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2 Effects of water chestnut (*Eleocharis dulcis*) extract on the shelf-life of refrigerated catfish (*Pangasius* sp.) fillet

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Abstract. 2 This study was aimed to determine the effect of water chestnut (*Eleocharis dulcis*) extract on catfish (*Pangasius* sp.) fillet stored at cold temperatures. Several stages were carried 2 including sampling, sample preparation, sample extraction, and fish filleting. Extraction of multilevel maceration with a sequence of solvents n-hexane, ethyl 17 late, and 70% ethanol was carried out. The parameters observed were protein content, TVBN, total plate count (TPC), and whiteness. The results showed that protein content decreased while TVBN and TPC levels increased during storage. The addition of water chestnut extract reduced the levels of TVBN, TPC of the fillet, and whiteness.

Keywords: catfish, fish fillet, shelf life, water chestnut

1. Introduction

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Water chestnut (*Eleocharis dulcis*) is a weed that grows and develops on muddy tidal swamps. Water chestnut belongs to the family Cyperaceae or group of puzzles. Water chestnut is a source of organic material that provides benefits for both soil and plants due to its content (Ariwibawa 2001). Each plant contains several bioactive components which are natural chemicals in plants that can give taste, aroma, and color to plants (Winarti 2010).

Bioactive components are components of natural compounds in plants that can be used and utilized. The contents of bioactive components possessed by water chestnut are steroids, terpenoids, tannins, saponins, flavonoids, and phenols (Baehaki *et al* 2018). One of the benefits of the content of the bioactive of water chestnut is as an antibacterial compound. According to Zhan *et al* (2013), tuber skin extraction and fractionation of water chestnut (*E. Dulcis*) showed antibacterial activity against three common foodborne pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*. Water chestnut juice containing puchiin antibiotics was effective against *S. aureus*, *E. coli*, and *Aerobacter aerogenes* (Asikin and Thamrin 2012).

Preservation is a process to maintain longer shelf life. Preservation of common food is done using cold temperatures. However, this method is considered to be less effective, so that it is necessary to add 3 compounds that can maintain the shelf life of catfish fillets during storage. The bioactive content of



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water chestnut extract is expected to be a new alternative as a natural antibacterial substance which is effective against the shelf life of catfish fillets. So that it can inhibit the process of quality deterioration during storage and the storage capacity of catfish fillets can be maintained longer. Water chestnut contains compounds that can prevent and inhibit microbial growth and activity so that they can be used to maintain the shelf life of catfish fillets.

According to Baehaki *et al* (2018), water chestnut extract could inhibit several pathogenic bacteria (*Vibrio cholera*) and food decay bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*). This study was aimed to determine the effect of the use of water chestnut extract (*E. dulcis*) on the shelf life of catfish fillet (*Pangasius* sp.) stored in cold temperatures.

1 2. Materials and methods

2.1. Materials

The materials used in this study were catfish (*Pangasius* sp.), water chestnut (*E. dulcis*), boric acid (Merck), NaOH (Merck), buffered peptone water, nutrient agar (Merck) and nutrient broth (Merck).

The tools used include a pH meter, analytical balance (OHAUS), an incubator, micropipette (Single Channel Capp 10-100 μ L, USA), an autoclave (Hirayama, Japan), a hotplate (Cimarec, United Kingdom), colorimeter model JP7100F, and a spectrophotometer.

2.2. Methods

2.2.1. *Water chestnut extract preparation (E. dulcis)*. Sampling was carried out in Indralaya. Sampling of water chestnut plants (*E. dulcis*) was done manually down directly to swamp water. Swamp plants were washed with running water to remove impurities such as mud, wood, twigs, other types of plants and other foreign objects. The clean sample was then cut into small parts (1-2 cm) and dried under the sun for \pm 3-4 days. Dried samples with a moisture content below 10% were mashed using a blender to prepare powder (simplicia).

