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Incorporation of spices on physical properties, biochemical parameters, microbial loads and sensory attributes of fish ball from the climbing perch (*Anabas testudineus*) fish during chilled storage

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Climbing perch (*Anabas testudineus*) fish was one of the most important fresh-water fishes as it contained a good source of minerals, essential fatty acids, amino acids with excellent textural properties. However, numerous spines on its flesh limited it from cuisine. People commonly utilized this fish for processing fish ball. Consumption demand of fish ball greatly increased due to its convenience in preparation. This research evaluated the supplementation (0.3%) of black pepper, onion, garlic and chilli on the physical characteristics, biochemical parameters, microbial loads and sensory attributes of fish ball prepared from climbing perch fish during 12 days of chilled storage. Results showed that among different spices incorporated into fish ball, 0.3% garlic revealed the best option to maintain water holding capacity ($92.65 \pm 0.00\%$), texture profile (hardness 39.84 ± 0.02 N, springiness 0.80 ± 0.01 , cohesiveness 0.67 ± 0.01 , chewiness 29.15 ± 0.00 N), total volatile base nitrogen (18.42 ± 0.16 mg/100 g), Thiobarbituric acid reactive substances (0.46 ± 0.01 mg malonaldehyde/kg), total plate count (4.52 ± 0.02 log CFU/g), *Enterobacteriaceae* (1.89 ± 0.01 log CFU/g) and overall acceptance (8.09 ± 0.03 score) during preservation. It was suggested that garlic would be considered as potential candidate to extend shelf life of fish ball from climbing perch and other seafood products.

Keywords: *Anabas testudineus*, biochemical, fish ball, incorporation, microbial, physical, sensory, spice

INTRODUCTION

Climbing perch (*Anabas testudineus*) was fresh-water fish widely distributed in Vietnam and other Asian countries. It was a rich source of minerals (copper and iron), poly-unsaturated fats and amino acids (Ahmadi, 2019). Apart from natural origin, it could be reared in semi-intensive, intensive or industrial farming models for 3-4 months in farm, cage, tank and pond (Long et al. 2006; Mondal et al. 2010; Kumar et al. 2013). It adapted well at temperature fluctuation, low dissolved oxygen and low alkalinity (Kohinoor et

al. 2009; Sarma et al. 2010; Be et al. 2017). It was also highly value due to its high yield of carcass, balance-nutritional proximate and textural characteristic.

Fish ball or scraped meat was a common seafood product with a great consumer's preference in the world (Nowsad et al. 2000). After cooking, steaming, dip-frying, the scraped meat achieved an elastic structure and a pleasant mouth-feel without the fishy feeling. Fish ball could be processed from different fish sources of marine and fresh-water fishes such as skipjack

tuna (*Sarda orientalis*), horse mackerel (*Megalaspis cordyla*), jeweled shad (*Ilisha filigera*), sea cat fish (*Tachysurus thalassinus*), red jewfish (*Johnius argentatus*) (Hoque et al. 2007), Spanish mackerel (*Scomberomorus maculatus*) (Tee and Siow, 2017), bronze feather back (*Notopterus notopterus*) (Nguyen et al. 2019; Nguyen, 2021), tilapia (*Oreochromis niloticus*) (Jayasinghe et al. 2013), pangasius (*Pangasianodon hypophthalmus*) (Rathod et al. 2018). Preparation of scraped meat from blended mince of fish would enhance usage efficiency, minimize post-harvest damage and improve commercial value. Scraped meat was commonly prepared from the blended mince of fish with food additives and herbal spices.

Spices were rich in bioactive constituents beneficial for human health (Martinez-Gracia et al. 2015; Mir et al. 2017). They were widely utilized in culinary as natural antioxidants with a great interest to stimulate the palatable taste and flavor, prevent degradation of nutritional quality, prolong the stability of foodstuffs (Kenawi et al. 2011; Velasco and Williams, 2011; Munekata et al. 2020). Black pepper (*Piper nigrum*) was well-known by piperine with antiseptic, antibacterial and anti-inflammatory capacities due to its essential oil with excellent antioxidant ability (Zhang et al. 2016). Black pepper was commonly used for seasoning with a strong, pungent flavor of various dishes (Rezvanian et al. 2016). Garlic (*Allium sativum*) contained abundant flavonoids, phenols and terpenoids with a strong antioxidant potential and inhibiting effect against free radicals (Melania et al. 2019). Garlic was also rich in organosulfur substances responsible for its flavor, aroma, and potential health benefits (Ranendra et al. 2015). Diversified beneficial advantages such as antitumor, antimicrobial, hypoglycemic, antithrombotic, antiarthritic, hypolipidemic and antioxidant properties were reported on garlic (Duyar et al. 2019). Onion (*Allium cepa*) included numerous highly valuable phytochemical constituents such as flavonoids, fructans, and organosulfur elements useful for human health (Greeshma et al. 2020). It was commonly utilized as a spice and seasoning vegetable (Khawaja et al. 2017). Chilli or red pepper (*Capsicum* spp) was one of the most common vegetables due to its spicy flavor and high nutritional value. It included a great amount of capsaicinoids, terpenoids and vitamins with antiseptic, anti-diarrhea, emmenagogue, anti-inflammatory, antirheumatic properties (Lopez-Valdez et al. 2016). It was used to cure various ailments such as bronchitis,

asthma, cough, sore throat and other respiratory syndromes (Ranajit et al. 2013).

Purpose of our research examined the incorporation of black pepper, onion, garlic and chilli on the physical characteristics (water holding capacity, texture profile), biochemical parameters (total volatile base nitrogen and Thiobarbituric acid reactive substances), microbial loads (total plate count and *Enterobacteriaceae*) and overall acceptance (sensory score) of fish ball prepared from climbing perch fish during 12 days of chilled storage.

