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Food-Grade Vinegar Production from the Extract of Sake Lees Obtained by Subcritical Water Treatment

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Abstract

In our previous study, it was found out that extracts obtained from food processing waste (rice bran and sake lees) of vinegar after subcritical water treatment contained sources of abundant nutrients. Based on these results, the objective of this study was to develop a method to further utilize the extract obtained from sake lees through the subcritical water treatment for the production of a food-grade vinegar, this method being the further fermentation by acetic acid of the subcritical water extract. The nutrient content was then compared with existing vinegar products in terms of general composition, amino acids, and mineral contents. In general, vinegars derived from the extract obtained by subcritical water had higher protein content compared to existing vinegars; as for amino acids, the same or higher amount were observed, together with 50% content of essential amino acids. Furthermore, the amount of total nitrogen (TN) in the critical water extract was almost twice as that of the existing vinegars. These findings suggest that the subcritical water extract of sake lees may be used not only to produce food-grade vinegar but also as an umami component and a nutritional supplement during fermentation.

Keywords: Biomass; Green Chemistry; Subcritical Water Extraction; Separation Recovery; Zymology

Introduction

Vinegar is a popular condiment in Japan and it is also used widely in health food. Vinegar has a variety of health benefits, including antimicrobial properties and energy, fat-burning, and blood-flow promotion effects [1-6].

Vinegar is brewed using a process such as that described in Figure 1. During this process, a large amount of brewing residue is generated as a by-product of brewing and processing vinegar.

First, the raw material for vinegar is rice, and subsequently rice bran is obtained in the process of polishing the rice. Next, saccharification is performed by alcoholic fermentation and squeeze the brewing alcohol. Sake residue is obtained in this process. In Japan, this brewing residue primarily consists of sake lees and rice bran, which are rich in nutrients such as carbohydrates and amino acids. However, most of these residues are discarded as industrial waste at a great expense. In the case of Maruboshi Vinegar Co., Ltd. (Fukuoka, Japan), three tons of rice bran and eighty tons of sake lees are discarded annually.

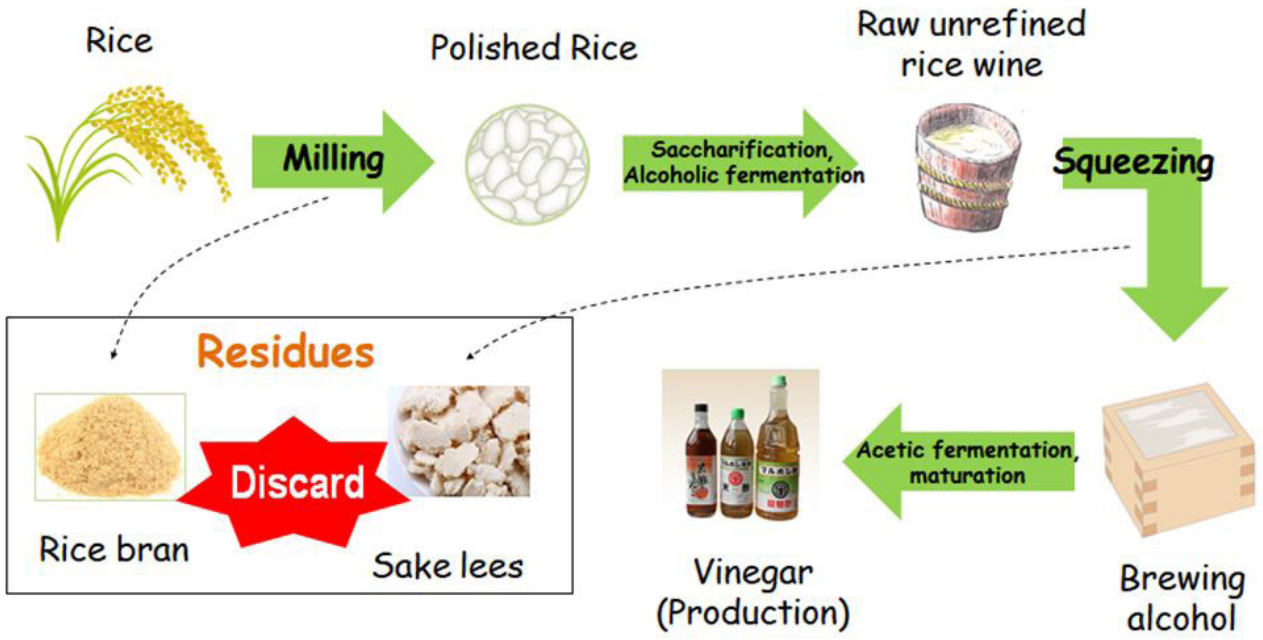


Figure 1: Process of producing vinegar and brewing residues

The deterioration of the global environment in recent years has become a social issue, and consequently, it is well known that residues of chemical and organic compounds in the field of chemical technology pose a growing concern as causes of environmental pollution.

