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Increasing Cassava Jerky Protein Through The Fermentation Process Using Saccharomyces Cerevisiae and Rhizopus Oryzae

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ARTICLE INFORMATION

Journal of Science and Technology – Volume 27 Number 2, December 2023

Page:
131 – 140
Date of issue:
December 20, 2023

DOI:
10.31284/j.iptek.2023.v27i2.5055

ABSTRACT

Cassava is a plant that is easy to grow in Indonesia. People generally use cassava as a food source of carbohydrates, while the leaves are used as a vegetable by processing it into curry or stir-fry. Cassava leaves have high nutritional value but have yet to be used optimally; most become agricultural waste. This study aims to increase cassava leaf jerky's nutritional value (protein) using microbial fermentation. The experimental design used in this study was a factorial, Completely Randomized Design with two factors: the type of microbe (Saccharomyces cerevisiae, *Rhizopus oryzae*) and fermentation time (2, 3, 4 and 5 days). The parameters analyzed were protein content, ash content, fat content, carbohydrate content, water content and consumer preference level. From the results of this study, there was an increase in protein levels in cassava leaves for up to 120 hours (5 days), 15.395% using *Saccharomyces cerevisiae* and 16.165% using *Rhizopus oryzae*. The ash content significantly differs from treatments with shorter fermentation times of 96 hours, 72 hours or 48 hours. There is no significant difference in the percentage of water content in the various treatments between the treatments. The highest score in the organoleptic test is A2B4 (jerky with the addition of *Rhizopus oryzae* with a fermentation time of 120 hours).

Keywords: *Cassava leaves; jerky; R. oryzae; S. cerevisiae.*

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ABSTRACT

Ubi kayu merupakan tanaman yang mudah ditumbuh kembangkan di Indonesia. Masyarakat umumnya memanfaatkan ubi kayu sebagai bahan pangan sumber karbohidrat, sedangkan daunnya digunakan sebagai sayur dengan diolah menjadi gulai atau tumis. Daun ubi kayu mempunyai nilai gizi yang tinggi namun belum di manfaatkan secara optimal sebagian besar menjadi limbah pertanian. Penelitian ini bertujuan untuk meningkatkan nilai gizi (protein) dari dendeng daun ubi kayu menggunakan fermentasi dengan mikroba. Rancangan percobaan yang digunakan pada penelitian ini adalah RAL (Rancangan Acak Lengkap) faktorial dengan 2 faktor yaitu jenis mikroba; *Saccharomyces cerevisiae*, *Rhizopus oryzae* dan lama fermentasi: 2, 3, 4 dan 5 hari. Parameter yang dianalisa adalah kadar protein, kadar abu, kadar lemak, kadar karbohidrat, kadar air dan tingkat kesukaan konsumen. Hasil penelitian menunjukkan bahwa terjadi peningkatan kadar protein pada daun singkong setelah difermentasi selama 5 hari, yakni 15.34% untuk *S. cerevisiae* dan 16.16% untuk *Rhizopus oryzae.* Hasil analisa kadar abu berbeda signifikan pada perlakuan dengan lama waktu fermentasi 96 jam, 72 jam dan 48 jam. Tidak ada perbedaan yang nyata untuk hasil analisa kadar air. Hasil uji organoleptik menunjukkan bahwa dendeng daun singkong yang paling disukai adalah A2B4, yakni dengan penambahan *Rhizopus oryzae* dengan lama fermentasi 120 jam.

Keywords: *Daun singkong; Dendeng; R. oryzae; S. cerevisiae.*

INTRODUCTION

The cassava plant is a type of tuber widely grown in Indonesia and processed into products with economic value. The parts of plants that are generally used as food are the tubers and young leaves (shoots). Wood tubers are very popular with Indonesians as a staple and additional food. However, cassava leaves are not utilized optimally and have become an agricultural waste. According to Askar [1], cassava leaves are a source of protein because they contain the amino acid. 100 g of cassava leaves contain 6.8 g of protein, 1.2 g of fat, 13.0 g of carbohydrates, 2.4 g of fiber, 165 mg of calcium, 54 mg of phosphorus, 2 mg of iron, and several minerals [2]. Cassava leaves are generally consumed as a vegetable, either processed into curry or stir-fried. Utilization for economic improvement purposes has yet to be optimal. The high nutritional potential and abundance of cassava leaves require efforts to diversify processing to increase its economic value and as an alternative source of nutrition.

