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Modification of purple sweet potato variety antin via fermentation process: Physicochemical Study

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Abstract

Purple Sweet Potato (PSP) is a valuable food ingredient due to its numerous benefits for human health. However, in terms of function, PSP has low physicochemical properties. To overcome these limitations, research was conducted on the effect of PSP (var. Antin) fermentation on the characteristics of flour, using the bio-activator Bimo-CF and *Lactobacillus plantarum*. This research aims to determine the physicochemical characteristics of fermented Purple Sweet Potato Flour (PSPF) of Antin Variety. Peeled and sliced PSP were separated into three treatment groups: fermentation with Bimo-CF, *L-Plantarum*, and no fermentation. The materials were dried, ground, and then analyzed for their physicochemical properties. Results data were analyzed by one-way analysis of variance (ANOVA), using SPSS 16 (SPSS Inc., USA). The total content of phenolics, anthocyanins, protein, and crude fiber decreased during the fermentation process. The fermentation process also increased the water absorption capacity (WAC), peak viscosity, breakdown viscosity, and setback viscosity of PSPF. Modifying PSPF through fermentation can change the structure of PSPF into a material that is more suitable for industrial ingredients such as noodles, bread, and vermicelli.

Keywords: Bio-activator, Fermentation, Lactobacillus, Modification, Purple sweet potato flour.

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1. Introduction

The Antin variety of purple sweet potato (PSP) which is widely planted in Kuningan Regency (West Java, Indonesia) has a high yield potential of 25 tons/ha, a carbohydrate content of 24.01%, and bioactive components for human health such as anthocyanin pigments of 45.58. mg/100 grams. PSP in Kuningan Regency is being developed to be processed into flour and exported to Japan. The yield potential and high starch content make PSP widely used in the food industry, though some weaknesses in its original form. This is where flour modification technology is needed [1].

A simple way to modify flour is through a fermentation process. Fermentation is an ancient method used in food processing to increase shelf life, palatability, digestibility, and nutritional value [2]. Fermentation increases the peak viscosity value, breakdown, and setback values, but reduces the maximum viscosity-temperature of all types of sweet potato flour (SPF) [3]. Factors that influence the quality of flour through modification with fermentation techniques include temperature, fermentation time, and type of bacteria [1, 4-6].

The role of bacteria in the fermentation process, both single and mixed applications, can affect the quality of the flour produced [7] and also the cooking time [8]. One of the bacteria that can improve the functional and physicochemical properties of flour is a species of lactic acid bacteria, such as *L. plantarum* which is also contained in Bimo-CF [9, 10]. Lactic acid fermentation changes the properties of flour by spontaneously changing the starch granules. Its application to SPF results in improved flour characteristics for industrial products such as soups, breads, and artisanal snacks [11]. However, there are no research specifically aimed at flour products from the Antin variety. Therefore, this research aims to determine the physicochemical characteristics of fermented Purple Sweet Potato Flour (PSPF) Antin Variety.

2. Material and Methods

The PSP used in this study was the local variety Antin (*Ipomoea batatas* antin) obtained from Kuningan Regency. They were harvested at the age of 4-5 months. The tubers were brought to the laboratory within one week after harvest and stored in a ventilated room (T = 18-22ºC). The chemicals used included: sodium metabisulfite, two types of starter (Bimo-CF and *L. Plantarum*) obtained from an online shop in Jakarta, free radical Diphenyl-2-picrylhydrazyl (DPPH) produced by Sigma, HCl pa., ethanol pa., methanol pa., and ammonium thiocyanate manufactured by Merck. The equipment used was a stove, slicer, soaking container, scales, drying oven, flour tool, 80 mesh filter, Shimazu UV-visible spectrophotometer, glassware, and Memmert oven.

2.1. Preparation of Purple Sweet Potato Flour

PSPF is made from sliced PSP. PSP was peeled and washed before slicing, soaked in sodium metabisulfite solution, rinsed, and then separated into three treatment groups. The first group was soaked in water containing Bimo-CF with a concentration of 1 gram per 1 liter for 1 kg PSP. The second was soaking in *L.plantarum* solution with a concentration of 3 mL per liter for 1 kg PSP. The third treatment was control, without further immersion. The first and second groups were soaked for 12 hours for the fermentation process at room temperature (25-27°C). After the process is complete, it is rinsed thoroughly and dried in a blower-drying oven at a temperature of 50-60°C for 12 hours, until it reaches a water content of 6 to 10%. The dried slices were ground using a hammer mill into flour with a size of 80 mesh. Then the PSPF samples were packaged in polypropylene plastic bags and stored in a desiccator at room temperature for further analysis.