The shift to green chemical processing [7] as a technological innovation for building a sustainable society is beginning to thrive in Japan. Biomass resources are roughly classified into resource crops and waste products. In particular, approximately 18 million tons of food residue is generated as waste biomass on an annual basis in Japan; this not only includes food loss but also food processing waste discarded in the process of manufacturing food products [8]. Technologies to effectively recycle these resources will both reduce the volume of solid waste such as food residue and possibly also have other benefits, such as mitigating industrial waste disposal costs paid by companies.

The objective of this study is to develop a method to further utilize the extract obtained through the subcritical water treatment of vinegar brewing residues produced as by-products of food processing. Therefore, based on the research results so far [9], vinegar derived from the subcritical water treatment of sake lees was first produced by acetic acid fermentation, which was one of the brewing residues. The nutrient content was then compared with existing vinegar products in terms of general composition, amino acids, and mineral contents.

Amino acids are components that are deeply related to the taste and umami of vinegar as well as health benefits. In particular, glutamic acid is contained in ordinary vinegar at an average of about 150 ml / L [10]. And, if it is rich in minerals, it can be expected not only to have a positive effect on the human body but also to be used for purposes other than food.

Experiment and Analysis

Raw materials for extraction

Sake lees, a residue from rice vinegar brewing, provided by Maruboshi Vinegar Co., Ltd. (Fukuoka, Japan), was used as the raw material. Figure 2 shows an experimental sample of sake lees, and Table 1 shows the composition thereof.

Figure 2: Experimental sample of sake lees

Experimental apparatus and procedure

The experimental equipment and methods are published in our previous paper [10]. An autoclave was to generate high temperature and high pressure (Figure 3).

The reactor was constructed from SS 316 steel and has an internal volume of 500 mL. The reactor was charged with 45 g of raw material and 300 mL of distilled water, mixed with a stirrer, and then sealed. Thereafter, the temperature was raised to a predetermined setpoint (160–225°C) by a band heater installed in the reactor. The heating time was 15 to 30 min. After reaching the predetermined temperature, the contents were reacted for 15–120 min while stirring at 300 rpm. The pressure in the reactor varied from 1.3 to 2.6 MPa depending on the vapor pressure of water and the product gas evolved during processing. After the subcritical water treatment, the band heater was removed from the reactor and a fan was used to quickly quench the reactor. After the reaction solution was sufficiently cooled (hereinafter this solution will be referred to as a sub-critical water treatment solution) was collected and separated into filtrate and water-insoluble components by suction filtration.

Table 1: Composition of sake lees[1]

Moisture content (%)	Total nitrogen (ppm-N)	Water-soluble amino acid content (ppm-N)	Total organic carbon (ppm-C)	Ammonic nitrogen content (ppm-N)	Mineral content (ppm)
56.2	2,510	127.1	8,835	21.3	96.5

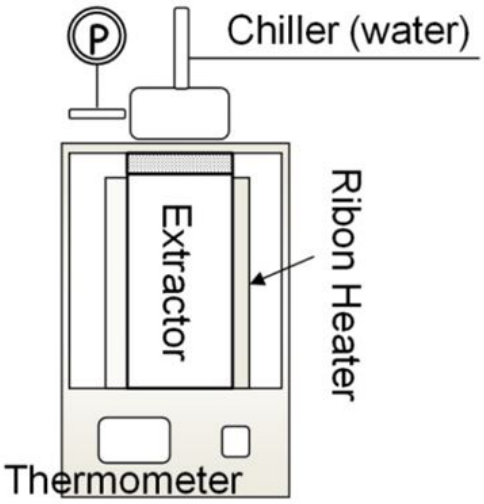


Figure 3: Schematic diagram of experimental equipment for Sub-critical water treatment

Subcritical water treatment experiment of sake lees

Table 2 shows the experimental conditions of the sub-critical water treatment experiment.

