Research Article

Effect of nanoemulsion-fish protein hydrolysate supplementation on selected physicochemical parameters of yogurt

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Abstract

The aim of the present study was to evaluate the physico-chemical criteria of yogurt fortified with fish protein hydrolysate (FPH) obtained from Fresh Abu mullet (*Planiliza abu*) fish weighing 40 g during 21-day refrigeration. A hundred milliliters of final milk were inoculated with starter culture, and fermented for 4-5 hours until the pH reached to 4.6. The yogurts were divided in two groups in triplicate and each group was prepared in triplicate. Yogurts supplemented with nanoencapsulated hydrolysates exhibited a slight reduction in pH and augmented acidity particularly up to three weeks of refrigeration. The pH of nanoemulsion-FPH yogurt was initially 4.52 and reached 4.01 in third week with a significant difference ($p<0.05$) compared with that of the control at the same time (3.80). The pH value of the fortified yogurt showed acceptable limit on day 7 (4.35) but it was remarkably decreased on day 14 (4.19, $p<0.5$). The upmost and the least values of viscosity of nano-FPH yogurt samples were respectively 4187.3 and 4046.6 (cps) on days 1 and 21. The viscosity values of control were ranged from 3716.0 to 4042.0, respectively in 21 and 1 days of refrigeration. Moreover, the maximum and minimum water holding capacity (WHC) value of nano-FPH yogurt samples 92.5% and 86.2%, respectively on days 1 and 21. It is concluded that the incorporation of the FPH in the form of nanoencapsulation offered superior physico-chemical advantages than those of control yogurt samples.

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Keywords: Yogurt, Nanoemulsion, Fish protein hydrolysate, Physico-Chemical parameters

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Introduction

The rising demand for healthy food has led to the emergence of new kinds of foods and products on the market that, that not only provide necessary nutrition, but also promote well-being and reduce the risk of certain diseases (Bayarri *et al.*, 2010). Milk and its dairy products such as yogurt, are widely consumed globally due of their taste and functional criteria (Jaster *et al.*, 2018). However, despite the nutritional benefits of yogurt, it is generally not considered to be a significant source of bioactive food (Ozturkoglu-Budak *et al.*, 2016). Thus, compensatory studies have been conducted in recent decades to enhance the nutritional value of yogurt. Several studies have investigated yogurt fortified with bioactive compounds, such as Microencapsulated Stripped Weakfish (*Cynoscion guatucupa*) protein hydrolysate (Lima *et al.*, 2019), sturgeon gelatin hydrolysates (Gheshlaghi *et al.*, 2021), fish collagen-derived bioactive proteins (Ayati *et al.*, 2022), Cod (*Gadus morhua*) protein hydrolysates (Farvin *et al.*, 2014), date palm pollen (*Phoenix dactylifera* L.) encapsulation (El-Kholy *et al.*, 2019), carrot powder (Madora *et al.*, 2016) and psyllium (*Plantago ovate*) husk (Bhat *et al.*, 2018). Selection of yogurt for functionality food could be due to that people of all ages extensively agree to take and consume yogurt daily. Consumers are increasingly interested in food products that make health claims beyond the provision of essential nutrients. These functional foods are believed to possess bioactive compounds that can confer health benefits beyond basic nutrition (Ahmad *et al.*, 2022). Scientific studies have shown that certain functional foods can improve physiological functions and reduce the risk of chronic diseases. As a result, the demand for such products has been growing steadily (Mousavi *et al.*, 2019; Agarry *et al.*, 2023). Such products are believed to contribute to reduced morbidity and mortality rates and improved quality of life among the general population. Scientific evidence suggests that the consumption of these functional foods can confer health benefits beyond those provided by essential nutrients alone. Therefore, their inclusion in the diet can be an effective strategy for optimizing overall health and well-being (Jones & Jew, 2007). Compatibly, proteins derived from fish Abu mullet characterize a very demanding source of bioactive peptides due to its low cost and the recognized requirement of dropping agro-industrial waste (Hemker *et al.*, 2020). Fish protein hydrolysates (FPH) are a rich source of peptides that exhibit strong antihypertensive, antioxidative, and anticancer activities (Ishak & Sarbon, 2018). Seafood protein hydrolysates containing low weight peptides have been shown to enhance various biological functions such as immunomodulatory activities (Gheshlaghi *et al.*, 2021). In addition, antioxidants action of FPH can also postpone and reduce reactive oxygen species (ROS), which increased in many stress and diseases, resulting in hypertension and aging (Nimse & Pal, 2015). According to the afore-mentioned findings, the objective of this study was to assess the physico-chemical properties of yogurt

