CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CHARACTERISTICS OF YOGHURT ENRICHED WITH PROTEIN HYDROLYSATE FROM PARROTFISH (*CHLORURUS SORDIDUS*) HEAD

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https://doi.org/10.34302/crpjfst/2023.15.3.14

Article history:

Received August 16 2023

Accepted October 6 2023

Keywords:

Chlorurus sordidus;

Fish protein hydrolysate;

Yoghurt.

ABSTRACT

Production of parrotfish (*Chlorurus sordidus*) fillets has increased significantly in Indonesia. The processing only uses fish meat, thus producing plenty of by-products. Fish by-products contain valuable protein that can still be processed into fish protein hydrolysate (FPH). FPH can be used to improve yoghurt's physicochemical and organoleptic. This study aimed to determine the effect of protein hydrolysate from parrotfish head addition on the characteristics of yoghurt. The FPH concentrations used were 0%, 0.1%, 0.15%, 0.2%, 0.25 and 3%. This research was conducted by an experimental method using a completely randomized design with six treatments and five replications. The results showed that the variation of FPH concentration significantly affected the pH, total acid, viscosity, syneresis, water holding capacity, colour L*, a*, b*, appearance, aroma, and taste of yoghurt. However, the addition of FPH did not significantly affect the a* colour and texture of yoghurt. The best treatment was observed from the addition of 0.15% FPH with a viscosity value of 2.432 N•s/m^2, syneresis 49.67%, WHC 37.4%, pH 4.36, TTA 0.76%, colour L* 74.55, colour a* -3.76, colour b* 8.2, taste score 5.39, aroma score 5.33, texture score 5.23, and appearance score 5.8.

1. Introduction

Parrotfish (*Chlorurus sordidus*) is one of the Indonesian export commodities with high economic value (Harms-Tuohy, 2021). The total export value of Indonesian parrotfish exceeds \$200M, with China as the primary export destination (Tridge, 2021). Most parrotfish caught are processed into fillet for consumption as it has smooth and soft meat fibres. Nevertheless, the high consumption of parrotfish produces many fish processing by-products (Silovs, 2018). Fish processing makes more than 60% of by-products, which are rich in protein but susceptible to microbial spoilage and must be handled immediately. In addition, bone, head, skin, offal, and other by-products can be converted to valuable products such as fish protein hydrolysate (Benjakul *et al.,* 2014). Many researchers have reported fish protein hydrolysate production from various fish by-products, such as the head, offal, and skin of black scabbard fish (*Aphanopus carbo*) and lemurus, parrotfish fish head, by-products of Siamese catfish, and salmon bones (Batista *et al.,* 2010; Prihanto *et al.,* 2019; Thuy *et al.,* 2015; Idowu *et al.,* 2018). Prihanto *et al.,* (2019) mentioned that the protein, fat, moisture content of parrotfish were 20.37%, 3.92% and 71.68, respectively.

Protein hydrolysate is obtained from protein cleavage into peptides (He *et al.,* 2013). The total essential amino acids of FPH parrotfish heads were up to 41.69%. The difference in amino acid composition between hydrolysates depends on differences in enzyme specificity and hydrolysis conditions (Prihanto *et al.,* 2019). The hydrolysis process can be divided into two methods: chemical (acid and base) and biochemical. Biochemical/enzymatic hydrolysis may occur using proteolytic enzymes naturally present in fish tissue (autolysis) (Petrova *et al.,* 2018). Previous studies reported the production of protein hydrolysate using the enzymatic method on 'sardines' by-products with pepsin, carp by-products with papain, and yellowfin tuna with alcalase (Benhabiles *et al.,* 2012; Saputra and Nurhayati, 2016; Siddik *et al.,* 2020). The use of flavourzyme as an enzyme that accelerates the hydrolysis process has been carried out in the production of protein hydrolysates of Atlantic salmon, carp, and scad (Kristinsson and Rasco, 2000; Dong *et al.,* 2008; Thiansilakul *et al.,* 2007).

Fish protein hydrolysates (FPH) are rich in amino acids or peptides, have good functional properties and offer fortification material to provide high-protein food. It has a balanced amino acid profile, easy to digest and absorb, and contains bioactive peptides (Chalamaiah *et al.,* 2012). In addition, fish protein hydrolysate may improve the characteristics of food products (Asare *et al.,* 2018). For example, Chen *et al.,* (2018) reported using chickpea flour to enhance the quality of yoghurt. Yoghurt fortified with microcapsules of bigeye fish protein hydrolysate (*Ilisha megaloptera*) was also reported by Jamshidi *et al.,* (2019).

Yoghurt is a dairy product rich in protein, lactose, water-soluble minerals, and vitamins (Ozturkoglu-Budak *et al.,* 2016). Yoghurt can be thick, slightly thick, or liquid in texture. The weakness of yoghurt products is that the binding power of water and whey molecules in casein gel is relatively weak. Therefore, yoghurt with a pH value of 4.7 -5.0 usually has poor quality, resulting in low solubility, low viscosity, and increased syneresis, which affects the final quality of yoghurt products (Annisa and Radiati, 2018). Numerous publications related to yoghurt enrichment and improvement have been carried out, including the addition of salmon oil, nuts, mushrooms white oyster, and spirulina (Estrada *et al.,* 2011; Ozturkoglu-Budak *et al.,* 2016; Anissa and Radiati, 2018; Barkallah *et al.,* 2017). Nevertheless, research on improving yoghurt products added with protein hydrolysate from fish by-products is still limited. Therefore, it was necessary to conduct this study to determine the effect of adding Parrotfish protein hydrolysate (*Chlorurus sordidus*) to yoghurt products.

2. Materials and Methods

2.1. Materials

In this study, the protein hydrolysate was made of the Parrotfish fish (*Chlorurus sordidus*) heads obtained from PT. Alam Jaya, Surabaya, East Java. The yoghurt used was Biokul Plain Stirred Yoghurt (DIAMOND). The materials used for the research were 0.2 N NaOH (German Merck, Grade AR/PA) and Flavorzyme 200,000 AU/g (Novozymes, Novo Alle, DK-2880 Bagsvaerd, Denmark). The equipment used was a digital pH meter (Model PH-9, Biobase, China), viscometer (Model LP-74, High-Performance Rotational Viscometer, Gilson, USA), centrifuge (Model PLC-05, Taiwan), and colour reader (Colorimeter CHN Spec CS-10, China). All materials used in this experiment were of analytical grade and were purchased from Merck (Darmstadt, Germany, USA).