Preliminary experiments were conducted with several patterns to determine the optimum conditions for this experiment. The results are shown in Figure 4 and Figure 5. The notation of the sample means that, for instance, in the case of 180S30, sub-critical water treatment was performed at a temperature of 180 ° C for 30 minutes; the same applies to other samples. These conditions were decided so that a certain amount of amino acids and minerals could be obtained without being overly hydrolyzed.

Therefore, we decided on the conditions shown in Table 2, which contained amino acids and nitrogen on average. Good results were also obtained from the 120S300 sample, but it was not used as the experimental conditions for this experiment since the experiment time was long and the results were not much different from the others.

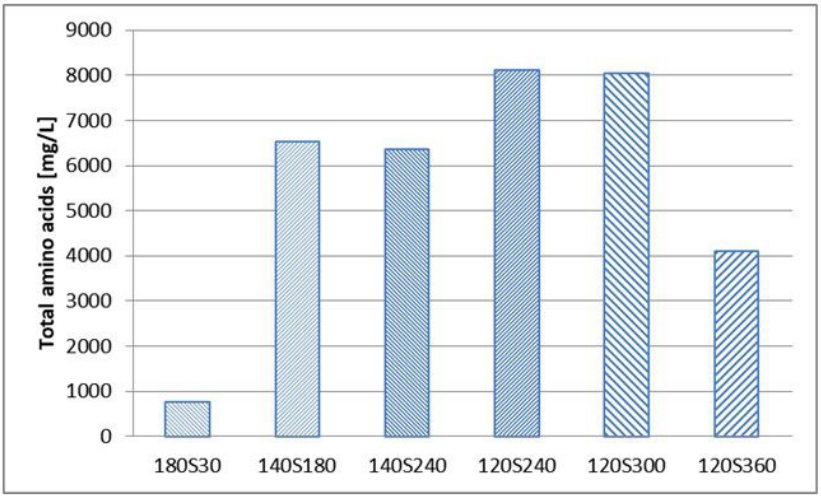


Figure 4: Total amino acid concentration in subcritical water treatment solution

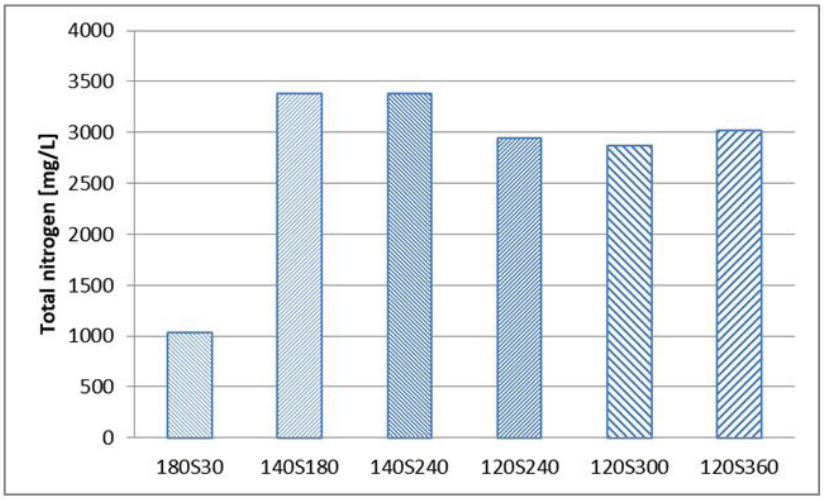


Figure 5: Total nitrogen in subcritical water treatment solution

Table 2: Experimental conditions

	Treatment time (min)	Reaction temperature (°C)
Sake lees A	240	140
Sake lees B	240	120

Analysis

The concentrations of amino acids, nitrogen, phosphorus, and minerals contained in the subcritical water treatment solution were quantitatively analyzed. The analysis method is the same as in our previous report [10].