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fortified with fish protein hydrolysate (FPH) obtained from Fresh Abu mullet (*Planiliza abu*) fish during 21-day refrigeration. The study aimed to compare the properties of yogurt supplemented with nanoencapsulated FPH and control yogurt samples. The parameters evaluated included pH, acidity, viscosity, and water holding capacity. By investigating the effects of FPH fortification on the physico-chemical properties of yogurt, this study aimed to provide insights into the potential applications of FPH as a functional ingredient in dairy products.

Materials and methods

Materials

Fresh Abu mullet (*Planiliza abu*) fish of 40 g for each, a species of rivers of South of Iran (Jorfipour *et al.*, 2022), were prepared from a fishing company, transferred to the lab with enough ice, washed with tap water and the carcasses and trimmings were treated in a meat–bone separator (High Tech, Brazil) and ultimately discarding the skin and bones. They were mixed and stored at refrigerator until use. Flavourzyme ® 1000 L enzyme (Sigma-Aldrich, Germany) was purchased for fish protein hydrolysis.

Protein hydrolysate

The fish muscle protein hydrolysate, with a degree of hydrolysis of 5%, was produced using the enzyme Protamex at 50 ºC and pH 7 as previously described in Lima *et al.* (2019). The lyophilized hydrolysate was stored at −18 ºC until use. Hydrolysate characteristics (amino acid profile, Fourier transform infrared spectroscopy, morphological and biological activity) were previously described in (Lima *et al.*, 2021).

The mixed fish muscle was melted at 4°C for 24 hours, combined with isopropanol solution then heated for about 25-35 min. Hydrolysate was produced following the method of Lima *et al.* (2019). For enzymatic hydrolysis process, a commercial peptidase, Flavourzyme ® 1000 L (Sigma-Aldrich, Germany) was purchased and applied at 1% concentration for an hour up to 60% degree of hydrolysis. The resultant combination was then heated at 90 °C for 20 min to deactivate the enzyme. Finally, the obtained mixture was centrifuged at 4 °C and 2000 g for 10 min.

Nanoemulsion of fish protein hydrolysates

Nanoemulsions of FPH were prepared by forming of oil in water. For this goal, first, a 3% solution of alginate was set by partying sodium alginate at medium viscosity with distilled water using a stirrer. It was then autoclaved at 120 °C for 12-17 min and cooled at room temperature, eventually. The resultant was followed the method of Zhou *et al.* (2022) with minor modification to obtain nanoemulsion-FPH .

Yogurt preparation

The pasteurized whole milk was applied in this research. Initially, the milk heated at 45 °C and addition of nanoemulsion to the glasses containing whole milk was achieved. The mixture was homogenized at 60-65 °C. The resultant milk was heated at 90 °C for 5-10 min. They were cooled at room temperature for 10 min. A hundred milliliters of final milk were inoculated with starter culture, which had been

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previously set and fermented for 4-5 hours until the pH reached to 4.6. Yogurts were designed in two groups in triplicate. The samples were then cooled in refrigerator until further process (El-Sayed *et al.*, 2022).

pH and titratable acidity

The pH was measured using a digital laboratory scale pH-meter (Methrom, Switzerland), at 20-30 °C. The titratable acidity (TA) of the yogurt samples was measured by titrating 9 g of the sample with 0.1 M NaOH and an indicator named phenolphthalein, which was expressed as % lactic acid in triplicates (Bondia-Pons *et al.*, 2007).