2.2. Determination of Moisture Content

Moisture content was measured using the Gravimetric method with a drying oven [11]. A sample (3-5 grams of PSP) was weighed and put into a cup that had been dried and the weight was known. Then the sample and cup were dried in an oven at 105 °C for 6 hours. The cup was cooled and weighed, then dried again until a constant weight was obtained.

2.3. Determination of Carbohydrate Content

Carbohydrate levels were measured based on SNI 01-2891-1992 [12]. 5 g of purple sweet potato flour sample was put into a 500 mL Erlenmeyer flask, 200 mL of 3% HCl solution, boiled in an upright cooler for 3 hours, cooled, neutralized with 30% NaOH solution (with litmus or phenolphthalein), and then a little CH_3COOH 3% to make a slightly acidic solution. then transferred into a 500 mL measuring flask, filtered, and 10 mL taken into a 500 mL Erlenmeyer flask. The solution was then added to 25 mL of luffa solution, some boiling stones, and 15 mL of distilled water. It was heated to a constant temperature within 3 minutes and allowed to boil for 10 minutes. Then immediately cool in a tub filled with ice. After cooling, 15 mL of 20% KI solution and 25 mL of 25% H_2SO4 were slowly added. The solution was immediately titrated with 0.1 N thio solution (using 0.5% starch solution as an indicator). The same method also applies to blank samples.

2.4. Determination of Protein Content

Protein content was measured using the semi-micro Kjeldahl method [12]. The flour sample was carefully weighed as much as 0.51 g and put into a 100 mL Kjeldahl flask. The sample was then added with 2 g of selenium and 25 mL of concentrated H_2SO4. The mixture was boiled with a heater or electric burner until the solution became clear with a greenish color (± 2 hours). The solution was allowed to cool, then diluted and placed in a 100 mL measuring flask. 5 mL of solution was put into a distiller, then 5 mL of 30% NaOH was added along with a few drops of PP indicator. The solution was then distilled for approximately 10 minutes and collected in a container with a mixture of 10 mL of 2% boric acid solution and indicator. After the cooling tip was rinsed with distilled water, the solution was titrated with 0.01 N HCl solution. The same method also applies to blank samples.

2.5. Crude Fiber Determination

Crude fiber content was measured by the Extraction method [12]. 2-4 flour samples were degreased by extraction using a Soxhlet and then dried. The sample was put into a 500 mL Erlenmeyer. Add 50 mL of 1.25% H_2SO4 solution. Then boiled in the upright cooler for 30 minutes, 50 mL of 3.25% NaOH was added, and boiled again for 30 minutes. The hot sample was filtered using a Bucher funnel containing Whatman 54.41 or 541 ash-free filter paper, which had been dried, and the weight was measured. Furthermore, the precipitate on the filter paper was washed successively with 1.25% hot H_2SO4, hot water, and 96% ethanol. The filter paper is removed with its contents and put into a weighing box whose weight is known. Then it was dried at 105 °C until the weight was constant.

2.6. Total Phenolic Content Determination

The total flavonoid content was determined by using the Folin-Ciocalteu Assay [13]. An aliquot (1 mL) extract or standard solution of gallic acid (100, 200, 300, 400 dan 500 μg/mL) was added to 25 mL of the volumetric flask, containing 9 mL of distilled water. A reagent blank using distilled water was prepared. 1 mL of Folin-Clocalteuphenol reagent was added to the mixture and shaken. After 5 minutes, 10 mL of 7% Na_2CO3 solution was added to the mixture. The volume was then made up to the mark. After 90 minutes of incubation at room temperature, the absorbance of the reagent blank was determined at 550 nm with a UV-visible spectrophotometer. The total phenolic content was expressed as mg of gallic acid equivalents per gram of sample (mg GAE/g).

