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Storage Stability of Fish Waste Peptone at Ambient Temperature

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Abstract

Peptones from fish waste has been widely studied, however information about its shelf life is still limited. This study aims to test the storability of dried peptone from tuna and shrimp waste produced through hydrolysis using alcalase enzyme. Peptone powders were packed in HDPE plastic bottles and plastic coated aluminum foil, stored at room temperature, and periodically observed in quality (moisture content, a_w, color and appearance). A test was also performed on their ability to support the growth of *Staphylococcus aureus* bacteria; all were compared to commercial peptone (Difco). Shrimp waste peptone had the highest moisture, ash calcium contents, while tuna peptone has the highest fat content. During five month storage at ambient temperature, all peptones experienced a slight decrease in quality. Aluminum foil performed better than HDPE bottles as a packaging material for peptones, i.e., able to maintain the moisture content, water activity, and appearance. Although the ability to support bacterial growth after five months of storage was slightly affected, the tested peptones were still able to be used as bacterial growing media. It can be concluded that fish waste peptones had comparable quality and shelf-life at ambient temperature to commercial peptone.

Keywords: growth media, fish waste, peptone, *Staphylococcus aureus*, storage stability

1. Introduction

The high demand for substrates to grow microbes is in line with the rapid development of biotechnology both at research and commercial levels. Nitrogen is an essential element contained in the substrate for microbial growth, as well as being the most expensive component in the production of microbial cell mass. One of the most important sources of nitrogen in microbiological media is peptone. Peptone is a secondary derivative of protein, which can be obtained by hydrolyzing animal protein such as meat, internal organs, gelatin, milk, and casein (Clausen, Gildberg, & Raa, 1985).

Fish waste contains various nutrients that are not different from the main ingredients and can be utilized into various products (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013; Skorupa & Sikorski, 1992, 1993). The protein of fish waste may reach 58% on dry matter basis (Ghaly et al., 2013) and has the potential to be converted into peptones (Annadurai, Sadeeshkumar, Vijayalaksmi, & Pirithiviraj, 2012; Najim, Al-Noor, & Al-Waely, 2015; Vieira, Vieira, Macrae, & Sousa, 2005). Some studies have reported the optimum conditions for liquid peptone production from fish waste, through ensilation (Poernomo & Buckle, 2002; Shirahigue et al., 2018) or enzymatically (Ariyani, Heruwati, Murdinah, Susetyo, & Wibowo, 2001; Fallah, Bahram, & Javadian, 2015; Husin, Kamal,Chuan, & Muhammad, 2015; Nurhayati & Desniar, 2013) and the drying of peptones from fish waste (Kosasih, Ratnaningrum, Endah, Pudjiraharti, & Priatni, 2018; Poernomo, Subaryono, Saleh, & Siswanto, 2000). The utilization of fish waste as a nitrogen source for the production of microbial enzymes has also been reported elsewhere (Ben Rebah & Miled, 2013). Studies using underutilized fish as raw materials for peptones have also been reported (Pere, Mbatia, Muge, & Wekesa, 2017). Tests

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of fish waste peptones for supporting the growth of various microorganisms have also been reported by the above authors which concluded that those peptones were able to support microbial growth comparable to commercial peptones such as those from Difco and Oxoid.

Peptones are usually used in small quantities in the laboratories and the package is frequently opened during usage. Peptones are mostly kept at ambient temperature, and their quality is very much affected by the environment, especially its moisture content as peptones are hygroscopic. Thus, proper packaging is then necessary. However, studies on the storage stability of fisheries waste peptones are minimal as so far there is only one report available (Klompong, Benjakul, Kantachote, & Shahidi, 2012). The report concluded that yellow stripe trevally hydrolysate could be stored for 12 weeks at ambient temperature and no significant changes were observed in their quality as a growing microbial media. A study to fill the gap has been conducted on the stability of peptones from fish waste (tuna and shrimp heads) during storage, including their ability to support the growth of *Staphylococcus aureus*. Tuna head is approximately 8% of total body weight containing 14.7-15.5% protein (Kasmiran, 2018), while for shrimp head is 34-45% containing up to 65% protein (Barratt & Montano, 1986; Limam, Saloua, & El Abed, 2008), and could be converted into peptones.