Amino acids

Amino acids were derivatized with OPA (o-Phthalaldehyde by Wako) and FMOC (9-Fluorenylmethyl Chloroformate by Wako) and separated by a column (2.6μ EVO C18 100×3mm by Kinetex) for ultrahigh-speed analysis and then analyzed by High performance liquid chromatography (HPLC: NEXERA X2 manufactured by SHIMADZU) with a fluorescence detector (RF-20AXS manufactured by SHIMADZU). The details are as follows: Mobile phase A consisted in 17 mmol / L potassium hydrogen phosphate and 3 mmol / L dipotassium hydrogen phosphate water solution; mobile phase B consisted in 15/45/40 (v / v / v) = water / acetonitrile / methanol with concentration of 10.5 to 100% (gradient method). Column temperature was set at 35 ° C while mobile phase flow rate was set at 0.85 mL / min. Chosen mixer capacity was 0.18 mL with a sample injection volume of 1 ; wavelength detection was done by excitation wavelength 350 nm, fluorescence wavelength 450 nm, and at last eighteen amino acids were isolated, as shown in Table 3.

Table 3: Amino acids quantitatively analyzed in this study

1. Aspartic acid	2. Glutamic acid	3. Serine	4. Histidine
5. Glycine	6. Threonine	7. Arginine	8. Alanine
9. GABA	10. Tyrosine	11. Valine	12. Methionine
13. Cysteine	14. Tryptophan	15. Phenylalanine	16. Isoleucine
17. Leucine	18. Lysine		

Total nitrogen concentration

Quantitative determination was made by dividing nitrogen into total nitrogen and ammonia-nitrogen, the Kjeldahl method [11] was used for total nitrogen.

Ammonia nitrogen concentration

Analysis of ammonia-nitrogen was carried out using the Indophenol method [12].

Phosphorus content

Phosphorus content was determined using the molybdenum blue method [13].

Minerals

Three minerals: potassium, calcium, and magnesium were quantitatively determined by atomic absorption spectrometry (AA-7000 by SHIMADZU).

Definitions of formulas

Solubilization ratio of raw material

Raw material solubilization ratio = water-soluble nitrogen yield = TN concentration [ppm-N] / total amino acid concentration treated with HCl [ppm-N] (1)

Water-soluble peptide yield

$Water_{Soluble}$ Peptide Yield =$Water_{Soluble}$ Nitrogen Yield-Amino Acid Yield Ammonia Yield (2)

Amino acid yield

Amino acid yield = Amino acid concentration [ppm - N] / HCl Total amino acid concentration treated [ppm - N] (3)

Ammonia yield

Ammonia yield = Ammonia concentration [ppm - N] / HCl Total amino acid concentration treated [ppm - N] (4)

Total organic carbon (TOC)

TOC = Total Carbon (TC)-Inorganic Carbon (IC) (5)

Mineral amount

Mineral amount = K concentration + Ca concentration + Mg concentration (6)

Acetic acid fermentation of the subcritical water extract

Static fermentation process

Acetic acid bacteria provided by Maruboshi Vinegar Co., Ltd. (Fukuoka, Japan) were added to the subcritical water extract obtained in the previous section, and vinegar was produced by static fermentation, which is explained below.

The static fermentation method used in the present experiment is an ancient Japanese brewing method; although it takes longer time to produce vinegar compared to other methods, the resulting vinegar has more *umami*. In this process, the acetic acid bacteria on the surface of the raw material come in contact with air, and the heat generated from fermentation at that time creates a convection current that further enhances fermentation.

Static fermentation experiment

To bring the acidity to 1.5%, 90 mL of 15% vinegar was added to 800 mL of subcritical water extract. The alcohol concentration was then increased to 3.9% by adding 40 mL of 95% alcohol, and acetic acid bacteria were then added. The mixture was then allowed to ferment for 4 days at room temperature to produce vinegar. Static fermentation typically requires several months to produce vinegar; however, in experimental cases such as ours, the vinegar was produced relatively quickly, perhaps due to the small liquid volume. After fermentation was completed, the solution was heated to 85°C, cooled naturally to room temperature about 25°C, and then collected after filtration. Figure 6 shows the vinegar produced by static fermentation at Maruboshi Vinegar Co., Ltd. The white film on the surface is the film of acetic acid bacteria.