2.2. FPH Preparation

Preparation of Parrotfish protein hydrolysate (*Chlorurus sordidus*) referred to the method of He *et al.* (2013) with some modifications. FPH production was carried out enzymatically using flavourzyme. Briefly, 150 g of finely chopped parrotfish head was mixed with 300 mL of distilled water (1:2). The pH of the sample was adjusted to neutral (pH 7) by adding 0.2 N NaOH solution, then conditioned at 50°C for approximately five minutes.

Flavourzyme (0.2 AU/g) was added as a catalyst to the reaction mixture. The samples were hydrolyzed by incubating at 50°C using an incubator shaker for 0, 3, 6, and 12 hours at 150 rpm. Afterwards, the sample was heated at 90-95 °C for 10 minutes for enzyme inactivation. The next stage was the recovery stage. Samples were centrifuged at 4,500 rpm for 30 minutes to separate layers of different fractions, such as lipid layers, light lipoproteins, soluble proteins, fine particles, and coarse particles. The layer of soluble proteins was separated and would be tested for the degree of hydrolysis (DH). FPH samples with the highest DH value were then dried to obtain FPH in powder using a spray dryer (BUCHI B-290, Labortechnik AG, Flawil, Switzerland) with an inlet temperature of 180 °C, an output temperature of 100 °C, and a flow rate of 40%. The process of making FPH powder from parrot fish head by-product is described in Figure 1. FPH powder was used for the fortification of commercial yoghurt products with various concentrations (A= 0%, B= 0.10%, C= 0.15%, D= 0.2%, E= 0.25%, and F= 0.3%) and subjected to further characterization.

2.3. Degree of Hydrolysis (DH)

The Kjeldahl method was used to measure %N in determining the DH value, according to Hoyle and Merritt (1994). The soluble protein fraction sample was divided into two parts. The first sample (A) was analyzed directly to calculate %N. 2 mL of the second sample (B) was mixed with 2 mL of 20% TCA, and then centrifuged at 4000 rpm for 20 minutes at room temperature. The supernatant collected was used to calculate %N. Finally, the value of the degree of hydrolysis was calculated using the following formula:

$$\text{DH (\%)} = \frac{\%\ \text{Nitrogen B}}{\%\ \text{Nitrogen A}} \times 100 \quad (1)$$

2.4. Analysis Method

2.4.1. pH test

The pH test was carried out using a pH meter based on AOAC (2005). pH test was performed using 10 mL of FPH yoghurt as the sample.

2.4.2. Total titratable acid test

A total titratable acid test was done by referring to Nielsen (2017). Briefly, a total of 10 mL of yoghurt samples was put in a measuring flask and diluted with distilled water. After dilution, 5 mL of the sample was taken and put into a 100 mL Erlenmeyer. Three drops of 1% phenolphthalein indicator were then added. The yoghurt sample was titrated with 0.1 N NaOH until the colour changed to pink. The formula for calculating total titratable acid (%) was as follows:

$$\text{TTA (\%)} = \frac{\text{V NaOH} \times \text{N NaOH} \times \text{MW organic acid}}{\text{V sample}} \times 100 \quad (2)$$

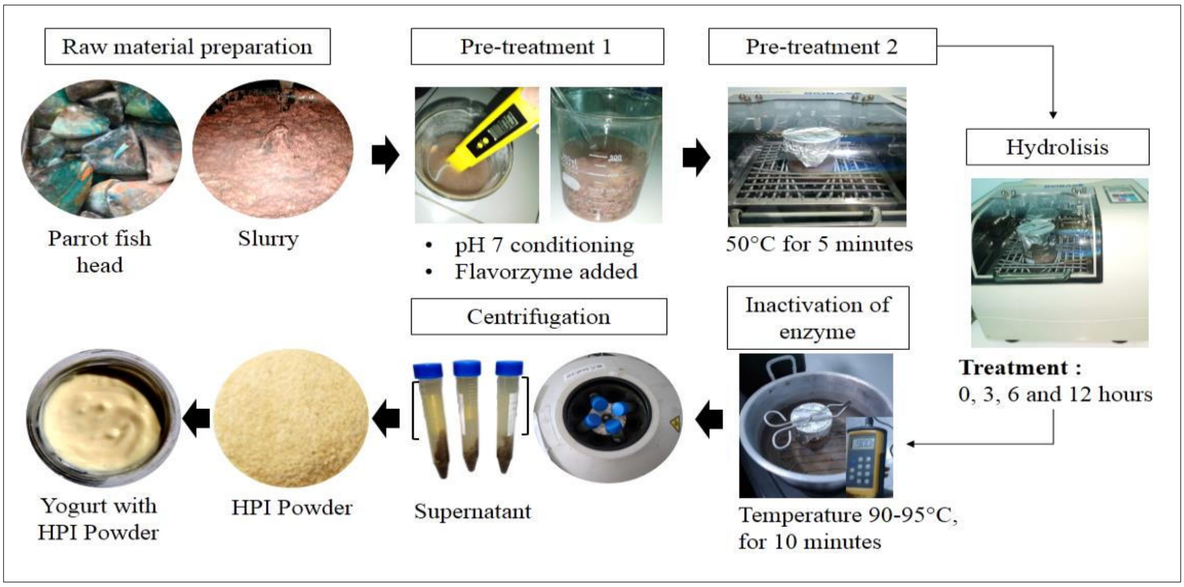

Figure 1. FPH preparation and fortification of yoghurt

2.4.3. Viscosity test

This test aimed to determine the level of yoghurt viscosity using a viscometer (Model LP74, High-Performance Rotational Viscometer) (Usmiati *et al.,* 2022). First, 100 mL of FPH yoghurt was placed into the tester glass. Next, the viscometer spindle was lowered until the yoghurt touched the 'spindle's edge. Finally, the speed was set to 20 rpm. The viscosity level was recorded as a number shown on the viscometer.