2. Materials and Methods

2.1. Materials and Pepton Preparation

Tuna (*Thunnus* sp.) and shrimp (*Penaeus* sp.) heads were obtained from freezing plants in Muara Baru, Jakarta, Indonesia and transported in ice to the laboratory. Upon arrival, they were then cut into small pieces with a chopper. After the materials were homogeneous in size, they were then macerated in distilled water with a ratio between water and material 1 : 4. The next process was hydrolysis using Alcalase 2.4 L enzyme (Novo Nordisk), which had an activity of 2.4 AU/mL. The ratio of enzyme and tuna head was 0.2% (v/w) while the hydrolysis temperature was 50° C for 4 hours. For shrimp head, the enzyme to shrimp head ratio was 0.3% (v/w) and the hydrolysis was at 50° C for 6 hours. The temperature of the liquids was then raised to 85° C for 15 minutes to deactivate the enzyme and then filtered with a cloth and centrifuged. Centrifugation was carried out at 4 °C and speed of 10,000g (± 13,000 rpm) for 10 minutes. The liquid phase was then stored at 4 °C overnight, then the floating fraction on the surface was removed. This solution was then dried using a spray dryer with an inlet temperature of 180 °C, the outlet temperature of 100 °C and the nozzle pressure of 2,000 millibars.

Peptone powders were then packed in 2 types of packaging materials; HDPE bottles (white) and plastic coated aluminum foil, and stored at ambient temperature (Figure 1). The aluminum foil sheet was firstly made into pouches, then filled with peptone and heat-sealed. Samples were taken every month during 5 (five) months for quality observation and test of ability to support the growth of *S. aureus*. This bacteria was chosen as it is a pathogenic bacteria from human reservoirs during handling, processing and packaging, which is frequently found in fish and fish products (Bujjamma & Padmavathi, 2015; Ghanem, Samaha, & Nossair, 2019; Karimela, Ijong, & Dien, 2017; Obaidat, Bani Salman, & Lafi, 2015; Riski, Fakhrurrazi, & Abrar, 2017; Saito, Yoshida, Kawano, Shimizu, & Igimi, 2011), and has been used to tests fish waste peptones in the previous studies (Fallah, Bahram, & Javadian, 2015; Klompong, Benjakul, Kantachote,& Shahidi, 2009; Nurhayati & Desniar, 2013; Poernomo & Buckle, 2002). Another reason is that *S. aureus* was the only pure bacterial culture available in the laboratory when the present experiment was conducted. Commercial peptone (Bacto Peptone, Difco) packed in a similar manner was used as a comparison. All peptones were individually packed for each observation time. In addition to the ability test to grow microbes, evaluation of chemical (moisture content, a_w and amino acid content) and physical (color and appearance) properties were also carried out.

2.2. Analysis

The chemical analysis was conducted by methods as described by Miwa and Ji (1992) and the a_w value was measured using an a_w meter (Shibaura WA-360 Digital). The physical analysis of peptone during storage was carried out visually.

Analysis of peptone's ability to support microbial growth was carried out by inoculating microorganism in liquid media containing the tested peptones. The liquid media was made by dissolving 0.5% (b/v) powdered peptone and 0.1% (b/v) dextrose into distilled water, then the pH of the media was set to 7.0 (neutral) before sterilizing. Pure culture of *S. aureus* (ATCC 25923) was propagated in 100 ml of nutrient broth at 37 °C for 24 hours after which 0.1 ml of the culture was put into 9.9 ml growth media containing the tested peptones, then incubated at 37 °C for 24 hours. Bacterial growth was predicted by measuring the turbidity of the media using a spectrophotometer at a wavelength of 650 nm every hour in the first 3 hours, then every 3 hours to 12 hours, then every 6 hours to 24 hours. All measurements were done in three replicates and corrected against the respective original liquid media blank. Growth rate (absorbance/ hour) was described by the slope of the exponential

Figure 1. Storage of tested peptones in HDPE bottles (top) and plastic coated aluminum foil at ambient temperature (bottom)

growth phase, while the total growth (absorbance) was calculated from the difference in absorbance at the end and the beginning of incubation.

