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Optimization Formula of Collagen Nanoemulgel from Waste Milkfish (*Chanos chanos*) Bones as Antiaging

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Abstract

Climate change has negative impacts throughout the countries in the world, especially Indonesia, which has a tropical climate. Successful aging can be achieved by building individual self-confidence through prevention and skin aging. Indonesia is a producer of milkfish waste, reaching 5.4 tons/year, which is in line with the increasing center of "Otak-otak" milkfish souvenirs found in Gresik. The waste, especially fishbones, can be synthesized into collagen. The characteristic fishbone collagen waste are in the form of large porous sheets, while particle size is an essential factor in the absorption effectiveness of cosmetics. Therefore, Nanoemulgel is able to overcome this problem. This research aims to produce best collagen nanoemulgel from milkfish bone by physical evaluation with varying ratios of surfactant: cosurfactant in collagen nanoemulgel. The research method was done experimentally by comparing the components Tween 80: PEG 400 F1 (30:30), F2 (40:20), and F3 (35:15) through qualitative and quantitative physical characteristics using one-way anova. Evaluations whose results differ significantly are pH on the 7th day, spreadability, and viscosity. Research results show F2 is the best formula and meets physical stability specifications : particle size 3.89 nm, PDI 0.1906, zeta potential -0.2 mV, pH 4.7±0.04, viscosity 890.1± 4.26 cps, spreading 5.93±0.23 cm.

Keywords : Antiaging, Collagen, Milkfish, Nanoemulgel, Physical Characterization

INTRODUCTION

Climate change has negative impacts throughout the countries in the world, especially Indonesia, which has a tropical climate. It is reinforced by the average temperature in Indonesia reaching 31.1^{o}C and increasing by 2% until 2099 (Ahmad, 2018). The high temperature is caused by exposure to UV rays, which start to penetrate the thinning ozone layer, causing an increase in premature aging of society (Indonesia Climate Risk Country Profile, 2021). WHO states that productivity in successful aging can be achieved by building individual self-confidence through prevention and skin aging (Ramos-e-Silva et al., 2018). One of the active ingredients that can prevent skin aging is collagen (Sagi et al., 2019).

Collagen is a complex protein that builds human skin structure (Wijaya, 2021). Collagen can be obtained from various land and marine animal sources and is also found in all parts of the body, such as skin, bones, and muscles (Soekandarsi & Evi, 2019). Indonesia produces milkfish waste, reaching 5.4 tons/year because of the increase in the Otak-otak milkfish souvenir center in Gresik City (Lutfiana et al., 2019). One way to reduce the impact of this waste on climate change is the use of collagen from fishbone waste. In line with the research focus on the commodification of local wisdom to overcome health problems and supported by the green economy concept, collagen can be utilized from milkfish bone waste as a source of active ingredients.

The dosage form used for an active ingredient influences the effectiveness of those materials (Setyowati & Setyani, 2015). The characteristic properties of collagen from fishbone waste are the form of porous sheets of large sizes (Muralidharan et al., 2014). Particle size is an essential factor for the main application of collagen, especially in the topical use of cosmetic preparations (Desmelati et al., 2020). One of the solutions to this problem is to reduce particle size to form a nanoemulgel preparation.

Nanoemulgel is a colloidal system consisting of a mixture of emulsion and gel. Emulsion could protect the drug from enzymatic degradation hydrolysis and increase permeation like other nanocarriers. The nanoscale measures between 1-100 nm, but nowadays, the criteria is extended to larger particles up to a maximum size of 500 nm (Hudson & Margaritis, 2014). With this size, the nanoemulgel formulation aims to increase drug penetration through the skin. It can maintain therapeutic concentrations of the drug for a sufficient period (Donthi et al., 2023). The gel portion increases viscosity and spreadability, thereby increasing retention time. It also reduces surface and interfacial tension, thereby increasing thermodynamic stability. Nanoemulgel has various advantages, such as high drug delivery capacity, penetration, better diffusion, and low skin irritation compared to other nanocarriers (Azeez & Alkotaji, 2021). With many advantages of this delivery system, it is hoped that collagen will easily penetrate through the skin if it is formulated into a nanoemulgel.