MATERIALS AND METHODS

2.1 Material

Fish ball was prepared from *Anabas testudineus* fish which was caught in Dong Nai river, Vietnam. Black pepper, garlic, onion and chilli were purchased in local market. Garlic and onion were peeled, washed and cut into small pieces. Chilli was also washed and cut into small pieces. Black pepper was finely ground into powder by grinder. Chemical reagents were all analytical grade. Petrifilm plates were supplied from 3M-Vietnam. Lab equipments and utensils included UV-Vis spectrophotometer, texture analyzer, stomacher, vortex mixer, weigh balance, incubator, colony counter, autoclave, test tube, micro pipette.

2.2 Researching method

Each fish had weight approximately 1.5 ± 0.1 kg. It was bled, gutted, washed and filleted by a sharp knife. From that scraped meat could be collected by spoon. Fish ball was prepared by adding sodium chloride 2.5%, sugar (0.5%), monosodium glutamate (0.1%), and different formulas of fine spices from black pepper (0.3%), garlic (0.3%), onion (0.3%) and chilli (0.3%) in individual batches. These separated mixtures were thoroughly mixed by scoop for 5 minutes before keeping in zipper bags at 4°C during 12 days. In 3 day-interval, fish ball was sampled to determine physical properties (water holding capacity, texture profile), biochemical parameters (Total volatile base nitrogen or TVB-N, Thiobarbituric acid reactive substances or TBARS), microbial loads (total plate count, *Enterobacteriaceae*) and overall acceptance (sensory score).

Water holding capacity (%) was estimated following procedure described by Nopianti et al. (2012). Texture profiles such as hardness (N), cohesiveness, springiness and chewiness (N)

were determined using a texture analyzer described by Nurkhoeriyati et al. (2012). Total volatile base nitrogen or TVB-N (mg/100 g) was determined following AOAC (1990). Thiobarbituric acid reactive substances or TBARS (mg malonaldehyde/kg) was determined following the 2-thiobarbituric acid spectrophotometric method (Anna et al. 2017). Total plate count (log CFU/g) and *Enterobacteriaceae* (log CFU/g) were enumerated by 3M-Petrifilm plates. These plates were incubated at 37°C for 24-48 h. Overall acceptance (sensory score) was determined by a group of specialists using 9-point Hedonic scale.

2.3 Statistical analysis

The experiments were run in triplicate with different groups of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTS

3.1 Effect of black pepper, garlic, onion, chilli incorporation on physical properties of *Anabas testudineus* fish ball during cool storage

Water holding capacity expressed as the capacity of fish ball to hold its inner moisture under gravitational force, cutting or squeezing (Yang and Park, 1998). It was a key variable of muscle protein gel because it not only affected the yield of manufacture but also its physical property. In our research, significant difference in the water holding capacity was observed among control and treated samples (figure 1). The initial water holding capacity of fish ball was observed from 93.84±0.02 to 93.90±0.04%. Fish ball supplemented with 0.3% garlic maintained the highest water holding capacity (92.65±0.00%) while the control showed the lowest one (81.13±0.03%) at the end of 12 days of chilled storage. Water holding capacity of the control had a steep reduction while the samples treated with spices had a gradual reduction of water holding capacity during chilled storage. Water holding capacity commonly represented for the degree of denaturation of the protein (Kristinsson and Liang, 2006).

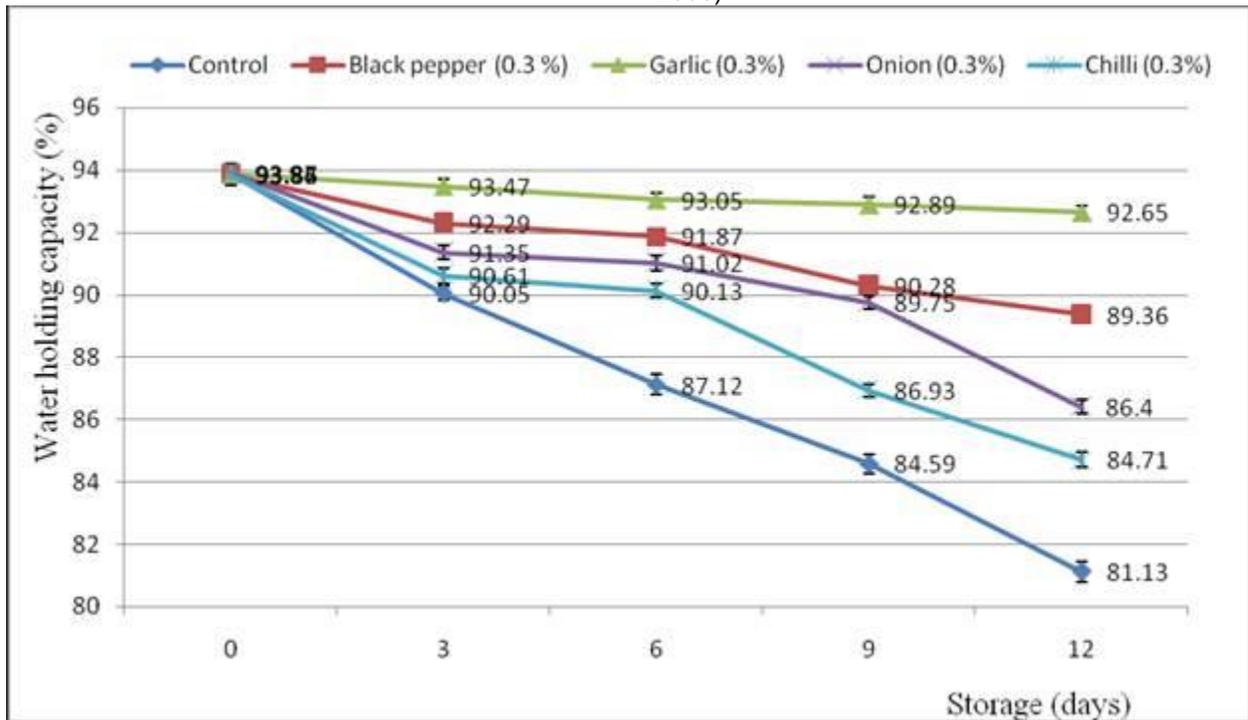


Figure 1: Effect of spice supplementation to water holding capacity (%) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

