

JIPK (JURNAL ILMIAH PERIKANAN DAN KELAUTAN)

Scientific Journal of Fisheries and Marine

Research Article

Optimization of Effervescent Tablets in Sensory Acceptance from the Active Ingredients of Fish Protein Hydrolysate and Microalgae *Chlorellae* sp. powder

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ARTICLE INFO

Received: March 14, 2024 Accepted: August 10, 2024 Published: August 15, 2024 Available online: Feb 11, 2025

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Keywords:

Effervescent Tablets Fish Protein Hydrolyzate Microalgae Design Expert



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Abstract

Effervescent tablets are a promising delivery system for functional food ingredients, offering convenience and enhanced consumer appeal. This study aimed to optimize effervescent tablet formulations incorporating Fish Protein Hydrolysate (FPH) and Chlorella sp. powder using the d-optimal mixture design method. Thirteen formulations were evaluated for sensory attributes (color, aroma, taste, aftertaste, hedonic scores) and chemical properties (protein, lipid, water content). The optimal formulation, consisting of 5.873% FPH and 9.127% Chlorella sp. powder, significantly improved sensory acceptance (p < 0.05), validated by a robust statistical model ($R^2 = 0.93$). Proximate analysis confirmed the nutritional value, with protein contents of 19.60% and 47.68% in FPH and Chlorella sp., respectively, along with flavonoids (2.69%) in Chlorella sp.. These results demonstrate the successful integration of bioactive compounds into effervescent formulations, addressing consumer demand for sustainable and health-promoting products. This research provides a framework for developing innovative functional food products, contributing to the global advancement of nutraceuticals and sustainable food solutions. Future studies should focus on long-term stability, bioavailability, and consumer acceptability to enhance the commercialization potential of these effervescent tablets.

Cite this as: Yuniarti, T., Putri, N.A., Dharmayanti, N., Mulyono, M., Sugiwati, S., Martosuyono, P., Dewi, I. J. P., Maulani, A., & Hidayat, T. (2025). Optimization of Effervescent Tablets in Sensory Acceptance from the Active Ingredients of Fish Protein Hydrolysate and Microalgae *Chlorella* sp. powder . *Jurnal Ilmiah Perikanan dan Kelautan*, 17(1):223–237. https://doi.org/10.20473/jipk.v17i1.56019

1. Introduction

Effervescent is a beverage tablet that is easy to carry and pampers the taste buds with varied flavors. Effervescent tablets, a widely preferred pharmaceutical dosage form, release carbon dioxide when acid-base reactions occur in aqueous solutions. This reaction enhances sensory appeal by providing a sparkling sensation, making them an attractive medium for delivering functional ingredients (Meisner et al., 2023). Effervescent tablets are primarily composed of acid and base components, such as citric acid and sodium carbonate, which react to generate carbon dioxide. This formulation principle enables the incorporation of bioactive compounds, such as Fish Protein Hydrolysate (FPH) and microalgae Chlorella sp., to develop functional and organoleptically enhanced products. As a beverage in tablet form, the ingredients required should be readily soluble in water (less than 15 minutes) (İpci et al., 2016). Modern effervescent formulations aim to balance sensory appeal with health benefits by incorporating bioactive compounds. This study explores the integration of FPH and *Chlo*rella sp. powder into effervescent tablets, emphasizing optimization for sensory acceptance and nutritional value (Yanti et al., 2023). Some of the health-promoting ingredients that can be added to beverages are dissolved proteins and bioactive compounds.

As a nutrient, protein is a substance needed as a building material. Protein in a simple form, such as protein hydrolysate, not only acts as a building agent but also as an active ingredient that has a role in health. Fish protein hydrolysate (FPH) is produced by degrading fish protein into smaller fragments through hydrolysis using protease enzymes such as Alcalase under controlled pH, temperature, and hydrolysis time (Yuniarti et al., 2021). FPH is rich in essential amino acids such as lysine, arginine, leucine, valine, isoleucine, and threonine (Prayudi et al., 2020). Stripe trevally protein hydrolysate produced by enzymatic hydrolysis using local protease for six hours had potential as a functional ingredient, particularly as an antioxidant, ACE inhibitor, and antihypertensive agent. Among those methods, enzymatic hydrolysis is preferable because enzymatic reactions are not extreme, easy to control, minimize by-product formation, and do not reduce the nutritional value of the protein. The hydrolysis result is a shorter protein, peptide, and free amino acid mixture (Putalan et al., 2018). Fish protein hydrolysate has a high solubility in water at pH 2-10 but aggregation at pH 4, has good emulsion capacity, considerable foaming ability, and is easily absorbed by the body (Dinakarkumar et al., 2022).