The experiment was designed in a three-factorial completely randomized design with two replications, of which the factors were types of peptones, types of packaging, and storage time. Tukey Test was exercised if the differences were significant for the factors tested.

3. Results and Discussion

3.1. Proximate Composition

The proximate composition of the tuna head, shrimp head, and commercial peptones (Difco) is shown in Table 1. Fat was found highest in tuna head peptone, clearly, because tuna belongs to large pelagic fish, which is characterized by high-fat content (Nazir, Diana, & Sayuti, 2017; Kasmiran, 2018). On the other hand, calcium was highest in shrimp head peptones and this is understandable as calcium content (in the form of calcium carbonate) is high in shrimp shells as the main component of the ash fraction (Dechapinan, Judprasong, On-nom, & Tangsuphoom, 2017; Ibrahim, Salama, & El Banna, 1999; Rødde, Einbu, & Varum, 2008).

The protein content of Difco peptone was the highest, but not significantly different from tuna peptone, while that of shrimp head was the lowest. According to the Product Catalog, Difco peptone is made from meat, while the amount of meat in tuna head was still high. On the other hand, the amount of meat in the shrimp head was not high. The protein content (96.2% db) of tuna head peptone was higher than the tuna head hydrolysates (Nguyen, Pérez-Gálvez, & Bergé, 2012), i.e., 87-88% db. The latter was produced using protamex enzyme from novozyme, and was freeze-dried. On the other hand, the protein content of shrimp head peptone was slightly higher than that of shrimp head hydrolysate produced by 0.1% (by weight) trypsin, as reported by Limam et al. (2008). The protein content of shrimp head peptone in this study is 82.8% wb, while that of Limam et al. (2008) is 79.2% wb. The above different results in tuna and shrimp heads peptones were probably due to the differences in the mechanisms of hydrolysis by the two enzymes. According to Nguyen,

Table 1. Proximate composition (% wb) of fish waste and commercial peptones

Components	TP*	UP	DP
Moisture	6.59 ± 0.04[a]	9.12 ± 0.04[b]	3.36 ± 0.07[c]
Protein	89.86 ± 0.17[a]	82.83 ± 1.10[b]	91.36 ± 0.00[a]
Fat	1.15 ± 0.04[a]	0.37 ± 0.00[b]	0.14 ± 0.00[c]
Ash	3.66 ± 0.13[a]	8.56 ± 0.06[b]	5.94 ± 0.37[c]
Ca	0.13	0.75	0.06
Mg	0.04	0.02	0.02
Mn	nd	0.01	nd
Fe	0.004	0.004	0.009
Cu	nd	nd	0.003

Note: TP= Tuna head peptone; UP= Shrimp head peptone; DP= Difco peptone; nd= not defined
Different letters in one data row (proximate composition) show significant differences ($p < 0.05$).
Minerals were analysed once due to limited samples availability.

(2015), Shu et al. (2015) and Linh, (2018), alcalase enzyme produced more soluble protein than Protamex, Flavourzyme, and trypsin.

3.2. Physical Properties

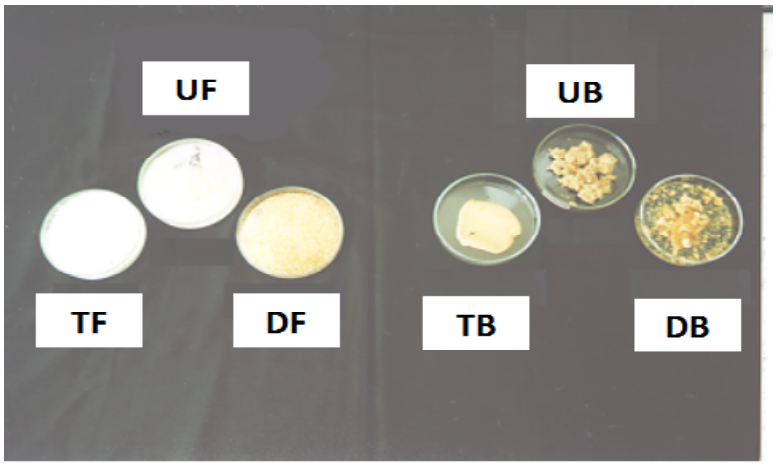
The raw materials influenced the appearance and color of the tested peptones. Peptones from tuna head and shrimp head had a lighter color than commercial peptone (Difco) which was slightly yellowish. Difco peptone has larger grains, while the tuna peptone is white and has fine grains. Slightly different from Difco and tuna head peptones, the shrimp head peptone was grayish with a somewhat rough texture.

