Jurnal Teknologi Perikanan dan Kelautan Vol. 16 No. 3 August 2025: 244-254 *ISSN 2087-4871*
DOI: https://doi.org/10.24319/jtpk.16.244-254 *EISSN 2549-3841*

FORMULATION DEVELOPMENT OF A PREGNANCY SUPPLEMENT BASED ON TUNA EYE OIL USING MIXTURE DESIGN

PENGEMBANGAN FORMULASI SUPLEMEN KEHAMILAN BERBASIS MINYAK IKAN MATA TUNA MENGGUNAKAN *MIXTURE DESIGN*

Wini Trilaksani[1], Silva Fauziah[1*], Utami Dyah Syahfitri[2], Bambang Riyanto[1]
[1]Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, IPB University, Jl. Agatis, IPB Dramaga Campus, Bogor 16680, Indonesia
[2]Department of Statistics, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Meranti, IPB Dramaga Campus, Bogor 16680, Indonesia
*Corresponding author: fauziahsilva688@gmail.com

(Received October 2, 2024; Revised December 6, 2024; Accepted May 21, 2025)

ABSTRACT

Stunting is a chronic nutritional problem with a high prevalence in Indonesia, with inadequate intake of protein and DHA during pregnancy being among the primary causes. This study aims to develop and optimize a pregnancy supplement formulation based on tuna eye oil and katsuobushi protein hydrolysate using a mixture design approach. The research stages included the extraction and characterization of fish oil from tuna eyeballs, the enzymatic hydrolysis of katsuobushi protein using papain, and the formulation of an emulsion using egg yolk as the emulsifier. Optimization was conducted using a simplex-lattice {3,1} design with axial points via Minitab 19 software. The optimal formulation consisted of 40.40% tuna eye oil, 31.02% katsuobushi protein hydrolysate, and 28.58% egg yolk, resulting in a pH of 4.29 and a viscosity of 3,880 cPs. The final product delivered 210 mg DHA per day, fulfilling the recommended daily intake for pregnant women. Nutritional profile and microbial safety tests also met established standards. These findings highlight the potential of tuna eye oil and katsuobushi-based supplements as an innovative nutritional intervention for stunting prevention.

Keywords: DHA, katsuobushi, mixture design, pregnancy supplement, tuna eye oil

ABSTRAK

Stunting merupakan permasalahan gizi kronis yang masih tinggi prevalensinya di Indonesia, dengan salah satu penyebab utamanya adalah kekurangan asupan protein dan DHA selama kehamilan. Penelitian ini bertujuan mengembangkan dan mengoptimalkan formula suplemen kehamilan berbasis minyak mata tuna dan hidrolisat protein katsuobushi menggunakan pendekatan *mixture design*. Tahapan penelitian meliputi ekstraksi dan karakterisasi minyak mata tuna, hidrolisis protein katsuobushi dengan enzim papain, serta formulasi emulsi menggunakan *eggyolk* sebagai emulsifier. Optimasi dilakukan dengan rancangan *simplex-lattice* {3,1} *with axial points* menggunakan *software* Minitab 19. Formula terbaik terdiri dari 40,40% minyak mata tuna, 31,02% hidrolisat protein katsuobushi, dan 28,58% *eggyolk*, dengan pH 4,29 dan viskositas 3.880 cPs. Formula ini menghasilkan kandungan DHA sebesar 210 mg per hari, mencukupi rekomendasi untuk ibu hamil, serta memiliki profil gizi dan keamanan mikrobiologi yang sesuai standar. Hasil ini menunjukkan bahwa suplemen berbasis minyak mata tuna dan katsuobushi berpotensi sebagai solusi inovatif dalam mendukung pencegahan stunting secara nutrisi.

Kata kunci: DHA, katsuobushi, minyak mata tuna, *mixture design*, suplemen kehamilan

INTRODUCTION

Stunting is defined as a child's growth disorder, characterized by height, weight, and IQ that are below average for children of the same age (WHO 2012). Basic health research data in 2018 stated that the prevalence of stunting in Indonesia reached 30.8%. The main cause of stunting is malnutrition during pregnancy, especially protein and iron intake. The government, in addressing this, has launched various national nutrition fulfillment programs through the First 1,000 Days of Life program, including the provision of iron and folic acid supplements for pregnant women (Kemenkes 2016).

Omega-3 contained in fish has important benefits for body health, vision, and brain development (Swanson *et al.* 2012). Koletzko *et al.* (2007) of the World Association of Perinatal Medicine stated that the daily requirement of DHA for pregnant and lactating mothers is at least 200 mg. Tuna eyes are a source of omega-3, and even in Japan, they are often consumed as a luxury food. The EPA and DHA content in tuna eyes is 7% and 35%, respectively (Gamarro *et al.* 2013). The fatty acid composition of tuna eye oil is dominated by unsaturated fatty acids, especially the polyunsaturated fatty acids (PUFA) group, with docosahexaenoic acid (DHA) as the main fatty acid (Riyanto *et al.* 2021). Trilaksani *et al.* (2020) succeeded in developing an environmentally friendly extraction method to produce tuna eye oil. Tuna eye oil supplementation for pregnant women offers an innovation in improving public health.

