Total Calcium Content of Enhalus Acoroides Milk Produced by Different Sugar Level and Storage Time

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Abstract
Seagrass seeds Enhalus acoroides is chosen as an alternative to milk production because of its great abundance and good nutrition for the body. One of the main nutrients of grain is calcium, so nutrient of E. acaroides seed will be more easily digested and absorbed by the body when processed into vegetable dairy products. The process of processing vegetable milk requires additional ingredients such as sugar and must pass through the storage process. This study was conducted to determine the dynamics of E. acaroides milk calcium content due to the addition of 50 g, 75 g, and 100 g of sugar and storage for 6 hours, 12 hours and 18 hours during the production process. Calcium content is determined by Atomic Absorption Spectroscopy (AAS) method. The test results of calcium content with AAS showed the calcium percentage range for all treatment types was 76.4-84.7 μg per 7 g of milk. The highest calcium content is a product added with 75 g of sugar - stored 6 hours and the lowest calcium in the product plus 50 g of sugar - stored 18 hours. The storage period had an effect on the calcium content of E. acaroides milk with variation of fluctuating trend (6 hours), decrease (12 hours), and increase (18 hours). Decreased calcium content occur with increasing storage duration of E. acaroides milk products.

Keywords: calcium content, Enhalus acoroides seed, storage duration, sugar addition, vegetable diary product

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Introduction
Enhalus acoroides is one of the most abundant seagrass species in Indonesian waters with morphological features ie rhizomes buried in substrates, green leaves, can produce flowers, fruits and seeds (Bujang, 2012). E. acoroides are easy to find in Indonesian waters, including in the waters of Maluku. This is supported by Citra et al. (2012), which states that E. acoroides and Thalassia hemprichii are common seagrass species found in Indonesian waters. Stated that the spread of seagrass ecosystems in Indonesia is quite extensive in the waters of Java, Sumatra, Bali, Kalimantan, Sulawesi, Maluku and Irian Jaya (Mulyaningisih et al., 2015). In Maluku, especially on Ambon Island, E. acoroides can be found in Tanjung Tiram Teluk Ambon Dalam (Latuconsina & Ambo-Rappe, 2013), Liang beach, Wai Waai beach and Rutong village (Wakano, 2014; Muskita, 2016).

Chemical content of E. acoroides are 13.8% crude protein, 20.3% crude fiber and 1.4% crude fat (Montener and Campos, 2015). In addition, E. acoroides has phytochemical content of flavonoids, steroids, triterpenoids, tannins, and saponins as well as high antioxidant activity and it can inhibit gram-positive bacteria (Santoso et al., 2012). However, the processing of seagrass as an innovative product that has economic value has not been utilized and developed properly. In fact, based on nutrient content of seagrass can be developed into food products such as vegetable milk, MP-ASI, and tempeh.

Potential chemical components possessed by E. acoroides cause the species to have a feature that can be used as food (BTNKpS, 2007). Food products that can be developed from seagrass seeds include vegetable milk, MP-ASI and tempeh, as a food diversification business. Nutrient content in E. acoroides such as protein, fat, carbohydrates, minerals, and vitamins can be easily digested and absorbed perfectly, so it can be processed for milk (Ressang & Nasoetion, 1989).
Milk is one of many drinks containing protein and vitamins, and is therefore widely consumed by almost all age groups. One of the requirements of good milk quality is protein content of at least 3%. Seagrass protein levels can reach these needs so that it can be used as raw material for making vegetable milk (Muskita 2010). Therefore, Seagrass seeds *E. acoroides* was chosen as an alternative to milk production because it is considered to have a fairly high nutritional content. The purpose of this study was to analyze the calcium content of vegetable milk based on seagrass seeds of *E. acoroides* through on the addition of sugar content and storage time.