According to Alfaruqi [3], cassava leaves, which are processed into beef jerky, can be an alternative to consuming vegetables, which contain protein, minerals, vitamins and fibre, which is suitable for the development of children who do not like consuming vegetables, and suitable for adults who do not consume enough fibre but consume high fat. Jerky is a food with lean cuts into strips, generally made from meat, seasoned, and then dried. Jerky usually tastes spicy and can be stored for several weeks at room temperature [4]. The nutritional value of beef jerky is higher than jerky made from cassava leaves. Therefore, the fermentation process can increase the nutritional value of cassava leaf jerky.

In the fermentation process, microbes will produce enzymes that will degrade complex compounds into simpler ones; microbes will also synthesize protein, which is a protein enrichment process. According to Anggorowati [5], *Saccharomyces Cerevisiae* can increase the protein content and the protein-rich microbial mass, while fermentation using *R. oryzae* will cause an increase in protein and fatty acids. According to Ninis [6], increasing the protein content in cassava skin using Saccharomyces yeast for seven days can increase the protein content from 1.93% to 10.5%. Anggorowati [5] stated that the protein content of young jackfruit before the fermentation process was 5,506%, then the protein content after fermentation continued to increase.. Unfortunately, research related to chemical and organoleptic analysis on cassava leaf jerky has never been reported. Hence, this study aimed to investigate cassava beef jerky's protein, ash, moisture and organoleptic test.

LITERATURE REVIEW

Cassava Leaves

Cassava leaves contain protein, Amino acids and chlorophyll, which positively affect our body [7]. The high nutritional content in cassava leaves can be an alternative nutritious food. Generally, people consume cassava leaves as a vegetable by boiling, steaming, or processing them into soupy foods such as vegetable curry stir-fries. Sometimes, the leaves are thrown away, and only the tubers are processed. Product diversification of cassava leaves for processed food includes formulations with seaweed into Nori products [8], cassava leaf sticks [9], cassava leaf beef jerky [7].

Jerky

Jerky is a traditional processed meat product that many Indonesians have used for a long time. Jerky can be classified as Intermediate Moisture Meat because the water content of jerky is in the range of Intermediate Moisture Food, which is 25% [10]. Jerky is a famous Indonesian food and has become a prevalent alternative side dish because it is practical and has high nutritional value. Jerky is usually made from raw beef, chicken and goat. Animal jerky rots quickly and contains relatively high cholesterol. The vegetable ingredients commonly used to make beef jerky are banana blossoms, cassava leaves and oyster mushrooms. Based on SNI-2908-2013 regarding beef jerky, the minimum protein content is 18% [11].

Food Fermentation

Fermented food is processed with the help of microorganisms or other biological components such as enzymes. In fermentation, microbes produce enzymes that will degrade complex compounds into simpler ones. *Saccharomyces cerevisiae* can increase the protein content in young jackfruit material due to the long fermentation time [5]. According to the content of microorganisms in the yeast, the fermentation process is divided into two stages: changing starch into sugar and changing sugar into alcohol. Treatment with longer incubation times showed an increase in the yeast population. This is because yeast takes longer to break down food substances. The treatment had the highest population, with an incubation time of 96 hours. Microbes can thrive, remain dominant, or die based on intrinsic, processing, extrinsic, implicit, and material factors.

Fermentation with *Rhizopus oryzae* in tempeh only takes 48 hours to break down the food. At that time, the microbes will thrive, remain dominant, or die at the intrinsic, processing, extrinsic, implicit and material times. Where the intrinsic time occurs, the decay or advanced fermentation phase (50-90 hours of fermentation) causes an increase in the number of bacteria and the amount of free fatty acids, fungal growth decreases. The fungal growth stops at a certain water content, and flavour changes due to further protein degradation, which forms ammonia [12].