Syneresis

The syneresis of the yogurts was evaluated based on the method of Santillán-Urquiza *et al.* (2017). Approximately 8-10 g of yogurt samples was centrifuged at 175× g for 15-20 min at 10°C. The syneresis, expressed as percentage, was achieved in triplicate and measured as the weight of the supernatant released over the weight of the initial yogurt × 100.

Results

pH and titratable acidity

The results concerning the pH at fortified yogurts during three weeks of cold storage is depicted in Figure 1. The pH of nanoemulsion-FPH yogurt was 4.52 at the first day and gradually decreased ($p > 0.05$), reach 4.01 in third week, which showed a significant difference ($p < 0.05$) compared with that of the control at the same time (3.80). The pH value of the fortified yogurt showed acceptable limit on day 7 (4.35) but it was remarkably decreased on day 14 (4.19, $p < 0.5$). The trend pattern of pH in control yogurt was similar to that of yogurt supplemented with FPH. In control, the pH was begun at 4.62 and ended at 3.80 after three weeks of cold storage (Fig. 1).

The change of TA was presented in Figure 2. The value of TA was 0.47% in yogurt fortified with FPH on day 1 and increasingly reached 0.64% a little more than the value of control after a week refrigeration but showed no significant difference ($p > 0.05$). This value (0.74%) in fortified yogurt significantly ($p < 0.05$) greater than that of control (0.55%) after two weeks (Fig. 2).

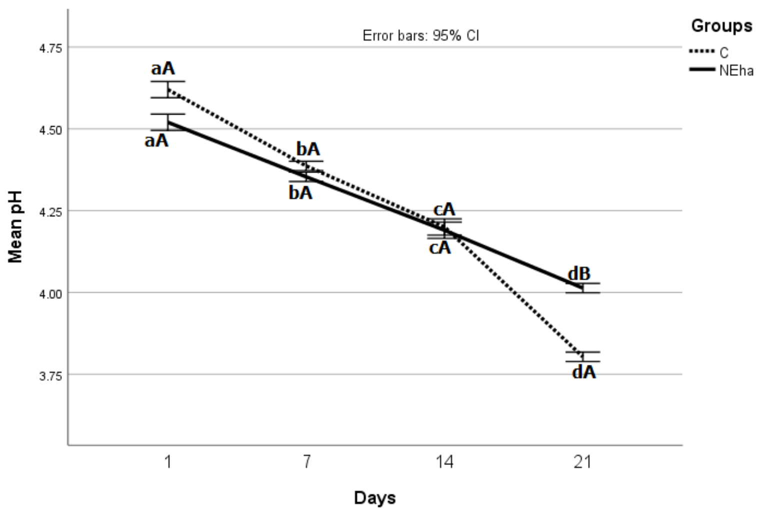

Figure 1. pH of Alg-Na nanoemulsion-FPH fortified yogurt and control yogurt during cold storage, C: Control, NEha: Alg-Na nanoemulsion-FPH yogurt containing More than 10 KDa protein

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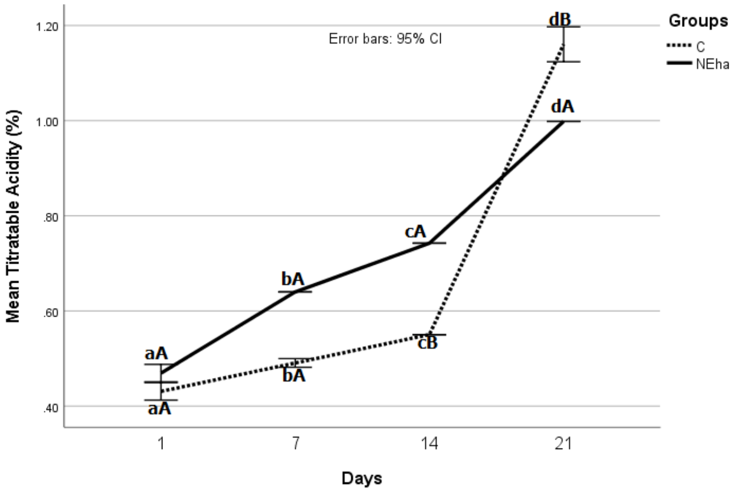