Omega-3 (EPA and DHA) are very sensitive to high temperatures and begin to degrade at 50°C (Hădărugă *et al.* 2016). Cold chain handling in fish processing is an important factor in preventing oxidation. Fish handling in Indonesia still faces many obstacles, resulting in low-quality fish. The heating process can also damage the omega-3 content, so to overcome this, antioxidants are often added to fish oil. Indonesian fishery resources that have the potential as natural antioxidants are mangrove fruit (Trilaksani *et al.* 2020). In addition, the simple structure in the form of amino acids and peptides from katsuobushi hydrolysate also has the potential to contain active antioxidant compounds (Noman *et al.* 2017; Chalamaiah *et al.* 2012; Lee *et al.* 2015). Some active components, for example. ACE inhibitors have additional potential to meet daily nutritional needs and help prevent preeclampsia in pregnant women (Abachi *et al.* 2019; Darewicz *et al.* 2014).

Anggraini (2018) reported that Indonesian katsuobushi hydrolysate hydrolyzed using 3% (w/v) papain enzyme had ACE inhibitor activity of 82.21±2.7%. This hydrolysate contains various types of active peptides, such as Alanine-Isoleucine-Tyrosine-Lysine, Aspartic Acid-Phenylalanine-Glycine, and Tyrosine-Histidine, which are known to have potential as ACE inhibitors.

Supplementation for pregnant women requires an appropriate nutritional composition (Permenkes 2019), especially in terms of determining the dose, proportion of ingredients, and composition of the formulation. The mixture design method allows the combination of two or more components to produce a more optimal formulation (Bezerra *et al.* 2020). This method can produce a final product with better properties than the use of a single component (Cornell 1990). This study aims to optimize the pregnancy supplement formula based on tuna eye oil and katsuobushi protein hydrolysate using a statistical mixture design approach.

METHODS

Materials and tools

The fish oil used in this study was obtained from tuna eyeballs, produced from the tuna loin processing industry in Bitung, North Sulawesi. Skipjack tuna katsuobushi (*Katsuwonus pelamis*) was produced by PT. Omereso Foods in Makassar, South Sulawesi. The protein hydrolysis process was carried out using the papain enzyme (Papain®, Merck, 300,000 U/mg). Ingredients for the emulsion formulation included chicken egg yolk, lemon, sugar, sodium benzoate, and coconut oil. Additional chemicals used included potassium iodide (KI) (Sigma), p-anisidine, and 2-2 azinobis (Sigma). Various laboratory instruments were used in this study, including centrifuge (Hitachi, Model Part No. R12A6904357D0), gas chromatograph (Shimadzu GC 2010 plus), water bath shaker (Yamato, Japan), homogenizer (Wiggen Hause), Minitab 19 software for statistical analysis, viscometer (Toki Sangyo, Model TV-10), and Dino Eye microscope with 4×10 magnification.

Research procedures

Preparation, extraction, and characterization of tuna eye oil

Tuna (*Thunnus albacares*) eyes were visually analyzed to measure their proportion, size, and weight. Freshness evaluation was carried out based on SNI 2729:2013 standards, which include assessment of the eyeball, cornea, and pupil. Analysis of the chemical composition of tuna eyes included measuring the content of moisture, fat, protein, ash, and carbohydrates using the by-different method (AOAC 2005). Oil extraction was carried out using the centrifugation method (11,200 g for 30 minutes at 4°C) based on Clodoveo and Hbaeib (2013). The oil obtained was stored at -20°C. The quality parameters evaluated included peroxide value (AOAC 2005), free fatty acid content (AOCS 1998), p-anisidine analysis (IUPAC 1987), total oxidation (TOTOX) (AOCS 1997), fatty acid content (AOAC 2005), and heavy metals (Cd, Pb, Hg, As) (SNI 2354-23 2021).

Hydrolysis and characterization of katsuobushi protein

Katsuobushi used as raw material was analyzed for its moisture, fat, protein, ash, and carbohydrate content (AOAC 2005). The polycyclic aromatic hydrocarbon (PAH) content was analyzed using EPA 610. The hydrolysis process was carried out by mixing katsuobushi powder with distilled water in a ratio of 1:5 (w/v). Papain enzyme 3% (w/v) was used at pH 7 and a temperature of 55°C for 4 hours, then the process was stopped by heating at a temperature of 90°C for 15 minutes (Nikoo *et al.* 2023). Hydrolysate powder was produced through a freeze-drying process at a temperature of -49°C and a pressure of 13.4 Pa. The hydrolysate was then analyzed for the degree of hydrolysis (Hoyle and Merritt 1994), molecular weight (Laemmli 1970), and antioxidant activity using the ABTS method (Re *et al.* 1999).

Optimization of pregnancy supplement emulsion formulation

Oil from tuna eyes, which is rich in DHA (Trilaksani *et al.* 2020), katsuobushi protein hydrolysate as a source of antioxidants (Lee *et al.* 2015), and egg yolk as an emulsifier were used as the main variables in this experiment. Fixed variables included coconut oil (38 g), lemon (3 g), sugar (5 g), salt (0.20 g), and sodium benzoate (0.05 g). The experimental design used was a mixture experiment {3,1} simplex-lattice design with axial points, using Minitab 19 software to determine the optimum formula (Figure 1).

There were seven formulas tested with two replications. The range of the main variable values was based on the daily nutritional adequacy figures for pregnant women aged 19-29 years. The amount of tuna eye oil ranged from 0.3 to 0.6 g, katsuobushi protein hydrolysate between 4.5 to 7.5 g, and egg yolk 6.5 to 9.5 g. The final formula was expressed in percentage and converted to weight (g). The recommended consumption dose is 3 times a day, 2 tablespoons, according to the AKG for protein (Permenkes 2019) and EPA/DHA recommendations for pregnant women (Koletzko *et al.* 2007). The parameters tested included pH value, viscosity, and sensory preference to determine the best formula.