Microalgae are microscopic photosynthetic organisms containing various essential nutrients such as protein, vitamins, minerals, and omega-3 lipids acids (Ibrahim *et al.*, 2022). *Chlorella* sp. is a type of

microalgae that contains bioactive ingredients. Chlorella sp. has been produced on an industrial scale to food-grade quality. Microalgae are considered healthy, safe, and promising as potential value-added ingredients for the functional food industry (Ampofo and Abbey, 2022). However, the antioxidant production in microalgae Chlorella sp. is usually determined using samples harvested at either the exponential or stationary phase (Yusuf et al., 2022). Vignaud et al. (2023) studied the potential antioxidant effects of microalgae and their biomolecules on mitochondrial activities and skeletal muscular oxidative stress during exercises or in musculoskeletal diseases, such as sarcopenia, chronic obstructive pulmonary disease (COPD), and Duchenne muscular dystrophy (DMD), through the enhancement and control of antioxidant pathways and protein synthesis. The primary antioxidants found in microalgae are carotene, astaxanthin, and tocopherol (Yang et al., 2023). The next challenge is making beverage-effervescent tablets enriched with fish protein hydrolysate, Chlorella sp., and organoleptically acceptable in the development of functional food or beverages and nutraceutical products.

A number of studies have been carried out to explore the potential for using protein and microalgae hydrolysates in effervescent tablets. These studies aim to optimize tablet formulation, evaluate its physical and chemical stability, and assess its release profile and bioactivity (Koyande *et al.*, 2019; Tan *et al.*, 2019). Several literatures have also discussed the production process of protein and microalgae hydrolysates, as well as the health benefits that may be provided by the combination of these two materials (Sun *et al.*, 2020; Aktsoglou *et al.*, 2021).

The Design Expert13.0® Program uses the d-optimal mixture design method to design optimal formulations to meet consumer tastes by producing superior products, nutrition, and organoleptic, to be accepted on the market. Mixture design is a Response Surface Design (RSM) method that combines independent variables or factors to produce responses that vary according to factor combinations (Sahin et al., 2016). This method has been widely applied in the food industry to optimize product formulas, walnut oil emulsions for water-based beverages (Gharibzahedi et al., 2012), such as coffee-soy beverages (Felberg et al., 2010), rose flower beverages (Mashkour et al., 2013), and the Persian yogurt beverages (dough) (Khodashenas and Jouki, 2020). Optimization steps include selecting the optimal concentration of the two active ingredients, selecting appropriate additional ingredients, and determining the optimal mixture ratio. In addition, the tablet's physical and chemical characteristics, release profile, and product stability will also be evaluated. The novelty of this research is the formulation of effervescent tablets made from FPH

and Microalgae that consumers or panelists can accept. This research aims to optimize the formulation of effervescent tablets by using FPH and microalgae *Chlorella* sp. powder as active ingredients organoleptically acceptable by the panelists.

2. Materials and Methods

2.1 Materials

The primary tools used include an inkjet tablet printing machine (IIM-Labo), a tablet hardness tester (ERWEKA GmbH), and a spectrophotometer (Mettler Toledo) for granule and tablet evaluation. Additional standard laboratory equipment was utilized for sample preparation and analysis. The main ingredient used is FPH powder obtained from the postgraduate laboratory collection of the Jakarta Technical University of Fisheries. FPH is made from snapper hydrolysed using Yuniarti et al. (2021) method. The Chlorella sp. powder food-grade type of microalgae is obtained from the marketplace. Additional food-grade ingredients are distilled water, citric acid, tartaric acid, sodium bicarbonate, saccharin, PVP, PEG 6000, and maltodextrin. All chemicals for chemical analysis, such as NaOH, FeCl₃, H₂SO₄ NaNO₂, and AlCl₃ have a pro analyst grade from Merck (Singapore).

Table 3. Concentration of dependent variable effervescent tablets (85%).

Ingredients	Concentration %
Citric acid	18
Tartaric acid	14
Sodium bicarbonate	32
Saccharin	1.5
PVP	3
PEG 6000	4
Maltodextrine	12.5

2.1.1 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

2.2.1 Qualitative and quantitative phytochemical analysis of microalgae

Qualitative phytochemical analysis of *Chlorella* sp. powder includes flavonoid, saponin, steroid, terpenoid, and alkaloid tests. Meanwhile, quantitative phytochemical analysis includes determining flavonoid

Table 1. The percentages of active ingredient formulation composition at the upper limit and lower limit of effervescent tablets.

Active ingredients	Lower limit	Upper Limit	Total active ingredients
FPH powder	5%	10%	15%
Chlorella sp. powder	5%	10%	15%

Table 2. Concentration of independent variables effervescent tablets (15%).

Run	FPH powder (%)	Chlorella sp. powder (%)
F1	5	10
F2	7.5	7.5
F3	7.5	7.5
F4	10	5
F5	6.67	8.33
F6	5	10
F7	10	5
F8	7.5	7.5
F9	6.25	8.75
F10	8.33	6.67
F11	8.75	6.25
F12	10	5
F13	5	10

levels in *Chlorella* sp. powder. All phytochemical analysis procedure refers to Rajkumar *et al.*, (2022).

2.2.2 Determination of upper limit, lower limit, and runs of effervescent formulation

The upper and lower limits for each independent variable are determined subjectively to influence the variation of effervescent tablet composition. FPH and microalgal hydrolysates refer to research on the formulation and physical evaluation of noni fruit extract effervescent tablets (*Morinda citrifolia* L). The total active ingredients used is 15% (Tanjung and Puspitasari, 2019). The formulations of the ingredients are based on subjective observations using the method of trial and error. The percentages of active ingredient formulation composition at the upper limit and lower limit of effervescent tablets are presented in Table 3.