2.7. Anthocyanin Content Determination

Analysis of anthocyanin content in this study used spectrophotometric techniques [14]. Anthocyanins were extracted using water and 1% HCl (sample to solvent ratio, 1:5) w/v. The extract was then separated using a vacuum filter and thickened using a vacuum evaporator at 60 °C to form a paste. Furthermore, the extract was dissolved in 75% ethanol (concentration 10% w/v). The extract solution was then stored in a dark bottle and blown with nitrogen to remove oxygen in the headspace. The vial was closed and stored in the refrigerator at 4 ^oC. The absorption of the purple anthocyanin color in the ethanol was measured at a wavelength of 535 nm.

2.8. Physical Properties Determination

The sticky properties of PSPF were analyzed with a Rapid Visco Analyzer (Perten 4500) using the method of Marta, et al. [15]. 2.50 ± 0.01 g of starch (14% moisture) was added to the water surface. A paddle is placed into the canister and vigorously moves the blade through the sample up and down until no lumps remain on the surface of the water or stick to the paddle. The paddle was then inserted into the canister, and the canister was inserted into the instrument. The measurement cycle began by pressing the instrument motor turret. Then, the canister was removed after the test was completed with isolation gloves and discarded. Finally, peak viscosity was recorded as the Sweet Potato Starch Index RVA™ [15].

The color of purple sweet potato flour samples was measured using -300 (Chromameter). Color measurements include L* (brightness, 0 = black/100 = white), a* (+a* = reddish/-a* = greenish), b* (+b* = yellowish/-a* = bluish).

The swelling power (SP) of the PSPF was measured according to the procedure of Kaur, et al. [16]. 0.1 g of starch plus 10 mL of distilled water was stirred until homogeneous. The mixture was put into a test tube and placed in a beaker at 60°C for 30 minutes, then transferred to a centrifugation tube and centrifuged at 1,500 RCF for 15 minutes. Discard the supernatant and weigh the sediment. The swell power value is taken from the weight of the paste divided by the dry sample weight (g/g) [16].

The Water Solubility Index (WSI) was measured according to the procedure of Diniyah, et al. [17]. 1 g of sample was put into a centrifuge tube and mixed with 10 mL of distilled water. Stirred for one minute and left for 1 hour before centrifuging at 4,000 rpm for 15 minutes. The supernatant was separated from the precipitate. The precipitate was put into a petri dish and heated in an oven at 100-105 ^oC for 1 hour or until only solid starch powder remained. Then the powder is weighed and dried again. The solubility value was the percentage of dry starch weight per initial sample weight [17].

Water Absorption Capacity (WAC) was measured according to the procedure of Subagio et al. (2003). The empty centrifuge tube was weighed (a). Then 1 g of starch is added (b) along with 30 g of water into a centrifuge tube. Then shake with a vortex for 10 seconds every 5 minutes for 30 minutes. Then the tube was centrifuged at 1,500 RCF for 5 minutes. The water was separated from the precipitated starch, then the precipitate was weighed along with the tube (c). WAC was the percentage of water absorbed by starch in g/mL with the following formula: ((c-a)-b)/b x 100% [17].

2.9. Statistical Analysis

Experimental data were analyzed by one-way analysis of variance (ANOVA), using SPSS 16 (SPSS Inc., USA). Multiple comparisons between treatments used the Tukey test at $P<0.05$.

3. Results and Discussions

3.1. Physicochemical Properties

The results of the PSPF physicochemical (content of water, carbohydrate, protein, fiber, phenolic, and anthocyanin) are presented Table 1. It showed that the fermentation treatment did not affect water and carbohydrate content; However, it had a significant effect on the content of protein, fiber, phenolics, and anthocyanins.

Table 1.
Physico-chemical properties of fresh purple sweet potato (PSP) and flour.