Hardness was defined as the optimal strength of the initial compacting power (Szczesniak, 1963). There was significant ($p \geq 0.05$) difference of hardness on the control and treated samples (figure 2). The initial hardness of fish ball was observed at around 40.03 ± 0.03 to 40.07 ± 0.02 N. Fish ball supplemented with 0.3% garlic maintained the highest hardness (39.84 ± 0.02 N) while the control showed the lowest one (35.24 ± 0.00 N) at the end of 12 days of chilled

storage. Hardness of the control had a steep reduction while the samples treated with spices had a gradual reduction of hardness during chilled storage.

Springiness was estimated as the proportion of zone by the arch behind the initial compacting to the zone by the arch behind the extra compacting (Szczesniak, 1963). There was not significant ($p \geq 0.05$) difference of springiness on the control and treated samples (figure 3).

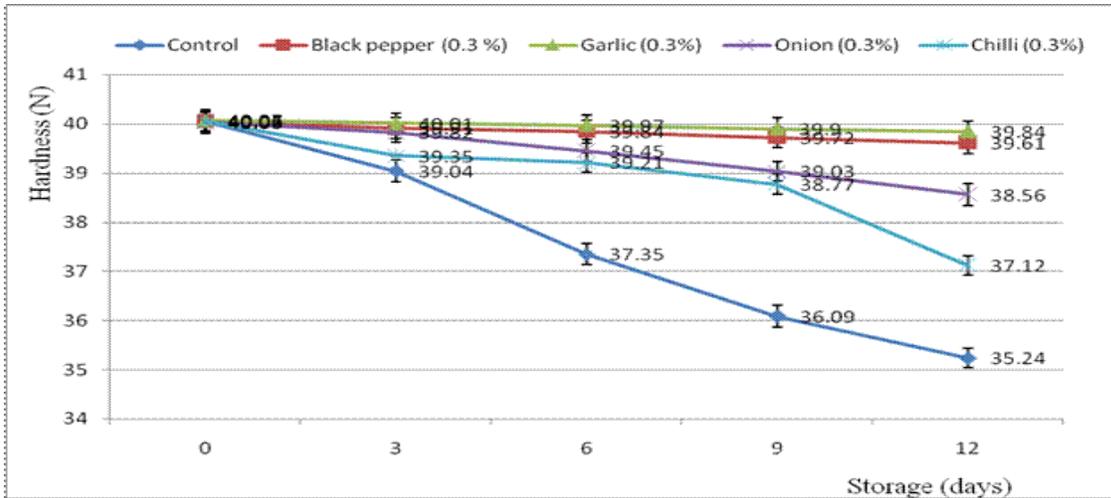


Figure 2: Effect of spice supplementation to hardness (N) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

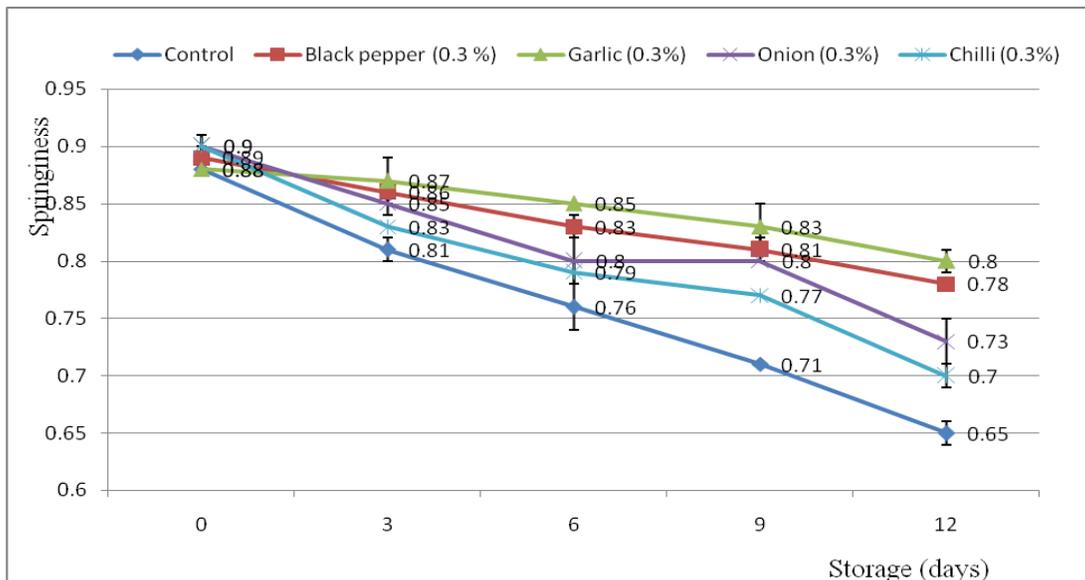


Figure 3: Effect of spice supplementation to springiness of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

The initial springiness of fish ball was observed from 0.88 ± 0.00 to 0.90 ± 0.01 . Fish ball supplemented with 0.3% garlic maintained the highest springiness (0.80 ± 0.01) while the control showed the lowest one (0.65 ± 0.01) at the end of 12 days of chilled storage. There was a slight reduction of springiness on both on the control and treated samples during 12 days of chilled storage at 4°C.

