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Techno-Economic Evaluation of Hyaluronic Acid Nanoparticles Production Through Extraction Method using Yellowfin Tuna Eyeball

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ABSTRACT

The purpose of this study is to determine the feasibility of a hyaluronic acid fabrication project by extraction and isolation of the fish eyeball, and we evaluated the results from an engineering and economic perspective. Engineering analysis was analyzed based on tools and raw materials that are available on e-commerce. The economic analysis was done using several economic parameters, such as gross profit margin, the length of time required for an investment to return the total initial expenditure, the condition of a production project in the form of a production function in years, project benefits, and so on. The production of Hyaluronic Acid nanoparticles has good prospects. Technical analysis of producing 50.4 g/L of Hyaluronic Acid nanoparticles per day shows the total cost of the equipment purchased to be 20,726 USD. The investment will be profitable after more than four years and compete with the capital market standard. To ensure the feasibility of a manufacturing project, the project is estimated from ideal conditions to the worst conditions in production activities, including raw materials, utilities, sales, labor, and external conditions. The project is prospective and profitable in the small-scale industry, confirmed by positive values in all economic parameters).

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1. INTRODUCTION

Hyaluronan (HA) is a biological substance that has been found in various sources, such as rooster combs (Boas, 1949). HA can also be produced from various chemical processes such as isolation, extraction, fermentation, separation, and also purification (Maharani *et al.*, 2021). The chemical structure of hyaluronan is a natural linear polymer composed of disaccharide repeating unit [(1 -4)-O-(b-D-glucopyranosyl uronic)- (1 - 3)-O-(2-acetamido-2-deoxy-b-D-glucopyranosyl)] (Meyer, 1958). HA has important structural, rheological, physiological, and biological functions. With its unique moisture retention and viscoelasticity, as well as lack of immunogenicity and toxicity. HA can be used in cosmetics, biomedicine, and food industries (Chong *et al.*, 2005).

The internal space of the eyeball that the vitreous body occupies between the lens and the retina is free of cells, suspended matter, and blood vessels, which can block or scatter light. Although I don't know the details of how to achieve this, the schematic is. Vitreous is a stable bio gel that contains hyaluronic acid. Even high molar mass hyaluronic acid does not form the gel found in the vitreous, and the stiffness of the tissue is increased by adding a very sparse fine mesh of collagen fibers (Scott, 1992).

The previous method for extracting hyaluronan from large ocular vitreous is time-consuming, and the yield and molecular weight of the extracted hyaluronan are relatively low (Mizuno *et al.*, 1991). To obtain pure hyaluronic acid with higher molecular weight, the extraction procedure is improved as follows: use frozen eyeball vitreous to avoid contamination of blood, muscle, tissue, and other contaminants; extraction is performed under cold conditions for a long time; after passing reagents Before fat and protein removal, cetylpyridinium chloride (CPC) was used in the initial procedure to separate mucopolysaccharides containing hyaluronic acid. Although CPC can not only bind with hyaluronic acid, but also with other glycosaminoglycans (ie, chondroitin sulfate, heparin sulfate, dermatan sulfate, etc.), it can be dissolved in 0.4 M NaCl to combine hyaluronic acid with The separation of the CPC complex is critical to the concentration of NaCl (Matsumura *et al.*, 1979). The process of the method is shown in **Figure 1**.

