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EVALUATION OF CHEMICAL QUALITY, ANTIOXIDANT CAPACITY, AND MICROBIOLOGICAL ACTIVITY OF BALI BEEF MARINATED WITH SUGAR PALM NEERA SOLUTION (Arenga pinnata)

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Abstract
This research analyzes the chemical quality, antioxidant capacity and microbiology of Bali beef marinated in sugar palm neera solution. This study employed a Completely Randomized Design (CRD) consisting of four treatments and four repetitions. For each repetition, a loin weighing 100 g was used and soaked for 40 minutes. The treatments were P0 (meat without marinating/control), P1 (meat marinated with 15% sugar palm neera solution), P (meat marinated with 20% sugar palm neera solution), and P3 (meat marinated with 25% sugar palm neera solution). The study discovered that marinating Bali beef with sugar palm neera solution at varying concentrations did not result in any significant differences in the chemical and microbiological characteristics of the meat ($P > 0.05$). The antioxidant capacity of Bali beef marinated with sugar palm neera solution resulted in a significantly increased value. The higher the concentration of sugar palm neera, the stronger its antioxidant capacity. Bali beef marinated with sugar palm neera solution with a concentration of 15%, 20% and 25% was able to maintain the nutritional content of the meat. However, it did not result in a total reduction in pathogenic microbes. Marinating Bali beef with a solution of sugar palm neera can enhance its antioxidant activity.

Keywords: beef, sugar palm neera, marinade, antioxidants

Introduction
One method of processing or preserving meat is by soaking the meat in a marinade. Marination is soaking meat in marinade ingredients before further processing (Nurwantoro *et al*., 2012). A marinade is a liquid mixture containing certain components that serve as a solution for soaking meat. It aims to enhance meat yield, flavor, juiciness, shelf life, tenderness, and water retention and minimize shrinkage during cooking (Alvarado and Sams, 2003). The advancement of current technology has enabled meat marination to serve as a preservative, reduce the bacterial content, and improve the taste and organoleptic properties of the meat. According to Syamsir (2010), the composition of marinade is not only salt but also oil (olive oil), acid (lemon), and other spices. One ingredient that can be used to marinate Bali beef and, at the same time, to improve organoleptic quality is sugar palm neera (*Arenga pinnata*). Sugar palm neera is commonly used as a marinade ingredient in roast pork to add a savory impression by the people of Pagindar, Pakpak Bharat, North Sumatra.

The sugar palm plant, similar to the coconut tree, is versatile as all its parts are utilizable for human sustenance. Sugar palm neera, derived from *the Arenga pinnata*, is highly favored by the general population due to its usefulness as both a direct consumable and a culinary ingredient. It possesses a delightful sweetness and pleasant aroma and is completely devoid of color. Fresh sugar palm neera contains sucrose, ash, protein, and vitamin C. The sugar palm neera is rich in antioxidants, including neeraonins, phenols, triterpenoids, and alkaloids, which are secondary metabolite chemicals (Putri *et al*., 2021). According to Lempang and Mangopang (2012), sugar palm neera contains organic acids such as lactic acid, malic acid, acetic acid, citric acid, and ascorbic acid. These acids contribute to enhancing the flavor of the neera. Marination is expected to maximize the absorption process of antimicrobial substances in Bali beef, and the use of sugar palm neera with different concentrations will influence the chemical, microbiological quality, and antioxidant capacity of Bali beef. Josefa *et al*. (2019) found that soaking skipjack tuna in 15% sugar palm neera could improve the quality and shelf life due to the lower water content, lower pH value, lower total fungi, lower total bacterial colonies, and higher organoleptic properties (taste, aroma or odor, tenderness, color).

The meat will experience spontaneous physiological changes. These changes are usually accompanied or followed by chemical and microbiological changes. Beef is a protein-rich food that provides an ideal environment for bacterial growth. In addition to posing health risks, these bacteria can adversely affect meat by compromising its nutritional composition and chemical quality. Marinating is the action used to reduce bacterial activity and chemical damage to the meat. This research was carried out to evaluate the effect of marination

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using sugar palm neera (*Arenga pinnata*) solution on the microbiological chemical quality and anti-oxidant capacity of Bali beef.