2.2.3 Design of effervescent tablet formula

The formulation process utilized the d-optimal mixture design method in Design Expert 13.0® software to optimize the proportions of active ingredients (FPH and *Chlorella sp.* powder) while maintaining consistent dependent variables such as citric acid and sodium bicarbonate. This approach generated 13 formulations to identify the optimal balance of sensory and nutritional properties.

The formula obtained from Design Expert 13.0® is a combination of the proportion of the upper and lower limits of each factor previously determined.

2.3. Determination of the Quality of Effervescent Tablets

2.3.1 Physical testing of granules and effervescent tablet

The characteristics of effervescent tablets are determined to evaluate the quality of the tablets by comparing the evaluation results with the tablet quality requirements from the Ministry of Health of the Republic of Indonesia (DEPKES RI) for tablets with a size of more than 300 mg. Granule physical properties tests include flow rate tests, angle of repose tests, compressibility tests, and drying shrinkage tests; while effervescent tablet physical properties tests include tablet size and thickness tests, weight uniformity tests, tablet firmness tests, solubility tests referring to Indonesian Pharmacopoeia, edition V, 2014 on page 148.

2.3.2 Determination of sensory and hedonic quality of effervescent tablets

Sensory evaluation was conducted using the Rate-All-That-Apply (RATA) method with 30 un-

trained panelists, supported by a focus group discussion (FGD) to verify and refine sensory attributes. This approach ensured accurate measurement of sensory parameters, such as color, aroma, taste, and aftertaste, to assess consumer acceptance. The RATA method can overcome this weakness since the panelists provide intensity values for specific attributes (Ares et al., 2014). The advantage of the RATA method is that the panelists are allowed to describe the intensity of the product's sensory attributes. One of the studies using the RATA method is research regarding the specific profile for comparison of taste profiles for different sweeteners in black tea, chocolate milk, and natural yogurt (Tan et al., 2020). RATA testing is generally accompanied by a hedonic rating test to see consumer preferences for the tested product. The hedonic sensory assessment criteria are (1) very weak, (2) somewhat lipid, (3) moderate, (4) somewhat strong, and (5) very strong. The attributes in assessing the application of fish protein and microalgae hydrolysate in effervescent tablets are color (green), aroma (plant-specific and sweet), taste (sweet and sour), and aftertaste in the form of a score sheet. The hedonic test results obtained from this research will be tabulated using the mixture design application, and the quality value will be determined by finding the average for each panelist at a 95% confidence level.

2.3.3 Analysis of the chemical composition of effervescent tablets (proximate)

The analysis of the chemical composition of the active ingredients (FPH and *Chlorella* sp.) in powder form and effervescent tablets includes the product's ash, water, lipid, and protein content referring to SNI 01-2981-1992.

2.4 Analysis Data

The best formulation using the Design Expert 13.0® program with the d-optimal mixture design method aims to determine the right formulation for making effervescent tablets with FPH and *Chlorella* sp. powder. The response data is entered into the design expert, then analyzed by ANOVA which is already available in the Design Expert 13.0® software, and the output data is an optimal formula.

3. Results and Discussion

3.1 Proximate Measurement Results of the Active Ingredients FPH and Chlorella sp. Powder

The chemical composition of food ingredients can be determined by proximate analysis, which includes protein, lipid, water, ash, and fiber. It determines the approximate nutritional con-

tent of the product to be made. The chemical composition of the active ingredients consisting of FPH and *Chlorella* sp. powders are shown in Table 4.

Table 4. Chemical composition of FPH and *Chlorella* sp. powder (%).

	Protein	Lipid	Water	Ash	Fiber
FPH Powder	19.6	0.15	0.06	1.15	79.04
Chlorella sp. powder	47.68	0.15	0.06	1.15	38.31

The protein content of the FPH powder is 19.60%, while that of the *Chlorella* sp. powder is 47.68%. The protein content of this FPH powder is smaller than the FPH powder of yellow fish scads (Selaroides leptolepis) by 31.71-33.97% (Martosuyono et al., 2019), tilapia by 30.17%, milkfish by 27.98%, and shark by 28.27% (Annisa et al., 2017). According to Purbasari (2008), the increase in protein content in hydrolyzed products is due to the conversion of insoluble protein into soluble nitrogen compounds during the hydrolysis process, which is then broken down into simpler compounds such as peptides and amino acids to be easily absorbed by the body. The Chlorella sp. protein content in this study was lower than commercial products made from powdered Chlorella sp., i.e., 51-58% (Nguyen et al., 2022). However, this protein content was higher than the Chlorella sp. protein studied by Hussein et al. (2020), i.e., 6.9%. Petrova et al. (2018), the lipid content in hydrolysate products is believed to be influenced by the characteristics of the raw materials used and the process of separating the lipid after hydrolysis. The FPH products stored at low temperatures and filtration using filter paper or calico commonly use lipid separation methods. The lipid content of *Chlorella* sp. powder was 0.5%, which is lower than the lipid content of commercial *Chlorella vulgaris* powder, i.e., 5-40% (Safi et al., 2014).