**Method**

The samples used in this study were taken from Rutong-Ambon Village, South Leitimur Subdistrict in March 2017. Calcium level analysis was performed at Basic Chemical Laboratory of Faculty of Mathematics and Natural Sciences (FMIPA) Pattimura University. The type of research used is comparative research to see the calcium content of vegetable milk *E. acoroides* based on sugar and storage time. Treatment of adding sugar is 50 grams, 75 grams and 100 grams and storage time of 6 hours, 12 hours and 18 hours.

*E. acoroides* seed (500 grams) washed to clean and boiled for 15 minutes and then soaked with clean water. Seagrass seed is then washed to remove the epidermis. Seagrass grass is then mixed with hot water. The mixture of seagrass seeds is stirred and filtered so as to obtain seaweed milk solution. Brownish, brown, and salt sugars are added to the solution and heated to boiling (Tri Radyati, 1992).

Vegetable milk *E. acoroides* then analyzed calcium by Atomic Absorbtion Spectroscopy (AAS) method (Sudarmadji, 1997). The 7 grams of sample are fed into the destructive flask, add 15-20 ml of nitric acid and 0.5 ml of sulfuric acid is left overnight. Preheat the destructive flask over the original electric bath at a temperature of 200-250 °C to the non-smoked solution, add 30 ml of hydrochloric acid and then warm it again at 300-400 °C until the solution inside the destructive flask becomes clear. The heating is terminated after the liquid becomes clear. The solution is then cooled to room temperature and filter with filter paper and make a 100 ml volume in a measuring flask which is then measured absorbansi by AAS method. The data obtained will be analyzed in the form of quantitative descriptive that is by counting in the sample research. Calcium levels are calculated using the equation:

\[
\text{Calcium weight} = \frac{\text{Sample weight} \times 100\%}{X}
\]

After calcium levels are obtained, then calculated average calcium levels. The average calcium content is calculated by using the formula:

\[
X = \frac{U_1 + U_2 + U_3}{N}
\]

Keterangan :

- **X** = Mean of calcium content
- **N** = Sum of repetition
- **U_1, U_2, U_3** = Sum of calcium content at whole repetition

Measurement result of calcium content by using AAS instrument that described in table and graphic form.
Result and Discussion

Seagrass seed-based milk *E. acoroides* is one of diversified food product based on Maluku commodity. Food diversification aims to increase self-sufficiency of food especially for coastal communities in the Ambon city and surrounding areas. The results of data analysis of the highest levels of calcium in vegetable milk *E. acoroides* showed different results in each treatment. Calcium content of *E. acoroides* milk stored for 6, 12, and 18 hours are shown in Table 1, Table 2 and Table 3, respectively.

Table 1. Calcium content of *E. acoroides* milk with 50 g, 75 g, and 100 g sugar addition in 6 hours storage time

<table>
<thead>
<tr>
<th>No</th>
<th>Amount of sugar (g)</th>
<th>Calcium weight (μg)</th>
<th>Calcium percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50</td>
<td>83,477</td>
<td>1,176 ± 0,006</td>
</tr>
<tr>
<td>2.</td>
<td>75</td>
<td>84,666</td>
<td>1,192 ± 0,001</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>82,778</td>
<td>1,166 ± 0,002</td>
</tr>
</tbody>
</table>

Addition of sugars with amounts of 50 g, 75 g, and 100 g in the manufacture of vegetable milk *E. acaroides* yielded different total calcium weights of 83.477 μg, 84.666 μg, and 82.778 μg (Table 1), respectively. The highest calcium content obtained from the addition of 75 g of sugar in the process of making vegetable milk *E. acaroides*, which is 1.192%. An increase in the amount of sugar in the process of producing vegetable milk *E. acaroides* stored for 6 hours has not shown an increase in the percentage of calcium. The process of storing vegetable milk of *E. acaroides* for 12 hours with the addition of 50 g, 75 g, and 100 g of sugar was shown in Table 2. Calcium content from *E. acaroides* vegetable milk sample tended to decrease as the addition of sugar amounts to the production process, 81,340 μg, 80,735 μg, and 79,424 μg. Indirectly, the percentage of calcium from *E. acaroides* milk also decreased, ie 1.145%, 1.137%, and 1.118% respectively.