2.2.2. *Water chestnut extraction*. Extraction of water chestnut was carried out with modified multilevel maceration (Aulifa *et al* 2015), using three types of solvents namely n-hexane (nonpolar), ethyl acetate (semipolar), 70% ethanol (polar). Four hundreds grams of mashed water chestnut were added to a erlenmeyer flask and mixed with 4 L of n-hexane (1:10) b/v. Samples were macerated for 2x24 hours at room temperature then filtered using Whatman 42 paper to produce n-hexane and residual filtrate. The residue was then soaked again with ethyl acetate (1:10) b/v. The residue was filtered with Whatman 42 paper to produce ethyl acetate and residual filtrate. The residue was further soaked with 70% ethanol (1:10) b/v to produce 70% ethanol and residue filtrate. The filtrate obtained was then evaporated using a vacuum rotary evaporator at a temperature of 45°C until dried. After evaporation, the extract was weighed to find out the extracted content, and stored in a refrigerator (3-5°C) in a light-tight bottle until used.

2.2.3. *Fillet preparation (Zega et al 2017)*. Catfish with initial weights (430-460 g) were washed clean and drained. Then the fish was sliced (fillet) by cutting the meat parallel to the spine so that the skinless fillet was obtained, then weighed (100-120 g).

2.2.4. *Fillet storage of fish at cold temperatures*. The pellet fillet with additive treatment (A1) was simultaneously immersed in a solution of water chestnut extract with a concentration of 2% for 15 min. Then all fillets were packed in PP (polypropylene) plastic packaging and sealed. Fillet which has been packaged was directly stored in a refrigerator at a cold temperature of 3-5°C for 28 days with an interval of observation every 7 days.

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2.2.5. *Protein content (AOAC 1995)*. Five grams of samples was added in to a 100 mL Kjeldahl flask, then 10 mL of selenium was added and 3 mL of concentrated H₂SO₄. Destruction for approximately 1 hour was employed until the solution was clear and then cooled. After cooling, into the Kjeldahl flask of 50 mL, distilled water and 20 mL of 40% NaOH were added. The distillation process was carried out with a temperature of 100°C. The distillation results were accommodated in a 125 mL Erlenmeyer flask containing a mixture of 10 mL boric acid (H₃BO₃) 2% and 2 drops of the indicator pink bromocresol green-methyl red. After the distillate volume reached 40 mL and bluish-green, the distillation process is stopped. Then the distillate was titrated with 0.1 N HCl until the mixture turned pink. The volume of the titrant was read and recorded. Blank solutions were analyzed. Protein levels were calculated as follows: % Protein content = % N × conversion factor (6.25).

2.2.6. *TVBN test*. The test was carried out in accordance with SNI 2354: 2009 (BSN 2009). A sample of 10 g was inserted as much as 50 mL into a distillation tube and added a few drops of phenolphthalein indicator (a colorless solution and in an acidic state), then add a few drops of anti-foaming silicone. The distillation tube was attached to the steam distillation apparatus and added 10 mL of 20% NaOH. To an Erlenmeyer flask, 100 mL of 3% H₃BO₄ and 3-5 drops of the tashiro indicator (purple solution) were added. Then steam distillation took approximately 10 min to obtain distillation of 100 mL.

2.2.7. *Microbiology analysis*. TPC testing is carried out in accordance with SNI 2897: 2008 (BSN 2008), as for how it works are as follows: A sample of 25 grams was weighed and then put in a container. A solution of BPW (Buffered Peptid Water) was added as much as 225 mL and homogenized. This was a 10-1 dilution solution, 1 mL of this solution with a sterile pipette was put into 9 mL BPW to get a 10-2 dilution. Then the solution was made a 10-3 dilution, 10-4, 10-5 and so on in the same way as in item 1 as needed. Then 1 mL of the suspension from each dilution is inserted into the petri dish in duplicate. Then 15 mL of PCA was added after cooling to a temperature of 45°C ±1°C on each dish containing suspension. After that, the cup was incubated at temperatures of 34°C-36°C for 24 h by means of the cup placed in the upside-down position.