The water content of FPH and *Chlorella* sp. was the same at 0.06%. This low water content classifies FPH and *Chlorella* sp. powder as hygroscopic food products (Enriquez, et al., 2017). The FPH and *Chlorella* sp. powder had a high ash content of 1.15%. This value is lower than FPH made from Fish Discards and By-Products from the North-West Spain Fishing Fleet by 15% (Henriques et al., 2021).

3.2 Result of Phytochemical Measurement of Chlorella sp. Powder

The results of the qualitative phytochemical test of *Chlorella* sp. powder showed strong positive flavonoids, tannins, saponins, and triterpenoids but did not contain alkaloid, saponin, and steroids (Table 5). Flavonoids are very effective for use as antioxidants and antibacterial. This is in line with the results of the study of Pietta (2000), showing flavonoids are high-class antioxidants because flavonoids work byscavenging free radicals and SOR (reactive oxygen compounds), such as superoxide anion radicals and

Table 5. Results of qualitative and quantitative measurements of phytochemicals in *Chlorella* sp. powder.

Sample	Parameter		Result	Unit	Analytical technique
	Phytochem	ical:	,		
	Flavonoid		Positive	-	Visualization of color
		Wagner	Negative	-	
	Alkaloid	Mayer	Negative	-	
C1.1		Dragendorf	Negative	-	
Chlorella sp. powder	Tanin		Positive	-	
powder	Saponin		Positive	-	
	Quinon		Negative	-	
	Steroid		Negative	-	
	Triterpenoid		Positive	-	
	Total of Fla	avonoid	2.69	% (w/w)	Spectrophotometry

The lipid content of FPH in this study was 0.15%. The lipid content in this study meets the standards for commercial fish FPH, which is less than 19-22% (Das *et al.*, 2021). This is due to differences in the characteristics of the raw materials and the process of separating the lipid after hydrolysis. According to

hydroxyl free radicals. Flavonoid compounds are multifunctional because they can react as reducers, capture free radicals, chelate metals, and reduce the formation of singlet oxygen. The quantitative phytochemical, especially for determining the total flavonoids of *Chlorella* sp. powder, contained 2.69%.

According to Sankhalkar and Vernekar (2016), flavonoids are polar compounds because they have hydroxy groups that bind to sugars and tend to solubilize in polar compounds. Flavonoids were tested using 10% AlCl₃ (aluminum chloride) as a stain. The formation of yellow spots indicates positive results for the presence of flavonoid compounds after spraying with 10% AlCl₃ and blue when viewed under 254 nm UV light. Research conducted by Abdel-Karim *et al.* (2020) explained that phytochemical components detected qualitatively in *Chlorella* include alkaloids, flavonoids, and phenols, where different extraction techniques produce different phytochemical components depending on the type of solvent.

3.3 Response of Mixture Design

The results of sensory and chemical quality response analysis on effervescent tablets with active ingredients of FPH and *Chlorella* sp. powder in mixture design are presented in Table 6. The results of the quadratic ANOVA test (Table 6) show a significant influence between FPH and *Chlorella* sp. powder on the intended response, where p-value <0.05. The lack of fit p-value for each response shows a non-significant effect with a p-value >0.05. Each response differs between the predicted R² and the adjusted R² values of 0.0157-0.2601. The test results show an agreement between the appearance response data and the model (independent variable) as a condition for the continuation of the optimization process (Şahin *et al.*, 2016).

3.4 Optimization of the Response of Effervescent Tablets

3.4.1 Color

Color is a material property resulting from the breakdown of the light spectrum. The addition of the more dominant Chlorella sp. powder to formula 13, with a response value of 3.83, caused the color to follow the dominance of this microalgae. Chlorella sp. has carotenoid compounds that produce yellowish-green pigment (Agustini and Winarni, 2017). The ingredient variables in formulas 1-13 and the color response (not shown), inform the use of Chlorella sp. powder was more dominant over FPH. The F13 consists of the independent variables of 5% FPH and 10% Chlorella sp. powder as a result of the best color response given. Meanwhile, FPH has a yellowish, slightly brownish color, which, if used too much, will change the tablet's color and make it cloudier. Therefore, the F13, which used more *Chlorella* sp. powder composition than FPH, is the most suitable formula for mixed two active ingredients.

3.4.2 Plant flavor and fishy flavor

Two flavor indicators, plant, and fishy, measure the flavor of effervescent tablets. *Chlorella* sp. powder produces the plant flavor, while the fishy flavor is made using FPH powder to manufacture effervescent tablets. Adding microalgae, like *Chlorella* sp. powder, to the effervescent tablet formulation can have the characteristic flavor of the microalgae itself. The more microalgae added, the more characteristicthe microalgae plant flavor will be, while using the FPH composition will influence the fishy flavor. The formula that produces the strongest typical plant flavor and the lowest fishy flavor is found in Formula 1, with an FPH concentration of 5% and 10% *Chlorella* sp. powder with values of 4.03 and 2.27. Microalgae has

Table 6. Results of the ANOVA test on the responses to the formulations of effervescent tablets.