2.4.4. Syneresis test

The syneresis test was conducted based on Varnaite *et al.* (2022). This test was carried out by centrifuging a 15 mL yoghurt sample at 2,000 rpm for 20 minutes. After centrifugation, the supernatant was weighed and calculated using the following formula:

$$\text{Syneresis (\%)} = \frac{\text{Supernatant Weight (g)}}{\text{Sample Weight (g)}} \times 100$$

2.4.5. Water holding capacity test

Water holding capacity test referred to Wang *et al.* (2022) with modification. 10 mL of the sample was homogenized using a vortex at room temperature for one minute, then centrifuged at 3.000 rpm for 30 minutes. The pellet or sample residue was weighed. The formula for calculating the water holding capacity (WHC) is as follows:

$$\text{WHC (\%)} = \frac{\text{Weight of sample before centrifugation}}{\text{Weight of sample after centrifugation}} \times 100 \quad (3)$$

2.4.6. Colour test L, a*, b**

Colour testing was carried out using a colour reader (Colorimeter CHN Spec CS-10) (Murda *et al.,* 2021). Measurements were made by placing a yoghurt sample in front of the colour reader sensor. Set the reading button to L*(lightness), a*(redness), and b*(yellowness), and press the target button. The results would come from the reading of the tool and then be recorded.

2.4.7. Organoleptic test

This test used the Hedonic Scale Scoring method, expressing 'panellists' preference level (Putri *et al.,* 2018). This method used a numerical scale, with a score of 1 to 7 (1 = dislike very much, 2 = dislike, 3 = somewhat dislike, 4 = neutral/ordinary, 5 = somewhat like, 6 = like, 7 = very much like) (Lestari and Susilawati, 2015). The test parameters included appearance (colour), aroma (specific aroma of fish, aroma of fermented milk, sour aroma), texture (thickness), and taste (typical taste of fish, taste of milk, sour taste of yoghurt, and sweet taste of yoghurt).

2.5. Data analysis

All data were analyzed by using SPSS Statistical software. The data obtained were analyzed through a one-way analysis of variance (ANOVA), followed by DMRT (Duncan Multiple Range Test) significance level of $\alpha=0.05$. Data were presented as the mean from the results of five independent experiments ± SD. The organoleptic test data were analyzed using the Kruskal Wallis (Non-Parametric) test to determine the best treatment using the effective index method (De Garmo, Sullivan and Canada, 1984).

3. Results and Discussions

3.1. Degree of Hydrolysis (DH) of FPH

The results showed that the degree of hydrolysis of Parrotfish fish protein hydrolysate ranged from 2.13 to 36.51% (Figure 2). The highest average was obtained at the incubation or hydrolysis duration of 12 hours at 36.51 ± 0.7%. The FPH with the highest degree of hydrolysis result was used for further analysis.

3.2. Physical Characteristics of Enriched Yogurt

The results showed that the addition of hydrolyzed parrot fish protein powder had a significant effect ($p<0.05$) on the viscosity, syneresis, Water Holding Capacity (WHC) and colour values of the yoghurt product enriched with FPH parrotfish (*Chlorurus sordidus*). The physical characteristics of FPH-enriched yoghurt are presented in Table 1.

3.3. Chemical Characteristics of Enriched Yogurt

The results showed that the addition of parrotfish protein hydrolysate powder had a significant effect ($p<0.05$) on the pH and TTA values of yoghurt (Table 2).

3.4. Sensory Characteristics of Enriched Yogurt

The addition of FPH Parrotfish powder had a significant effect ($p<0.05$) on the appearance, aroma, and taste values but had no significant effect ($p>0.05$) on the texture value (Table 3). The appearance of yogurt with the addition of FPH Parrotfish powder for each treatment can be seen in Figure 3.

3.5. Discussion

Based on the results, the longer the incubation time, the higher the degree of hydrolysis. A longer hydrolysis process increases the number of peptides or small proteins (Hau *et al*., 2018). The degrees of hydrolysis (DH) of protein could be influenced by the type of protease used, enzyme concentration, temperature, pH, and hydrolysis time (Restiani, 2017). Slizyte *et al*. (2016) mentioned that the peptide bond cleavage was more active at a longer incubation time and produced large amounts of essential amino acids dissolved in TCA. In addition, large amounts of amino acids were also associated with a longer contact time between the catalytic enzyme and fish substrate at optimal enzyme conditions (Ariyani *et al.,* 2017).

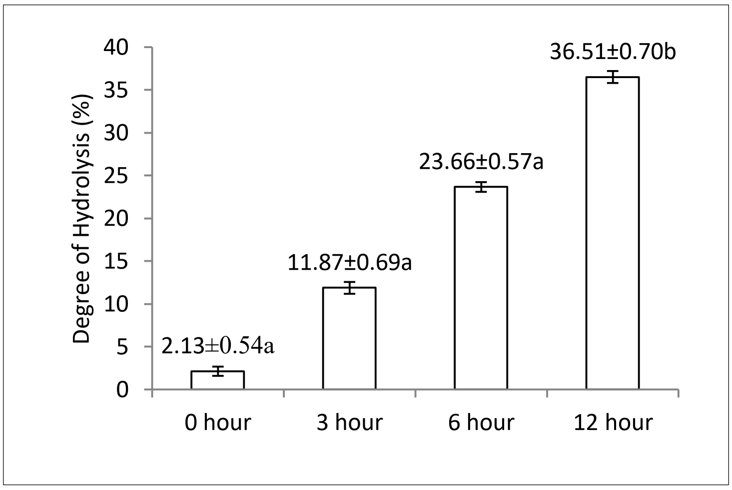

Figure 2. Degree of hydrolysis of FPH from parrotfish head at various incubation times.