Parameters	Fresh PSP	Control/without fermentation	Bimo-CF	*L. Plantarum*
Moisture **(%)**	-	6.23 ±0.19[a]	6.01 ±0.53[a]	5.84 ± 0.11[a]
Carbohydrate (%)	24.01±0.05	69.13± 0.14[a]	70.50 ±1.18[a]	69.90±1.20[a]
Protein (%)	1.25 ±0.02	0.28± 0.01[a]	0.19 ±0.01[b]	0.29 ±0.01[a]
Dietary Fiber (%)	5.53 ±0.01	3.06± 0.48[a]	1.82 ± 0.01[b]	2.51 ± 0.33[ab]
TPC (mg GAE/g)	-	182.65 ± 6.19[a]	70.57± 4.77[c]	82.72 ± 2.05[b]
Anthocyanin (mg/100 g)	-	45.86 ± 1.15[a]	19.93 ± 0.93[b]	15.01 ± 0.89[c]

Note: Numbers followed by the different letters are significant according to the Tukey test at a 95 % confidence level ($P < 0.05$).

Water content is an important parameter that determines the shelf life of a product. The lower the water content, the longer the shelf life of food products [18]. The water content in the fermented PSPF treatment was lower than in the non-fermented treatment. This was due to an increase in the activity of enzymes that degrade starch and reduce its ability to hold water. As a result, the carboxyl groups and the number of hydrogen bonds between and within the molecules were released and evaporated during drying [2].

The water content obtained in all treatments was quite low, namely 5.84-6.23% with an average of 6.03%. It was lower than fermented SPF in a wheat composite that was used in white bread (6.76 – 9.93%) [3]. Based on water content alone, the quality of PSPF flour in this study was lower than SPF in Yuliana, et al. [3] since the flour specification limit is usually around 14% or less. It's best to be closer to 14%; more than that will make the flour unstable at room temperature [19].

The carbohydrate content of control PSPF, and those fermented with Bimo-CF and L. plantarum were 69.13± 0.14, 70.50 ±1.18, and 69.90±1.20% respectively. All three were equivalent because the carbohydrate content of PSPF was not influenced by fermentation but by the carbohydrate content of the raw material [20].

The crude fiber content was influenced by the type of bio-activator. The fermentation process using bio-activators Bimo-CF and *L. plantarum* was able to break down sweet potato fiber up to 0.55 – 1.24 % lower than the control. The crude fiber content of sweet potato flour has decreased due to the activity of lactic acid bacteria. These bacteria produce various depolymerization enzymes that can hydrolyze proteins, fibers, and fats [3]. Nevertheless, in this study, the protein decrease in controls was still greater than PSPF fermented with *L. plantarum*. This may be because, in some cases, fermentation with L. plantarum can increase protein [21-23] due to its ability to reduce protein surface hydrophobicity [22].

The total phenolic content (TPC) of PSPF and anthocyanin content were influenced by fermentation treatment. The TPC values obtained in fermentation with Bimo-CF and L. plantarum bio-activators were, respectively, 61.4 and 54.7% lower than the control. The results of this study are different from the research of Chintha, et al. [24] which produced equivalent TPC between control and fermented sweet potato [24]. This is because there are several differences between the two studies. First, material differences. Both use two different cultivars with different flesh colors. Colored potatoes (PSP) contain much higher TPC than regular potatoes [25]. Second, differences in fermentation methods, including different temperatures, air content, bacterial forms, and the number of inoculations. TPC is very sensitive to the processing method used. Very easily oxidized due to exposure to light and temperature differences [5]. The rate of change depends on the strain of the initial microorganism. The process of changing phenolic compounds can be caused by enzymatic or non-enzymatic oxidation, diffusion of dissolved phenolic compounds into fermented sweat (exudate), or condensation reactions with proteins [26]. In the case of fermentation with Lactobacillus strains, changes can occur due to the release of bioactive components from the phenolic compounds themselves [27].

Anthocyanins are water-soluble flavonoid pigments that are responsible for the color of some fruits, flowers, leaves, stems, tubers, and rhizomes [28]. The PSPF anthocyanin content in this study was significantly different between treatments. Total fermented PSPF anthocyanins were between 15.01 and 19.93 mg/100g, or 56.54 and 67.3 % lower than the control (Table 1). This result was lower compared to Nurdjanah, et al. [5] which was in the range of 50.4-63.2 mg/100g [5]. This was possible due to differences in the type of raw material used or the conditions at the time of analysis or extraction.

Anthocyanin content is strongly influenced by enzymatic and non-enzymatic factors. Enzymatically, the anthocyanin content can be damaged due to the browning process by anthocyanin enzymes and polyphenols [5, 29]. Meanwhile, non-enzymatically, the stability of anthocyanin is affected by pH, light, and temperature. The anthocyanin value corresponds to the a* color value, which will be discussed further in the next paragraph.