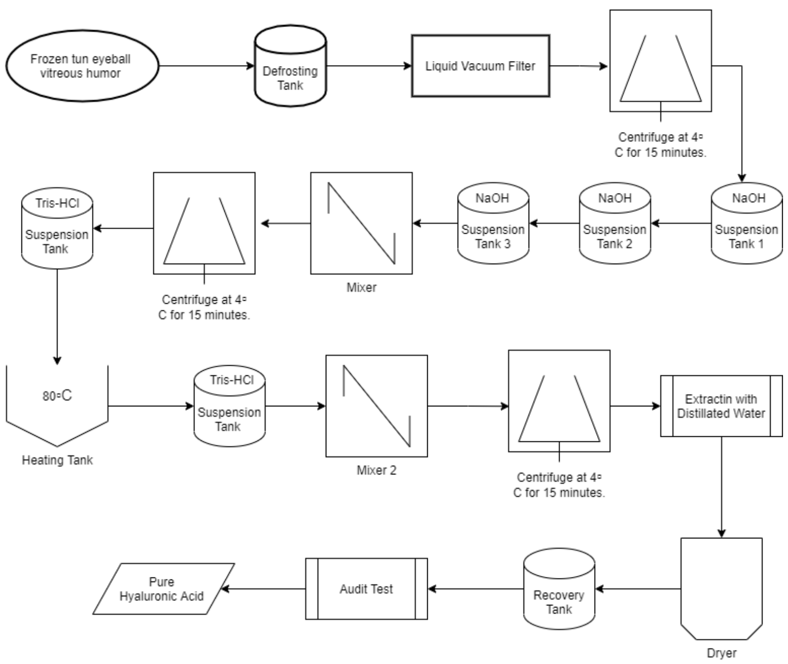

Figure 1. The synthesis process of HA using extraction and isolation of Fish eyeball.

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The purpose of this study is to determine the feasibility of a hyaluronic acid fabrication project by extraction and isolation of the fish eyeball and then evaluating it from an engineering and economic perspective. This research has carried out a transformation from laboratory scale to industrial scale in the number of raw materials and equipment. We have made several economic changes to taxes, sales, raw materials, labor, and utilities.

2. METHOD

2.1. Material and Method

The method that was used in this study was based on the analysis of the price of equipment, equipment specifications, and materials that are available on e-commerce. Each data is calculated based on mathematical calculations using Microsoft Excel. The economic evaluation of this manufacturing project is confirmed by the economic evaluation parameters. The parameters used in this economic evaluation are based on the literature. (Nandiyanto, 2018).

This study utilizes a strategy dependent on the investigation of the cost of materials, gear, and equipment specifications that are accessible on the internet shopping web. All data is calculated utilizing Microsoft Excel. The monetary assessment of the undertaking is affirmed by the financial assessment boundaries. There are a few economic evaluation parameters used based on the literature (Nandiyanto, 2018).

Economic evaluation parameters that are presented in this paper are:

(i) Gross Profit Margin (GPM) is an analysis that determines the profitability of the project by using raw material costs to reduce the cost of selling the product.

(ii) The payback period (PBP) is a calculation used to predict how long it will take for an investment to repay the initial cost. PBP is calculated when CNPV is zero for the first time.

(iii) The break-even point (BEP) is the minimum number of products that must be sold at a given price to cover the production costs used. BEP can be calculated by dividing the fixed cost by (total sales price minus total variable cost).

(iv) The current net accumulated value (CNPV) is a value that predicts the situation of production projects in the form of production functions for several years. CNPV is obtained by adding a CNPV value from the first project at the end of the operation of the plant.

(v) The profitability index (PI) is an indicator to identify the costs of projects and impact relationships. Pi can be calculated by dividing CNPV by total investment costs (ICT). If I PI is more than one project, the project can be classified as a highly profitable project, and if IP is less than 1, the project can be classified as an unprotected project.

In this article, some assumptions based on this process are in the following. This hypothesis represents the stoichiometric calculation after the expansion of the project, which results in approximately 50.4 g / L of hyaluronic acid. The assumptions are:

(i) All materials used are of high purity.

(ii) The conversion rate of the hyaluronic acid formation process is 100%. A few presumptions are utilized to affirm the monetary examination.

These presumptions are utilized to investigate and anticipate the potential outcomes that happen during the task. The assumptions are:

(i) All analysis is in USD. 1 USD = 14,460 rupiahs.