The type of packaging material turned out to influence the physical properties of the packaged peptone. Peptones which were packed in plastic bottles quickly changed their physical properties. After three months, the color of the tuna head peptone tended to be slightly brownish, began to clot and the texture was hard, so it must be crushed or cut when used as microbial growth media. Likewise, with shrimp peptone packed in the same material, the color tended to be browner, and its appearance was somewhat clumpy. Commercial peptone packed in the same material did not show any changes in appearance or color, and only after four months storage it began to clump (Figure 2).

On the other hand, all peptones in aluminum foil pouch did not show physical changes even though they have been stored for five months. This showed that this type of packaging is more impermeable to surrounding moisture than a plastic bottle, as also stated by Behringer (1970) and Zeppelzauer (1970). This was supported by permeability analysis that showed that aluminum foil was less permeable to water vapor than plastic bottles, of which the permeability to water vapor was 0.06 and 2.64 g/m^2/ 24h for aluminum foil sheet and plastic bottles, respectively. Also, it occurred that the double cap to seal the bottle, i.e., inner and outer (screwed) caps failed to protect the peptones in the bottle, probably due to minor leakage. On the other hand, it seems that aluminum foil was able to do the job as they were heat-sealed thus ensured no leakage. This was probably the reason why commercial peptone is packed in a bottle with a screwed cap and aluminum foil seal.

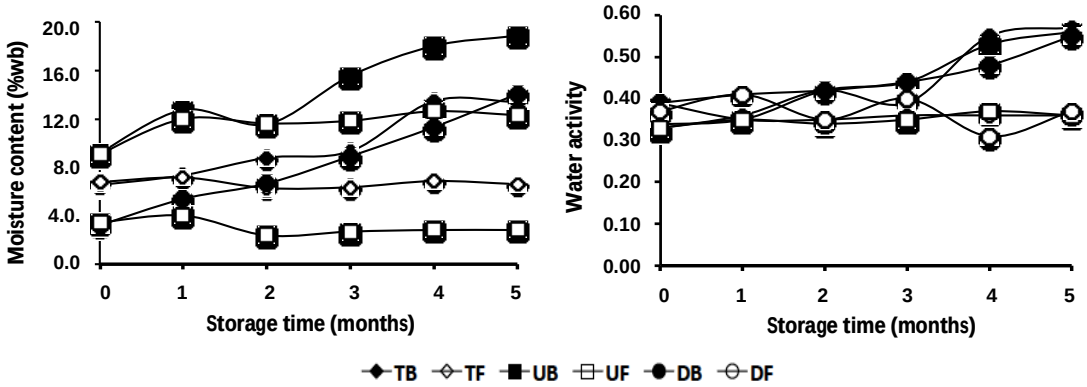
The color of fish waste peptone changed from creamy white and grayish-white to brownish after four months storage and was possibly due to the high permeability of plastic bottle packaging materials to oxygen. Thus with increasing storage time, contact between peptone fisheries waste and oxygen was prolonged, resulting in fat oxidation on the peptone. Considering that the tuna head peptone contained the highest level of fat (Table 1), it is understandable if its browning rate is the highest compared to other peptones.

Commercial and fish waste peptones in plastic bottles have increased moisture content during storage, namely 3.2-9.2% at the beginning of storage to 13.4 - 18.9% after five months of storage, while the



Note: TB/TF: Tuna head peptone in plastic bottle/aluminium foil; UB/UF: Shrimp head peptone in plastic bottle/aluminium foil; DB/DF: Difco peptone in plastic bottles/aluminium foil

Figure 2. Appearance of tested peptone after 4 months storage at ambient temperature