Cohesiveness was defined as the proportion of zone behind the extra compacting to the zone behind the initial compacting (Szczesniak, 1963). There was not significant ($p \geq 0.05$) difference of cohesiveness on the control and treated samples

(figure 4). The initial cohesiveness of fish ball was observed from 0.70 ± 0.01 to 0.72 ± 0.01 . Fish ball supplemented with 0.3% garlic maintained the highest cohesiveness (0.67 ± 0.01) while the control showed the lowest one (0.57 ± 0.00) at the end of 12 days of chilled storage. There was a slight reduction of cohesiveness on both on the control and treated samples during 12 days of chilled storage at 4°C

Chewiness was estimated as the power necessary to bite a sturdy pattern to a stable status of oral taking (Tee and Siow, 2017).

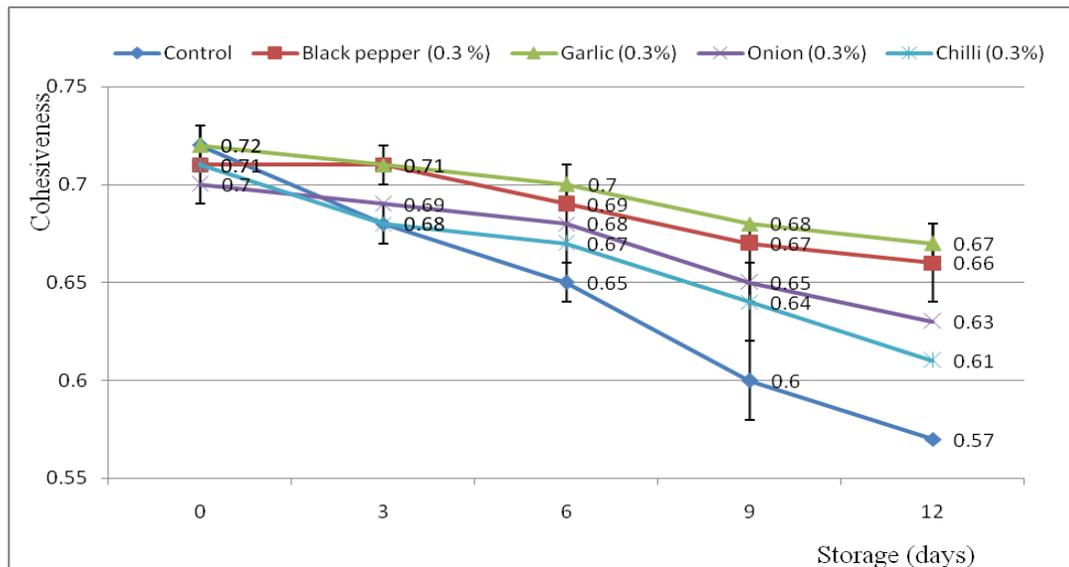


Figure 4: Effect of spice supplementation to cohesiveness of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

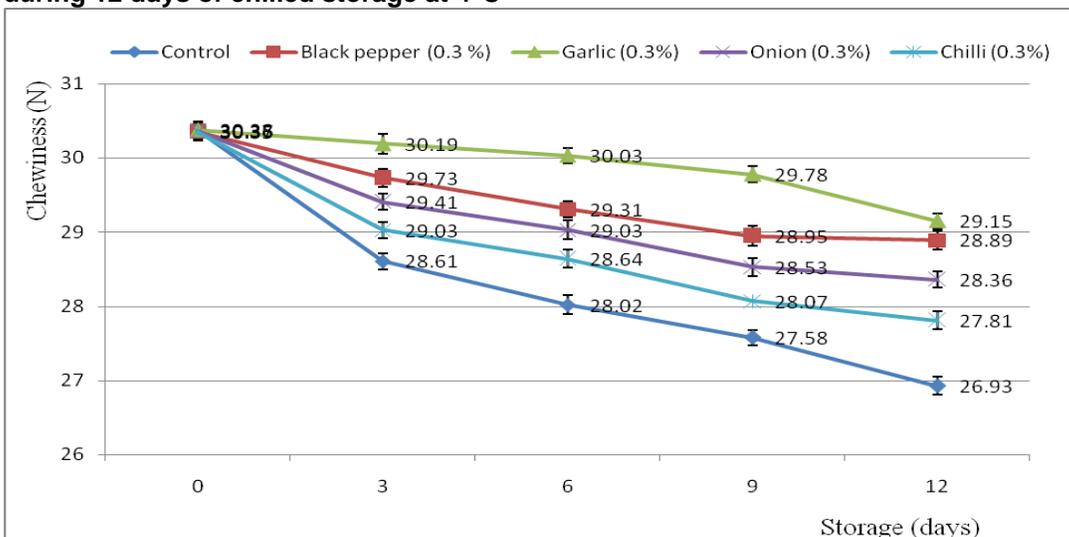


Figure 5: Effect of spice supplementation to chewiness (N) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

There was significant ($p \geq 0.05$) difference of chewiness on the control and treated samples (figure 5). The initial chewiness of fish ball was observed from 30.35 ± 0.00 to 30.38 ± 0.01 N. Fish ball supplemented with 0.3% garlic maintained the highest chewiness (29.15 ± 0.00 N) while the control showed the lowest one (26.93 ± 0.02 N) at the end of 12 days of chilled storage. Chewiness of the control had a steep reduction while the samples treated with spices had a gradual reduction of chewiness during chilled storage. In other reports, Thai pangas surimi treated by garlic extract had higher water holding capacity with a stronger protein network formation. Hardness, adhesiveness and gumminess were significantly influenced with the supplementation of garlic extract (Ranendra et al. 2015). More cross links formed after inclusion of garlic extract decreased the flexibility of protein aggregates; the gels became less springy and more rigid (Ngapo et al, 1996; Ranendra et al. 2015).