Table 1. The physical characteristics of yoghurt enriched with FPH parrotfish

Code	Physical characteristics					
	Viscosity ($N{\bullet}s/m^2$)	Syneresis (%)	WHC (%)	Colour		
				L*	a*	b*
A	2.231±6.658^{a}	53.53±0.030^{d}	34.50±1.353^{a}	86.18±0.272^{c}	-3.13±0.206	7.06±0.071^{a}
B	2.330±3.215^{ab}	52.80±0.557^{d}	34.57±1.002^{a}	75.61±2.199^{b}	-4.46±1.991	7.79±0.636^{ab}
C	2.432±4.509^{c}	49.67±1.528^{c}	37.40±0.700^{b}	74.55±3.006^{b}	-3.76±0.485	8.20±0.478^{abc}
D	2.389±78.409^{b}	44.13±1.343^{b}	41.33±1.201^{c}	72.64±2.507^{b}	-4.78±0.326	8.81±0.082^{bc}
E	2.487±10.263^{d}	45.63±1.069^{b}	43.17±0.513^{d}	68.88±0.742^{a}	-5.60±0.522	9.53±0.631^{cd}
F	2.524±12.097^{e}	30.60±0.964^{a}	43.50±0.436^{d}	66.63±1.106^{a}	-5.47±0.979	10.86±1.557^{d}

Note: FPH concentration; A=0%, B=0.1%, C=0.15%, D=0.2%, E=0.25, F=3%. WHC= Water Holding Capacity. L*(lightness), a*(redness), b*(yellowness). Data were presented as mean±S.D. Different letters showed a significant difference between treatments.

Table 2. The Chemical characteristics of yoghurt enriched with FPH parrotfish

Code	Chemical Parameters	
	pH	TTA
A	4.31±0.015^{a}	0.78±0.030^{c}
B	4.33±0.015^{ab}	0.77±0.015^{c}
C	4.36±0.010^{b}	0.76±0.015^{bc}
D	4.40±0.010^{c}	0.71±0.061^{ab}
E	4.45±0.030^{d}	0.69±0.010^{a}
F	4.50±0.015^{e}	0.67±0.015^{a}

Note: FPH concentration; A=0%, B=0.1%, C=0.15%, D=0.2%, E=0.25, F=3%. TTA= Total Titratable Acid. Data were presented as mean±S.D. Different letters showed a significant difference between treatments.

Table 3. Organoleptic of yoghurt enriched with FPH parrotfish

Code	Organoleptic Test			
	Appearance	Aroma	Flavour	Texture
A	5.78±0.185^{ab}	5.47±0.188^{bc}	5.63±0.119^{c}	5.10±0.135^{a}
B	5.67±0.035^{ab}	5.59±0.051^{c}	5.27±0.142^{b}	5.09±0.150^{a}
C	5.8±0.030^{b}	5.33±0.035^{b}	5.39±0.051^{c}	5.23±0.115^{a}
D	6.03±0.035^{c}	5.21±0.101^{b}	5.52±0.068^{c}	5.14±0.191^{a}
E	5.54±0.103^{a}	5.13±0.035^{b}	5.06±0.081^{b}	5.06±0.511^{a}
F	5.28±0.017^{a}	4.76±0.081^{a}	4.61±0.185^{a}	5.26±0.103^{a}

Note: FPH concentration; A=0%, B=0.1%, C=0.15%, D=0.2%, E=0.25, F=3%. Data were presented as mean±S.D.

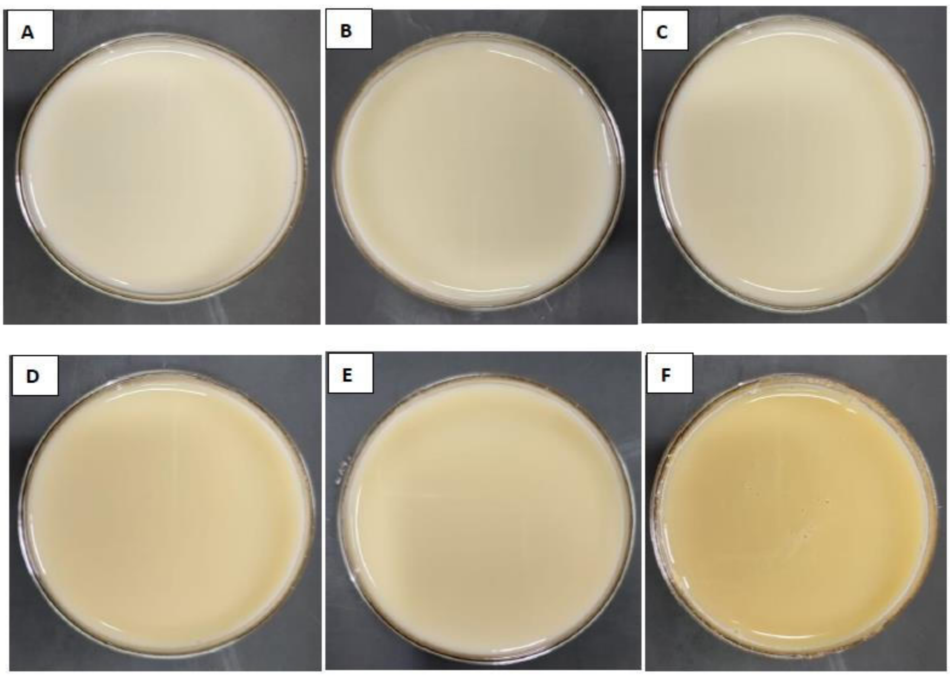

Figure 3. Yoghurt enriched with various concentrations of parrotfish FPH

The viscosity of yoghurt enriched with various concentrations of Parrotfish FPH ranged from 2.231 to 2.524 (N•s/m^2). The highest viscosity was obtained in sample F, with the parrotfish FPH concentration of 0.3%. The average viscosity of yoghurt in this study was similar to the results of a previous study by Chen *et al.* (2018) at 2.230– 2.900 (N•s/m^2). Based on the results, adding Parrotfish protein hydrolysate powder can increase the viscosity of yoghurt. It might be due to the increase in total solids in yoghurt, so the texture becomes more compact and thicker. As explained by Unnikrishnan *et al.* (2019) and Dibyanti *et al.* (2014), the increase in viscosity was directly proportional to the amount of protein and the total solids contained, and the ability to bind water (WHC). Yoghurt viscosity increased due to the formation of lumps or curds of fat globules due to the acidic atmosphere and low temperature. Sakul *et al.* (2020) state that these clots result from denatured proteins, so coagulation occurs in milk proteins. Hau *et al.* (2020) showed that the viscosity of mayonnaise fortified with yellowfin tuna protein hydrolysate was higher than the control. It might be due to the interaction between lipophilic and hydrophobic groups (peptide chains) with proteins in mayonnaise (Unnikrishnan *et al.,* 2019). Enhancing yoghurt with fibre enrichment components increased water retention ability and thickness (Marand *et al.,* 2020).