Table 2. Calcium content of *E. acoroides* milk with 50 g, 75 g, and 100 g sugar addition in 12 hours storage time

<table>
<thead>
<tr>
<th>No</th>
<th>Amount of sugar (g)</th>
<th>Calcium weight (μg)</th>
<th>Calcium percentage (%)</th>
</tr>
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</tr>
</tbody>
</table>

*E. acaroides* milk are also produced with a storage period of 18 hours. Calcium content and their percentages showed Table 3. The calcium content *E. acaroides* milk with the addition of 50 g, 75 g, and 100 g of sugar were 76.424 μg, 77.424 μg, and 78.424 μg, respectively. The calcium content *E. acaroides* milk was 1.076%, 1.090%, and 1.104%, respectively, in addition to 50 g, 75 g, and 100 g of sugar.

Table 3. Calcium content of *E. acoroides* milk with 50 g, 75 g, and 100 g sugar addition in 18 hours storage time

<table>
<thead>
<tr>
<th>No</th>
<th>Amount of sugar (g)</th>
<th>Calcium weight (μg)</th>
<th>Calcium percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50</td>
<td>76,424</td>
<td>1,076 ± 0,006</td>
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</tr>
<tr>
<td>3</td>
<td>100</td>
<td>78,424</td>
<td>1,104 ± 0,006</td>
</tr>
</tbody>
</table>

Based on the results of weight analysis and calcium percentage in vegetable dairy products *E. acaroides*, it is known that the highest calcium percentage is in the process of adding 75 g of sugar with the process of storage for 6 hours. The production process with the addition of 50 g sugar along with 18 hour storage showed the lowest percentage value. Generally, calcium content in *E. acaroides* milk tend to be affected by storage duration. It can
be known from the range (minimum-maximum value) percentage of calcium produced by adding sugar 50, 75, and 100 g. *E. acaroides* beverage storage for 6 hours has a calcium percentage range of 1.16% - 1.19%; 12 hours storage with a range of 1.12% - 1.15%; and 18 hours storage with a range of 1.08% - 1.10%. Distribution of calcium concentration from 9 types of production process can be seen in Figure 1.

The Atomic Absorbption Spectroscopy (AAS) method is a standard calcium estimate of dairy products and milk derivatives. Sowmya et al. (2015) showed that there was a calcium content in products such as milk produced from the processing of khoa seed and paneer by atomic absorption spectroscopy (AAS) method. *E. acaroides* seeds that are processed into products such as milk also contain calcium. The AAS instrument presents data in the form of calibration curves, which express the relationship between concentration and absorbance of Ca$^{2+}$. This directly shows the weight of the calcium molecule per wet unit of milk sample. The greater the concentration used, the greater the absorbance value. It is suspected to directly affect the percentage distribution of calcium in each type of treatment. The percentage distribution of calcium shows a trend that varies based on storage period, ie fluctuation (6 hours), decrease (12 hours), and increase (18 hours). The variation in the percentage of calcium is thought to be due to the protein and fat characteristics contained in *E. acaroides* milk.

According to the International Dairy Foods Association (2016), milk is 87% water and 13% solid (solute) obtained from mammary gland secretions from cows, buffalo, goats, and other types of mammals. *E. acaroides* milk are processed from *E. acaroides* seeds, so they are not classified into dairy products, but plant proteins. Therefore, the process of degradation of protein, fat and minerals content is not linear with dairy products based on animal protein. *E. acaroides* seed’s extract are thought to be synonymous with soybean extract products because they are obtained from monocotyl plant seeds of Angiospermae, otherwise known as leguminosae (Wu et al., 2003). Based on the production process terminology, *E. acaroides* milk can be categorized as Whey Protein Isolate (WPI), as well as soy protein isolates (Zhu et al., 2008).