(ii) Based on the prices of commercially available materials, prices for yellowfish tuna, Hyaluronic Acid, sodium chloride, acetic acid, Ethanol, potassium acetate, trichloroacetic acid, hexadecyl pyridinium chloride monohydrate, 2-amino-2-

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hydroxymethyl-1,3-propanediol, Pyridine, formic acid, alcian blue 8GX are 20.00 USD/ kg, 14.86USD/ kg, 15.00 USD/ kg, 0.09 USD/ kg, 0.90 USD/ kg, 10.00 USD/ kg, 30.00 USD/ kg, 15.00 USD/ kg, 2.00 USD/ kg, 0.02 USD/ kg, 20.00 USD/ kg.

(iii) The quantity of material is estimated based on stoichiometry.
(iv) The total investment cost (TIC) is calculated based on the Lang Factor.
(v) The TIC is prepared in two steps. The first step is 40% of the first year and the second step is the rest (during the development of the project).
(vi) Land purchase. Therefore, the land cost increases at the beginning of the factory construction year and is recovered at the end of the project.
(vii) Depreciation is estimated using a direct calculation method.
(viii) The freight is borne by the buyer.
(ix) Hyaluronic acid sells for 5 USD/pack.
(x) A one-year project is 240 days (the rest are days dedicated to cleaning and organizing processes).
(xi) To simplify utility, utility units can be described and converted into electric units, such as kWh (Nandiyanto, 2018). Then, the electricity unit becomes cost. The expected cost of utility is 30,1970 USD / KWh (5 days).
(xii) 150 USD / Day Workers Total Salaries / Workers are set to 15 workers.
(xiii) The discount rate is 15% per year.
(xiv) The income tax is 10% per year.
(xv) The operating period of the project is 5 years. Economic evaluation is being carried out to test the viability of the project.
(xvi) This economic evaluation is carried out by changing the value of taxes, sales, raw materials, labor, and utilities under some conditions. Fiscal variations are performed at 10, 25, 50, 75, and 100%. Sales, raw materials, effort, and business fluctuations of public interest were carried out at 80, 90, 100, 110, 120%.

3. RESULTS AND DISCUSSION

3.1. Engineering Perspective

Figure 2 illustrates the steps of the isolation Hyaluronic acid synthetic process. The synthetic process begins with vitreous removal from the eye of the tuna and then the vitreous is liquefied and filtered at 4 degrees celsius to obtain a precipitate by 3% CPC. Then, the sample centrifugation at 2.22 x 10^3g for 15 min at 4C. Furthermore, the precipitate was resuspended with 0.4 M NaCl and centrifuged, then the supernatant was obtained. This process is repeated up to three times. The supernatant was mixed with a solution of 10% potassium acetate and 95% ethanol and then centrifuged again. The precipitate obtained was resuspended with 0.1 M Tris-HCl (pH 7.7) containing myosin and held for 24 hours at 37°C. The mixture was heated at 80°C for 15 minutes, and 30% trichloroacetic acid was added to twice the volume of the resuspended solution. After stirring, the solution was centrifuged, then the supernatant was obtained. After adding acetone, the supernatant was centrifuged at 2.22 x 10^3g for 15 min at 4C. The precipitate obtained was resuspended with distilled water and dialyzed for 2 days against distilled water. The precipitate obtained was dried under vacuum and recovered in a freeze-dried state. Then, the sample was audited to compare the mobility of the hyaluronan between bigeye tuna and the references. Next, the viscosity of the hyaluronan was calculated by dissolving overnight in a 0.5 M NaCl solution at 4°C, and this solution was clarified by filtration through a 0.45-lm nylon filter. Then, Hyaluronan molecular weight distribution was measured by using gel permeation chromatography. As a result, the extraction time of this method improved for

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obtaining hyaluronic acid is about half of the previous method. This hyaluronic acid (42 mg/g) from a dry source shows the same high value as a dry comb (50 mg/g) (Swann, 1968). As shown in **Table 1**, the molecular weight of hyaluronic acid in the eyeballs of tuna obtained by the improved method is the same as that of comb and streptococcus.