3.2. Color Properties

Color is a parameter of food quality. PSPF color measurements were carried out using a Chromameter. PSPF color parameters vary greatly depending on the type of fermentation (Table 2). The type of fermentation significantly affected the a* and b* values, not the L* PSPF value. All treatments in this study gave a high L* (Lightness) value (range 62.13-63.88), which indicated a light color or faded purple color.

The chroma value indicates the purity of the color of the material [30]. The higher the chroma value, the darker the color [31]. Chroma values consist of two values of color, a* for redness and b* for yellowness. Fermented PSPF had a* and b* values that were significantly different compared to the control. They had a lower a* value but a higher of b* value. This showed that the fermentation tends to reduce the redness but increases the yellowness of PSPF. These results were slightly different from the comparison between steamed, boiled, and fresh PSP (control) by Mahmudatussa'adah, et al. [29] where the a* and b* values for the steamed treatment were higher and the boiled treatment lower than the control [29].

Table 2.
Color value of purple sweet potato flour from different fermentation.

Sample	L*	a*	b*
Control	62.13 ± 0.55[a]	9.48 ± 0.17[a]	10.34 ± 0.12[b]
Bimo-CF	62.19 ±1.11[a]	7.21 ±0.32[b]	13.30 ±0.28[a]
L. plantarum	63.88 ± 0.48[a]	6.83 ± 0.03[b]	12.89 ± 0.06[a]

Note: Numbers followed by the different letters are significant according to the Tukey test at a 95 % confidence level ($P < 0.05$).

The steaming process can strengthen the color because it breaks the bonds between protein and dye [32]. The steaming treatment causes enzymes that play a role in the browning process, such as anthocyanase enzymes, peroxidase enzymes, and polyphenol oxidase enzymes, to become inactive [1]. This also occurred in the fermentation process. However, in this study, the a* PSPF value was lower than the control because the form of PSP was flour. Control flour can undergo a non-enzymatic process during drying, resulting in a redder color than fermented PSPF [1].

3.3. Functional Properties

The functional characteristics of PSPF can be seen in Table 3. It consisted of swelling power (SP), water solubility index (WSI), and water absorption capacity (WAC). SP shows the ability of starch to absorb water and swell [33]. This ability is influenced by the amylopectin content. Crystallites in the amylopectin molecule determine swelling but also gelatinization. Polymerization degree ⩾35 in amylopectin contributes to increased swelling [34]. WSI is the amount of polysaccharide released from granules at the excess water solubility index [35], and WAC is the amount of water absorbed by flour to achieve the desired quality of food products [36].

Table 3.
Functional properties of purple sweet potato flour from different fermentation.

Sample	Swelling Power (%)	Water Solubility Index (g/g)	Water Absorption Capacity (g/g)
Control	84.87 ± 6.06[a]	0.21 ± 0.01[a]	1.30 ± 0.06[b]
Bimo-CF	100.33 ± 8.14[a]	0.26 ± 0.03[a]	1.58 ± 0.05[a]
L. plantarum	100.67 ± 8.14[a]	0.25 ± 0.01[a]	1.49 ± 0.02[a]

Note: Numbers followed by the different letters are significant according to the Tukey test at a 95 % confidence level ($P < 0.05$).

SP and WSI were not affected by the fermentation, but WAC was affected by the treatment. The SP resulting from the fermentation of Bimo-CF and *L. plantarum* bio-activators was higher than the control, although it was not significantly different. This is in line with other studies with the same fermented object and starters [37]. Modification of starch with *L. Plantarum* and Bimo-CF bacteria will produce amylase enzymes, which affect the porosity and structure of starch granules. The weaker the bond between the starches, the more water is absorbed by the starch granules, so that the swelling of the material increases [37].

The WSI values for all treatments were also not significantly different because the WSI value is very dependent on the type of starch granule structure [37]. As long as the raw materials for all treatments are the same, the WSI value will remain the same. However, this is different from the research results of Yuliana, et al. [1] where the fermentation treatment produced lower WSI compared to the control [1]. This may be due to differences in WSI measurement methods between the two studies. Both use different temperature levels for the heating process.