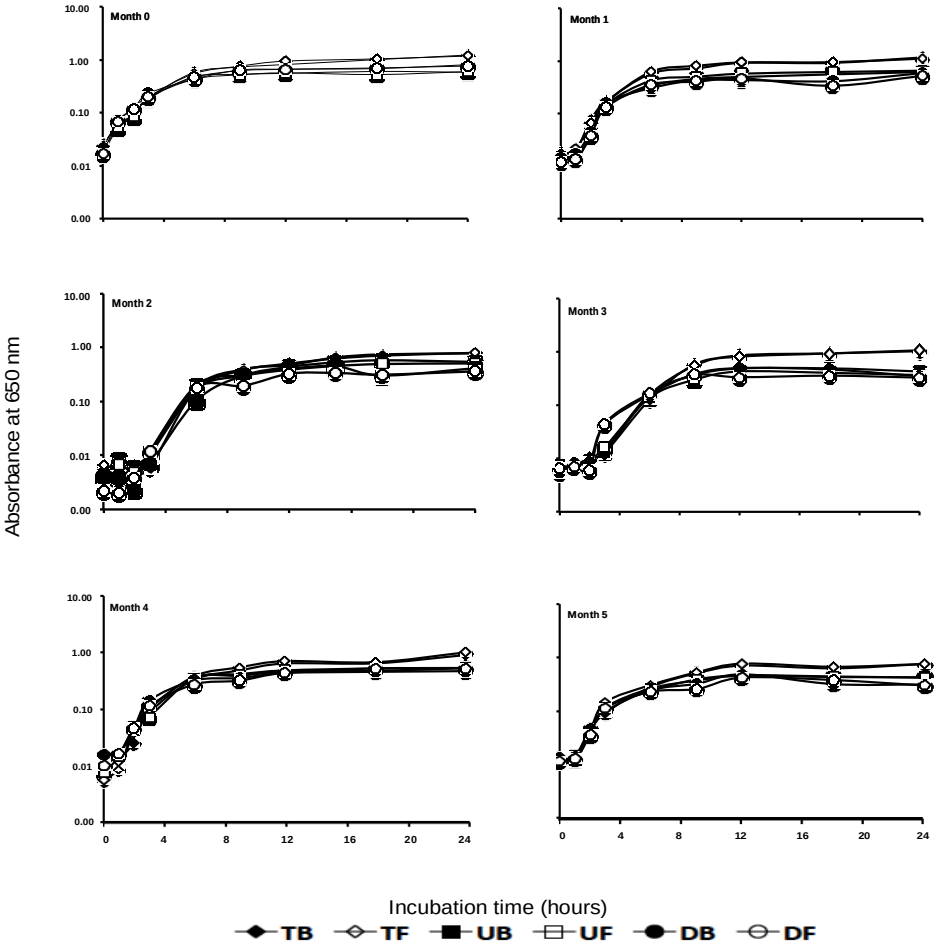


Note: TB/TF: Tuna head peptone in plastic bottle/aluminium foil; UB/UF: Shrimp head peptone in plastic bottle/aluminium foil; DB/DF: Difco peptone in plastic bottles/aluminium foil

Figure 3. Moisture content and water activity of tested peptones during storage

moisture content of peptones in aluminum foil-coated plastic practically did not change (Figure 3). The difference in peptones moisture content packed with plastic bottles and aluminum foil-coated plastic turned out to cause differences in the physical properties of peptones during storage. As previously described, the difference in permeability of packaging materials and the presence of leakage in the bottle cap promoted differences in moisture content and physical properties. From the types of raw materials, peptone made from shrimp head has the highest moisture content, followed by peptone made from tuna head and commercial peptones. The low moisture content of commercial peptones is probably due to the different raw materials (meat), and a more controlled drying process so that the produced peptone powder with granules/crystals is rather large compared to the peptones of fish waste. Shrimp head peptones have the highest moisture content, which was probably because shrimp head had higher ash content than other peptones, as well as its calcium content so that shrimp head peptones absorbed water more quickly than other peptones.

As with the moisture content, the a_w of peptones packaged in plastic bottles also increased quite significantly, while those packaged in aluminum foil-coated plastic pouch did not undergo much change (Figure 3). This happens because of differences in the properties of the packaging material, which results in differences in chemical and physical properties.