Response	Model	P-Value	Lack of Fit	Difference between <i>Predicted R</i> ² and <i>Adjusted R</i> ²	Adeq Precision
Color	Linear	0.000	0.128	0.087	9.745
Plant flavor	Linear	< 0.0001	0.418	0.016	28.101
Fishy flavor	Linear	< 0.0001	0.179	0.056	12.519
Sweet	Quartic	0.014	0.992	0.228	5.405
Sour	Quadratic	< 0.0001	0.054	0.039	10.389
Aftertaste (sweet)	Quadratic	0.029	0.156	0.068	9.970
Hedonic	Linear	0.002	0.075	0.127	7.766
Protein content	Linear	0.012	0.535	0.156	5.958
Lipid content	Linear	0.037	0.505	0.158	4.657
Water content	Linear	0.000	0.831	0.062	10.937
Ash content	Quadratic	0.033	0.983	0.260	5.035

a hexanal group, which gives a grass aroma; while the hexanal compound in this microalga has a percentage of 20.21% (Agustini and Winarni, 2017). This compound will mask the flavor of other ingredients added to the formula 13 effervescent tablets. Therefore, F13 is the closest to a positive response.

3.4.3 Sweet and sour taste

Taste is one of the sensory attributes evaluated by adding saccharin, citric acid, and tartaric acid to effervescent tablets to impart a sweet and sour taste to effervescent tablets with active ingredients FPH and Chlorella sp. powder. Adding a sweet and sour taste increases the palatability of effervescent tablets. Using citric acid and sodium bicarbonate significantly affects carbonated beverages' color (clarity), aroma, acidity, and taste. The higher the concentration of sodium bicarbonate used, the lower the level of transparency and smell of the carbonated beverage. Meanwhile, the more citric acid and sodium bicarbonate added, the higher the acidity but the lower the sweetness of carbonated beverages (Yamamoto et al., 2020). The tongue is vital in detecting and tasting the taste of beverages. The tongue's influence on cold carbonated beverages results from their components, such as sour and sweet tastes, so there is a refreshing sensation from effervescent tablets containing FPH and Chlorella sp. powder. The best formula for sweet and sour taste response is found in the F13 with a total sweet taste response value of 2.9 and sour taste of 2.57, containing a concentration of FPH 5% and microalgae 10%.

3.4.4 Aftertaste (sweet)

Aftertaste response analysis involves evaluating the taste experience after consumption of a food or beverage. Aftertaste refers to the taste sensation that continues to appear after the substance is consumed, even though the food or beverage is no longer in the mouth. In aftertaste response analysis, several factors can be evaluated, including intensity, type of taste, duration, consistency, and harmony with the primary taste (Ramdani, 2015). The best analysis results on the aftertaste response were in the F1 with a composition of 5% FPH and 10% Chlorella sp. powder at a value of 3.07 with a quadratic model. Aftertaste responses can provide insight into the post-consumer taste characteristics of an ingredient and provide valuable information for food or beverage product development. This information can be used to understand how aftertaste influences the overall taste experience, consumer satisfaction, and preference for a particular product.

3.4.5 Hedonic

The best formula from the hedonic analysis was found in the F13, with a value of 3.76. This effervescent formula contains a concentration of 5% FPH and 10% Microalgae. Adding active ingredients, FPH and *Chlorella* sp. powder increases the nutrition of beverages in effervescent tablets, significantly increasing the protein content. The development of food technology requires the industry to make food or beverage products that are not only delicious on the taste buds but also beneficial for health (van der Heijden et al., 2020). Adding FPH and *Chlorella* sp. powder gives a level of liking at level 3.76 (including category 4=somewhat). This result contradicts a paradox circulating in society that unhealthy food or beverages taste better than healthy food or beverages.

3.4.6 Protein content

The protein content in FPH, which was not made into effervescent tablets, was 19.60%. Meanwhile, the protein content made into effervescent tablets decreased by 6-8%. Analysis of protein content shows that effervescent tablets had the best protein content, F1, with a composition of 5% FPH and 10% microalgae. Decreased protein levels in effervescent tablets were due to the addition of other ingredients. The protein content in these effervescent tablets resembles effervescent tablets made from egg powder at 8% (Wulandari et al., 2021).

3.4.7 Lipid content

The analysis results show that the response to the lipid content of 13 effervescent tablet formulations was very low or even non-existent. Most effervescent tablets are designed to dissolve in water before being consumed. Hence, the presence of lipids is unnecessary because it can reduce the solubility level of the ingredients. The ANOVA results show that the effervescent tablet with the best lipid content was the F13, with a value of 10.91, FPH concentration of 5%, and *Chlorella* sp. powder of 10%. The lipid content response analysis can be seen in a linear model with a p-value of 0.0002, i.e., <0.05, meaning significant model results. In contrast, the lack of fit value shows insignificant results.

3.4.8 Water content

Analysis of the response to water content examined the effect of water content in effervescent tablets on various aspects, such as taste, freshness, texture, product durability, and storage conditions (Suparman et al., 2021). The ANOVA results show that the effervescent tablet with the best water content was the F13, with a value of 10.91%, FPH concentration of 5%, and Chlorella sp. powder of 10%. The sweet and sour taste

response analysis can be seen in a linear model with a p-value of 0.0002, i.e., <0.05, meaning significant model results. In contrast, the lack of fit value shows insignificant results.