From an engineering point of view, the total cost of purchasing raw materials a year is 20,726 USD. One year's sales are 240,000 USD. The profit obtained is 43,332.32 USD. The price of the equipment cost analysis is 20,726 USD. TIC must be less than 22,176.82 USD. This project requires considerable investment funds, and the PBP was reached in the second year.

Table 1. Hyaluronan molecular parameters obtained from viscometry and GPC*.

Origin	[η] (100 cm^3 / g)	$\bar{M}_w$ x 10^{-6}	$\bar{M}_w$ / $\bar{M}_n$
Tuna eyeball	2.87	1.04	2.51
Streptococcus	14.14	3.81	1.47
Comb	7.06	7.64	1.52

*Gel permeation chromatography

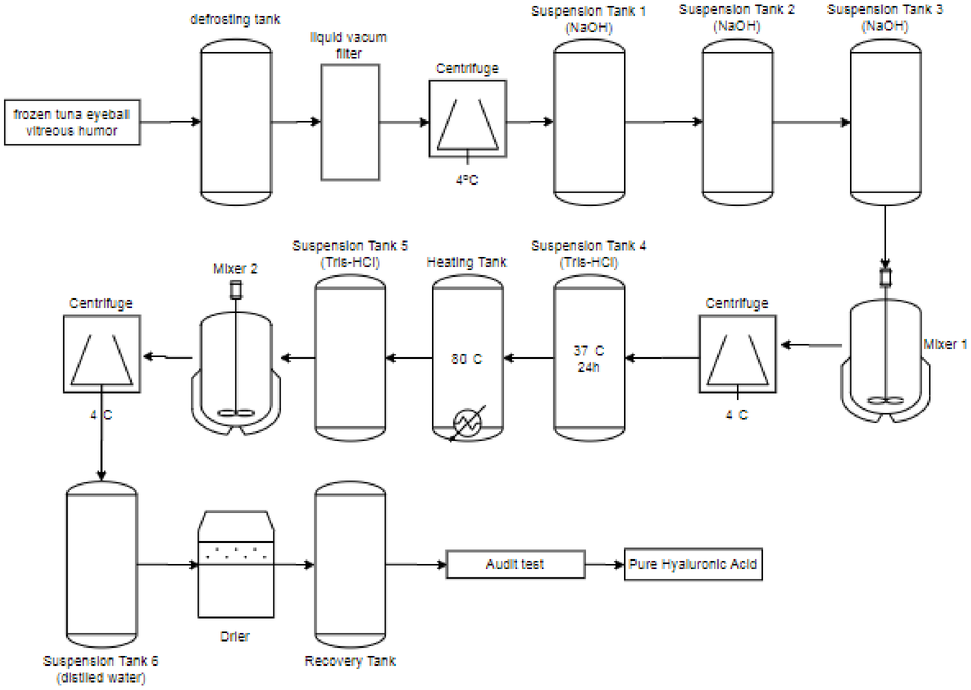

Figure 2. Process flow diagram of hyaluronic acid extraction from tuna eyeball.

3.2. Economic Evaluation

3.2.1. Ideal condition

Figure 3 shows a curve of the relationship between the CNPV / TIC value on the y-axis and the lifetime (year) on the x-axis. n the y-axis which is CNPV / TIC (%) will have a negative value in the first year to the second year. This may be caused by a new company that is developing its business so that it has not yet received a return on investment. The lowest CNPV/TIC value occurred in the second year, which was -0.9746 as shown in **Table 2.** However, when going to the third year, the company experienced a return on investment and the following years were like the fourth and fifth years. Although in the third year the company only made a profit of 0.1676, in the fifth year the company experienced a significant profit to reach 20,246. From this curve, it can be said that the company can be run well.