Methodology

Material

The research utilized fresh, unfermented sugar palm neera obtained directly from neera crafters Loin sourced from Mambal RPH, Badung Regency, Bali Province. In order to inhibit fermentation during transportation from the point of purchase, the neera was stored in a container equipped with ice flasks. Upon arrival at the laboratory, the neera was promptly transferred into a refrigeration unit.

Research Design

The research employed a Completely Randomized Design (CRD) with four treatments and four repetitions. Each iteration utilizes a loin with a weight of 100 g.

P0 : Meat was not marinated (control)
P1 : Meat marinated with 15% sugar palm neera
P2 : Meat marinated with 20% sugar palm neera
P3 : Meat marinated with 25% sugar palm neera

Research Procedure

This research began with preparing samples of Bali beef. The meat was cut into 16 pieces, weighing 100 g each, for each repetition. The meat cut was divided into four groups according to the treatment administered. The beef that had been grouped was then soaked in the marinade solution. The marinade solution was prepared by combining distilled water with neera, following the indicated treatments (P0, P1, P2, P3). Treatment P0, as a control, was not marinated. Treatment P1 with a sugar palm neera concentration of 15%, namely 15 mL of sugar palm neera mixed with 85 mL of distilled water. Treatment P2 with a sugar palm neera concentration of 20%, namely 20 mL of palm neera mixed with 80 mL of distilled water. P3 treatment with a sugar palm neera concentration of 25%, namely 25 mL of palm neera mixed with 75 mL of distilled water. The meat was soaked in sugar palm neera for 40 minutes, then drained and left to rest for 15 minutes at room temperature. Next, sample testing was carried out to evaluate the chemical quality, antioxidant capacity, and microbiological quality of the meat.

Meat Quality Testing

Moisture Content

Moisture content was determined directly using an oven at 105°C. First, the empty cup was dried in an oven at 105°C for 15 minutes and cooled in a desiccator, then weighed. A total of 1.5 g of sample was placed in a weighted cup and then dried in an oven at 105°C for 3 - 4 hours. The cup containing the dried sample was then transferred to a desiccator, cooled for 30 minutes, and weighed. Moisture content was calculated using the following formula:

$$\%\ Moisture\ Content = \frac{(Initial\ wight\ of\ sample - Final\ weight\ of\ samplel)(g)}{nitial\ wight\ of\ sample\ (g)} \times 100$$

Protein Content

A total of 0.3 g of the material were introduced into a Vapodest tube, with 1 selenium catalyst and 5 mL of concentrated H_2SO_4. Then, digestion (heating while boiling) was carried out for 1.5 hours until the solution was clear. After cooling, add 50 mL of distilled water and 20 mL of 40% NaOH and continue to the distillation process. The distillation results were collected in an Erlenmeyer flask containing 20 mL of H_3BO_3 and 2 drops of pink-green bromine cresol. Once the reservoir volume reached 100 mL and acquires a bluish hue, the distillation process was halted. Subsequently, the distillate was titrated with 0.1 N HCL until it turned pink. The identical procedure was likewise executed on blanks. With this method, crude protein content was obtained, which was calculated using the formula:

$$\%\ Crude\ Protein\ Content\ = \frac{(S - B)\ x\ 0{,}1\ x\ 14\ x\ 6{,}25}{W\ x\ 1000} \times 100\%$$

Note:

S: Titrant volume for sample
B: Titrant volume for blank
W: Weight of dried sample

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Fat Content

Fat content was determined using the Soxhlet method. A total of 2 g of meat sample (A) were weighed and enclosed in filter paper, then positioned within a container and subjected to a 9-hour drying process in an oven set at 105°C. The Soxtherm tube was dried in an oven for 3 hours at 105°C, cooled in a desiccator, and weighed (B). The lead containing the sample was put into a Soxtherm tube and then filled with 200 mL n-Hexane until the sample was fully immersed. Extraction was carried out for 4 hours on a Soxtherm apparatus. After that, the Soxtherm tube was dried in a forced oven for 15 minutes and continued with drying for 3 hours in a dry oven at 105°C. After that, cooling was continued in a desiccator for 30 minutes. The Soxtherm tube containing the fat extract was then weighed (C).