3.5 pH Value

Food with a low pH usually cannot grow bacteria but can become damaged due to the growth of yeast and mold. The pH value of effervescent tablets ranges from 5 – 6. The lowest pH value of 5 was found in formulations 1, 5, 6, 9, and 13. Meanwhile, the highest pH value of 6 was found in formulations 2, 3, 4, 7, 8, 10, 11, and 12. Thus, the higher the concentration of microalgae containing FPH, the higher the pH. This pH value is considered good and still meets the requirements, close to neutral pH (Reddy *et al.*, 2017).

3.6 Physical Characteristics of Granules and Effervescent Tablets

3.6.1 Evaluation of physical characteristics of granules

Granule evaluation refers to 4 indicators: flow rate, repose angle, compressibility, and drying loss. Granules result from the extraction of a commodity in the form of tiny flakes such as sand. The first indicator in evaluating granules is to measure the flow rate. Several factors influence flow rate, including particle size, shape, and cohesiveness. Different flow rates are caused by the body, density, and size of each particle being different (Parajuli-Baral, 2023). Evaluation results of effervescent tablet granules are shown in Table 7.

3.6.2 Flow rate test

The flow rate for each formulation shows all values are above 4 g/sec. The highest flow rate was at point F4, meaning that F4 had the fastest flow rate, i.e., 6.3 g/second. Based on the flow rate test category, test value results in the 6-10 g/second range are categorized as good. Meanwhile, the lowest flow rate test value known was F3, meaning that F3 had the slowest flow rate compared to the other formulations. The flow rate value for F3 was 4.08; according to the flow rate results category, F3 is between the difficult and good categories. However, adding active substances from natural extracts will have poor flow properties (Adi-Dako et al., 2021).

Table 7 shows that apart from F4, included in the highest flow rate, is F2, F8, and F12, respectively 5.6; 5.4; 5.9. The four formulations had a good flow rate category because they were still within the specified good standard range. The average flow rate for all formulations was 4.89 g/second, or between difficult and good categories. The flow rate is also influenced by the tablet's mass or weight, which is almost the same (Wulandari *et al.*, 2021).

3.6.3 Repose angle test

Another indicator that can be measured in pellet evaluation is the angle of repose. The rise of relaxation is a parameter of pellet evaluation, which is a comparison between the physical properties of the pellet mixture and the cotangent of the height of the cone that forms the pellet and gives the size of the angle that includes it (Rajani *et al.*, 2017). The highest

Table 7	7. Eva	luation	results	of	granul	les.
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Formula	Flow rate	Condition	Repose angle	Condition	Compressibility	Condition	Drying loss	Condition
F1	4.8	Good	27.7	Excellent	9.66	Excellent	3.56%	Good
F2	5.6	Good	25.03	Excellent	14.79	Good	3.98%	Good
F3	4.08	Good	25.69	Excellent	14.69	Good	3.83%	Good
F4	6.3	Good	27.65	Excellent	15.71	Fair	4.87%	Good
F5	4.75	Good	26.29	Excellent	11.54	Good	4.95%	Good
F6	4.2	Good	30.41	Excellent	6.94	Excellent	3.74%	Good
F7	4.4	Good	27.79	Excellent	17.24	Fair	4.56%	Good
F8	5.4	Good	25.83	Excellent	9.66	Excellent	3.56%	Good
F9	4.4	Good	25.5	Excellent	10.61	Good	4.70%	Good
F10	4.2	Good	30.11	Excellent	16.18	Fair	3.46%	Good
F11	4.5	Good	26.06	Excellent	8.82	Excellent	3.77%	Good
F12	5.9	Good	26.24	Excellent	17.57	Fair	4.95%	Good
F13	5.1	Good	30.88	Excellent	8.57	Excellent	3.08%	Good

angle of repose was found at F13, with an angle of repose of 30.88°. According to the standard angle of repose category, this value falls into the relatively poor category. The flatter the cone resulting from the formation of powder or granules, the smaller the angle of repose, the better the properties of the granules, making it easier to make tablets (Martinez et al., 2023). The highest value of the angle of repose shown at F13 is interpreted as a relatively poor category. Meanwhile, the lowest angle of repose value was 25.03° for F2. This means that F2 had the best angle of repose category among the other formulations.

3.6.5 The compressibility test

The compressibility test is a test to assess the strength of the granule "bridge" (power bridge strength). Low compressibility values indicate better flow properties. The higher the compressibility level of the effervescent tablet, the worse the quality of the effervescent tablet. Factors that influence the level of compressibility are density and particle size (Martinez et al., 2023). The lowest compressibility value was F6 at 6.94. This value is in the excellent category. Meanwhile, the highest compressibility value was F12 at 17.57, which means it is in a good category. Formulations that fall into the excellent compressibility category apart from F6 are F1, F5, F8, F9, F11, and F13.

Table 8. Physical characteristic of effervescent tablet.