Characterization of the optimal formula of pregnancy supplement emulsion

Analysis to determine the optimal formula of pregnancy supplement emulsion includes analysis of moisture, fat, protein, ash, and carbohydrate content using the by-difference method (AOAC 2005). In addition, fatty acid content was analyzed using the GC-MS technique following the AOAC 2005 No.969.33 standard. Evaluation of nutritional value was carried out based on the nutritional adequacy rate (AKG) guidelines from the Permenkes (2019), and microbiological testing to ensure product safety was carried out following the BSN standard (2009a).

Data analysis procedures

A mixture experiment {3,1} simplex-lattice design with axial points used in the Minitab 19 application. The model is mapped in a contour plot in the form of an image (2D) and a response curve in the form of a graph (3D) to provide an overview of the test variables.

$$E(Y) = \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3$$

Description:
E(Y) = Response estimate
βi = Value of the i-th coefficient

βij = Value of the combined response coefficient of i and j
x1 = Proportion of tuna eye oil
x2 = Proportion of katsuobushi protein hydrolysate
x3 = Proportion of egg yolk

RESULTS AND DISCUSSION

Characteristics of tuna eye oil

The composition of the tuna eye analyzed showed a water content of 67.933 ± 1.371%, fat 18.437 ± 0.473%, ash 1.13 ± 0.049%, protein 11.43 ± 0.294%, and carbohydrate 1.09 ± 0.715%. Evaluation of the quality of tuna eye oil was carried out based on the Codex Alimentarius 329-2017 standard regarding the quality and safety of consumable fish oil. The results of the tuna eye oil quality parameter test are presented, showing that all measured parameters are within safe limits according to international standards.

Free fatty acid (FFA) levels are an early indicator of fat degradation due to triglyceride hydrolysis into free fatty acids and glycerol. The analysis results showed an FFA value of 0.56 ± 0.01% (w/w), far below the maximum threshold of <3.5%, indicating that the oil produced is still fresh and has minimal enzymatic and microbial degradation. The p-anisidine number measures the content of secondary aldehydes formed during the secondary oxidation process of the oil. The value obtained was 3.65 ± 0.01 meq/kg, still far below the maximum limit of <20 meq/kg, indicating that the oil has not undergone significant further oxidation.

Peroxide value (PV) was used to assess the level of primary oxidation, especially the formation of peroxides and hydroperoxides as the initial compounds of unsaturated fat oxidation. The results of the PV analysis reached 4.30 ± 0.00 meq/kg, slightly close to the Codex maximum limit of <5.0 meq/kg, but still in the safe category and indicating relatively good oil stability in the early stages of storage. The last parameter, total oxidation (TOTOX), was calculated as a combined indicator of peroxide value and p-anisidine to describe the overall level of oxidative damage to the oil. The TOTOX value obtained was 12.26 ± 4.49 meq/kg, lower than the Codex maximum threshold of <26 meq/kg, so the oil can be categorized as having good oxidative quality and is safe for consumption. Overall, the four quality parameters indicate that the tuna eye oil extracted in this study has met international quality standards and is suitable for use as a raw material in the formulation of DHA-rich pregnancy supplements.

Heavy metal content, such as lead (Pb), cadmium (Cd), and mercury (Hg), was not detected, while arsenic (As) was detected at less than 0.005 mg/kg (Table 2). These results are following Codex Standard 193-1995, which regulates the limits of heavy metal and toxin contamination in food, so that the oil is safe for consumption.

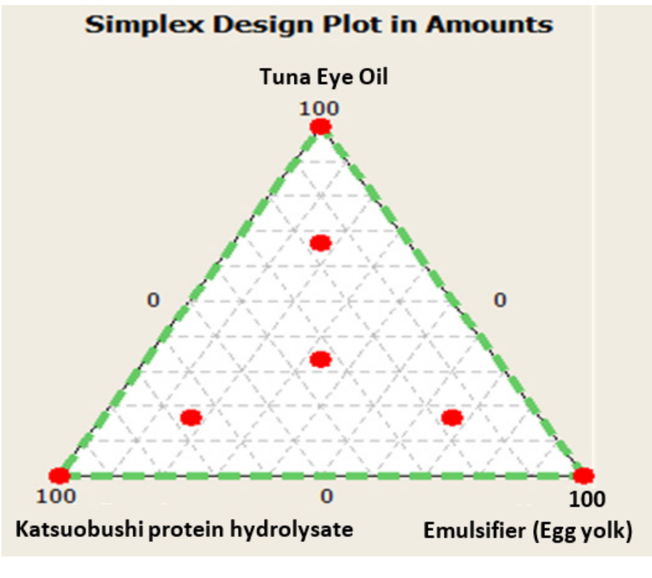


Figure 1. Mixture experiments {3,1} simplex-lattice design with axial points, pregnancy supplement; ■ : design point formula, 0-100: percentage formula.

The dominant fatty acid component in this oil was docosahexaenoic acid (DHA), with the highest percentage of 32.12%, followed by palmitic acid (C16:0) at 19.13%. Palmitic acid is known to play a role in forming retinol esters (retinol palmitate), which support maintaining eye moisture (Diao *et al.* 2017). DHA, which contributes to the function of the retina as a photoreceptor, also has a significant role in eye health (Jeffrey *et al.* 2001).