The percentage of fat content was calculated as follows:

$$Fat\ Content\ (\%)\ = \frac{C\ -B}{A}\ x\ 100\%$$

Note:

A: Weight of sample (g)
B: Weight of soxtherm tube (g)
C: Weight of soxtherm tube + fat extract (g)

Ash Content

The porcelain cup was heated in an oven at 100–105°C for 30 minutes, then left in a desiccator and weighed to obtain a constant weight. One gram of beef was placed in a porcelain cup and weighed. It was then burned until it stopped producing smoke and subjected to a temperature of 600°C in a kiln for 3 hours until it turned white and its weight remained consistent. The furnace was turned off and left for 12 hours. After that, the sample was cooled in a desiccator for 30 minutes and then weighed.

$$Ash\ Content\ (\%)\ = \frac{Weight\ of\ ash}{Weight\ of\ sample}\ x\ 100\%$$

Antioxidant Capacity Test

Antioxidant capacity was measured using the DPPH method (Yun, 2001).

Microbiological Quality Test

Total Plate Count (TPC)

The beef sample was ground and weighed 5 g. The dilution process started by making a sample solution of 10 mL (a mixture of 1 mL/gram of sample and 9 mL of peptone solution). From this solution, 1 mL was taken and put into the next test tube to obtain the desired dilution. Next, the solution was taken from the last 2 test tubes (10-7 and 10-8) and poured into a Petri dish. Next, agar media was added and rotated like number 8 so that the sample and media were evenly mixed and solidified. Samples were incubated at 37°C for 2 x 24 hours. The number of bacterial colonies was calculated using the following formula:

$$CFU\ = \frac{Number\ of\ bacterial\ colonies}{Dilution\ factor} \times poured\ sample$$

Total Coliform and *Escherichia coli*

The spread plate technique was employed to obtain the total count of *Escherichia coli* and Coliform bacteria (Fardiaz, 1989) utilizing an EMBA medium. A total of 5 g of beef were added to an Erlenmeyer tube with 45 mL of a 0.1% peptone water solution, resulting in a dilution of 10-1. Subsequently, the 10-1 dilution was thoroughly mixed and further diluted by transferring 1 mL using a pipette and adding it to a test tube containing 9 mL of peptone solution. This resulted in dilutions of 10-2 and 10-3.

From the 10-1 dilution, 0.1 mL was taken using a sterile pipette and poured onto the surface of the solidified EMBA media. Next, the sample was incubated at 37°C. Results can be calculated after 24 – 48 hours. Planting was performed at dilution levels of 10-1, 10-2 and 10-3. Bacterial colonies that grew were counted using the plate counting method, namely by selecting the number of colonies that grew in Petri dishes ranging from 30 - 300 colonies (Fardiaz, 1989).

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$$Colonies/gram = Number\ of\ colonies\ per\ Petri\ dish \times \frac{1}{dilution\ factor}$$

Statistic Analysis

Variance analysis was conducted to assess data on chemical quality, antioxidant capacity, and meat microbiology. If there is a statistically significant difference ($P < 0.05$) between the treatments, the analysis proceeds with Duncan's multiple range test as outlined by Steel and Torrie (1993). Prior to analysis, meat microbiological data was initially transformed into logarithmic values. Data analysis was facilitated using the SPSS 22 software.

Results And Discussion

Chemical Quality of the Meat

The statistical analysis of the chemical quality of the meat (water content, protein content, fat content, ash content, carbohydrate content, and antioxidant capacity) of Bali beef treated with marination with sugar palm neera is presented in Table 1.