Figure 2. Titratable acidity of Alg-Na nanoemulsion-FPH fortified yogurt and control yogurt during cold storage, C: Control, NEha: Alg-Na nanoemulsion-FPH yogurt containing More than 10 KDa protein

Viscosity and water holding capacity

The superficial viscosity (centipoise) of yogurt samples during 21d refrigeration were depicted in Figure 3. The upmost and the least values of viscosity of nano-FPH yogurt samples were respectively 4187.3 and 4046.6 (cps) on days 1 and 21. The viscosity values of control were ranged from 3716.0 to 4042.0, respectively in 21 and 1 days of refrigeration, which were significantly ($p<0.05$) lower than those of each sampling day of yogurt samples fortified with nanoemulsion Abu mullet protein hydrolysate.

The WHC fluctuation was depicted in Figure 4. The maximum and minimum WHC value of nano-FPH yogurt samples 92.5 and 86.2%, respectively on days 1 and 21, greater than those of control yogurt samples at the same time. The WHC values of control yogurt samples were ranging from 88.5% on day 1 to 81.6% after three weeks.

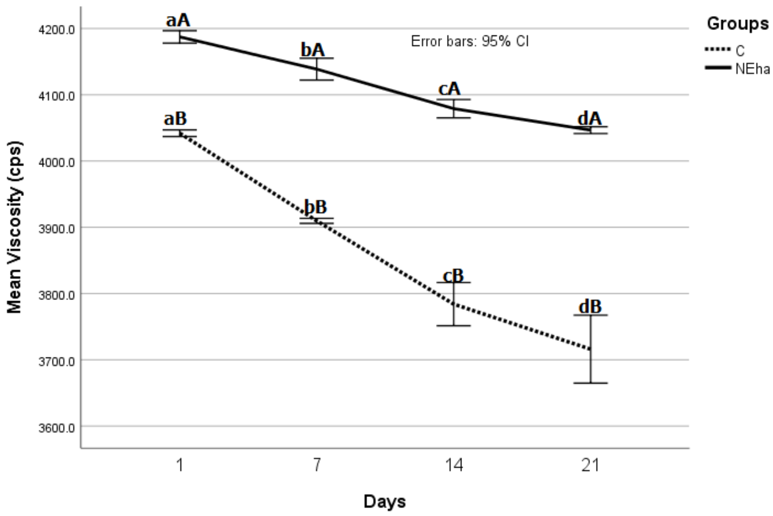

Figure 3. Viscosity (cps) of Alg-Na nanoemulsion-FPH fortified yogurt and control yogurt during 21 days of cold storage, C: Control, NEha: Alg-Na nanoemulsion-FPH yogurt containing More than 10 KDa protein.

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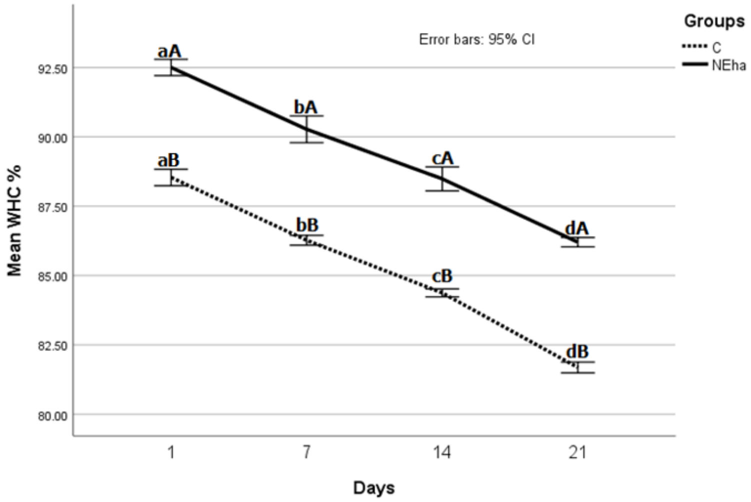