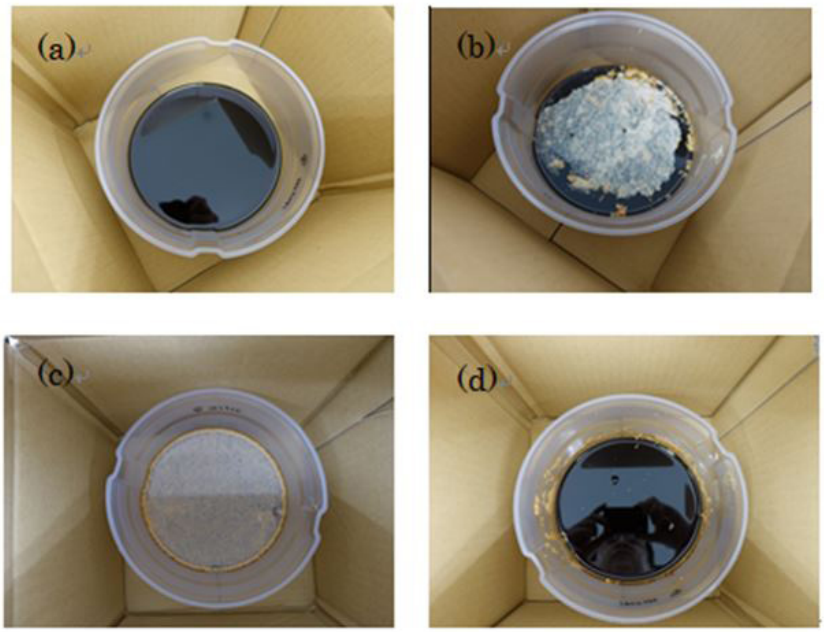


Figure 6: Vinegar and acetic acid bacteria during static fermentation
(a) Subcritical water extract before static fermentation, (b) Subcritical water extract with acetic acid bacteria, (c) During fermentation, (d) After fermentation was completed

The composition of the vinegar derived from the subcritical water extract was compared with regular grain vinegar and black vinegar products manufactured by Maruboshi Vinegar Co., Ltd.

Results and Discussion

Comparison of general composition

The general composition[1] of grain vinegar, black vinegar, and vinegar derived from subcritical water is shown in Table 4. The results showed that macronutrient contents of vinegar derived from subcritical water extract was generally the same as that of grain vinegar and black vinegar. Of particular interest was the higher total protein content of both sake lees A and B, compared to the total protein content of the existing vinegar products. The protein in the sake lees, which is not water-soluble in normal vinegar, must have become water-soluble when the molecular weight was reduced during the subcritical water treatment of the sake lees, and remained after the fermentation. In other words, compared to normal vinegar, a vinegar with high

nutrient content was produced, and that is believed to be a positive sign. The difference between sodium of grain vinegar and other vinegar values is due to addition of salt to adjust the taste.

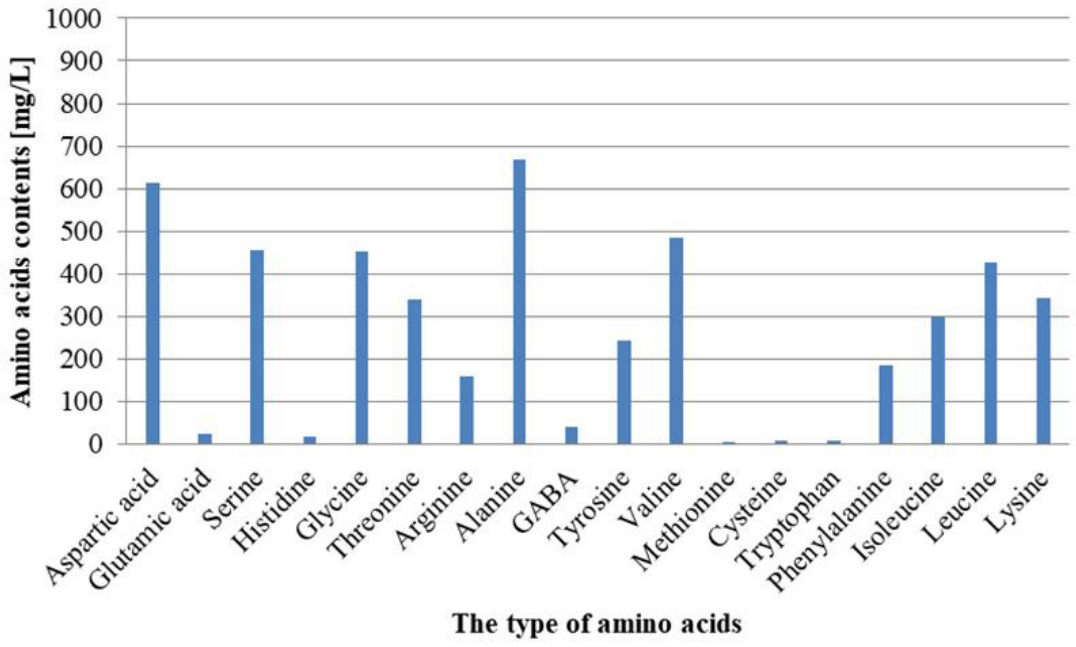
Comparison of amino acid content of different vinegars