2.2.8. *Physical analysis (white degrees)*. The color of white of catfish fillet was measured using a colorimeter model JP7100F. The values of L* (lightness), a* (redness / greenness) and b* (yellowness / blueness) were measured and calculated in white degrees based on the following formula: Whiteness = 100 - [(100-L*)² + (a*² + b*²)]^{1/2}.

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3. Results and discussion

3.1. Protein content

Protein is a very important component in the body. Proteins function as building substances for forming new networks and maintaining existing networks (Winarno 1997). Effects of water chestnut extract on protein content during storage can be seen in figure 1.

Figure 1 shows a decrease in protein content without the addition of water chestnut extract (A0) and by the addition of water chestnut extract (A1) during storage of day 0 to day 28. The protein content of catfish fillets without the addition of water chestnut extract (A0) ranged from 10.94%-11.84% while the protein content of catfish fillets with the addition of water chestnut extract (A1) ranged from 11.05%-11.90%. The lowest protein content was found in the treatment without extract (A0) at 28th storage day (10.94%) while the highest protein content was found in the treatment with the addition of water chestnut extract (A1) 11.90%.

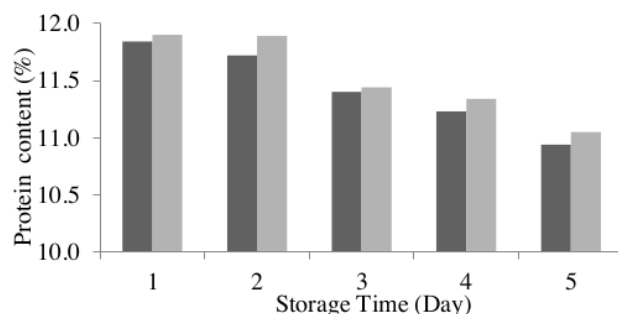


Figure 1. Protein content of catfish fillets without (dark grey) and with the addition of extracts (light grey).

3.2. TVBN test (total nitrogen volatile base)

TVBN test is one of the measurement methods to determine the freshness of fish based on the evaporation of basic compounds (Hong *et al* 2012). Histogram of TVBN content of catfish fillet with the addition of extract (A1) and without extract (A0) during storage is shown in figure 2.

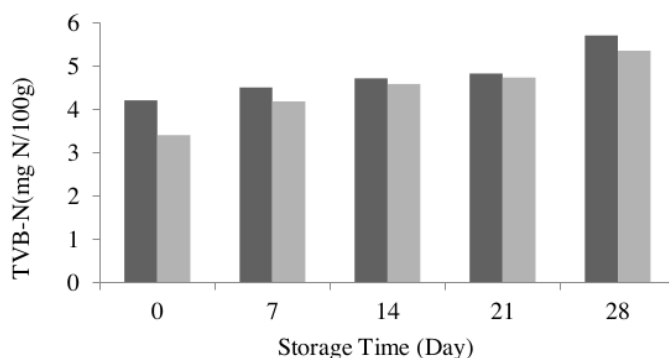


Figure 2. TVBN values in the fillet of catfish without (dark grey) and with the addition of extract (light grey).

The catfish fillet showed the highest TVBN value of 5.70 mg N/100 g. The TVBN value below 30 mg N/100 g, shows the catfish fillet with the addition of extract (A1) and without extract (A0) until 28th days was still suitable for consumption, based on Ermaria (1999), who claimed that the TVBN value standard is 30 (mg/100g). According to Santoso *et al* (1999), increasing TVBN content in fish meat during storage is due to compounds such as trimethylamine (TMA), ammonia, and H₂S because of the degradation of proteins and their derivatives by microorganisms that produce volatile bases. In addition, bacteria also play a role in hydrolyzing fat and causing rancidity (Gill and Newton 1978).

3.3. TPC (total plate count) test

TPC is a method of calculating microbes contained in the product. Determination of the number of microbes in the pangasius fillet can be used as a reference to determine the level of deterioration in the quality of fillets during storage. TPC values during storage is shown in figure 3.