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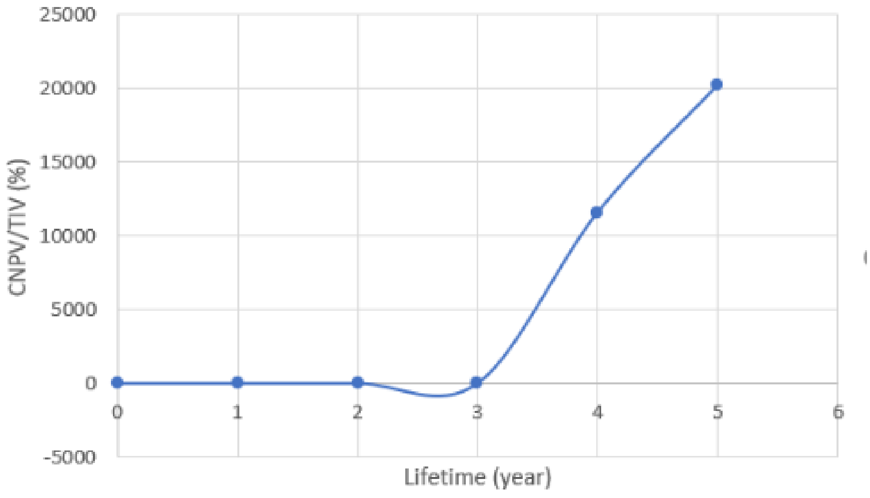

Figure 3. CNPV/TIC curve for a lifetime under ideal conditions.

Table 2. Annual CNPV / TIC values in ideal conditions.

CNPV / TIC	Lifetime (year)
0	**0**
-0.5916	**1**
-0.9746	**2**
0.1676	**3**
11.609	**4**
20.246	**5**

3.2.2. The effect of external conditions

Figure 4 shows the state of the company when tax rates varied. Figure 4 shows that the company will only run well if the tax price is 10 or 25% only. If it is 50, 75, or even 100% then the company will experience a loss without getting a return on investment. At 10%, the company will get a return on investment in the third year. While at 25%, the company will get a return on investment in the fourth year. In the fifth year, the 10% variation makes a bigger profit than the 25%.

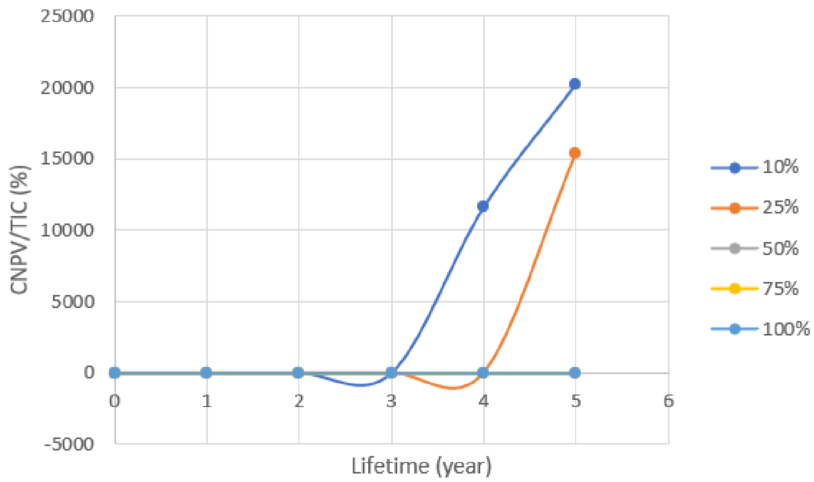

Figure 4. CNPV/TIC curve under tax variations.