![Figure 1. Calcium percentage (%) in *E. acaroides* milk processed with 6 hour, 12 hour, and 18 hour storage time and different sugar addition (■ = 50 g; □ = 75 g; dan ▼ = 100 g)](image-url)
Hydrolyzate products or vegetable protein isolates have a fairly high mineral content. Mineral content in 100 g of soy extract is 21.6 mg sodium (Na), 133.4 mg potassium (K), 21.6 mg calcium (Ca), and 1.2 mg iron (Fe) (2008). The calcium content in every 7 g of *E. acoroides* seed’s extract (in this study) for all treatments ranged from 76.4 to 84.7 μg. The range is still far below the value of Ca soy extract concentration, but higher than Ca concentration in starch *E. acoroides* (Montaño et al., 1999). Ca ion is a macro mineral element that serves as a marker agent in edosimbin plants and ATP transport during the process of photosynthesis of plants (Nomura and Shiina 2014). The calcium ion (Ca$^{2+}$) is thought to interact with one of the amino acid in proteins. Norimatsu et al. (2017) found that calcium pumps in the form of crystals mediated protein-phospholipid interactions. Therefore, increased Ca$^{2+}$ accelerate the bonds between proteins and phospholipids. Indirectly the percentage of calcium is an indicator of the percentage of protein content in the case of vegetable protein hydrolyzate, especially on *E. acoroides* seeds.

The storage of *E. acoroides* milk over a certain time interval may result in the reduction of nutrients. The processed products or derivatives of the main ingredients generally have less shelf life because they have undergone autocesidation reactions during the process and of course protein degradation (Lehninger 2008). Due to a good correlation between protein and calcium, it is suspected that the decrease in protein content is accompanied by a decrease in calcium content over a period of storage time.

In addition to storage time, there are various uncontrollable factors that affect the calcium content. Temperature is one of the physical factors that can change the quality of the material. Extracts of food stored at low temperatures can inhibit microbial activity and chemical reactions (Hudaya, 2002). Data analysis of calcium content in *E. acaroides* milk tends to decrease with increasing storage duration. Room temperature with unstable conditions can lead to the growth of microbes or bacteria. It can lower the calcium from milk because the milk is a medium that is preferred by bacteria and microbes for the process of growing. The other hand, short time milk become unfit for consumption (Hadiwiyoto, 1994).

According to Hamidah (2012) milk is a liquid medium that has a very complete composition. It can not last long when stored at room temperature. Milk stored at room temperature will be easily damaged if not treated like pasteurization, cooling / freezing, and heating. The higher the temperature the calcium level will decrease, because storage at room temperature will cause mold growth and damage to milk quality (Sudarmaji, 2009). Pelczar and Chan (1986) explain that storage at low temperatures aims to reduce or attract free water content. Low temperatures convert free water into ice crystals so they can not be used by microorganisms for their activity.

The milk storage factor also contributes to the shelf life of milk, especially in unheated milk (fresh) and pasteurized milk because the spores will grow and contaminate the milk, therefore milk should be stored at low temperatures. Based on the results of previous research, the calcium content *E. acaroides* milk was low but calcium levels are not much different. The ratio of calcium content in *E. acaroides* milk to some other levels of calcium milk is not much different, thus vegetable milk *E. acaroides* can be consumed.
Conclusion

Based on the results of the analysis it can be concluded that the highest calcium content of *E. acaroides* milk was obtained by the product given the addition of sugar 75 g with 6 days storage time. The product with the lowest calcium value is the product given the addition of 50 g sugar with 18 days storage time. There are differences in the levels of calcium stored for 6, 12, and 18 days. Calcium content of *E. acaroides* milk with the shortest storage time is higher than the longer storage. The storage period influenced the calcium content of *E. acaroides* milk. The longer the storage the smaller the calcium level in *E. acaroides* milk.

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