METHOD

This research is conducted from June 2023 to August 2023. The samples used in this study were cassava leaves grown in the Pasuruan area, East Java. *Rhizopus oryzae* and *Saccharomyces cerevisiae* were purchased from local distributors, other ingredients such as ground spices, pepper, coriander, onion, garlic), eggs, and tapioca flour were purchased from local distributors, H_2SO_4 (Merck), NaOH (Merck), HCl (Merck), and aquadest.

The Fermentation Process of Cassava Leaves

Cassava leaves are washed, mashed, steamed for 30 minutes, and then cooled so that the microorganisms do not die during fermentation. The *Saccharomyces cerevisiae* and *Rhizopus oryzae* starter was added and then fermented for 2, 3, 4 and 5 days with aerobic conditions. Fermented cassava leaves are added with other ingredients such as ground spices (pepper, coriander, onion, and garlic), eggs, and tapioca flour. The dough is steamed for 30 minutes, then let it cool. After that, the jerky is cut into thin slices with a thickness of 0.5 cm, then flattened and dried in the oven. The proximate analysis was done by following AOAC [13], which consisted of moisture, protein, and ash content measurement, while sensory descriptive analysis used organoleptic test parameters (hedonic test), which included aroma, taste, colour, and texture of cassava leaf jerky's.

Data An4alysis

The research data were analyzed statistically using the Minitab 17 program with analysis of variance (ANOVA), and if there was a difference, Tukey's test was carried out with a 95% confidence interval to determine the effect of each treatment.

RESULTS AND DISCUSSION

Protein Content

The analysis of variance (ANOVA) showed that the use of microorganisms *Saccharomyces cerevisiae* and *Rhizopus oryzae* in cassava leaf fermentation as jerky showed a significant difference. Likewise, the fermentation time of 48 hours, 72 hours, 96 hours and 120 hours also showed significant differences. . Table 1 shows the protein content of cassava leaves fermented with different microorganisms, and fermentation times a significant difference. Treatment using *Saccharomyces*

cerevisiae with a fermentation time of 48 hours (A1B1) showed a significant difference with treatment using the same microorganism with a fermentation time of 72 hours (A1B2). At 96 hours of fermentation, there was no significant difference. Then, there was a significant difference in the fermentation time of 120 hours.

Table 1. The effect of fermentation time on protein content at fermentation with two type of microorganism

Sample	Protein content (%)
S. Cerevisiae 48 hours	11.702[a]
S. Cerevisiae 72 hours	12.573[b]
S. Cerevisiae 96 hours	12.697[b]
S. Cerevisiae 120 hours	15.395[e]
R. oryzae 48 hours	13.846[c]
R. oryzae 72 hours	15.215[d]
R. oryzae 96 hours	15.394[d]
R. oryzae 120 hours	16.165[e]

The protein content of cassava leaves fermented using *Rhizopus oryzae* experienced a significantly different increase from cassava leaves fermented using Saccharomyces cerevisiae. Each treatment with a different fermentation time showed a significantly different increase [14].

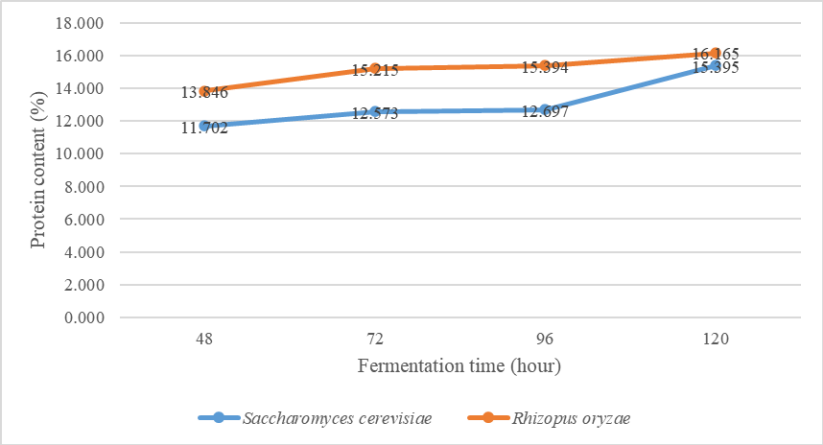

Figure 1. The effect of fermentation time on protein content at fermentation with two type of microorganism