Table 1. Chemical Quality and Antioxidant Capacity of Bali beef Marinated with Sugar Palm Neera (*Arenga Pinnata)*

Variable	Treatment				SEM[2]
	P0	P1	P2	P3	
Moisture content (%)	63.27[a1]	61.57[a]	63.00[a]	62.66[a]	0.36
Protein content (%)	26.47[a]	28.38[a]	26.28[a]	27.8[a]	0.53
Fat content (%)	2.46[a]	2.45[a]	2.22[a]	2.89[a]	0.19
Ash content (%)	1.08[a]	0.86[a]	0.99[a]	0.97[a]	0.03
Carbohydrate content (%)	6.74[a]	6.73[a]	7.49[a]	5.66[a]	0.48
Antioxidant capacity mg/L GAEAC	0.21[a]	1.53[b]	2.69[c]	2.94[d]	0.28

Note:

P0 : Meat was not marinated (control)
P1 : Meat marinated with 15% sugar palm neera
P2 : Meat marinated with 20% sugar palm neera
P3 : Meat marinated with 25% sugar palm neera

1. Values with the same letter on the same row are not significantly different (P>0.05)
2. SEM = Standart Error of Treatmeans

Statistical analysis (Table 1) shows that the water content of Bali beef marinated with sugar palm neera in all treatments was not significantly different ($P > 0.05$). The presence of antioxidants in sugar palm neera, such as neeraonins, phenols, triterpenoids, and alkaloids, could be the source of this phenomenon. These chemicals possess antibacterial properties, thereby inhibiting bacterial contamination in meat and ensuring the preservation of its quality. Meat protected from bacterial contamination will experience a minimal decrease in water content. This water content, which is not significantly different, also shows no osmotic pressure from the meat to the sugar palm neera solution. This condition also shows no protein denaturation in the sugar palm neera solution because the neera used is fresh neera that has not been fermented. Fermented neera typically has a low pH level, which will most likely cause protein denaturation. The absence of protein denaturation in this study can also be seen from the protein levels, which were not significantly different in all treatments. The results of a similar study using ingredients containing high antioxidants, namely garlic, also obtained a meat water content that was not significantly different (Rumondor *et al*., 2023).

The protein content of Bali beef marinated with sugar palm neera in all treatments was not significantly different ($P > 0.05$). This is caused by the concentration of sugar palm neera solution (15-25%) used not being able to hydrolyze protein, so there is no real change in the protein content of the meat. Protein hydrolysis can occur because the acid content can lower the pH and reduce the protein content of meat. Although sugar palm neera contains organic acids such as malic, ascorbic, citric, and fumarate, which are known to lower the pH of meat (Neerautra *et al*., 2015), at a concentration of 15 - 25%, it is possible that these organic acids will not be able to hydrolyze protein meat. Besides that, the pH value of fresh sugar palm neera ranged from 5.5 – 6.0, while the pH value of regular meat was 5.4 – 5.8. In addition, the pH range of raw sugar palm neera was between 5.5 and

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6.0, whereas that of regular meat was between 5.4 and 5.8. Therefore, the pH of sugar palm neera remains within the acceptable range, and it does not result in a decrease in the acidity level or pH value of the meat. The moisture content value is inversely proportional to the protein content. If the moisture content of a food item decreases, the protein content increases due to the salting-out process. In this study, the moisture content was not significantly different, which meant that the protein content was also not significantly different. According to Buckle *et al*. (2007), protein in beef ranges from 16 - 22%. The protein levels obtained in this study were relatively high, ranging between 26.28 - 28.38%. This is caused by the relatively low water content of the meat, ranging from 61.57 - 63.27%. Good quality meat typically has a moisture content ranging from 65% to 80% (Aberle *et al*., 2001). Diverse elements contribute to variations in the protein content of meat, such as the animal's age, farming practices, nutrition quality, pre-slaughter treatment, and post-slaughter treatment.

The fat percentage of Bali beef marinated with sugar palm neera is as follows: (P0) 2.46%, (P1) 2.45%, (P2) 2.22%, and (P3) 2.89%. The observed value does not exhibit a significant difference ($P < 0.05$). The marination process using sugar palm juice neera did not result in a notable reduction or increase of the moisture or protein levels in beef. The findings of this study remain within the average range, as shown by Backle *et al*. (2007), who said that the fat content of beef can vary from 0.5% to 13%. The fat content and protein content have an inverse relationship. As the protein content increases, the fat content decreases—similarly, the correlation with water content. As the water quantity increases, the fat content decreases. The study found no significant differences in water and protein content, leading to no significant differences in fat content.

The ash content of Bali beef marinated with sugar palm neera was measured to be 1.08% for P0, 0.86% for P1, 0.99% for P2, and 0.97% for P3, which were not significantly different ($P > 0$ .05). This is caused by the ash content being influenced by the water content of the meat, where in this study the water content of the meat was not significantly different.