AC indicates the ability of flour or starch to absorb water and swell. This is necessary for types of products that require dough swelling in their manufacturing process [33, 38]. The ability of flour to absorb water and swell can increase food uniformity [39]. The fermentation process in this study produced WAC values between 1.49 – 1.58 g/g or 14.6 -21.5% better than the control. They significantly increased WAC values compared to the control.

3.4. Pasting properties

The pasting properties of PSPF were determined by a Rapid Visco Analyzer (RVA), a tool for measuring the physiochemical properties of flour [40]. Pasting properties were affected by the size and shape of the starch granules, the amylose content, and the distribution of the branched chain lengths of amylopectin [41].

Pasting properties parameters are shown in Table 4. The fermentation treatment showed a significant effect on the pasting properties. Similar to the results of research by Yuliana, et al. [3]. Fermentation treatment increased the peak viscosity, peak temperature, peak time, and setback viscosity, but decreased the breakdown viscosity value [3].

Table 4.
Pasting properties of purple sweet potato flour from different fermentations

Sample	Peak Temp (°C)	Peak Time (s)	Peak Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)
Control	76.00 ±0.17[b]	553.33 ±2.31[b]	76.33 ± 2.89[b]	46.00 ± 2.65[a]	-33.67 ±2.52[c]
Bimo-CF	77.93±0.55[a]	583.03 ±15.15[a]	841.70 ± 26.70[a]	31.00 ± 1.00[b]	206.00 ± 2.00[b]
L. plantarum	77.77± 0.29[a]	574.00 ± 3.46[ab]	883.67 ±12.74[a]	15.33 ± 0.58[c]	260.33 ± 4.93[a]

Note: Numbers followed by the different letters are significant according to the Tukey test at a 95 % confidence level ($P < 0.05$).

The control treatment of PSPF had a lower peak temperature (76.00 ºC) than the fermentation treatment (77.77-77.93 ºC). Pasting temperature indicates a change in natural starch from a semicrystalline form to an amorphous form. The stability of starch crystals can be measured by knowing the gelation temperature; the higher the gelatinization temperature, the more stable the starch crystals [42].

The peak viscosity of the modified PSPF was higher than the control treatment and significantly different ($p<0.05$). The low peak viscosity indicates that the granule starch of the control PSPF was more rigid, resulting in instability [1]. The high PSPF peak viscosity also indicates that starch was more resistant during the heating process at high temperatures [42]. The fermentation process with lactic acid bacteria (Bimo-CF and *L. plantarum*) produces acids and enzymes, which affect the amorphous structure of the granules, and then provide more susceptibility. The growing microbes will also produce pectinolytic and cellulolytic enzymes in the fermentation process. These enzymes cause damage to cell walls and result in starch degradation. The greater the viscosity value, the more swollen the starch granules will be [43]. The peak viscosity shows the maximum swelling before the starch granules are ruptured [44]. Fermented sweet potato flour with high viscosity can be used as a thickening product [1].

Breakdown viscosity is an indicator of the paste's resistance to disintegration in response to heat and shear [39]. The breakdown viscosity decreases after frying and will increase when the viscosity sets back [45]. The breakdown viscosity and viscosity setback were significantly different between treatments. Fermented PSPF had a lower breakdown viscosity compared to the control treatment. The lower the breakdown viscosity, the more resistant the flour is to heating and shear stress. Therefore, fermented flour was more resistant to damage than unfermented flour [1]. The setback viscosity of fermented flour was higher than control flour. The higher the setback viscosity value, the more stable the starch and the more likely it is to form a gel when cooled. Flour with this quality is suitable for making noodles, bread, and vermicelli [46].

4. Conclusion

Fermentation treatment with Bimo-CF and L. plantarum bio-activators affected the protein content, fiber, WAC, phenolic content, anthocyanin content, and the pasting properties of the modified PSPF var. Antin. Fermented PSPF does not have better nutritional content than non-fermented, but is more resistant to environmental stress and produces better properties of flour for making noodles, bread, and vermicelli.

5. Practical Implication

In the food industry, food isn't just packaged and served to satisfy consumer preferences. It must also consider nutritional content and production costs. This research aims to provide input into the best processing methods for PSP, resulting in optimum quality as a food ingredient.

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