Making nanoemulgel consists of three stages, namely the formation of nanoemulsion, the development of gel mass, and a combination of nanoemulsion and gel mass. The essential point for the formation of globules is the addition of the surfactant and co-surfactant used (Imanto et al., 2019)). Therefore, optimization of surfactants and co-surfactants in the first phase, namely the formation of nanoemulsions, is vital, where collagen with a significant molecular weight can be reduced in this phase. In this study, three nanoemulgel formulas were made with a ratio of Tween 80 and PEG 400, F1 (30:30), F2 (40:20), and F3 (35:15). Based on this background, this research aims to produce the best formula which is based on the results of physical evaluation with variations in the ratio of surfactant and cosurfactant in collagen nanoemulgel preparations from milkfish bone waste as antiaging in the era of climate change. The evaluation carried out included nanoemulsion tests, namely homogeneity tests, particle size, polydispersity index, zeta potential, and emulsion type. Meanwhile, nanoemulgel characteristic tests include organoleptic tests, pH, viscosity, spreadability, physical stability, and feasibility tests.

METHODS

This research was experimental, with gel formula and formula components as fixed variables other than surfactant and cosurfactant in the preparation of collagen nanoemulgel from milkfish bone waste. The variable depended on the results of physical tests and the effectiveness of variations in the surfactant equal to the cosurfactant ratio in the collagen emulgel preparation from milkfish bone waste. Meanwhile, the independent variable was the comparison between surfactant and cosurfactant, which were Tween 80 equal to PEG 400.

Experimental Design

Product development is carried out in 3 stages. The first stage was making the active ingredient of collagen. The second stage was the formulation and evaluation of nanoemulsion and nanoemulgel. The final stage was testing the feasibility of the product on ten human subjects with several types of skin. The statistical analysis was carried out using the one-way ANOVA method to see whether there was an effect of comparing three groups of variations in the combination of surfactant and cosurfactant on physical tests and effectiveness. The design formula with the ratio of surfactant and cosurfactant is as follows:

Formula 1: Tween 80: PEG 400 = 30:30
Formula 2: Tween 80: PEG 400 = 40:20
Formula 3: Tween 80: PEG 400 = 45:15

Data Analysis

The data was collected through physical tests. The effectiveness of the three formulas was then analyzed statistically using the SPSS one-way anova method to see whether there were significant differences in each formula. Next, the best formula was determined by fulfilling the physical requirements for nanoemulgel preparations.

Materials and Instrumentation

Nanoemulgel from milkfish bone waste was prepared using sodium hydroxide (technical), milkfish bone waste, acetic acid (technical), bromelain enzyme, Aquadest, avocado oil (cosmetical grade), tween 80 (cosmetical grade), PEG 400 (cosmetical grade), carbomer 940 (cosmetical grade), TEA (cosmetical grade), glycerin (cosmetical grade), phenoxyetanol (cosmetical grade). The nanoemulsion and nanogel stability and physical evaluation will measured by Viskosimeter Brookfield, Particle Size Analyzer, binocular microscope, pH meter, sentrifuge, skin test analyzer, sonikator (GT Sonic), dan ultra turax (IKA T18 Basic).

A.5 Procedure

a) Collagen Extraction from Milkfish Bone Waste

The extraction method was adapted from previous research (Hariyati et al., 2019) with several modifications (Hariyati et al., 2019). After extraction, the quality of the dried collagen was checked in the form of water and ash content.

b) Emulgel Formula Optimization

The active and additional ingredients were processed through three stages: forming nanoemulsion, developing gel mass, and combining the nanoemulsion with the gel mass. The formula used in this research is shown in Table 1.