Figure 4. Water holding capacity (%) of Alg-Na nanoemulsion-FPH fortified yogurt and control yogurt during 21 days of cold storage, C: Control, NEha: Alg-Na nanoemulsion-FPH yogurt containing More than 10 KDa protein

Syneresis

The results of syneresis values (whey percent) of the yogurt samples were figured in Figure 5. This value was opposite of WHC and increased with increase of cold storage time. The syneresis of the nano-FPH yogurt samples was ranged from 10.17% on day 1 to 15.37% on day 21 of refrigeration. These values were 11.46%-18.31% for those of control yogurt samples at the same time and significantly ($p<0.05$) different from those of treated yogurt samples.

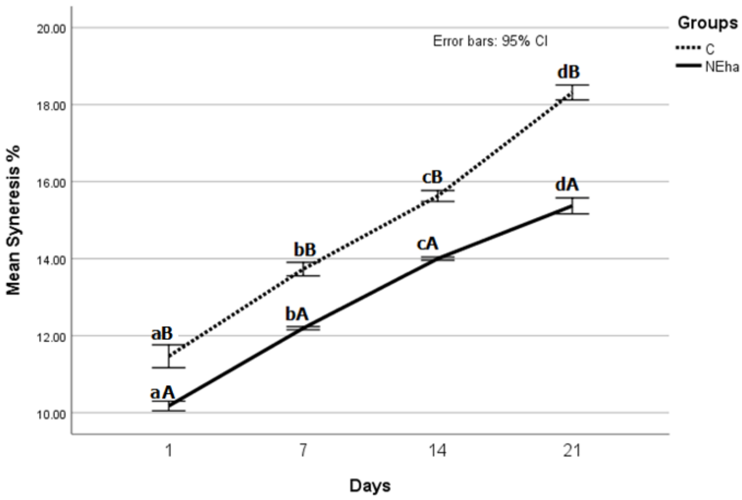

Figure 5. Estimated marginal means of syneresis (%) of Alg-Na nanoemulsion-FPH fortified yogurt and control yogurt during cold storage, C: Control, NEha: Alg-Na nanoemulsion-FPH yogurt containing More than 10 KDa protein

Discussions

The present study aimed to evaluate the physico-chemical properties of yogurt fortified with fish protein hydrolysate (FPH) obtained from Fresh Abu mullet fish during 21-day

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refrigeration. The results showed that supplementation with nanoencapsulated FPH had a significant impact on the pH, titratable acidity, viscosity, water holding capacity, and syneresis of the yogurt samples (Figs 1-5). These results (Fig. 2) indicated that yogurt supplemented with NE-FPH had remarkable properties on decreasing the pH and increasing of TA particularly after a week of cold storage (Figs. 1 & 2). This different of acidification could be due to the increase of viable microbes and fermentation process performed with nano FPH-fortified yogurt (Tseng & Zhao, 2013). The pH of the nanoencapsulated FPH yogurt was initially 4.52 and gradually decreased after three weeks of refrigeration, with a significant difference ($p<0.05$) compared to the control samples. This decrease in pH was likely due to the accumulation of lactic acid during fermentation. The pH value of the fortified yogurt was within an acceptable limit on day 7 (4.35) but was significantly lower on day 14 (4.19) compared to the control samples (Fig. 1). Similar to this study, yogurt supplemented with stripped weakfish (*Cynoscion guatucupa*) Protein Hydrolysate (3%) showed a tendency to slight increase of TA and minor decrease of pH. Their fortified yogurts presented a TA of 1.09% after a week, which was greater than that of this study (Fig. 2) at the same time (0.64%) indicating had less sour taste for consumers. According to the recommended aspects (Frye & Kilara, 2015), the pH acceptable level of yogurt should be less than 4.2-4.4. Thus, the yogurt fortified with nano-FPH of this study can be used after two weeks of production. The results showed that the value of TA increased moderately reached to 0.64% after one week of refrigeration. However, this increase was not significantly different from that of the control samples ($p>0.05$). After two weeks of refrigeration, the value of TA in the fortified yogurt samples significantly increased to 0.74%, which was significantly higher than that of the control samples (0.55%, $p<0.05$). In another research (Ayati *et al.*, 2022) in which the yogurt was supplemented with fish collagen-derived proteins, the TA reached 0.82% after 2 weeks refrigeration. It was greater than that of FPH-yogurt of this study showed 0.74% at the same time.