Characteristics of katsuobushi protein hydrolysate

The katsuobushi used had a blackish surface with an average length of 18 cm and a weight of 89.47 g. The water content was 20.44 ± 0.028%, which slightly exceeded the SNI 2691.1:2009 standard by 0.440 ± 0.028%. Other main components included protein (69.320%), ash (2.95 ± 0.021%), fat (7.18 ± 0.014%), and carbohydrates (0.10%).

The hydrolysis process using 3% (w/v) papain enzyme produced a degree of hydrolysis of 48.86%, while without the enzyme, it was only 12.68%. The molecular weight of the protein was detected at 19.5 kDa (Figure 2), with antioxidant activity (IC50) of 0.199 ± 0.18 mg/mL (199 ppm), which is classified as weak (Blois 1958). Several peptides have been identified as having potential antioxidant activity, such as Isoleucine-Lysine-Proline-Lysine and L-Histidine, which are thought to be able to ward off free radicals through a proton donor mechanism (Chalamaiah *et al.* 2012).

Characteristics of pregnancy supplement emulsion

Visually, this emulsion has a dark yellow color with a thick texture (Figure 3). Egg yolk is used as an emulsifier because its high phospholipid lecithin content (10%) is more stable than lecithin from soybeans (Palacios and Wang 2005). The average pH value of the entire formula is 4.24 ± 0.21, according to the McClements standard (2015), which is 2.5-4.5, to prevent microbial growth.

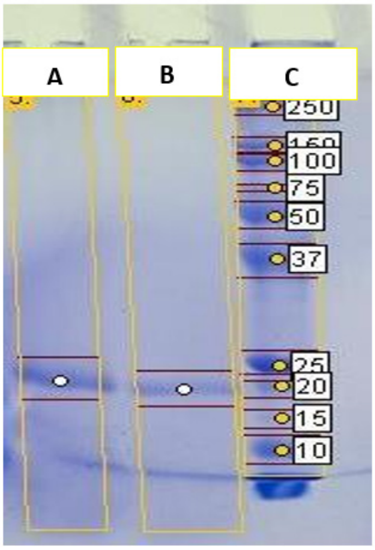


Figure 2. Banding pattern of katsuobushi protein hydrolysate. A, B first and second test, C marker.

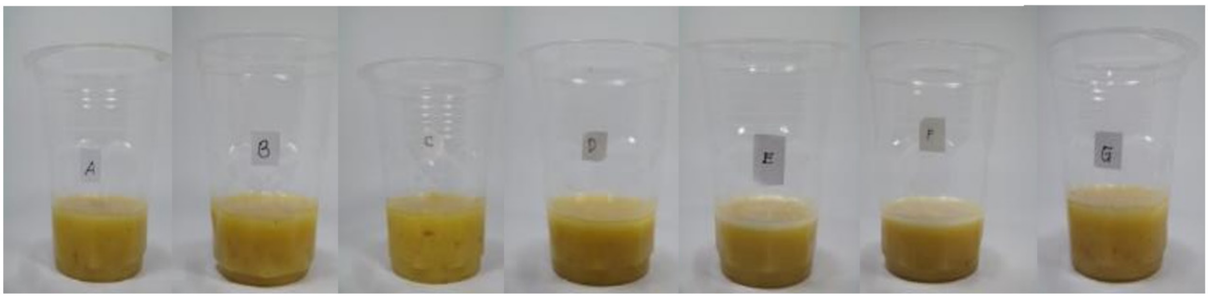


Figure 3. Visualization of the appearance of the pregnancy supplement emulsion.

The average viscosity of the formula reached 3,600 ± 0.6 cPs, which is comparable to commercial products (3,347 cPs) (Amertaningtyas and Jaya 2011). The optimal formula consisted of 40.40% tuna eye oil, 31.02% katsuobushi protein hydrolysate, and 28.58% egg yolk, with pH and viscosity of 4.29 ± 0.007 and 3,880 ± 1.13 cPs, respectively.

The surface plot graph (Figure 4A) shows the response of pH values with variations in height. The predicted maximum response (4.69 ± 0.3) is visualized on the contour plot graph (Figure 4B) with a dark green area, located in the combination region of tuna eye oil and emulsifier. The average pH value for all formulas produced is 4.24 ± 0.21, which is between the katsuobushi protein hydrolysate and egg yolk regions, with a light green color. This average pH value is in accordance with the range recommended by McClements (2015), which is 2.5-4.5 because this range is effective in preventing the growth of spoilage microbes. The visualization of the surface plot (Figure 4C) shows the difference in height as a representation of the response value. The higher the graph, the greater the response value achieved. Based on the graphical analysis, the minimum viscosity response is located on the surface plot graph with a value of 2,360 ± 0.5 cPs, which is visualized on the contour plot graph (Figure 4D) with a green area in the katsuobushi protein hydrolysate region. The estimated average viscosity is 3,600 ± 0.6 cPs, which is relatively close to the commercial product viscosity of 3,347 cPs according to Amertaningtyas and Jaya (2011).