The carbohydrate content of Bali beef marinated with sugar palm neera was measured to be 6.74% for P0, 6.73% for P1, 7.49% for P2, and 5.66% for P3, which were not significantly different ($P > 0$ .05). This means that the neera solution used to marinate Bali beef cannot increase the carbohydrate content of the meat. The reason for this is that the sugar palm neera contains low amount of glucose and fructose. The sweet taste of sugar palm neera is due to the presence of sugars such as sucrose, glucose, fructose and maltose. In addition to sugar, sugar palm neera also contains protein, fat, water, starch, ash, and organic acids such as citric, malic, cycinic, lactic, fumarate, and pyroglutamate, which contribute to the taste development (Lempang and Mangopang, 2012). The absence of an increase in the carbohydrate content of meat can be attributed to the low concentration of the neera solution and the suboptimal penetration of the neera solution into the meat. In general, beef is not considered a significant source of carbohydrates due to its low content of carbohydrates, specifically glycogen and glucose (Soeparno, 2015). However, carbohydrates play a role in meat characteristics, such as the texture and taste of beef.

The findings of this study demonstrated a statistically significant increase ($P < 0.05$) in the antioxidant capacity of Bali beef when marinated in a solution of sugar palm neera. As the content of sugar palm neera increases, so does its antioxidant capacity. The presence of bioactive chemicals in sugar palm neera is attributed to secondary metabolites that act as antioxidants. The composition of sugar palm neera includes many secondary metabolite chemicals, such as neeraonins, phenols, triterpenoids, and alkaloids (Putri *et al*., 2021).

Sugar palm trees are known to have many benefits, not only in their neera but also in their seeds, which contain antioxidant compounds (Rinda *et al*., 2019). Plant secondary metabolites can act as antioxidants in humans, which can be used as ingredients to eliminate free radicals. Adequate intake of antioxidants has been shown to decrease the occurrence of degenerative diseases, enhance immune function, and prevent the development of age-related degenerative diseases. The findings of this study suggest that beef soaked in sugar palm neera solution may have promising prospects as a functional foot. Functional food, as defined by the European Commission (EC), refers to food that has positive effects on specific health functions of the human body, promotes nutritional sufficiency, enhances overall health and fitness, and lowers the likelihood of disease. The following is a graph of the increase in the antioxidant capacity value of Bali beef marinated in sugar palm neera solution.

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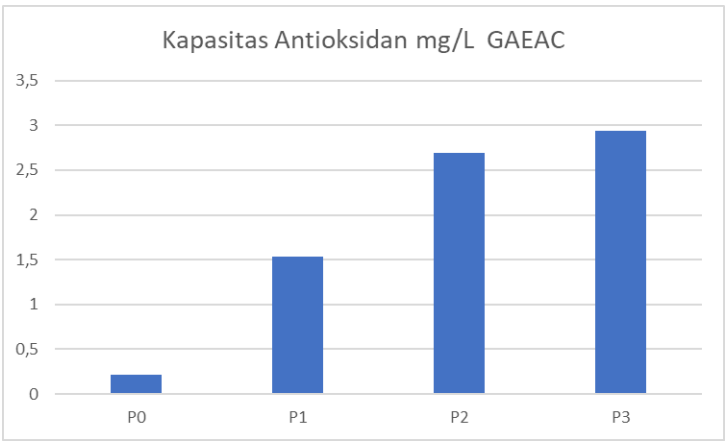

Figure 1. Graph of the antioxidant capacity of Bali beef marinated in sugar palm neera solution

Microbiological Quality of Meat

The statistical analysis of the microbiological quality of meat (TPC, Coliform, and *Escherichia coli*) from sugar palm beef that was marinated with sugar palm neera is presented in Table 2.