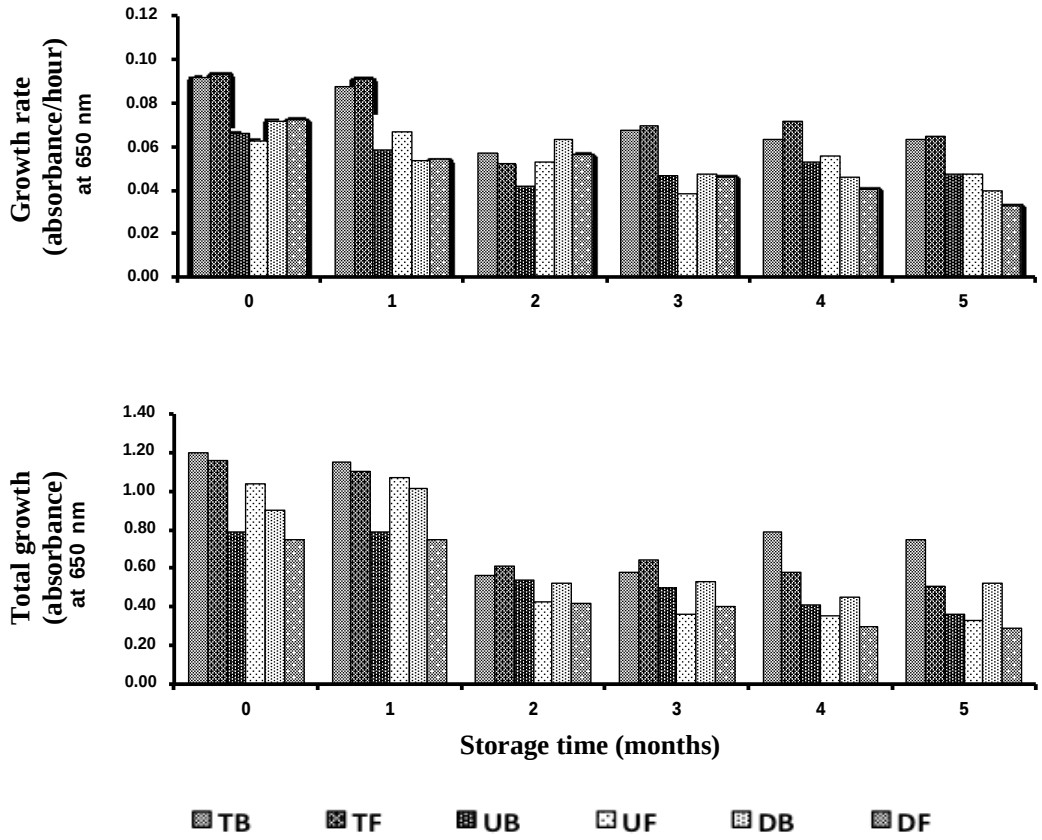
Note: TB/TF: Tuna head peptone in plastic bottle/aluminium foil; UB/UF: Shrimp head peptone in plastic bottle/aluminium foil; DB/DF: Difco peptone in plastic bottles/aluminium foil

Figure 4. Growth (log absorbance) of *S. aureus* on fisheries waste and commercial peptones which have been stored for up to 5 months at ambient temperature

3.2. Bacterial Growth

The growth of *S. aureus* on tested peptones is depicted in Figure 4 as absorbance at 650nm wavelength. It is shown that the curves resemble the normal growth of microorganisms, which started with a lag phase, followed by exponential and stationary phases. The death or declining phase was not observed as dead, and live cells were not separated during observation. In general, the lag phase finished by 1-3 hours after inoculation, followed by the exponential phase. The latter finished between 6 to 9 hours when the curves started to level off.