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3.2.3. Change in sales

Figure 5 shows CNPV curves with various sales variations. Variations in sales will greatly affect the condition of the company. The price set for the sale is $5. The greater the selling price, the greater the profit obtained by the company. The curve in **Figure 5** shows that sales with a variation of 80% will continue to experience losses without experiencing a return on investment. Thus, it is said that the company cannot be run well if the sales variation is only 80%. Then at a variation of 90%, the company will experience a return on investment in the fourth year and will make a profit in the fifth year. When the company sells with a 100% variation, the company experiences a return on investment in the second year and continue to make profits in the following year. However, in the 110% of variation, the company's condition fluctuated, where the return on investment occurred in the third year and then the fourth and fifth years benefited, the difference was with a 100% variation. Then, in the fifth year, the 110% of variation of the profit was lower than it should have been. This can be interpreted that the condition of the company is not stable. The last is when the 120% of variation, in which it experienced a return on investment in the second year and continued to benefit significantly the following year. From **Figure 5**, it can be concluded that the sales variation of 100 and 120% is a good variation for the company.

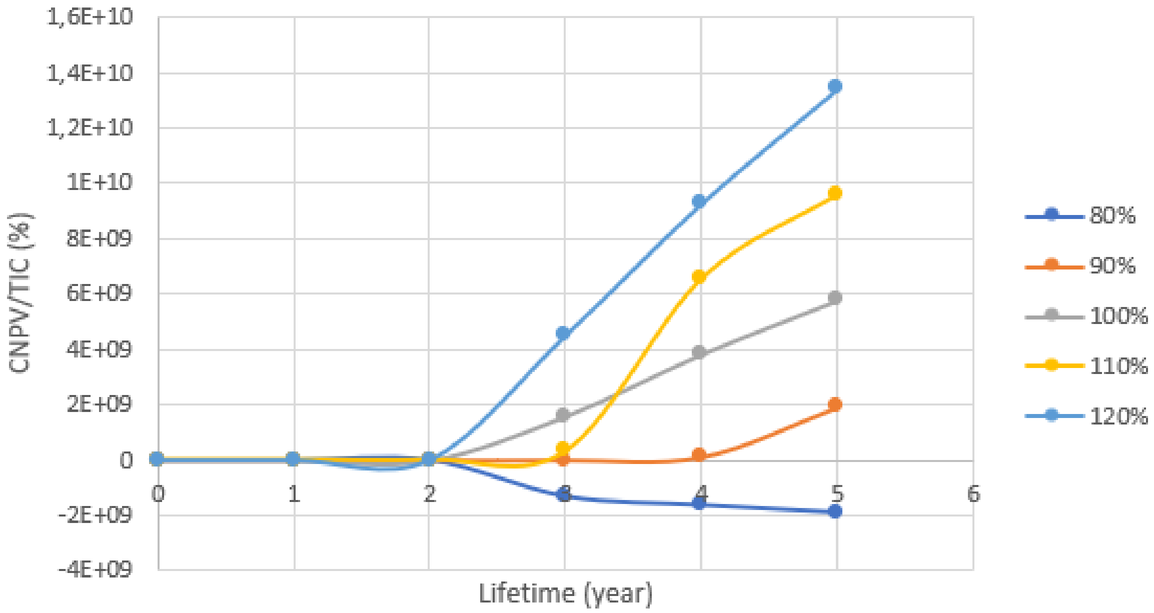

Figure 5. CNPV curve of sales variation.

3.2.4. Change in variable cost (raw materials, utility, and labor)

Raw material, labor, or utility is a variable cost for each company. These three things can have a great impact on the condition of the company. The greater the variable cost price obtained, the more the company will lose. **Figure 6** shows the variation of the price of raw materials. In variations of 80, 90, and 100% are the best variations of raw material prices, in this variation the company will experience very rapid profits after the return on investment occurs. The company will get a return on investment in the third year. While at the 110% variation, the company will experience a return on investment in the fourth year and the fifth year will benefit. However, in the 120% variation, the company will not experience a return on investment and continue to suffer losses.