Characteristics of the best formula for a pregnancy supplement emulsion

The optimal formula obtained based on pH and viscosity analysis consisted of 40.40% tuna eye oil, 31.02% katsuobushi protein hydrolysate, and 28.58% egg yolk (Figure 4). The maximum pH and viscosity values resulting from the simplex-lattice design {3,1} with axial points were 4.39 and 3,291.23 cPs, respectively. After being verified in the laboratory, the pH and viscosity values obtained were 4.29 ± 0.007 and 3,880 ± 1.13 cPs. The pH response graph showed a uniform pattern for all variables, while the viscosity showed an opposite response between katsuobushi protein hydrolysate with tuna eye oil and egg yolk.

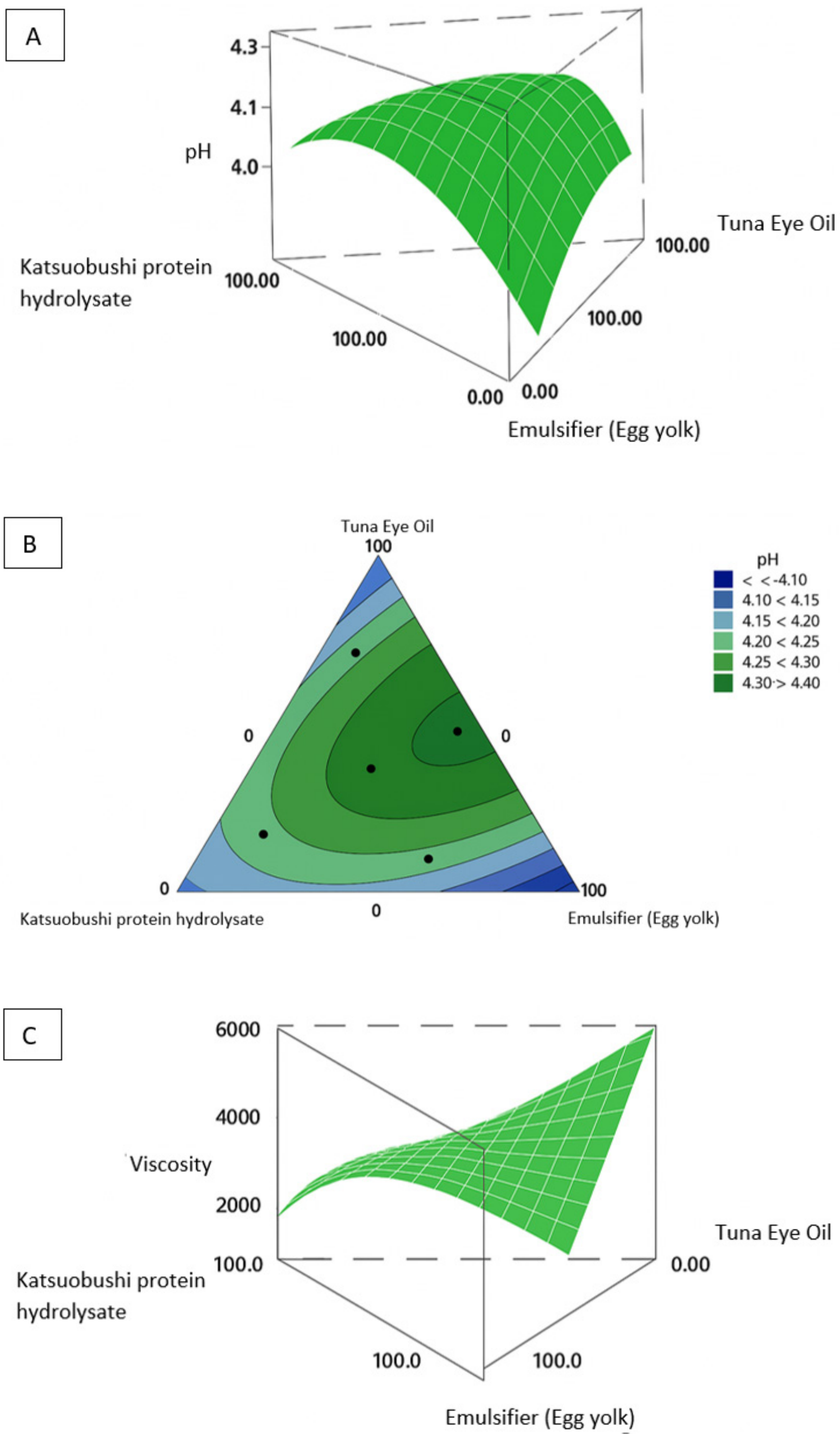
The nutritional characteristics of this best formula emulsion also show high quality, with a fat content reaching 74.45 ± 0.12%, which exceeds the minimum standard of 65% according to SNI 01-4473-1998 for mayonnaise products. The water content is 14.74%, carbohydrates 7.04%, protein 3.35%, and ash 0.42%. The addition of tuna eye oil and coconut oil significantly contributes to the high fat content. In terms of food safety, the results of the total microbial plate count test showed only 3×10^3, far below the maximum limit of 1×10^4 CFU/g, so this product is safe for consumption by pregnant women (BSN 1998).

Visualization in the graph shows a relatively consistent pH response pattern to variations in material composition. In contrast, the viscosity response shows a striking difference, where increasing the proportion of katsuobushi hydrolysate tends to decrease viscosity, while increasing the proportion of tuna eye oil and egg yolk actually increases the emulsion viscosity. This relationship is reflected in the interaction curve presented in the graph and the validation of the maximum point by the dashed blue line in Figure 5.