3.2 Effect of black pepper, garlic, onion, chilli incorporation on biochemical parameters of *Anabas testudineus* fish ball during cool storage

Total volatile base nitrogen (TVB-N) including trimethylamine, dimethylamine and ammonia was

considered as one of the key biochemical parameters to estimate the microbial decomposition of fish (Wu and Bechtel, 2008). Quality of fish was classified basing on TVB-N (mg/ 100 g) values as follows: 25 (very good), 30 (good), 30 - 35 (marketable), over 35 (spoiled) (Duyar et al. 2019). In our research, the initial TVB-N value was recorded 11.49 ± 0.14 to 11.63 ± 0.08 mg/100 g in fish ball, following a gradual increase during preservation. The highest TVB-N was noticed at 48.61 ± 0.18 mg/100 g on the 12th day of the control sample. Meanwhile, the most significant retardation of TVB-N was noticed at the sample incorporated by garlic (18.42 ± 0.16 mg/100 g) (figure 6). This could be explained by the antioxidant capacity of garlic. Marinated anchovy treated by 0.1% garlic oil had the minimum TVB-N during 6 months of storage at $4 \pm 2^\circ\text{C}$ (Kocatepe et al. 2018). TVB-N of smoked Atlantic Mackerel supplemented with garlic was lower than control group (Duyar et al. 2019). Garlic incorporation effectively controlled TVB-N accumulation in marinated anchovy during 7 months of storage at 4°C (Ficicilar and Genccelep, 2020). Anchovy fish burgers supplemented with garlic peel extract had lower TBARS value compared to the control (Fadiloglu and Ucak, 2020)

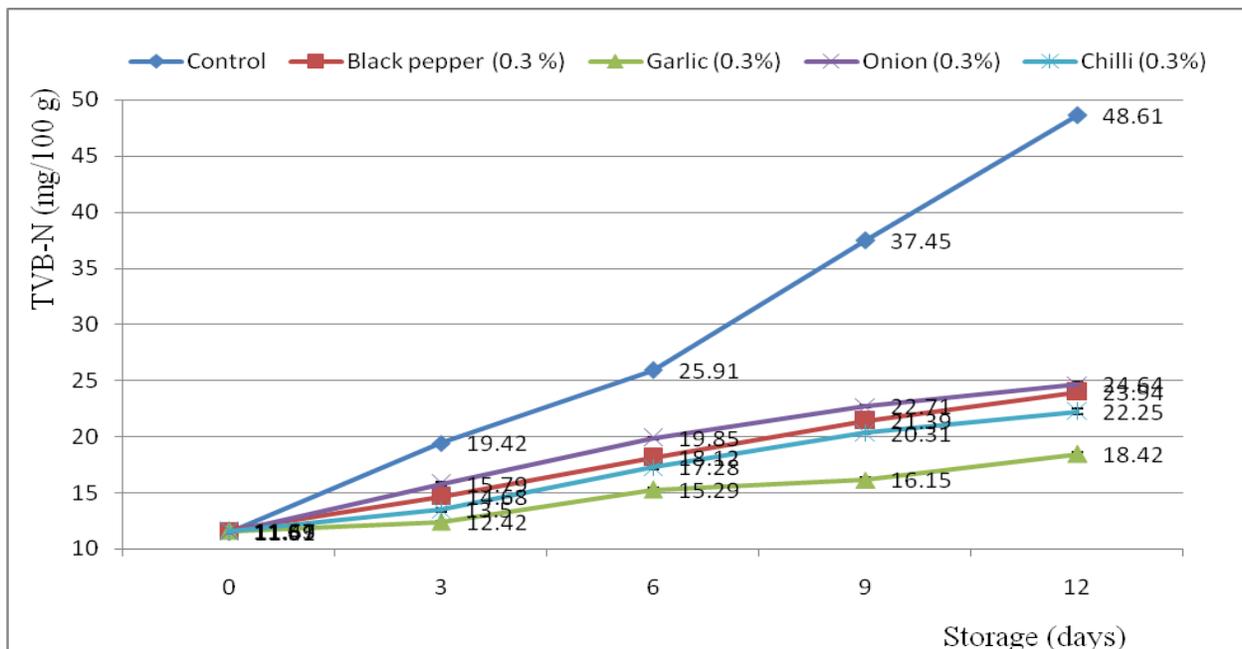


Figure 6: Effect of spice supplementation to TVB-N (g/100 g) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

Lipid oxidation commonly happened in fish products during storage. Thiobarbituric acid reactive substances (TBARS) was also one of the most important indicators to determine the oxidative rancidity of polyunsaturated fatty acids via formation of malonaldehyde facilitating to release ketones and aldehydes by peroxidase reaction (Bremner, 2002; Feliciano et al. 2010). TBARS should not over 2 mg malonaldehyde/kg fish to avoid bad smell and poor taste accumulation (Connell, 1990). In our research, the initial TBARS value was recorded at 0.12 ± 0.00 to 0.16 ± 0.01 mg malonaldehyde/kg in fish ball. During storage, this variable greatly increased on the control sample (3.74 ± 0.02 mg malonaldehyde/kg) while the lowest value was reported at sample supplemented with garlic (0.46 ± 0.01 mg malonaldehyde/kg) (figure 7). This could be explained by the antioxidant capacity of

garlic. In another report, black pepper revealed powerful antioxidant capacity against the oxidative rancidity (Danuta et al. 2021). Antioxidant elements in garlic were demonstrated to be effective to prevent oxidative rancidity in fish mince during cool preservation (Kumaraguruparan et al. 2005). Raw garlic had a strong capacity to limit the accumulation of TBARS on hot-smoked catfish during the 28 days of storage (Kumolu-Johnson and Ndimele, 2011). Spices with bioactive antioxidants were demonstrated to be applicable to retard the lipid oxidation in fish (Goswami et al. 2013). TBARS in Thai pangas surimi was limited by the higher content of garlic extract (Ranendra et al. 2015) similarly to finding by Yang et al. (1993). TVB-N and TBARS values in the round scad supplemented with onion or garlic was greatly inhibited during the chilling storage (Huynh et al. 2020)