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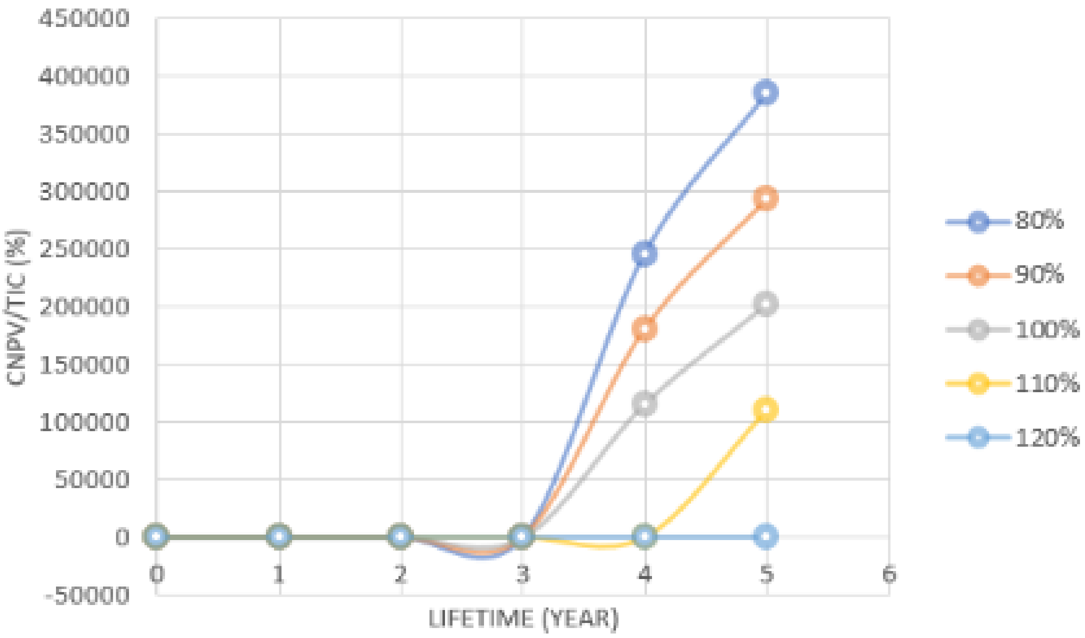

Figure 6. CNPV/TIC with raw material price variations.

Figure 7 shows that the higher the percentage of salary, the company will experience a longer return on investment. In the 80, 90, and 100% of variations, the company will experience a return on investment in the third year and continue to make profits in the following year, but the 80% variation gets a bigger profit than the other variations. While in the variation of 110% and 120% the company will get a return on investment in the fourth year and continue to earn profits until the fifth year. From this figure, it can be concluded that the 80% variation is the best variation for the company because it gets a bigger profit.

Figure 8 shows the CNPV/TIC curve analyzed with various utility prices. Figure 8 shows that regardless of the variation given by the company (80, 90, 100, 110, and 120%), there is no significant change, the return on investment will occur in the third year. Then in the following years, the company will experience rapid profits. From the five variations, the curves are almost the same, meaning that the company can run well.