The fatty acid profile in the pregnancy supplement emulsion formula is dominated by saturated fatty acids (SFA). The highest percentage of fatty acid is lauric acid (C12:0) with a percentage of 27.71%, followed by myristic acid (C14:0) at 11.49%. The percentage of polyunsaturated fatty acids (PUFA) is 1.79%, with a DHA content of 0.36% and an EPA of 0.04%. The ratio of omega-3 to omega-6 in this formula is 1:4.7. Simopoulos (2002) noted that the recommended consumption ratio of omega-6: omega-3 varies according to the desired function and health benefits, such as reducing cell proliferation in colorectal cancer, suppressing inflammation in rheumatoid arthritis, and helping manage asthma.

The best pregnancy supplement formula meets the recommended dietary allowance (RDA) with a protein content of 3.35%, MUFA 5.67 g, and PUFA 1.10 g per day. The total consumption of omega-6 and omega-3 achieved is 1.46 g and 230 mg per day. Koletzko *et al.* (2007) recommend daily DHA consumption for pregnant women of 200 mg, and this formula has met these needs with a DHA content of 210 mg per day.

The pregnancy supplement formula resulting from this study is designed to be consumed three times a day with a dose of two tablespoons per consumption (a total of 60 grams per day). The resulting nutritional composition shows that this supplement contains 3.35% protein, which plays an important role in supporting fetal tissue growth and the metabolic needs of pregnant women. The content of monounsaturated fat (MUFA) reaches 5.67 grams per day, while polyunsaturated fat (PUFA) reaches 1.10 grams per day. The total omega-6 fatty acids in this formula are 1.46 grams, consisting of 900 mg of linoleic acid (C18: 2n-6c) and 60 mg of arachidonic acid (C20: 4n-6), equivalent to 10.43% of the nutritional adequacy rate (RDA).



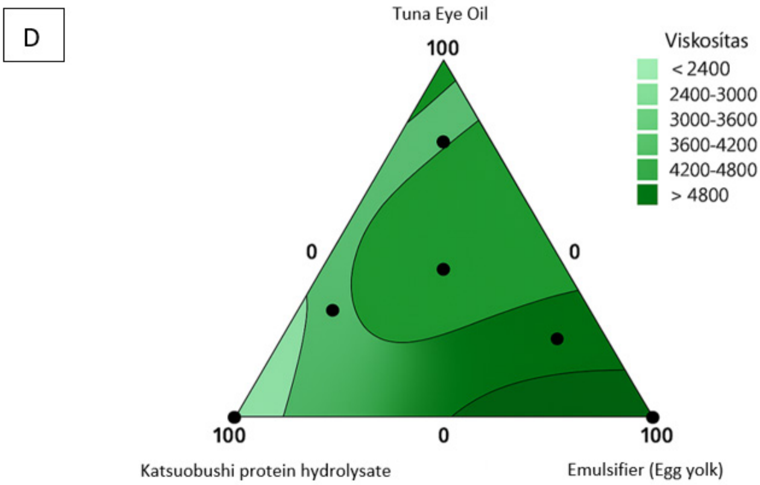


Figure 4. Mixture design graphs for pH (A, B) and viscosity (C, D) responses; (A, B) mixture surface plot, (C, D) mixture contour plot.

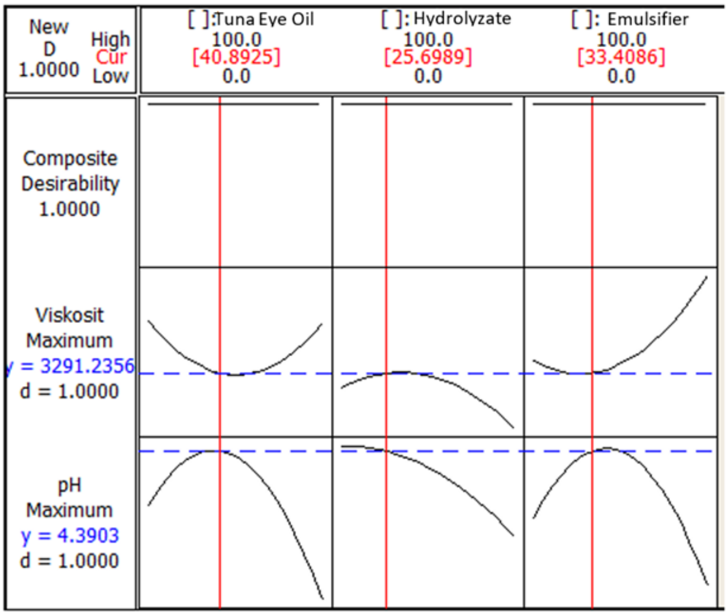


Figure 5. Pregnancy supplement emulsion plot with mixture experiment {3,1} simplex-lattice design with axial points.

Total omega-3 fatty acids reach 230 mg per day or 16.43% of the RDA, consisting of 10 mg linolenic acid (C18:3n-3), 20 mg eicosapentaenoic acid (EPA), and 210 mg docosahexaenoic acid (DHA). The DHA content has exceeded the minimum recommendation of 200 mg per day for pregnant women as determined by Koletzko *et al.* (2007). The ratio of omega-6 to omega-3 in this formula is 1:4.7, which is close to the ratio considered ideal in supporting cardiovascular health and fetal nervous system development. With a complete and balanced nutritional composition, this supplement has the potential to be an effective functional nutritional intervention in supporting healthy pregnancies and preventing stunting from the early stages of life.