The number of microbes from pangasius fillet without extract tend was higher than that with the addition of extracts. According to Zega *et al* (2017), the catfish fillet with the addition of the apu-apu extract showed a relatively lower TPC value compared to that without the addition of apu-apu extract.

This is because the apu-apu extract on the fillet contained several metabolites that could act as antibacterial substances.

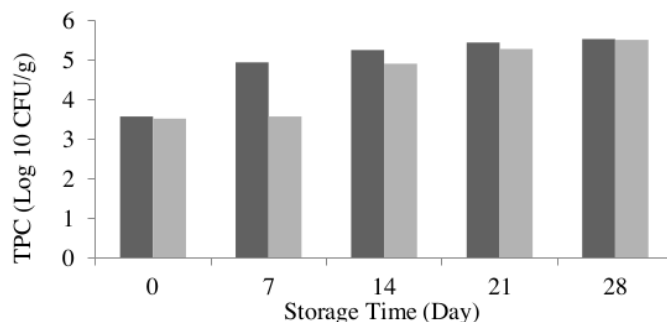


Figure 3. TPC values of catfish fillets without (dark grey) and with the addition of extracts (light grey).

The number of microbes from fillet without the extract was higher than that with the addition of extract. According to Baehaki (2018), compounds that have the potential antibacterial activity in water chestnut extract are terpenoids and flavonoids. The TPC of catfish fillets increased during cold storage. Based on BSN (2006), the number of microbes that are still permitted in fishery products is a maximum of 5×10^5 CFU/g. The fillet of catfish without the addition of extract (A0) on day 0 to day 28 amounted to $5.53 \log_{10}$ (3.4×10^5 CFU/g) and catfish fillet with the addition of extract (A1) water chestnut, catfish fillet on day 0 to day 28 equal to $5.51 \log_{10}$ (3.2×10^5 CFU / g). This indicates that the catfish fillet without extra extract (A0) and with the addition of extract (A1) can still be consumed until the 28th-day storage.

3.4. Whiteness

Color testing is one of the important parameters in assessing a product. According to Kusumamurni (2013), the color of a product can be an attraction for consumers before looking at the other properties of the product. The whiteness values of the catfish fillet without extract (A0) and with the addition of extract (A1) is shown in figure 4.

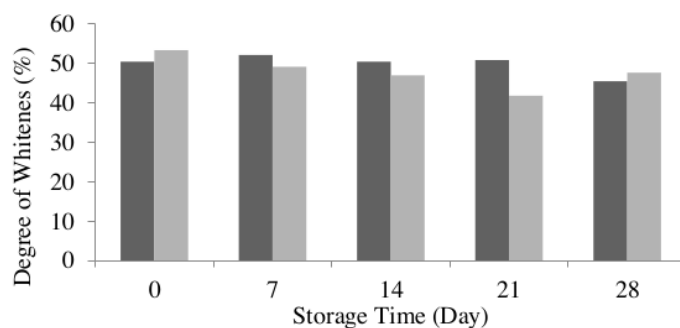


Figure 4. The whiteness values of catfish fillets without (dark grey) and with the addition of extracts (light grey).

Figure 4 shows the whiteness value of catfish fillets without water chestnut extract (A0) ranged from 50.79% to 45.43%, while with the addition of water chestnut extract (A1) ranged from 53.36% to

41.81%. The highest degree of whiteness in the treatment with the addition of water chestnut extract (A1) appeared on day 0 was 53.36% and the lowest value of white grade on day 21 was 41.81% with the addition of extract. The value without the addition of extract showed instability. This can be caused by the influence of myoglobin the fillet.

4. Conclusion

The water chestnut extract had effect on sensory values of catfish fillet. Treatment of catfish fillets with water chestnut extract (2%) and without the addition of extracts during cold storage is considered safe for consumption until the 28th day.

2 Acknowledgments

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