The results showed that the syneresis of yoghurt with the addition of various concentrations of FPH Parrotfish ranged from 30.6 to 53.53%. The lowest value was obtained in sample F (0.3% FPH). The average syneresis of yoghurt in this study was similar to the results of previous studies by Kwon *et al.* (2019) (30.40% – 36.47%) and Ahmed *et al.* (2021) (48% - 53%). Syneresis occurs because changes in the structure of the associated protein network result in a weak binding to curd protein so that it is released to the surface of yoghurt (Bahrami *et al.,* 2013). The results showed that Parrotfish protein hydrolysate powder added to yoghurt could reduce syneresis. It may be due to an increase in protein, fat and total solids levels, thereby improving the microstructure of yoghurt. In line with the statement of Delikanli and Ozkan (2016), yoghurt with protein enrichment can increase the number of bonds between proteins, resulting in a denser network due to an increase in solids in the protein matrix in the yoghurt gel microstructure. Ahmed *et al.* (2021) stated that yoghurt added with Argel leaf extract showed an interaction of Argel leaf content (polyphenols) with yoghurt protein, which made the yoghurt gel matrix firmer and increased the defence against curd release.

The range of water holding capacity (%) of yoghurt with the addition of various concentrations of FPH Parrotfish was from 34.50 to 43.50. The highest WHC was obtained in sample F, with a concentration of 0.3%. Similarly, Ozturkoglu-Budak *et al.* (2016) reported the average water-holding capacity of yoghurt, ranging from 35.57% to 48.97%. WHC is defined as the ability of a food component to bind water. In this study, it was found that adding Parrotfish protein hydrolysate powder could increase the water-binding capacity of yoghurt. It might be because fish protein hydrolysate contains peptide side chains with hydrophilic groups that are polar or can bind to water. Nurdiani *et al.,* (2016) and Benjakul *et al.* (2014), explained that the high solubility of fish protein hydrolysate was due to the opening of protein molecules during hydrolysis to produce peptides with amino and carboxyl groups. Lima *et al.* (2021) stated that ionic and dipole interactions relate to water retention or holding capacity. This might be due to the 'protein's hydrolyzed amino acid profile, which has a dominant hydrophilic amino acid (57.4%) and a negatively charged amino acid (64.4%). In addition, there might be a contribution from hydrogen bonding and dipole-dipole interactions. Bahrami *et al.* (2013) stated that the increase in water-holding capacity in yoghurt caused by hydrocolloids occurred in two ways: physically and chemically. Physically, water was trapped in increasing the protein 'network's density. At the same time, chemically, the hydrophilic nature of hydrocolloids facilitated the association of proteins with water molecules, thereby increasing the water-holding capacity of the gel.

The Commission Internationale de l'Eclairage (CIE) determined the colour measurement using L*, a*, b* codes. The value of L* (brightness) was ranged from 100 (white) to 0 (black). The a* value indicated a reddish (+) or greenish (-) colour, while colour b* indicated a yellowish (+) or bluish (-) (Wrolstad and Smith 2017). The results showed that the colour L* value of yoghurt with the addition of various concentrations of FPH Parrotfish ranged from 66.63 to 86.18. The lowest L* value was obtained in sample F (0.3% FPH). The average L* value in this study was not significantly different from the average L* colour of yoghurt in the study of Tamjidi *et al.* (2012), which was 89.75 – 91.50. The colour a* of yoghurt was obtained between 3.13 and -5.60. The lowest a* value was obtained in sample E (0.25% FPH). This study's average a* value was similar to other studies, i.e., -1.69 to -2.94 (Tamjidi *et al.,* 2012) and -4.61 to -5.14 (Sjaznar *et al.,* 2018). The value b* of yoghurt was obtained between 7.06 and 10.86. The lowest value was obtained in sample F. The average b* value in this study was similar to Raikos *et al.* (2018) of 7.03 – 7.76.

The colour of yoghurt with the addition of fish protein hydrolysate powder gives a darker, greenish, and yellowish colour. It may be influenced by genetics or the pigment possessed by the fish used as raw material. According to Madora *et al.* (2016), the colour of yoghurt decreased in brightness when carrot powder was added, as carrot powder contains carotenoid pigments, resulting in a darker colour in yoghurt. The greenish colour of yoghurt in this study could be derived from the turquoise pigment in the skin of the parrotfish head. Taheri *et al.* (2013) explained that the yellowish colour comes from fish protein hydrolysate powder because it contains higher haemoglobin, myoglobin, and brown pigment. The pigment resulted from aldol condensation of carboxyl groups produced from lipid oxidation in the reaction of protein groups.

The pH range of yoghurt with the addition of various concentrations of FPH Parrotfish was 4.31 to 4.5. The highest pH was obtained in sample F, with an FPH Parrotfish concentration of 0.3%. These results were still within the quality standard of yoghurt according to SNI 01-2981-1992, which is in the range of 4.1 – 4.5. The increased pH of yoghurt might be influenced by the addition of various concentrations of FPH Parrotfish, which has a pH of 7 or neutral. According to Unnikrishnan *et al.* (2019), the product will experience an increase in pH if protein hydrolysate is added. In general, the pH of a protein hydrolysate is around 6.24 or close to neutral. A decrease in total acid might also cause an increase in yoghurt pH. Hermiastuti *et al.* (2013) stated that the amino acids in fish protein hydrolysate are neutral in pH. Amino acids with a dipolar structure contain one carboxyl group (negatively charged, acidic) and one amino group (positively charged, primary). Throughout the storage period, a consistent reduction in pH levels was observed across all yoghurt variants, predominantly attributable to the generation of microbial metabolites (Lima *et al.,* 2021).