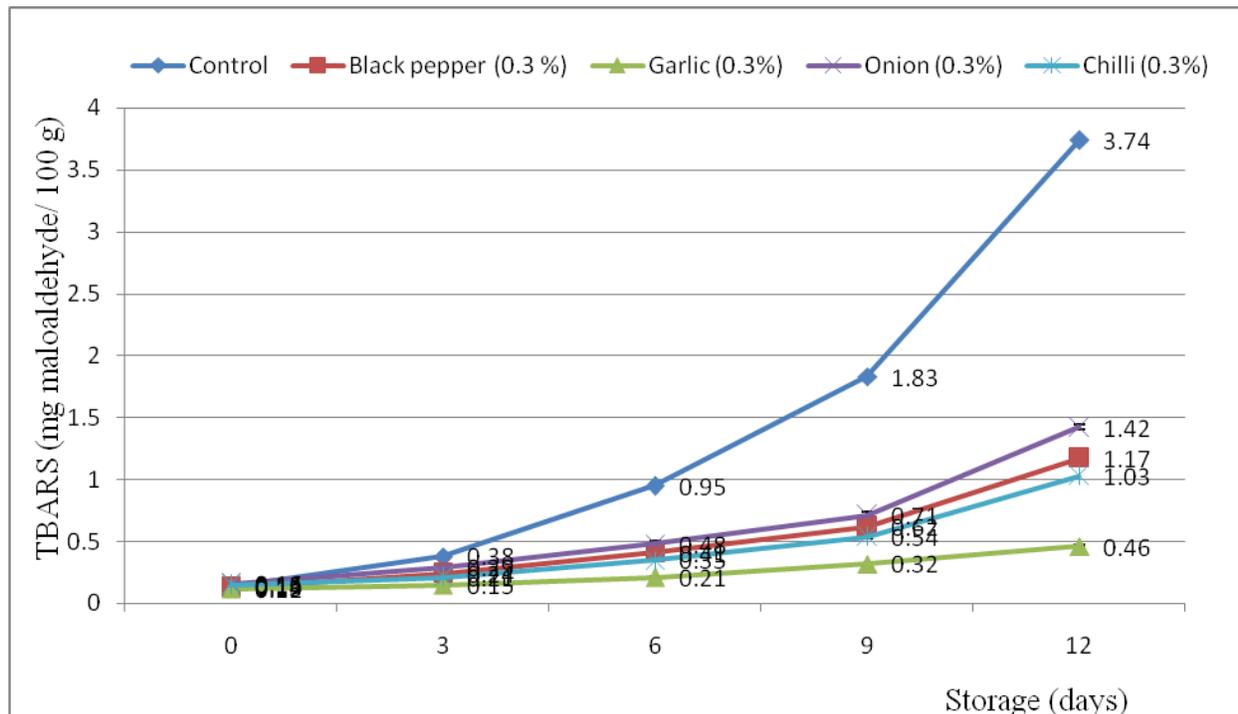


Figure 7: Effect of spice supplementation to TBARS (mg malonaldehyde/kg) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

3.3 Effect of black pepper, garlic, onion, chilli incorporation on microbial load of *Anabas testudineus* fish ball during cool storage

The initial total plate count (TPC) of fish ball was 3.49 ± 0.03 to 3.61 ± 0.01 log CFU/g. During preservation, the TPC remarkably increased in the control (7.08 ± 0.03 log CFU/g) while the lowest microbial proliferation was found at fish ball treated with garlic (4.52 ± 0.02 log CFU/g) (figure 8). *Enterobacteriaceae* was normally considered as an indicator of post-process contamination of fecal contamination (Verdos et al. 2019). The initial *Enterobacteriaceae* of fish ball was 0.89 ± 0.03 to 0.94 ± 0.01 log CFU/g. During preservation, the *Enterobacteriaceae* remarkably increased in the control (5.24 ± 0.02 log CFU/g) while the lowest microbial proliferation was found at fish ball treated with garlic (1.89 ± 0.01 log CFU/g) (figure 9). In one report, black pepper was demonstrated to be effectively control microbial load with excellent inhibitory effects against the proliferation of psychrotrophic microorganisms (Danuta et al. 2021). Garlic had the high

antimicrobial effect superior to thyme and basil against *Bacillus coagulans* in tomato sauce (Ergun and Baysal, 2016). Garlic was found to be highly effective better than onion and ginger against *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Micrococcus luteus* and *Salmonella enteritidis* (Ebenezer et al. 2005). It has been reported that chilli could prolong the stability of fish paste 2-3 days more (Yoon et al. 2001). Mackerel paste added with garlic had lower microbial load than mackerel paste alone (Malicki et al. 2010). Acceptable limit of total plate count on fresh fish was 7 log CFU/g (ICMSF, 1986). Marinated anchovy treated by 0.1% garlic oil had the lowest total plate count during 6 months of storage at $4 \pm 2^\circ\text{C}$ (Kocatepe et al. 2018). Garlic incorporation effectively retarded the microbial growth and proliferation in the marinated anchovy during 7 months of storage at 4°C (Ficicilar and Genccelep, 2020). 4% garlic peel extract kept the total plate count in anchovy burgers to the lowest load (Fadiloglu and Ucak, 2020).