The protein content of fresh cassava leaves before the boiling process was 20.271%. According to Hermanto and Fitriani [15], cassava leaves generally have a crude protein content ranging from 20-36% of dry matter. Protein levels will decrease after cooking or boiling. The cooking process will affect the protein content of cassava leaves; when boiling, the ingredients come into direct contact with water, so some of the protein will dissolve in it [7]. The protein content of cassava leaf jerky without adding tapioca flour (control) by the steam cooking process was 13.06% [7]. Figure 1 shows that each cassava leaf fermentation with *Saccharomyces cerevisiae* and *Rhizopus oryzae* with more than 48 hours of fermentation showed increased protein levels. Cassava leaves fermented with *Saccharomyces cerevisiae* amounted to 11.702%, while cassava leaves fermented with *Rhizopus oryzae* 13.846%. Increased protein levels occurred during fermentation using Saccharomyces cerevisiae, and *Rhizopus oryzae*, where the protein content of cassava leaves fermented using *Rhizopus oryzae* experienced a higher protein content than using Saccharomyces cerevisiae. The protein content of fermented using *Rhizopus oryzae* for 96 hours was 15.215%, while cassava leaves fermented using *Saccharomyces cerevisiae* for 96 hours was 12.573%. From the results of this study, there was an increase in protein levels in cassava leaves for up to 120 hours (5 days), 15.395% using

Saccharomyces cerevisiae and 16.165% using *Rhizopus oryzae*. Previous studies showed increased protein from young jackfruit, which had a protein content of 5.506% before fermentation, to 10.591% after being fermented for four days using *Saccharomyces cerevisiae* [5].

The fermentation process using *Saccharomyces cerevisiae* can increase the content of cassava skin protein from 10.03% to 20.91% on the fifth day of fermentation [16]. Research conducted by Hermanto and Fitriani 2018 also stated that the protein content of cassava leaves on the second day of fermentation was 7.59%, increased to 8.48% on the fourth day, and the 6th and eighth day, it decreased to 6 48%. and 6.81%. When cells in the static phase are transferred to the new media, the cells will carry out adaptation processes, including synthesizing new enzymes suitable for the media and recovering toxic metabolites. The fermentation time of 12 to 24 hours is the accelerated phase, where cells start dividing [17]. The increase in protein levels on day 2 was not optimal; this could be because *Saccharomyces cerevisiae* was still adapting to the new environment, and changes occurred—protein by proteolytic microorganisms, which lasted for the first 24 hours. On day 2 (48 hours), microorganisms have been able to adapt and start the growth phase (logarithmic) with a vast cell mass growth.

Ash Content

Ash is an inorganic substance left over from the combustion of an organic material whose content and composition depend on the material and method of ashing [18]. Ash content is related to the minerals of a material. Determining the ash content is done by oxidizing the material at a high temperature around 500 - 600 C and then weighing the substances left behind after combustion. Total ash content is a proximate analysis used to determine the nutritional value of a food ingredient and show the toxic minerals contained in the ingredient[18].

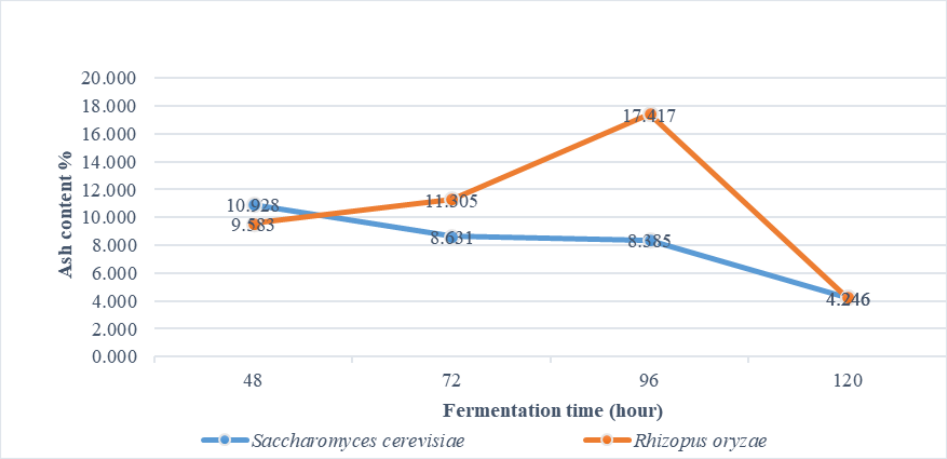

Figure 2. Effect of Fermentation Time and Type of Microorganisms on the Ash Content of Cassava Leaf Jerky