TTA (%) of yoghurt with the addition of various concentrations of FPH Parrotfish ranged from 0.67 to 0.78. The lowest TTA value was obtained in sample F, with an FPH Parrotfish concentration of 0.3%. The results were still within the quality standard of yoghurt acid according to SNI 01-2981-1992, which is 0.5 - 2.0%. The decrease in TTA as the FPH was added might be due to the decline in the number of hydrogen ions in yoghurt (Anissa and Radiati, 2018).

The yoghurt appearance score with the addition of various concentrations of FPH Parrotfish ranged from 5.28 to 6.03. The highest value was obtained in sample D, with an FPH Parrotfish concentration of 0.2%. The highest hedonic score was 6.03, which means that the panellists liked the appearance of yoghurt with the addition of 0.2% Parrotfish protein hydrolysate powder or accepted by consumers. Panellists prefer ivory white or milky white colour given by FPH Parrotfish powder to yoghurt. The lowest F score was because the panellists thought the yoghurt colour was too yellow. In this study, FPH Parrotfish powder's brown-yellow colour was caused by the spray-dry method in the production process.

The score of yoghurt aroma ranged from 4.76 to 5.59. The highest average was obtained in sample B with the FPH parrotfish concentration of 0.1%. It means that the panellists preferred the smell of yoghurt with the least addition of FPH parrotfish powder (0.1%). The higher the FPH parrotfish powder concentration and the yoghurt's aroma will be pungent. Tamjidi *et al.* (2012) stated that panellists preferred yoghurt with the slightest fishy aroma or still smells typical of yoghurt. According to Junianto *et al.* (2019), the fishy smell in FPH was caused by chemical compounds such as dipeptides and other amine compounds. Adding FPH Parrotfish powder caused a fishy aroma that was not strong in yoghurt. The weak fishy odour detected by the panellists was probably due to the low-fat content in fish raw materials (Bernadeta, 2012).

The yoghurt taste score was from 4.61 to 5.52. The highest score was obtained in sample D with the FPH Parrotfish concentration of 0.2%. In this study, yoghurt with FPH Parrotfish powder had a characteristic sour taste even though it had a slightly bitter taste at higher FPH powder concentrations. The atmosphere or sour taste of yoghurt was due to the lactose metabolism by lactic acid bacteria, where the role of *Lactobacillus bulgaricus* gave a sharp, distinctive flavour to yoghurt. According to Benjakul *et al.* (2014), 'FPH's bitter taste was caused by the formation of peptides containing hydrophobic groups in the amino acid chain. Compounds that could cause a bitter taste include valine, glutamic acid, polyphosphate, and glycine. The level of bitterness in fish protein hydrolysate was significantly influenced by the type of protease enzyme added during hydrolysis. Flavorzyme could minimize bitterness in fish protein hydrolysate by removing terminal hydrophobic amino acids (Dauksas *et al.,* 2008).

The yoghurt flavour score with the addition of various concentrations of FPH Parrotfish ranged from 5.06 to 5.26. The highest value was obtained in sample F, with a concentration of 0.3%. The change in texture was due to the loss of water or fat content, the breakdown of emulsion, and the hydrolysis of proteins and carbohydrates. Yoghurt would be thicker if more FPH Parrotfish powder was added. Asare *et al.* (2018) explained that texture was influenced by the proximate content (fat, protein, moisture, and carbohydrates) of fish protein hydrolysate.

The results of the optimization calculation showed that the best treatment was the addition of 0.15% parrotfish FPH. Generally, the quality of yoghurt produced with a 0.15% concentration of fish protein hydrolysate powder met the established standards for yoghurt. According to the Indonesian National Standard (SNI, 01-2981-1992), the properties of yoghurt should meet the following criteria: pH 4.1-4.5, TTA 0.5-2.0%, and sensory properties typical of fermented milk.

4. Conclusions

The addition of FPH Parrotfish (*Chlorurus sordidus*) powder with different concentrations significantly affected the characteristics of yoghurt (pH, total acid, viscosity, syneresis, water holding capacity, L* colour, b* colour, hedonic parameters appearance, aroma, and taste). The best treatment for all parameters was the 0.15% concentration of Parrotfish fish protein hydrolysate.

5. References

Ahmed, I.A.M., Alqah, H.A., Saleh, A., Al-Juhaimi, F.Y., Babiker, E.E., Ghafoor, K., Hassan, A.B., Osman, M.A., Fickak A. (2021). Physicochemical quality attributes and antioxidant properties of set-type yoghurt fortified with argel (*Solenostemma argel* Hayne) leaf extract. *Food Science and Technology,* 137, 110389. https://doi.org/10.1016/j.lwt.2020.110389

Anissa, D., Radiati, L.E. (2018). The effect of adding white oyster mushroom extract (*Pleurotus ostreatus*) on the production of yoghurt drink in terms of physical quality. *Journal Animal Production of Science Technology,* 13(2), 118125. https:// doi.org /10.21776/ub.jitek.2018.013.02.6

Ariyani, F., Saleh, M., Tazwir, T., Hak, N. (2017). Optimasi Proses Produksi Hidrolisat

Protein Ikan dari Mujair (*Oreochromis mossambicus*). *Jurnal Pasca Panen dan Bioteknologi*, 9(5), 11-21.

Asare, S.N., Ijong, F.G.I., Rieuwpassa, F.J., Setiawati, N.P. (2018). Penambahan Hidrolisat Protein Ikan Lemuru (*Sardinella lemuru*) Pada Pembuatan Biskuit. *Jurnal Ilmiah Tindalung,* 4(1), 10-18.

Bahrami, M., Ahmadi, D., Alizadeh, M., Hosseini, F. (2013). Physicochemical and sensorial properties of probiotic yoghurt as affected by additions of different types of hydrocolloid. *Food Science of Animal Resources*, 33(3), 363-368.

Barkallah, M., Dammak, M., Louati, I., Hentati, F., Hadrich, B., Mechichi, T., Ayadi, M.A., Fendri, I., Attia, H., Abdelkafi, S. (2017). Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yoghurt during fermentation and storage. *Food Science and Technology,* 84(1), 323-30.