CONCLUSION

This study successfully developed and optimized the formulation of pregnancy supplements based on tuna eye oil and katsuobushi protein hydrolysate using the simplex-lattice type mixture design method {3,1} with axial points. The optimum formula consisted of 40.40% tuna eye oil, 31.02%

katsuobushi hydrolysate, and 28.58% egg yolk, with characterization results showing a pH value of 4.29 and a viscosity of 3,880 cPs. The final product meets food safety standards and can provide 210 mg of DHA per day, according to the daily needs of pregnant women. Its nutritional composition shows a balanced fatty acid profile, with an omega-6 to omega-3 ratio of 1:4.7. These results indicate that this supplement has the potential as an innovative functional nutritional intervention in supporting the health of pregnant women and preventing stunting from early life.

ACKNOWLEDGEMENTS

Thank you to the Ministry of Education and Culture through the 2020 Student Creativity Program (PKM) and LPDP through the Productive Innovative Research (RISPRO) program for funding this research.

REFERENCES

Abachi S, Bazinet L, Beaulieu L. 2019. Antihypertensive and Angiotensin-I-Converting Enzyme (ACE)-Inhibitory Peptides from Fish as Potential Cardioprotective Compounds. *Marine Drugs*. 17(11): 613. DOI: https://doi.org/10.3390/md17110613.

Amertaningtyas D, Jaya F. 2011. Sifat Fisiko Kimia Mayonnaise dengan Berbagai Tingkat Konsentrasi Minyak Nabati dan *Eggyolk* Ayam Buras. *Jurnal Ilmu-Ilmu Peternakan*. 21(1): 1-6.

Anggraini VR. 2018. Aktivitas *Inhibitor ACE* dari Hidrolisat Peptida Katsuobushi Tradisional [Thesis]. Bogor (ID): Institut Pertanian Bogor.

[AOAC] Association of Official Analytical Chemist. 2005. *Official Methods of Analysis of the Association of Official Analytical of Chemist International*. Gaithersburg (US): AOAC International.

[AOCS] American Oil Chemists Society. 1997. *Official Methods and Recommended Practices of AOCS International*. Champaign (US): AOCS Press.

[AOCS] American Oil Chemists Society. 1998. *Free Fatty Acids. In: Official Methods and Recommended Practices of the American Oil Chemists Society*. Champaign (US): AOCS Press

Badan Penelitian dan Pengembangan Kesehatan. 2018. Data Riset Kesehatan Dasar (Riskesdas) 2018. Jakarta.

Bezerra MA, Lemos VA, Novaes CG, de Jesus RM, Filho HRS, Araújo SA, Alves JPS. 2020. Application of Mixture Design in Analytical Chemistry. *Microchemical Journal*. 152: 1-13. DOI: https://doi.org/10.1016/j.microc.2019.104336.

Blois MS. 1958. Antioxidant Determination by The Use of a Stable Free Radical. *Nature*. 181: 1199-1200. DOI: https://doi.org/10.1038/1811199a0.

[BSN] Badan Standardisasi Nasional. 1998. SNI 01-4473-1998. Mayones. Jakarta.

[BSN] Badan Standardisasi Nasional. 2009a. SNI 2691.1:2009. Ikan Kayu. Jakarta.

[BSN] Badan Standardisasi Nasional. 2009b. SNI 7388:2009. Batas Maksimum Cemaran Mikroba dalam Pangan. Jakarta.

[BSN] Badan Standardisasi Nasional. 2013. SNI 2729:2013. Ikan Segar. Jakarta.

[BSN] Badan Standardisasi Nasional. 2021. SNI 2354-23:2021. Cara Uji Kimia – Bagian 23: Penentuan Logam Berat: Merkuri (Hg), Timbal (Pb), Kadmium (Cd), Arsen (As), dan Timah Putih (Sn) pada Hasil Perikanan dengan *Inductively Couple Plasma Mass Spectrometer* (ICP-MS) secara Simultan. Jakarta.

Chalamaiah M, Kumar BD, 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. DOI: https://doi.org/10.1016/j.foodchem.2012.06.100.

Clodoveo ML, Hbaieb RH. 2013. Beyond the Traditional Virgin Olive Oil Extraction Systems: Searching Innovative and Sustainable Plant Engineering Solutions. *Food Research International*. 54(2): 1926-1933. DOI: https://doi.org/10.1016/j.foodres.2013.06.014.

Cornell JA. 1990. *Experiments with Mixtures: Designs, Models, and The Analysis of Mixture Data*. New York (US): John Wiley & Sons, Inc.

Darewicz M, Borawska J, Vegarud GE, Minkiewicz P, Iwaniak A. 2014. Angiotensin I-Converting Enzyme

(ACE) Inhibitory Activity and ACE Inhibitory Peptides of Salmon (*Salmo salar*) Protein Hydrolysates Obtained by Human and Porcine Gastrointestinal Enzymes. *International Journal of Molecular Science*. 15(8): 14077-14101. DOI: https://doi.org/10.3390/ijms150814077.

Diao Y, Deng A, Wang J, Hong J. 2017. Vitamin A Palmitate Eye Gel Improves the Density of Conjunctival Goblet Cells and the Production of Mucin-5 Subtype AC in Rabbits with Dry Eye Syndrome. *International Journal of Clinical and Experimental Medicine*. 10(12): 16181-16188.

[EPA] Environmental Protection Agency. 1984. *Method 610: Polynuclear Aromatic Hydrocarbons*. Washington DC (US): Environmental Protection Agency.