Fermentation using *Saccharomyces cerevisiae* with a fermentation time of 120 hours (A1B4) and fermentation using *Rhizopus oryzae* with a fermentation time of 120 hours (A2B4) did not significantly affect ash content. However, both significantly differ from treatments with shorter fermentation times of 96 hours, 72 hours or 48 hours. The ash content of cassava leaves fermented using *Rhizopus oryzae* increased greater than that of cassava leaves fermented using *Saccharomyces cerevisiae* (Figure 2). There was an increase in ash content from 48 hours to 96 hours of fermentation, then decreased at 120 hours. In general, the fermentation process changes the chemical composition significantly. According to Purwanto [19], the longer the fermentation process, the more influential it is in reducing ash content because the more microorganism reduces crude fibre. The decrease in crude fibre is closely related to the decrease in ash content in a feed ingredient. Wibowo [20] shows that crude fibre content and ash content have a positive relationship; high crude fibre will positively

affect the ash content of a feed ingredient. The decreasing ash content indicates that the organic material content is increasing, so a decrease in the ash content is desirable.

Moisture Content

Several parameters of jerky quality are greatly influenced by its water content, including appearance, taste, texture, product acceptability, freshness and durability [21]. Water content is the percentage of water content of a material that can be expressed on a wet or dry basis. The wet-weight water content has a theoretical maximum limit of 100%, while the dry-weight water content can be more than 100% [22]. From Figure 3, it can be concluded that there is no significant difference in the percentage of water content in the various treatments between the treatments because the existing samples have a dry appearance with low water content. The heating process also affects the water content of various samples because removing water by heating can cause water evaporation from the food due to the difference in water vapour content between the air and the product. The higher the air temperature, the more water vapour it can hold before saturation. The air that moves in the smoking process allows the collection and removal of water vapour from the surface of the jerky to be more efficacious [23]. The lower water content can slow down the growth of spoilage microbes, extending the product's shelf life for longer [24].

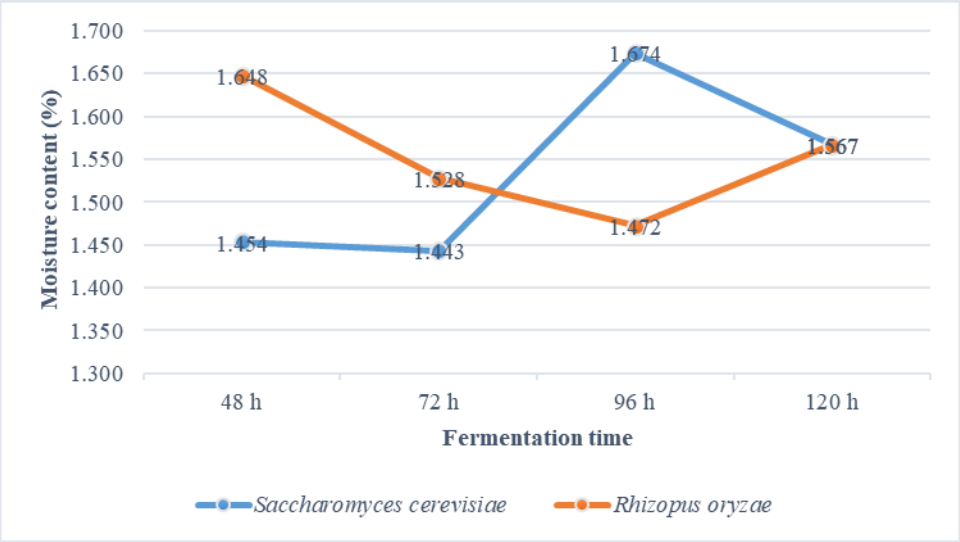

Figure 3. Effect of fermentation time and type of microorganisms on the moisture content of cassava leaf jerky