Batista, I., Ramos, C., Coutinho, J., Bandarra, N., Nunes, M. (2010). Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) by-products and antioxidative activity of the hydrolysates produced. *Process Biochemistry*, 45(1), 18–24.

Benhabiles, M.S., Abdi, N., Drouiche, N., Lounici, H., Pauss, A., Goosen, M.F., Mameri, N. (2012). Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit. *Materials Science and Engineering*, 32 (4), 922-8.

Benjakul, S., Yarnpakdee, S., Senphan, T., Halldorsdottir, S.M., Kristinsson, H.G. (2014). Fish protein hydrolysates: production bioactivities and applications. *Antioxidants and functional components in aquatic foods,* 237-281.

Bernadeta, P.A. (2012). Penentuan kondisi optimum hidrolisat protein dari limbah ikan ekor kuning (*Caesio cuning* berdasarkan karakteristik organoleptik. *Jurnal Kimia Khatulistiwa*, 1(1), 26-30.

Chalamaiah, M., Hemalatha, R., Jyothirmayi, T. (2012). Fish protein hydrolysates: proximate composition amino acid composition antioxidant activities and applications: A review. *Food Chemistry,* 135(4), 3020-3038.

Chen, X., Singh, M., Bhargava, K., Ramanathan, R. (2018). Yoghurt fortification with chickpea (*Cicer arietinum*) flour: physicochemical and sensory effects. *Journal of The American Oil Chemists Society,* 95(8), 1041-1048.

Dauksas, E., Slizyte, R., Rustad, T., Storro, I. (2008). Bitterness in fish protein hydrolysates and methods for removal. *Journal of Aquatic Food Product Technology,* 13(2), 101-114.

Delikanli, B., Ozcan, T. (2017). Improving the textural properties of yoghurt fortified with milk proteins. *Journal of Food Processing and Preservation,* 41(5), 13101.

Dibyanti, P., Radiati, L.E., Rosyidi, D. (2014). Effect of addition of various concentrations of culture incubation period on pH acidity levels viscosity syneresis set yoghurt. *Jurnal Ilmu Ternak,* 1-6.

Dong, S., Zeng, M., Wang, D., Liu, Z., Zhao, Y., Yang, H. (2008). Antioxidant and biochemical properties of protein hydrolysates prepared from Silver carp (*Hypophthalmichthys molitrix*). *Food Chemistry,* 107(4), 1485-93.

Estrada, J.D., Boeneke, C., Bechtel, P., Sathivel, S. (2011). Developing a strawberry yoghurt fortified with marine fish oil. *Journal of Dairy Science,* 94(12), 5760-9.

Harms-Tuohy, C.A. (2021). Parrotfishes in the Caribbean: a regional review with recommendations for management. FAO Fish Aqua Circ Rome.

Hau, E., Amiza, M., Mohd-Zin, Z., Shaharudin, N., Zainol, M. (2020). Effect of yellow stripe scad (*Selaroides leptolepis*) protein hydrolysate in the reduction of oil uptake in deep-fried squid. *Food Research*, 4, 1929-1936.

Hau, E., Mohd-Zin, Z., Zuraidah, N., Shaharudin, N.A., Zainol, M.K. (2018). Physicochemical properties of powdered protein hydrolysate from Yellowstripe scad (*Selaroides leptolepis*) fish. *International Food Research Journal,* 25(6), 2553-2559.

He, S., Franco, C., Zhang, W. (2013). Functions applications and production of protein hydrolysates from fish processing co-products (FPCP). *Food Research International,* 50(1), 289-297.

Hermiastuti, M. (2013). Analisis Kadar Protein dan Identifikasi Asam Amino pada Ikan Patin (*Pangasius djambal*). Faculty of Mathematics and Natural Sciences Universitas Jember.

Hoyle, N.T., Merritt, J.H. (1994). Quality of fish-protein hydrolysates from herring (*Clupea harengus*). *Journal of Food Science,* 59 (1):76-79.

Idowu, A.T., Benjakul, S., Sinthusamran, S., Sookchoo, P., Kishimura, H. (2018). Protein hydrolysate from salmon frames: Production characteristics and antioxidative activity. *Journal of Food Biochemistry*, 43(2), 12734.

Jamshidi, A., Shabanpour, B., Pourashouri, P., Raeisi, M. (2019). Optimization of encapsulation of fish protein hydrolysate and fish oil in W1/O/W2 double emulsion: Evaluation of sensory quality of fortified yoghurt. *Journal of Food Processing and Preservation,* 43(9), 14063.

Junianto, J., Khan, A.M., Rostini, I. (2019). Protein fortification in melarat crackers with protein hidrolyzate flour of tilapia (*Oreochromis niloticus*) Meat. *Jurnal Pengolahan Hasil Perikanan,* 22(1), 111-118.

Kristinsson, H.G., Rasco, B.A. (2000). Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle proteins hydrolyzed with various alkaline proteases. *Journal of Agricultural and Food Chemistry,* 48(3), 657-66.

Kwon, H.C., Bae, H., Seo, H.G., Han, S.G. (2019). Chia seed extract enhances physiochemical and antioxidant properties of yogurt. *Journal of Dairy Science,* 102(6), 4870-4876.

Lestari, S., Susilawati, P.N. (2015). Organoleptic test ofwet noodles made from beneng taro flour (*Xantoshoma undipes*) in an effort to increase local value added food ingredients in Banten. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia,* 1(4), 941-946.

Lima, K.O., da Rocha, M., Alemán, A., López-Caballero, M.E., Tovar, C.A., Gómez-Guillén, M.C., Montero, P., Prentice, C. (2021). Yoghurt Fortification by the Addition of Microencapsulated Stripped Weakfish (*Cynoscion guatucupa*) Protein Hydrolysate. *Antioxidants Journal,* 10(10), 1-19.

Ma, Y. S., Zhao, H. J., Zhao, X. H. (2019). Comparison of the effects of the alcalase-hydrolysates of caseinate, and of fish and bovine gelatins on the acidification and textural features of set-style skimmed yogurt-type products. *Foods*, 8(10), 501.

Madora, E.P., Takalani, T.K., Mashau, M.E. (2016). Physicochemical, microbiological and sensory properties of low fat yoghurt fortified with carrot powder. *Interntaional Journal of Agricultural and Biological Engineering,* 9(1), 118-24.