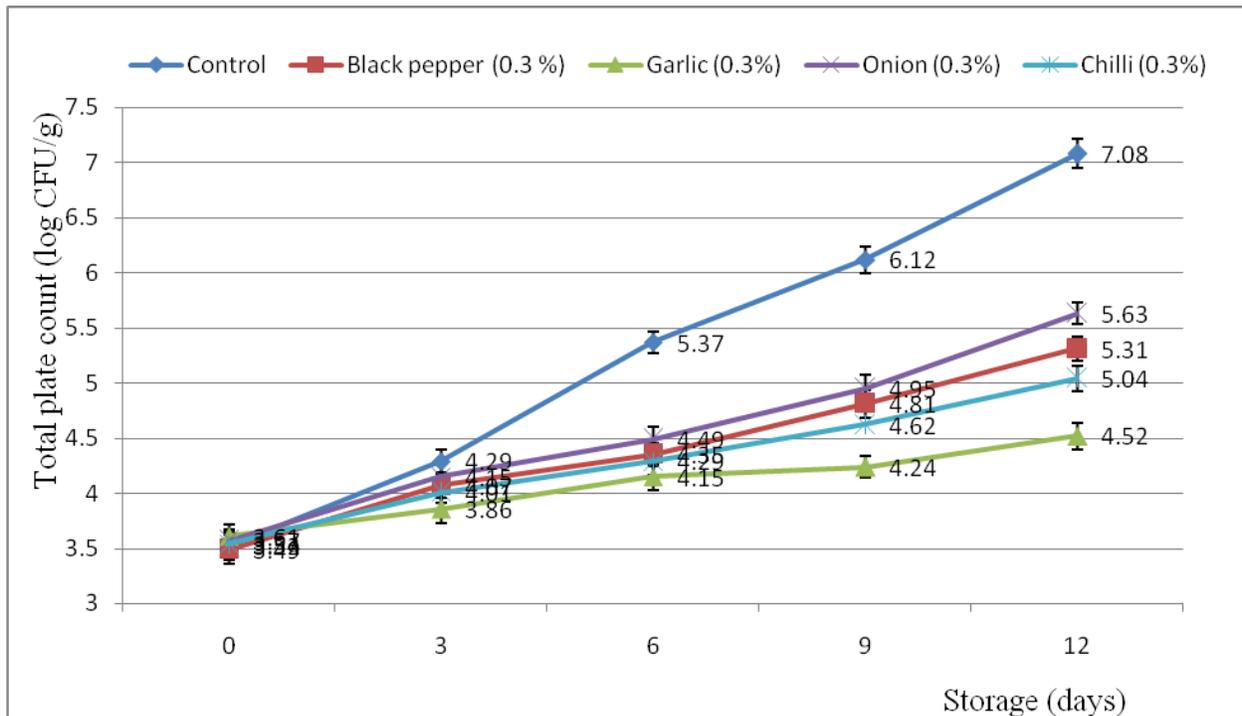


Figure 8: Effect of spice supplementation to total plate count (log CFU/g) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

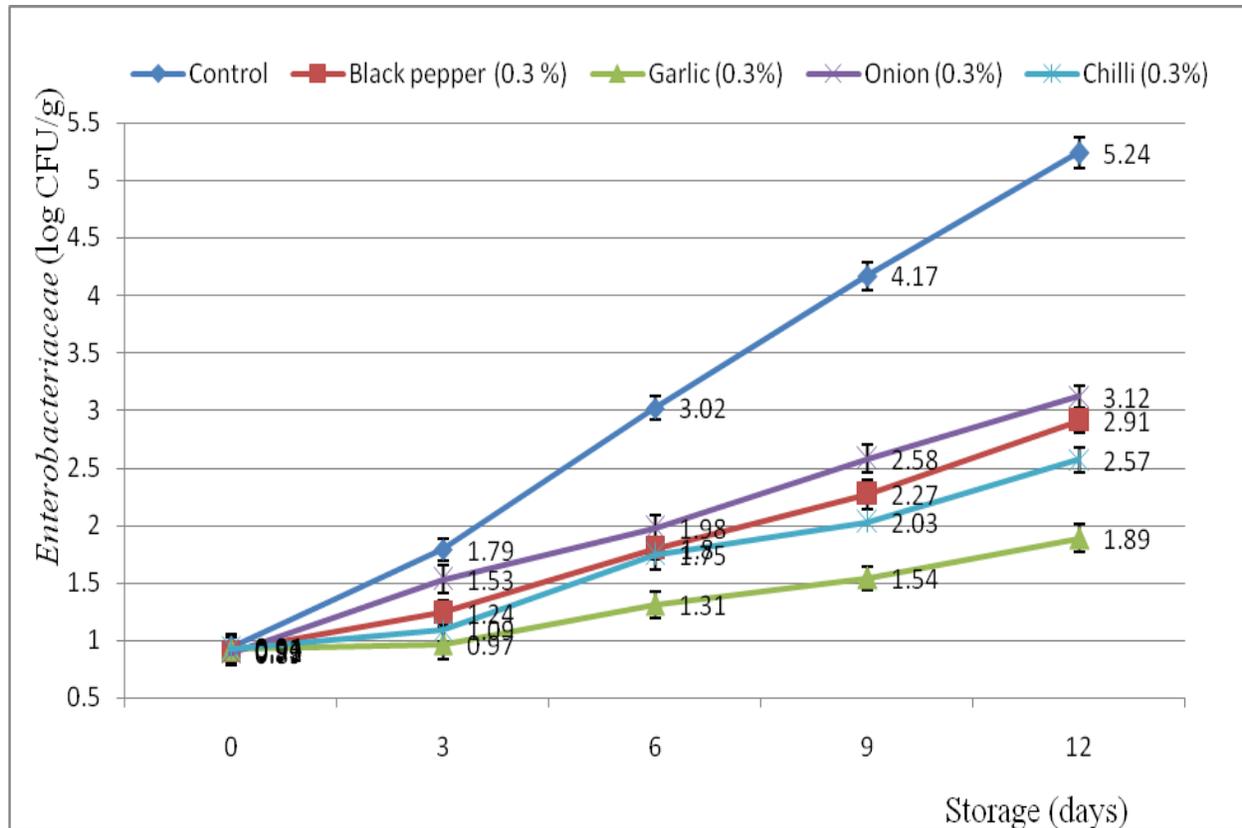


Figure 9: Effect of spice supplementation to *Enterobacteriaceae* (log CFU/g) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C