Table 1. Formulation of Nanoemulsion

Composition	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Collagen Solution	5	5	5
Avocado Oil	6	6	6
Tween 80	30	40	45
PEG 400	30	20	15
Aquadest	29	29	29

The first preparation was homogenized Avocado oil with turrax at 11.000 rpm and stirrer at 500 rpm. The second was adding Tween 80 slowly, then processing the mixture with turrax and stirrer until homogeneous. PEG 400 was added slowly and stirred with turrax and stirrer until it reached the homogeneous (oil phase) phase. Then, the collagen solution was added to distilled water and homogenized with a magnetic stirrer until homogeneous (water phase). The water phase was mixed into the oil phase and homogenized with turrax and stirrer until milky white was formed. The final stage was sonified for 1 hour at a temperature of 40 kHz until clear. Then, it was followed by adding the gel phase with ingredients, as shown in Table 2.

Table 2. Composition of Gel-Nanoemulsion

Composition	Amount
Carbomer	1 g
TEA	3 drops
Gliserin	5 g
Phenoxyetanol	0.8 g
Aquadest	80 mL
Nanoemulsi	64.5 ml
Fragrance	0.2 ml

The mixing step was done by adding the gel, nanoemulsion, carbomer, and TEA. Then, it was followed by stirring with a hand-blender, adding gel mass, adding glycerin, stirring with a hand-blender until homogeneous, adding phenoxyethanol, stirring with a hand-blender until homogeneous. The next was adding the fragrance, stirring with a hand-blender until it was homogeneous. Finally, nanoemulsion and water were added and sonicated for 60 minutes at 40^{o}C 40kHz.

c) Stability Evaluation

The physical evaluation of nanoemulgel comprises two parts: the evaluation of nanoemulsion characteristics and the physical evaluation of nanoemulgel preparations. Meanwhile, the evaluation of physical nanoemulgel preparations includes organoleptic tests, pH, spreadability, and centrifugation stability tests, observed every week at room temperature for 7 days.

d) PSA and Zeta potensial Assay

Measuring the size of nanoemulsion using a PSA (Particle size analyzer) tool uses the laser diffraction principle. The PSA method provides an overview of the particle size uniformity of the nanoparticles formed, including their distribution. Zeta potential analysis is a technique for determining the ion charge on colloidal nanoparticles. The general type of zeta potential value is +100mV to -100mV. In various journals, it is stated that the stable condition of nanoparticles is more than +30mV or less than -30mV (Gupta & Trivedi, 2018). The polydispersity index (PdI) is close to 0, indicating that the particle size distribution is homogeneous with a more uniform size, so it does not tend to agglomerate easily.

RESULTS AND DISCUSSION

Collagen stability evaluation tests are carried out through water and ash content tests, which are shown in Table 3 below:

Table 3. Moisture and Ash Content Of Collagen

Moisture (%)	Ash Content (%)
20.33± 5.51	3.48±1.52

The water and ash content results are better with the defatting process compared to previous research (Nasyanka et al., 2023). This is possible because most of the ash content results from burning carbon atoms from proteins still in fish bones.

Homogeneity Prepared Nanoemulsion

The results of the nanoemulsion homogeneity test can be seen in Table 4 and Figure 1. Based on the homogeneity test, the stability of the nanoemulsion in 7 days is still good, as proven by the results that it is still homogeneous on the seventh day.