Marand, M. A., Amjadi, S., Marand, M. A., Roufegarinejad, L., Jafari, S. M. (2020). Fortification of yogurt with flaxseed powder and evaluation of its fatty acid profile, physicochemical, antioxidant, and sensory properties. *Powder Technology*, 359, 76-84.

Murda, R.A., Maulana, S., Mangurai, S.U., Augustina, S., Bindar. Y. (2021). Wettability Properties of Heat-Treated Oil Palm Trunk Under Various Heating Times. *IOP Conference Series: Earth and Environmental Science,* 830(1), 012011.

Nielsen, S.S. (2017). Food analysis laboratory manual: Standard solutions and titratable acidity, Chapter 21. *Springer,* 179–184.

Nurdiani, R., Dissanayake, M., Street, W.E., Donkor, O.N., Singh, T.K., Vasiljevic, T. (2016). In vitro study of selected physiological and Physicochemical properties of fish protein hydrolysates from 4 Australian fish species. *International Food Research Journal,* 23 (5): 2029-2040.

Ozturkoglu-Budak, S., Akal, C., Yetisemiyen, A. (2016). Effect of dried nut fortification on functional Physicochemical textural and microbiological properties of yoghurt. *Journal of Dairy Science,* 99(11), 8511-8523.

Petrova, I., Tolstorebrov I., Eikevik, T.M. (2018). Production of fish protein hydrolysates step by step: technological aspects equipment used major energy costs and methods of their minimizing. *International Aquatic Research,* 10(3), 223-241.

Prihanto, A.A., Nurdiani, R., Bagus, A.D. (2019). Production and characteristics of fish protein hydrolysate from parrotfish (*Chlorurus sordidus*) head. *PeerJ*, 7, 1-16.

Putri, R. M. S., Hermiza, M. (2018). Uji Hedonik Biskuit Cangkang Kerang Simping (*Placuna placenta*) dari Perairan Indragiri Hilir. *Jurnal Teknologi Pertanian*, 7 (2): 11-29.

Raikos, V., S. B. Grant, H. Hayes, and V. Ranawana. (2018). Use of β-glucan from spent 'brewer's yeast as a thickener in skimmed yogurt: Physicochemical, textural, and structural properties related to sensory perception. *Journal of Dairy Science,* 101(7), 5821-5831.

Restiani, R. (2017). Hidrolisis Secara Enzimatis Protein Bungkil Biji Nyamplung (*Calophyllum inophyllum*) Menggunakan Bromelain. *Biota: Scientific Journal of Life Sciences,* 1(3), 103-110.

Sakul, S., Rosyidi, D., Radiati, L.E., Purwadi, P. (2020). The effect of different starter cultures on the fermentation of yoghurt added with aqueous extract of white oyster mushroom (*Pleurotus ostreatus*). *Jurnal Ilmu Teknologi Hasil Ternak,* 15(1), 46-51.

Saputra, D., Nurhayati, T. (2016). Production of fish hydrolysates protein from waste of fish carp (*Cyprinus carpio*) by enzymatic hydrolysis. *Comtech: Computer, Mathematics and Engineering Applications,* 7(1), 11-8.

Siddik, M.A.B., Howieson, J., Fotedar, R., Partridge, G.J. (2020). Enzymatic fish protein hydrolysates in finfish aquaculture: A review. *Reviews in Aquaculture,* 13(1), 406-430.

Silovs, M. (2018). Fish processing by-products exploitation and innovative fish-based food production. *Economics*, 2, 210-215.

Slizyte, R., Rommi, K., Mozuraityte, R., Eck, P., Five, K., Rustad, T. (2016). Bioactivities of fish protein hydrolysates from defatted salmon backbones. *Biotechnology Report,* 11, 99-109.

SNI (Standar Nasional Indonesia). (1992). Badan Standar Nasional SNI 01-2891-1992. Cara Uji Makanan dan. Minuman. Badan Standarisasi Nasional.

Taheri, A., Anvar, S. A. A., Ahari, H. & Fogliano, V. (2013). Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (*Onchorhynchus mykiss*) viscera. *Iranian Journal of Fisheries Sciences*, 12(1), 154-169.

Tamjidi, F., Nasirpour, A. Shahedi, M. (2012). Physicochemical and sensory properties of yoghurt enriched with microencapsulated fish oil. *Food Science and Technology International*, 18(4), 381–390.

Thiansilakul, Y., Benjakul, S., Shahidi, F. (2007). Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. *Journal of Food Biochemical,* 31(2), 266-87.

Tridge. 2021. Parrotfish. https://www.tridge.com/intelligences/parrot-fish1/ID.

Unnikrishnan, P., Kizhakkethil, B.P., Jadhav, M.A., Sivam, V., Ashraf, P.M., Ninan, G., Abubacker, Z.A. (2019). Protein hydrolysate from yellowfin tuna red meat as a fortifying and stabilizing agent in mayonnaise. *Journal of Food Science and Technology*, 57(2), 413-425.

Usmiati, S., Budiyanto, A., Yuliana, T. (2022). Optimizing Skim Milk Concentration and Type of Starters in The Processing of a Whey Probiotic Drink. *IOP Conference Series: Earth and Environmental Science*, 1024(1), 012013.

Varnaitė, L., Keršienė, M., Šipailienė, A., Kazernavičiūtė, R., Venskutonis, P.R., Leskauskaitė, D. (2022). Fiber-rich cranberry pomace as food ingredient with functional activity for yogurt production. *Foods*, 11(5), 758.

Wang, J., Zhang, H., Xie, J., Yu, W., Sun, Y. (2022). Effects of frozen storage temperature on water-holding capacity and physicochemical properties of muscles in different parts of bluefin tuna. *Foods*, 11, 2315.

Wrolstad, R.E., Smith, D.E. (2017). Color analysis: Food analysis. *Springer*, Chamical.

Acknowledgment

The authors thank the Ministry of Research, Technology, and Higher Education and Universitas Brawijaya for the research funding provided through the "Hibah Doktor Non Lektor Kepala" Scheme (Contract Number: 2446/UN10.F06/PP/2022).