3.4 Effect of black pepper, garlic, onion, chilli incorporation on overall acceptance of *Anabas testudineus* fish ball during cool storage

Sensory score was also one of the key parameters influencing to acceptance of consumer in commerce. Figure 10 showed the influence of black pepper, garlic, onion and chilli incorporation on overall acceptance of *Anabas testudineus* fish ball during 12 days of chilled storage. The control sample revealed the highest reduction of sensory score after 12 days of chilled storage (8.53 ± 0.02 down to 7.50 ± 0.00); meanwhile the samples treated by garlic showed the least reduction of overall acceptance (8.58 ± 0.01 down to 8.09 ± 0.03) after 12 days of chilled storage. Results expressed that spices effectively contributed to the extended shelf-life of fish ball during storage. Spices mainly contained essential oils, terpenes and aldehydes giving specific flavor and aroma as well as a strong fragrance for finished product (Szymandera-Buszka et al. 2020). Spices had pungency in their flavour and too much spicy flavour was not

accepted by the consumers because not all consumers accepted high spicy tastes (Meilgaard et al. 2006). Fried fish-paste supplemented by onion had better favorability in respect of flavor and taste (Park et al. 2004). Thai pangas surimi treated by 1% garlic extract was greatly preferred with shelf life up to 12 days (Ranendra et al. 2015). Extended stability and better sensory appreciation was reported on smoked Atlantic Mackerel with garlic supplement (Duyar et al. 2019). Round scad added with either onion or garlic extracts maintained its basic sensorial attributes longer than 4 days in cool storage, compared to the control (Huynh et al. 2020). 2% and 4% garlic peel extract prolonged the stability of anchovy burgers for three days (Fadiloglu and Ucak, 2020).

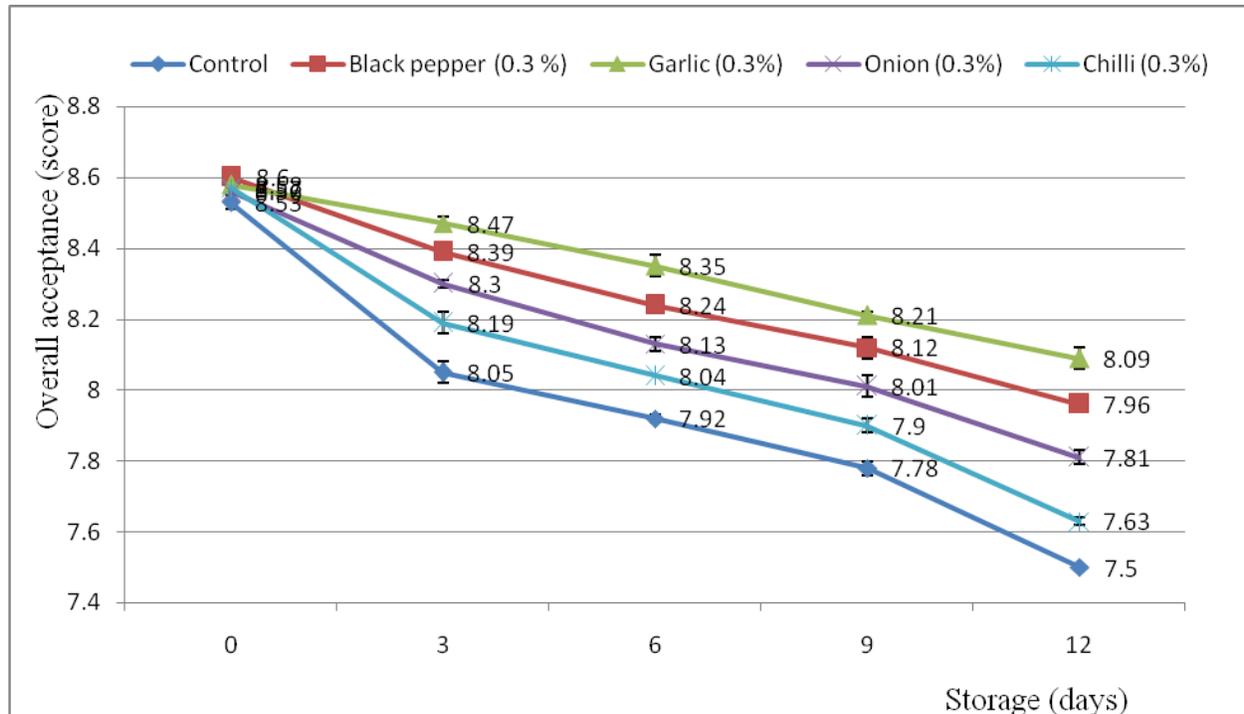


Figure 10: Effect of spice supplementation to overall acceptance (sensory score) of fish ball prepared from climbing perch during 12 days of chilled storage at 4°C Vietnam.

CONCLUSION

Climbing perch contained a great amount of minerals which were beneficial for haemoglobin biosynthesis. Findings of this research suggested that incorporation of spices like black pepper, garlic, onion and chilli caused significant impact on physical parameters (water holding capacity, texture profile), biochemical parameters (total volatile base nitrogen and Thiobarbituric acid reactive substances), microbial loads (total plate count and *Enterobacteriaceae*) and overall acceptance (sensory score) of fish ball prepared from climbing perch fish during 12 days of chilled storage. Garlic might be the best choice among 4 spices. Incorporation of spices into fish ball revealed the efficiency and safety of natural ingredients to replace chemical additives towards healthy food.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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