	Physical cl	haracteristi	c of effervescent tak	olets
Formula	Weight Uniformity (mg) Si	ze Uniform (cm)	ity Firmness (%) Di	issolve time (minute)
F1	4.415	2.53	0.74	7*
F2	4.410	2.54	0.7	8*
F3	4.468	2.53	0.79	7*
F4	4.450	2.53	0.41	6*
F5	4.505	2.53	0.77	7*
F6	4.495	2.53	0.72	4
F7	4.469	2.53	0.75	7*
F8	44,556	2.53	0.51	6*
F9	4.454	2.53	0.64	6*
F10	4.461	2.54	0.62	10*
F11	4.458	2.53	0.46	10*
F12	4.447	2.54	0.57	6*
F13	4.461	2.53	0.79	5

3.6.4 Drying loss test

Drying losses provide a maximum limit (range) regarding the number of compounds lost due to the drying process (Aklima et al., 2020). The highest drying loss values were F5 and F12 (4.95%), while the lowest was F13 (3.08%). According to the Indonesian Ministry of Health, the quality standard for water content in the standardization of medicinal ingredients is 10%. This means that if the drying loss is above 10%, it does not meet the standardization requirements. The entire F1-F13 effervescent tablet formulation above shows a value below 10%, which all meets the standard drying loss value for effervescent tablets.

3.7 Evaluation of Physical Characteristics of Effervescent Tablets

The evaluation of effervescent tablets' physical characteristics aims to measure effervescent tablets' quality by comparing the evaluation results with the tablet requirements in the Indonesian Pharmacopoeia, edition V, 2014, on page 148. The physical characteristics of the effervescent tablets in this study were carried out by measuring uniformity of size, uniformity of weight, firmness, and time. Below is the data presented in evaluating the physical properties of effervescent tablets (Table 8).

3.7.1 Weight uniformity

The uniformity of effervescent tablet weight in this study was determined by weighing all tablet formulations (mg). The uniformity of tablet weight in each formulation from F1 to F13 was not much different. The tablet size range is 4.4104-4.5045 mg. The weight of the tablets on the F1-F13 has fairly good weight uniformity. Weight uniformity is used to determine the dosage for an effervescent tablet (Nagashima et al., 2013).

3.7.2 Size uniformity

The uniformity of effervescent tablet size in this study was determined by measuring the tablet diameter and thickness. The uniformity of tablet size in each formulation from F1 to F13 was similar. The tablet size range is 2,530-2,540 cm. The tablet size will affect its hardness, thereby determining the level of tablet roughness. The more optimal size formed in tablet production will help increase the optimization of tablet formation, which has a smoother surface and looks perfect (Rajani *et al.*, 2017).

3.7.3 Firmness

Firmness tests were also carried out to support the physical evaluation of effervescent tablets in this study. The firmness test aims to determine the level of resistance of the tablet surface to friction during packaging, shipping, or storage. This test is influenced by the hardness of the tablet and the size of the tablet (Permadi *et al.*, 2021). The lowest firmness value was F4 (0.41%), while the highest was for two formulations, F5 and F13 (0.79%). The higher the firmness value in a tablet formulation, the higher the resistance level. The overall toughness test has an average of <1%. A toughness value below 1% is considered an acceptable formula (Kartikasari *et al.*, 2015).

3.7.4 Dissolve time

The porosity of the tablet influences the speed of dissolution in effervescent tablets in water, and porosity is influenced by the diameter size distribution and compressive force during pressing (Rajani et al., 2017). The average dissolution time for each formulation is 6 minutes, and this value only meets the requirements of 5 minutes. The highest dissolution time was in formulations 10 and 11; both had relatively longer solubility than other formulations. Meanwhile, the lowest dissolving time was in formulation 6, which had a dissolving time of 4 minutes. This is the only formula that meets the dissolving time requirements. The relationship between size distribution and weight uniformity influences the penetration of water, which

will be the medium for dissolving the tablet. Therefore, these three parameters will determine the physical characteristics that meet the requirements regarding the level of solubility (Dewi *et al.*, 2010).

3.7.5 Results of effervescent tablet optimization

Optimization results of effervescent tablet formulation enriched with FPH and *Chlorella* sp. powder and observed the response of organoleptic, hedonic, and chemical test results are in accordance with the safety and quality requirements of drugs, which were analyzed using the Design Expert application. The best formulation for effervescent tablets was obtained. The best formulation of effervescent tablets was at an FPH percentage of 5.873% and *Chlorella* sp. powder of 9.127% with a final tablet weight of 4.5 g. The results of optimizing effervescent tablets are shown in Table 9.

Table 9. Results of optimization formulation effervescent tablets.

Compound	Percentage %
FPH	5.873
Chlorella sp. powder	9.127
Citric acid	18
Tartaric Acid	14
Sodium bicarbonate	32
Saccharine	1.5
PVP	3
PEG 6000	4
Maltodextrine	12.5

3.8 Optimization of Organoleptic, Hedonic, and Chemical Results Conditions for Effervescent Tablets Formulation

This optimization was carried out to obtain a formula with an effervescent tablet composition with FPH and *Chlorella* sp., the suitable powder, to obtain the desired product and have an optimal response. Each component was weighed and responded to in the optimization process based on its importance to the resulting product. In this regard, effervescent tablets with FPH and *Chlorella* sp. powder had good physical characteristics and had functional value for health. Table 10 shows the optimization criteria for effervescent tablet formulas containing FPH and *Chlorella* sp. powder.