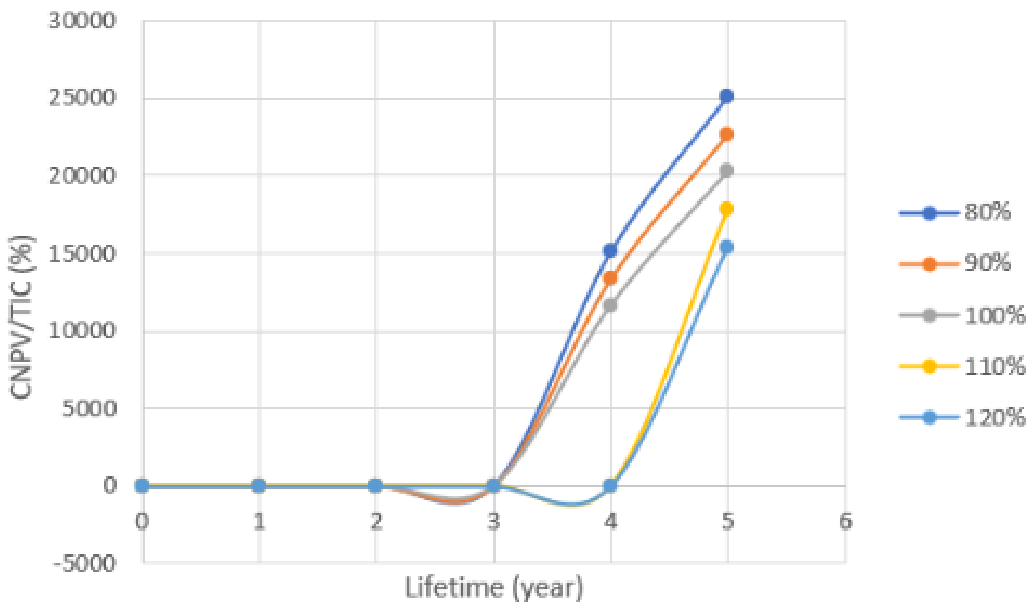

Figure 7. CNPV/TIC with worker's salary variation.

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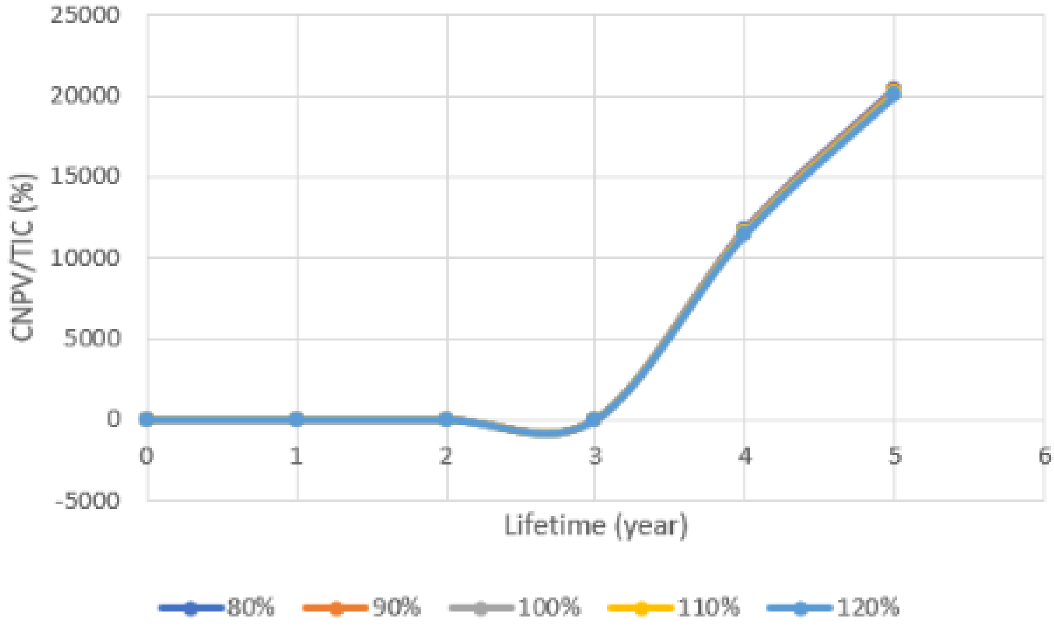

Figure 8. CNPV/TIC with utility variation.

4. CONCLUSION

Based on the analysis, the production of hyaluronic acid by extraction using tuna fish eyeballs is promising. The economic evaluation shows that the project is profitable, due to all economic parameters show positive values. Analysis by PBP shows that the investment is profitable after more than 5 years. This project can compete with PBP capital markets standards due to its short return on investment. Some of the things that influence this benefit include using the repeat batch method, as it is very simple, does not use hazardous materials. We can conclude that this project is feasible and the industry will become a promising project in the future based on the economic evaluation analysis.

5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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