Gamarro EG, Orawattanamateekul W, Sentina J, Gopal TKS. 2013. *By-Product of Tuna Processing*. Rome (IT): FAO, Globefish.

Hădărugă DI, Ünlüsayin M, Gruia AT, Birău C, Rusu G, Hădărugă NG. 2016. Thermal and Oxidative Stability of Atlantic Salmon Oil (*Salmo salar* L.) and Complexation with β-cyclodextrin. *Beilstein Journal of Organic Chemistry*. 12: 179-191. DOI: https://doi.org/10.3762/bjoc.12.20.

Hoyle NT, Merritt JH. 1994. Quality of Fish Protein Hydrolysates from Herring (*Clupea harengus*). *Journal of Food Science*. 59(1): 76-79. DOI: https://doi.org/10.1111/j.1365-2621.1994.tb06901.x.

[IUPAC] International Union of Pure and Applied Chemistry. 1987. *Standard Methods for the Analysis of Oils, Fats, and Derivatives, 7th*. Paquot C dan Hautfenne A (ed). Oxford (GB): Blackwell Scientific.

Jeffrey BG, Weisinger HS, Neuringer M, Mitchell DC. 2001. The Role of Docosahexaenoic Acid in Retinal Function. *Lipids*. 36(9): 859-871. DOI: https://doi.org/10.1007/s11745-001-0796-3.

[Kemenkes] Kementerian Kesehatan Republik Indonesia. 2016. Infodatin: Pusat Data dan Informasi Kementerian Kesehatan RI: Situasi Balita Pendek. Jakarta.

Koletzko B, Cetin I, Brenna JT. 2007. Dietary Fat Intakes for Pregnant and Lactating Women. *British Journal of Nutrition*. 98(5): 873-877. DOI: https://doi.org/10.1017/S0007114507764747.

Laemmli UK. 1970. Cleavage of Structural Protein during the Assembly of the Heat of Bacteriophage T4. *Nature*. 227: 680-685. DOI: http://dx.doi.org/10.1038/227680a0.

Lee JK, Jeon JK, Byun HG. 2015. Characterization of an Antioxidant Peptide from Katsuobushi (Dried Bonito) Protein Hydrolysates. *Journal of Marine Bioscience and Biotechnology*. 7(1): 19-27. DOI: https://doi.org/10.15433/ksmb.2015.7.1.019.

McClements DJ. 2015. *Food Emulsions: Principles, Practices, and Techniques, Third Edition*. New York (US): CRC Press.

Nikoo M, Regenstein JM, Yasemi M. 2023. Protein Hydrolysates from Fishery Processing By-Products: Production, Characteristics, Food Applications, and Challenges. *Foods*. 12(24): 1-28. DOI: https://doi.org/10.3390/foods12244470.

Noman A, Xu Y, Al-Bukhaiti WQ, Abed SM, Ali AH, Ramadhan AH, Xia W. 2017. Influence of Enzymatic Hydrolysis Conditions on the Degree of Hydrolysis and Functional Properties of Protein Hydrolysate Obtained from Chinese Sturgeon (*Acipenser sinensis*) by Using Papain Enzyme. *Process Biochemistry*. 67: 19-28. DOI: https://doi.org/10.1016/j.procbio.2018.01.009.

Palacios LE, Wang T. 2005. Egg-yolk Lipid Fractionation and Lecithin Characterization. *Journal of the American Oil Chemists' Society*. 82: 571-578. DOI: https://doi.org/10.1007/s11746-005-1111-4.

[Permenkes] Peraturan Menteri Kesehatan Republik Indonesia Nomor 28 Tahun 2019 tentang Angka Kecukupan Gizi yang Dianjurkan untuk Masyarakat Indonesia. Jakarta.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Evans CR. 1999. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical Biology and Medicine*. 26(9-10): 1231-1237. DOI: https://doi.org/10.1016/S0891-5849(98)00315-3.

Riyanto B, Sinulingga F, Trilaksani W, Fauziah S, Krisnawan WV, Fitradiansyah LO, Rahmat MZ. 2021. Formulasi Pangan Darurat Cookies dengan Pengkayaan Tepung Ikan Lele dan Minyak Ikan Mata Tuna. *Jurnal Teknologi Perikanan dan Kelautan*. 12(2): 175-181. DOI: https://doi.org/10.24319/jtpk.12.175-181.

Simopoulos AP. 2002. The Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acid. *Biomedicine & Pharmacotherapy*. 56(8): 365-379. DOI: https://doi.org/10.1016/S0753-3322(02)00253-6.

Swanson D, Block R, Mousa SA. 2012. Omega-3 Fatty Acids EPA and DHA: Health Benefits Throughout Life. *Advances in Nutrition*. 3(1): 1-7. DOI: https://doi.org/10.3945/an.111.000893.

Trilaksani W, Riyanto B, Azzahra F, Santoso J, Tarman K. 2020. Recovery of Tuna Virgin Fish Oil and Formulation as a Product Model of Emulsion Food Supplement. *The World Seafood Congress 2019 – "Seafood Supply Chains of the Future: Innovation, Responsibility, Sustainability" 9–11 September 2019, Penang, Malaysia*. IOP Conference Series: Earth and Environmental Science.

[WHO] World Health Organization. 2012. *Nutrition Landscape Information System (NLIS) Country Profile Indicators: Interpretation Guide*. Jenewa (CH): WHO.