When grown on fresh peptones (month 0), the exponential phase of *S. aureus* started very early, less than 1 hour after inoculation, and lasted for 6 hours. After one month storage or more of the peptones, the lag phase of *S. aureus* took longer time to cease and consequently delaying the exponential phase to commence, indicating that the microbe needed more time to adapt to the new environment. Slight changes have likely taken place in the properties or quality of the peptones due to storage. Similar results were reported by Klompong et al. (2012) who grew *S. aureus* on media containing yellow stripe trevally hydrolysate and found that the growth of *S. aureus* decreased as the storage time increased. The decrease in growth of *S. aureus* was probably due to the changes in the nutritive value of the peptones, especially amino acid loss. All tested peptones in the present study contained fat, and according to Klompong et al. (2012) reaction of amino acids with lipid in the media might lead to a browning reaction. Further, the changes in growth were in line with the increase in moisture contents of the peptones. However, the storage time of tested peptones in the present study was longer than those reported by Klompong et al. (2012) for yellow stripe trevally hydrolysate, i.e., 20 compared to 12 weeks.



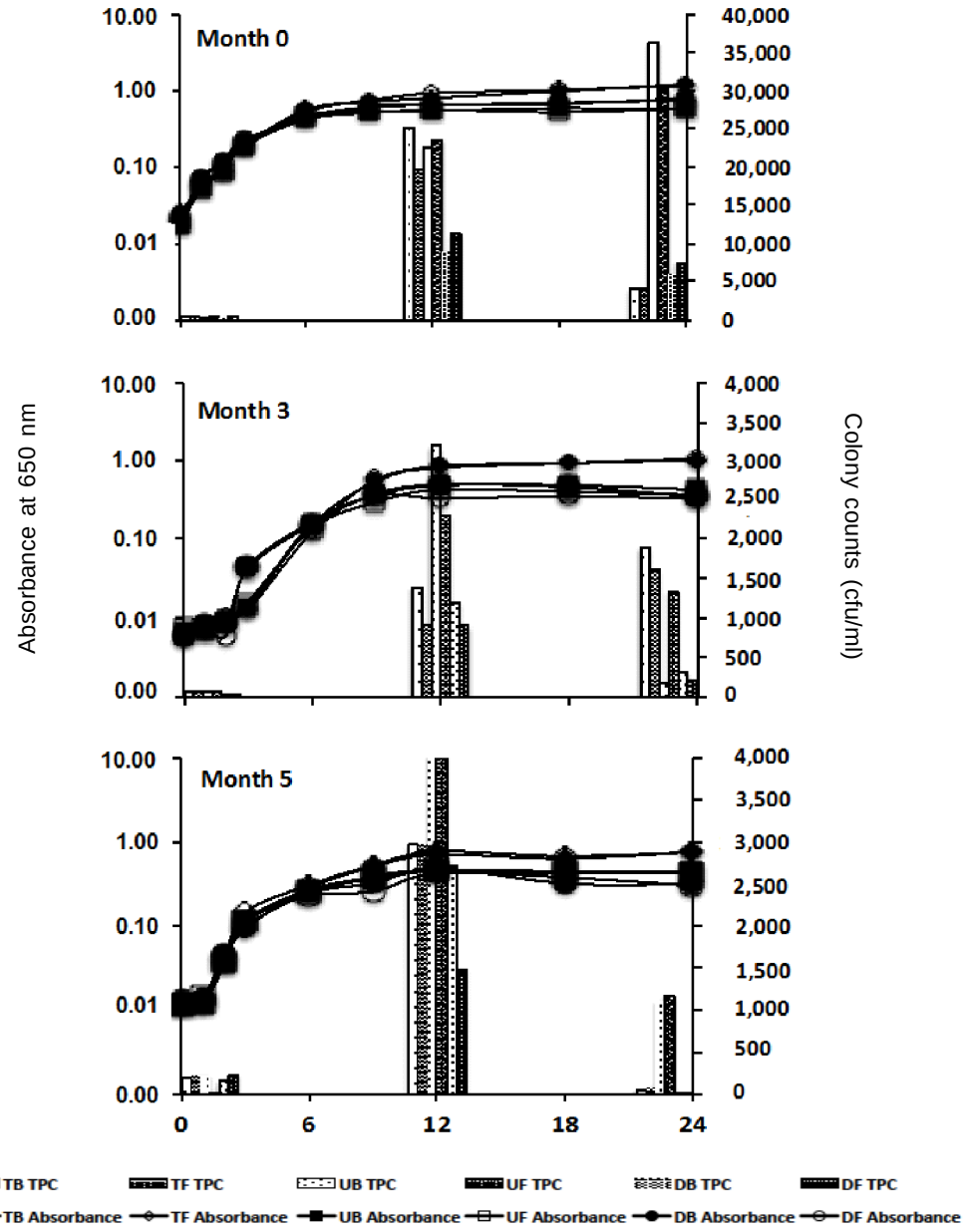
Note: TB/TF: Tuna head peptone in plastic bottle/aluminium foil; UB/UF: Shrimp head peptone in plastic bottle/ aluminium foil; DB/DF: Difco peptone in plastic bottles/aluminium foil.