From Figure 3, it can be concluded that samples with the addition of *Saccharomyces cerevisiae* show that the longer the fermentation process, the percentage of water content of the material tends to increase due to the aerobic metabolism of microorganisms that occurs during the fermentation process. Under aerobic conditions, *Saccharomyces cerevisiae* hydrolyzes sugar into water and CO_2 [25]. Meanwhile, samples with the addition of *Rhizopus oryzae* showed that the longer the fermentation process, the percentage of the water content of the material tended to decrease due to the formation of mycelium spreading [26]. Mycelium will increase the density of the jerky mass with each other, thus forming a compact mass and reducing the air spaces inside. At the end of the fermentation process, this air cavity can be filled with the mass of water resulting from the respiration of the tempeh fungus during fermentation.

Organoleptic Test

The Organoleptic Test is the most widely used test to measure the level of liking for a product. This level of liking is called a hedonic scale, for example, very like, like, neutral, somewhat

dislike, dislike, strongly dislike. This test is used to test consumer reactions to an ingredient or to determine consumer reactions to the samples tested [27]. The hedonic test used in this research involves as many as 40 untrained testers to determine the expected consumer acceptability. Organoleptic test results data are presented in Table 2.

Table 2. Organoleptic test results

Sample	Aroma	Taste	Colour	Texture
A1B1	3,286	3,029	3,000	3,393
A1B2	3,429	3,286	2,964	3,214
A1B3	3,286	2,964	2,786	3,143
A1B4	3,179	2,750	2,893	3,071
A2B1	3,071	3,321	2,964	2,86
A2B2	3,179	2,929	2,929	3,143
A2B3	3,107	3,179	3,107	3,107
A2B4	3,464	3,286	3,143	3,000

From Table 2, it can be concluded that the aroma with the highest score is A1B1 (jerky with the addition of *Saccharomyces cerevisiae* with a fermentation time of 48 hours); these results are in line with research by Juliana [28], which explains that increasing colour brightness and eliminating unpleasant aromas can be done with the fermentation process because the fermentation process can influence the physical properties of a food ingredient. Taste plays an essential role in determining a consumer's final decision to accept or reject a product [29]. Table 2 shows that the taste with the highest average is sample A1B1 (jerky with the addition of *Saccharomyces cerevisiae* with a fermentation time of 48 hours). Sample A1B4 (jerky with adding microorganisms of *Saccharomyces cerevisiae* with a fermentation time of 120 hours) had the lowest score. The distinctive aroma and taste can be caused by adding sugar, salt, tamarind extract and other spices. During the making of jerky, a caramelization process called the Maillard reaction occurs, which gives rise to the aroma of jerky [30]

In Table 3, sample A2B4 (jerky with the addition of microorganisms in the form of Rhizopus oryzae with a fermentation time of 120 hours) has the highest score, while sample A1B4 (jerky with the addition of microorganisms in the form of Saccharomyces cerevisiae with a fermentation time of 120 hours) has the lowest score. The longer the drying time, the greater the Maillard reaction can occur, causing the jerky to turn brown due to the non-enzymatic browning reaction (Maillard reaction) and caramelization [31]. Sample A1B3 (jerky with *Saccharomyces cerevisiae* with a fermentation time of 96 hours) has the highest score. Texture (hardness) is influenced by the even and rapid growth of mycelia from *Rhizopus oryzae* so that it covers the surface, thus providing a firm texture. In contrast, *Saccharomyces cerevisiae* produces alcohol and CO_2 compounds; from other metabolic processes, it produces H_2O and energy in heat [25]. With the formation of heat during the fermentation process, the temperature of the food will increase so that the water will evaporate, resulting in a decrease in water content [32].

CONCLUSION

The results of this study show that there was an increase in protein levels in cassava leaves for up to 120 hours (5 days), 15.395% using *Saccharomyces cerevisiae* and 16.165% using *Rhizopus oryzae*. The ash content significantly differs from treatments with shorter fermentation times of 96 hours, 72 hours or 48 hours. There is no significant difference in the percentage of water content in the various treatments between the treatments. The highest score in the organoleptic test is A2B4 (jerky with the addition of *Rhizopus oryzae* with a fermentation time of 120 hours).

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