)	20 (log cfu/g)
Raw material	2.96	3.67	4.81	6.14	6.79
Chitosan(0.4%) + acetic acid(0.1%)	2.85	3.16	4.03	5.16	6.23
Sorbate (2%)	2.04	3.58	3.49	5.22	5.58
Aceti acid(0.1%)	2.75	3.35	4.24	5.48	6.62

Table 4: Changes of TVB-N in mackerel according to storage days in each group.

Group \ Day	0 (mgN/100g)	5 (mgN/100g)	10 (mgN/100g)	15 (mgN/100g)	20 (mgN/100g)
Raw material	27.6	21.7	27.0	31.5	46.6
Chitosan(0.4%) + acetic acid(0.1%)	24.8	22.8	27.6	31.2	41.3
Sorbate (2%)	22.0	24.5	29.5	30.0	37.6
Aceti acid(0.1%)	22.6	25.0	30.4	30.7	37.2

Table 5: Changes of TBA in mackerel according to storage days in each group.

Day / Group	0 (μmol/kg)	5 (μmol/kg)	10 (μmol/kg)	15 (μmol/kg)	20 (μmol/kg)
Raw material	50.3	101.9	179.5	177.1	145.0
Chitosan(0.4%) + acetic acid(0.1%)	33.3	75.3	152.3	149.8	130.8
Sorbate (2%)	36.4	54.9	175.4	257.7	122.0
Aceti acid(0.1%)	42.2	86.5	147.5	213.7	145.7