Figure 5. Growth rate (top) and total growth (bottom) of *S. aureus* grown on fisheries waste and commercial peptones which have been stored at ambient temperature in different packaging

Data on growth rates (Figure 5) reflect the same trends of which the rate for *S. aureus* grown on fresh peptones (month 0) or peptones after one-month storage, were high then gradually decreased in the subsequent month. However, there were no differences between the types of packaging and storage time ($p<0.05$), while tuna head peptones produced a higher growth rate for *S. aureus* compared to other peptones.

For total growth (Figure 5) similar situation was shown where during the first month of storage, all peptones produced high growth as measured from the difference in absorbance after 24-h incubation, clearly due to the high growth rate during the same period. The total growth then decreased in the subsequent month, again, similar to the growth rates. Statistically, the total growth of *S. aureus* on tuna head peptones was higher than those on the other peptones ($p>5$). Similar results also, reported by Safari et al. (2012) who reported that for growing Lactobacillus peptone produced by alcalase hydrolysis of tuna head performed better in terms of growth rate and total growth of compared to commercial peptones. In contrast to the type of peptone, the type of packaging does not have a significant effect on both growth rate and total growth ($p <0.05$). Calcium and magnesium was reported to negatively affect the growth of *S. aureus* (Gultekin & Kucukates, 2019; Xie & Yang, 2016), and this could be observed from the growth rate and total growth of *S. aureus* on shrimp head peptones which were relatively lower than the others.

Absorbance basically measures all the soluble materials in the liquid, including dead cells, and thus does not reflect the number of the living cells. Total plate counts on agar were conducted to confirm the number of living cells in the liquid media. Figure 6 shows the colony counts with their respective absorbance during the growth of *S. aureus* on tested peptones which have been stored for up to 5 months. Samples were only taken on months 0, 3, and 5 as starting from month three, the peptones have changed their physical appearance.



Note: TB/TF: Tuna head peptone in plastic bottle/aluminium foilUB/UF: Shrimp head peptone in plastic bottle/aluminium foilDB/DF: Difco peptone in plastic bottles/aluminium foil

Figure 6. Growth (log absorbance) and related colony count of *S. aureus* grown on fisheries waste and commercial peptones which have been stored for 0, 3 and 5 months

On the hour of 12, living cells were present in a much higher count than the initial, on all tested peptone after storage, which was also reflected by their respective absorbance. The number of colonies decreased markedly on the hour of 24, although the values of absorbance were relatively the same or higher. This was because dead cells present in the liquid were included in the absorbance measurement. According to Erkmen and Bozoglu (2016), after such periods, the nutrients of the media have been used up while the toxic metabolites could add to the effect on the death of microorganisms.

As shown in Figure 6, it can be depicted that storage time affected the ability of tested peptones to support the growth of *S. aureus*. The longer the storage time of the tested peptones, the lower the counts of microorganisms grown on it. However, peptones packaged in aluminum foil performed better, indicating that this packaging was able to protect the peptones from the environment, which was supported by visual observation.

4. Conclusion

Tuna and shrimp heads were able to be converted into peptones through enzymic hydrolysis using alcalase and were able to support the growth of *S. aureus*, of which the ability was comparable to those of commercial peptones (Difco). Aluminum foil performed better than HDPE bottles in the protection of the peptones during storage at ambient temperature, i.e., moisture content, water activity, and appearance were relatively unchanged during 5-month storage. While after 3-4 months storage peptones kept in HDPE bottles began to change their physical properties, i.e., the appearance begins to clot, the color tends to become more brownish and moisture content, as well as water activity, increased. However, all peptones did not suffer a significant loss in their ability to support the growth of *S. aureus* after being stored for five months at ambient temperature.

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