3.9 Verification of Optimum Formula of Effervescent Tablets

At the verification stage of the optimum formula, the actual response value was compared with the

resulting response prediction. The optimum formula's chemical, hedonic, and physical analysis show that the predicted response and actual values were close. The actual value obtained for the elasticity response was in the confidence interval range. The organoleptic, hedonic, and chemical values of effervescent tablets

ment results were compared with the predicted value from the Design Expert 13.0® program. The measurement results show that the hedonic response exceeds the highest limit of the 95% prediction interval of 3.95 and the prediction of 3.67 to become 4.07. Confident interval (CI) is a range that shows the average expec

Table 10. Criteria for effervescent tablet formulas containing FPH and *Chlorella* sp. powder.

Response	Value target	Lower limit	Upper limit	Interest
FPH	Is in range	5	10	***
Chlorella sp. powder	Is in range	5	10	***
Color	Maximize	1.93	4.17	***
Plant flavor	Maximize	2.37	4.2	***
Fishy flavor	Minimize	2.27	3.13	***
Sweet	Maximize	2.41	3.43	***
Sour	Maximize	2.23	3.3	***
Aftertaste (sweet)	Maximize	2.47	3.17	***
Hedonic	Maximize	3.2	3.83	***
Protein content	Maximize	6.38	7.99	***
Lipid content	Maximize	0.2	0.39	***
Water content	Minimize	10.91	12.63	***
Ash content	Minimize	22.09	24.88	***

Table 11. Optimum formula verification results.

Dagnanga	Validation	Duadiation	95% Predi	ction interval
Response	Validation	Prediction	Low	High
Color	4.03±1.17	2.929	3.348	3.955
Plant flavor	4 ± 2.11	2.372	3.614	3.838
Fishy flavor	2.67 ± 1.17	2.622	2.309	2.528
Sweet	3.5 ± 2.11	3.128	2.917	3.324
Sour	3±1.17	2.906	2.789	3.085
Aftertaste (sweet)	3.27 ± 0.69	2.943	2.980	3.166
Hedonic	4.07 ± 2.12	3.393	3.581	3.777
Protein content	8.29 ± 0.02	4.397	7.319	7.957
Lipid content	0.3 ± 0.3	0.414	0.261	0.360
Water content	11.27 ± 0.31	11.442	10.979	11.456

were in the confidence interval and prediction interval range as shown in Table 11.

The verification stage is an optimization stage that aims to prove and ensure the match between the actual and predicted values of the optimum formula resulting from the Design Expert 13.0® program. At the verification stage, there is a prediction interval (PI) value, which shows the confidence interval of the response value at the 95% confidence level. The verification stage was done by reproducing fish and microalgae protein hydrolysate effervescent tablets with previously obtained optimum formula and continued with response measurements. The response measure-

tation of measurement results at a significant level of 5%. The prediction interval (PI) is a range that shows the expected results of the following response measurement under the same conditions at a significance level of 5% (Putra, 2015).

4. Conclusion

This research successfully developed an optimized formulation for effervescent tablets utilizing Fish Protein Hydrolysate (FPH) and *Chlorella sp.* powder as active ingredients, employing the d-optimal mixture design method. The optimal formula, compris-

ing 5.873% FPH and 9.127% Chlorella sp. powder, demonstrated enhanced sensory acceptance, including color, plant aroma, fishy aroma, sweet and sour taste, aftertaste, and hedonic scores. Chemical analyses revealed significant nutritional properties, such as high protein content (19.60% in FPH and 47.68% in Chlorella sp.) and bioactive compounds like flavonoids (2.69%). The statistical analysis confirmed the model's robustness, with an R2 value of 0.93, ensuring reliable predictions for formulation optimization. These findings contribute to the development of functional food products by providing a nutritionally enriched and sensory-appealing effervescent tablet. This study addresses a critical gap in integrating bioactive compounds into functional food systems, demonstrating the potential of FPH and Chlorella sp. as ingredients for sustainable and health-promoting applications. Future research should investigate the product's long-term stability, bioavailability, and consumer acceptability to expand its application in nutraceutical markets.

Acknowledgement

We thank all of the institutions who contributed to this research: LPDP BRIN, as the funder for the research, the Naval Pharmacy Laboratory (LAFIAL) was permitted to research making effervescent tablets, and the Management of Jakarta Technical University of Fisheries permitted the use of frozen storage facilities for storing chemicals and chemical and sensory testing.

Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author is as follows, Tatty; as a main author, and conceptor, wrote the manuscript with input from all authors. Nanda; carried out tests in the laboratory and wrote the manuscript. Niken; wrote the manuscript with input from all authors. Mugi; sampling data and assisting with statistical analysis. Sri; determination of hydrolysis degree and antioxidant activity. Pujoyuwono; made FPH powder. Ita; in charge of overall direction and planning. Aghitia; wrote the research funding proposal and manuscript. Taufik; determine procedure analyze, and interpret data.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The authors affirm that no artificial intelli-

gence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

Funding Information

This research was funded by the National Research and Innovation Agency (BRIN) based on Deputy Research and Innovation Facilitation decree 65/II.7/HK/2022 about Research and Innovation Program for Advanced Indonesia Batch 1 in 2022 on behalf of Dr. Tatty Yuniarti, M.Si.

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