This increase in acidity may be attributed to the release of free fatty acids from the FPH during fermentation, which can contribute to the production of lactic acid and increase the acidity of the yogurt, which explained with similar findings in another study (Huang *et al.*, 2020). The increase in TA observed in the fortified yogurt samples may have important implications for the nutritional and health properties of the product. Higher acidity levels can promote the growth of beneficial bacteria in the gut, which can improve digestion and overall health (Kailasapathy & Chin, 2000). Additionally, increased acidity levels can contribute to the preservation of the product, reducing the risk of spoilage and extending its shelf life (Deshwal *et al.*, 2021). Overall, the results suggest that the incorporation of FPH in yogurt can increase the acidity of the product, with significant differences observed after two weeks of refrigeration. Further studies are warranted to investigate the effects of FPH

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fortification on the sensory properties and consumer acceptance of yogurt.

The decrease slope of viscosity for nano-FPH yogurt samples was lower than that of control (Fig. 3), which showed that addition of fish protein hydrolysate to yogurts resulted in more stable of gel network in comparison than control during 21d refrigeration. This increase in viscosity may be attributed to the hydrocolloid properties of the FPH, which can enhance the gel-forming ability of the yogurt. yogurt fortified with fish collagen-derived proteins (Ayati *et al.*, 2022) showed viscosity of 1834.0 with negligible difference at different concentrations, which is lower than the result of this study (Fig. 3). Other researchers (Raftani Amiri *et al.*, 2016) exhibited that squid protein hydrolysate (*Sepia pharaonis*) at 1.5% concentration could increase viscosity of low-fat yoghurt to 3653.6 cps. Fish gelatin at 0.3 g/dL could perform a solider gel network during yogurt fermentation (Gheshlaghi *et al.*, 2021). Thus, addition of sturgeon gelatin hydrolysates, which enriched with a high share of hydrophilic amino acids such as aspartic acid, glutamic acid, arginine, and lysine, could be contributed to a better WHC and gel network results in modification of the viscosity (Gheshlaghi *et al.*, 2021). This positive changes in WHC value could be due to the peptide-concentration Increases (Barkallah *et al.*, 2017) indicating that FPH fortification can improve the water retention properties of yogurt. Fish collagen-derived bioactive Peptides (0.4%) increased WHC to 87.11% after 2-weeks refrigeration (Ayati *et al.*, 2022), which was similar to the result of Abu mullet protein hydrolysate supplemented yogurt samples in this study (88.4%, Fig. 4).

Syneresis happens in yogurt samples might be due to the contraction of the protein network, thereby ejecting water during cold storage (Gheshlaghi *et al.*, 2021). In one study, hydrolysates of fish gelatin encouraged whey (10.53%) and less gel texture than control yogurt after 7 days (Ma *et al.*, 2019), which was less than the result of nano-FPH yogurt samples of this study (12.2%) showing slightly more effectiveness of fish gelatin on yogurt firmness than nano-FPH. Fish protein hydrolysate obtained from Hyrcanian goby (1.5%) showed the least syneresis in low-fat yogurt fortification (Rezaei *et al.*, 2020).

In conclusion, fortification of yogurt with Alg-Na nanoemulsion-FPH containing more than 10 KDa protein showed a significant effect on the pH, titratable acidity, viscosity, water holding capacity, and syneresis of the yogurt during three weeks of cold storage. The results suggest that Alg-Na nanoemulsion-FPH can be used as a potential ingredient for fortifying yogurt with improved physicochemical properties.

Conflict of interest

The authors have no conflict of interest in this work.

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