Table 4. Homogeneity of Nanoemulsion

Week	F1	F2	F3
0	Homogenous	Homogenous	Homogenous
7	Homogenous	Homogenous	Homogenous

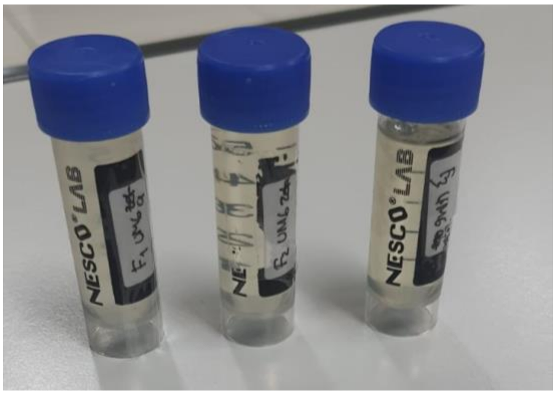

Figure 1. Nanoemulsion of F1, F2, and F3

Particle Size, Polydispersity Index, and Zeta Potential

Based on the test results, the three formulas (Table 5) meet the required nanoemulsion size range of 1-100 nm (Handayani et al., 2018). However, the smallest size in Formula 2 is 3.89 nm. A good polydispersity index for nanoemulsions is close to 0. All three formulas have a PdI close to 0, with the best index in Formula 2, which is 0.1906. A polydispersity index (PdI), which is close to 0, indicates that the particle size distribution is homogeneous with a more uniform size, so it does not tend to agglomerate easily. Meanwhile, a PdI value of more than 0.5 indicates that the particles are not homogeneous or have varying particle sizes (Avadi et al., 2010).

Meanwhile, the zeta potential values of the three formulas do not meet the specifications, namely more than +30 mV or less than +30 mV (Moacir Rossi Forim et al., 2012). The use of Tween 80 as a nonionic surfactant may tend to reduce the zeta potential value (Handayani et al., 2018) because this surfactant has no charge on its hydrophobic groups so that the surface of oil droplets covered with Tween 80 tends to be uncharged (Ode et al., 2023). The use of PEG 400 cosurfactant, which is also a nonionic surfactant, further reduces the zeta potential value of the formula (Maharini et al., 2020). The OH- group found in the Tween 80 and PEG 400 compounds can cause the zeta potential value to become negatively charged (Nurhidayati, 2020). This means that the nanoemulsion formula is not stable enough and that further optimization is needed to get better results.

Table 5. Particle Size, Polydispersity Index, and Zeta Potential Nanoemulsion Waste Milkfish (*Chanos chanos*) Bones

Formula	Particle Size (nm)	Polydispersity	Zeta Potential (mV)
F1	4.58	0.2978	-0.9
F2	3.89	0.1906	-0.2
F3	5.47	0.2309	-0.2

Type of Nanoemulsion

The results of the emulsion type test on F1, F2, and F3 all remained consistent with the oil-in-water emulsion type (o/w or w/a) until day 7, as shown in Table 6.

Table 6. Stability of Nanoemulsion Based on Emulsion Type

Formula	Before (Initially)	After 7 days
F1	o/w	o/w
F2	o/w	o/w
F3	o/w	o/w

After ensuring that the nanoemulsion results meet the requirements, it is mixed with the gel to form a slightly clear, milky white nanoemulgel, according to Figure 2.