Table 2. Microbiological Quality of Bali beef Marinated in Sugar Palm Neera Solution

Variabele	Tretament				SEM[2]
	P0	P1	P2	P3	
Total Plate Count cfu/g	9.4×10^{5a1}	1.9×10^{6a}	3.2×10^{6a}	3.7×10^{6a}	0.12
Coliform cfu/g	1.0×10^{5a}	6.3×10^{4a}	3.8×10^{4a}	2.3×10^{4a}	0.11
E. colli cfu/g	3.5×10^{3a}	3.2×10^{3a}	3.0×10^{3a}	2.3×10^{3a}	0.07

Note:

P0 : Meat was not marinated (control)
P1 : Meat marinated with 15% sugar palm neera
P2 : Meat marinated with 20% sugar palm neera
P3 : Meat marinated with 25% sugar palm neera
3. Values with the same letter on the same row are not significantly different (P>0.05)
SEM = Standart Error of Treatmeans

Beef marinated with sugar palm neera at room temperature with different concentrations showed a total TPC value of 9.4×105 cfu/g for P0, 1.9×106 cfu/g for P1, 3.2 x 106 cfu/g for P3, and 3.7 ×106 cfu/g for P3, which was not significantly different (P > 0.05). Although statistically, there was no significant difference, there was a tendency for TPC colonies to increase with increasing concentrations of sugar palm neera. It could be due to the fact that neera provides a rich environment for the proliferation of bacteria, such as *Acetobacter acetic* and yeast cells belonging to the Saccharomyces genus. In neera that undergoes natural fermentation, yeast cells from the genus Saccharomyces will be more active in synthesizing sugar (glucose) and producing alcohol and CO_2 gas (Lempang and Mangopang, 2012).

Beef marinated with sugar palm neera with different concentrations showed that in all treatments, P0 (1.0 ×105 cfu/g), P1 (6.3 ×104 cfu/g), P2 (3.8 ×104 cfu/g), and P3 (2.3 ×104 cfu/g) total Coliform values were not significantly different (P > 0.05), likewise, with total *Escherichia coli*. All treatments P0 (3.5 ×103 cfu/g), P1 (3.2 ×103 cfu/g), P2 (3.0 ×103 cfu/g), and P3 (2.3 ×103 cfu/g) statistically not significantly different (P>0.05). Quantitatively speaking, there was a noticeable trend towards reducing the quantity of Coliform and *Escherichia coli* colonies. The magnitude of this quantitative decline in the bacterial population is quite noteworthy, as even a minor drop can lead to a decrease in the likelihood of harm. Infections caused by coliform and *Escherichia coli* can lead to gastrointestinal problems. The onset of symptoms resulting from the infection occurs within 12 - 24 hours and is marked by lower abdomen pain, disorientation, diarrhea, vomiting, fever, and headache.

The quantitative decrease in Coliform and *Escherichia coli* content was caused by the activity of the bioactive components of sugar palm neera, namely neeraonins, phenols, triterpenoids, and alkaloids. Neeraonin functions

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by inducing protein denaturation, causing damage to the cytoplasmic membrane of cells. Due to its resemblance to detergent, neeraonin can function as an antibacterial agent by diminishing the surface tension of the bacterial cell wall and impairing the permeability of the bacterial membrane (Sani *et al*., 2014). Phenolic compounds can denature proteins and damage bacterial cell membranes (Neeratarini *et al*., 2016). Shan et al. (2007) stated that phenolic compounds can potentially inhibit the growth of *E. coli* bacteria. This compound has a certain mechanism to break down the lipopolysaccharide walls of *E. coli* bacteria. It is also stated that phenolic compounds can denature proteins and damage bacterial cell membranes (Neeratarini *et al*., 2016). Terpenoids are phenolic compounds that are lipolytic. The mechanism of action of terpenoids is to damage bacterial cell membranes (Makaadd *et al*., 2020). Alkaloids are chemical compounds that contain nitrogen, have pharmacological activity, and act as anti-bacterial by inhibiting the work of enzymes in synthesizing bacterial proteins so that bacterial metabolism is disrupted (Oktaviana *et al*., 2019).

Conclusion

Marinating Bali beef with sugar palm neera solution at concentrations of 15%, 20%, and 25% effectively preserves the nutritional content of the meat. However, it does not lead to a complete elimination of pathogenic microorganisms. The antioxidant capacity of Bali beef can be increased through marinating in sugar palm neera solution, thereby enhancing its potential as a functional food.

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