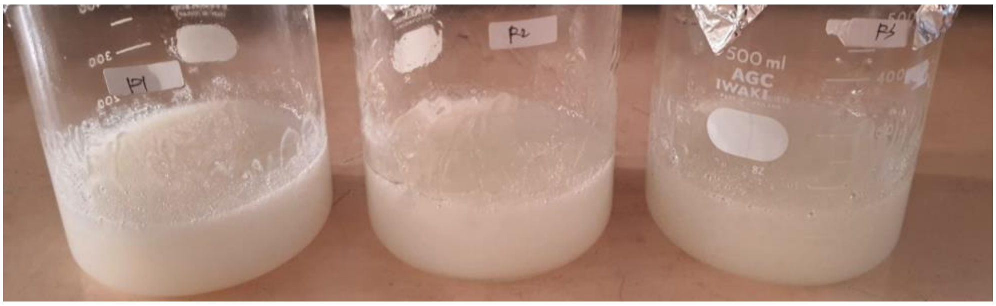

Figure 2. Nanoemulgel F1, F2, dan F3

Physical evaluations include organoleptic tests, pH, viscosity, stability, and product suitability on days 0 to 7 of each formula. The results of these tests are shown in Table 7. F1 and F2 have viscosity adjustments due to the use of carbomer so that the consistency becomes relatively thick, but in F3, there is no change. The three formulas have not changed much organoleptically, except for consistency. Based on the results of the pH test on the three formulas on the 0th and 7th days, it is found that there is a significant change ($p<0.05$). However, the changes for formulas 2 and 3 still meet the requirements, namely 4.5-7 for the semisolid preparation (Nasyanka et al., 2020). However, statistical tests using one-way ANOVA show no significant difference in pH on the 0th day among the formulas. However, pH significantly differs among the formulas on the 7th day ($p<0.05$). The spreadability test describes the ability of the preparation to be applied to the skin. Based on the results of the spreadability test on the 0th and 7th days, there is a significant difference among the formulas ($p< 0.05$).

However, only Formula 1 and 2 still have good spreadability for 5-7 until the 7th day. Based on the viscosity test results, the three formulas meet the requirements for gel preparations, namely between 500-10,000 cps (Andriani and Amin, 2023). Based on one-way ANOVA analysis, it is found that there is a significant difference between the viscosities of formula 1, formula 2, and formula 3 ($p<0.05$). Based on a centrifugation test at 4000 rpm for 30 minutes, stability is obtained from the 0th day to the 7th day. Therefore, the stability of the emulsion can still be maintained at both F1, F2, and F3. The organoleptic or hedonistic feasibility test results show that the formula with the average most preferred color is F1>F2>F3, respectively. The formulas are F1>F3>F2 according to the preferred smell. Meanwhile, the preferred consistency is F3>F1=F2. The irritation test results for the three formulations show "No signs of irritation" in the areas used. Therefore, it is worth testing the feasibility of the following product.

Table 7. Physical Studies Result of Nanoemulgel

	F1		F2		F3	
	0	7	0	7	0	7
			Appearance			
Color	Milky	Milky	Milky	Milky	Milky	Milky
Smell	Honey	Honey	Honey	Honey	Honey	Honey
Consistency	A bit runny	Semi-solid (Thick)	Semi-solid (Thick)	Semi-solid (Thick)	A bit runny	A bit runny
pH	5.01±0.10	4.08±0.06	4.08±0.06	4.7±0.04	4.76±0.06	5.44±0.51
Viscosity	602.1± 1.58	-	890.1± 4.26	-	919.6 ± 2.57	-
Spread Ability	5.80±0.1	6± 0.35	5.93±0.23	5.1±0.12	4.77±0.16	4.3 ± 0.15
Emulgel Stability	Stable	Stable	Stable	Stable	Stable	Stable
			Eligibility Product			
Color	4,1± 0,82	-	3,5± 0,79		3,2± 0,38	-
Smells	5± 0,00		3,5± 0,98		3,8± 0,95	
Texsture	4± 0,82		4 ± 0,69		4,2± 0,79	
Irritation Test	Not Irritated	-	Not Irritated	-	Not Irritated	-

The selection of the best formula is determined based on safety and physical test evaluation that meets the requirements. A comparison of the three formulas shows that all physical tests that meet the requirements are Formula 2 so that in future research, effectiveness testing can be carried out using the ratio Tween 80: PEG 400 = 40:20.

CONCLUSION

Based on the optimization results, the best formula for making collagen nanoemulgel from milkfish bone waste is Formula 2, with a ratio of Tween 80 and PEG 400, respectively, 40 and 20. Formula 2 is the best one because the physical characteristics meet all the specified specification requirements except zeta potential. Evaluations where the results are significantly different are the pH test on day 7, the spreadability test, and the viscosity of the nanoemulgel.

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