Table 3 shows the amino acids that were quantitatively analyzed in this study. The changes in amino acid content of (a) sake lees A and (b) sake lees B are shown in Figure 7 and comparison of the amino acid amounts of grain vinegar, black vinegar and sake lees A, B are shown in Figure 8.

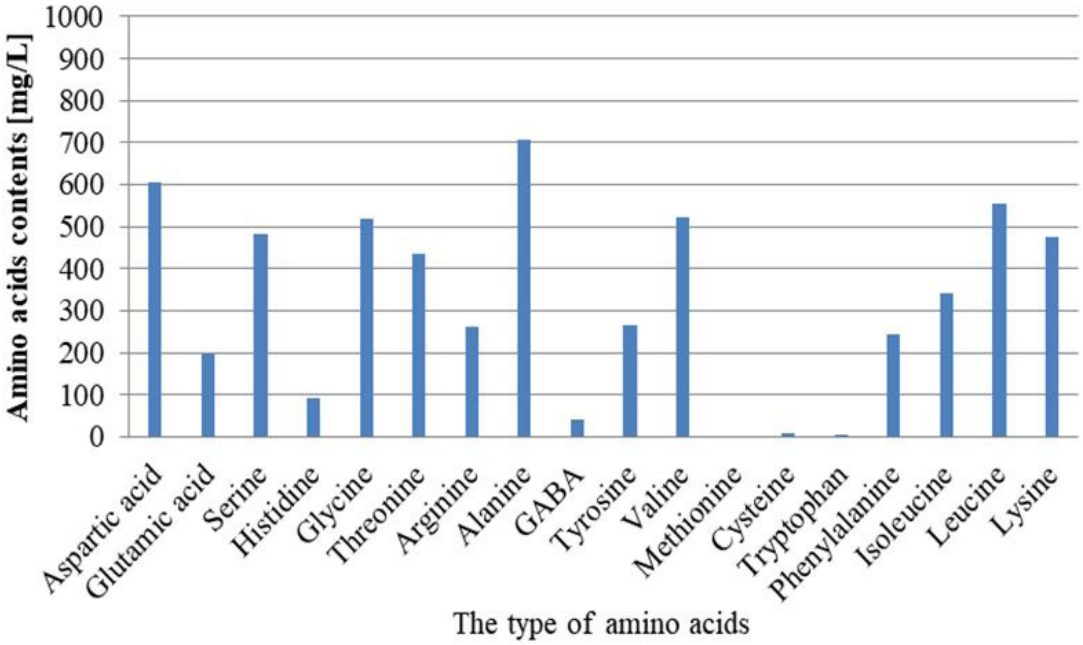
These figures show that free amino acids are present in both sake lees A and sake lees B, and that there is not much difference between these and existing vinegar products. Furthermore, the total amount of amino acids was higher in the vinegar derived from subcritical water extract compared to that of existing vinegar products. As described earlier, the subcritical water treatment of sake lees hydrolyzes many nitrogen-containing components such as proteins into amino acids. It was found out that these amino acids are not lost during the static fermentation of the extract, and that vinegar which retains a high nutrient content can be produced.

Table 4: Comparison of macronutrient content from different vinegars[2]

Parameter	Grain vinegar	Black vinegar	Sake lees A (This study)	Sake lees A (This study)
Energy (kcal)	21	18	26	25
Moisture (g)	94.7	96.4	92.4	92.6
Protein (g)	0.2	0.8	2.1	1.7
Lipid (g)	0.0	0.0	0.0	0.0
Carbohydrates (g)	6.2	4.8	5.4	5.6
Ash (g)	0.7	0.1	0.1	0.1
Sodium (mg)	282.0	3.0	2.0	2.0
Salt equivalent (g)	0.7	0.01	0.005	0.005
Acidity (%)	4.26	4.35	4.29	4.31



(a) Sake lees A



(b) Sake lees B

Figure 7: Amino acids contained in vinegar derived from sub-critical water of (a) sake lees A and (b) sake lees B

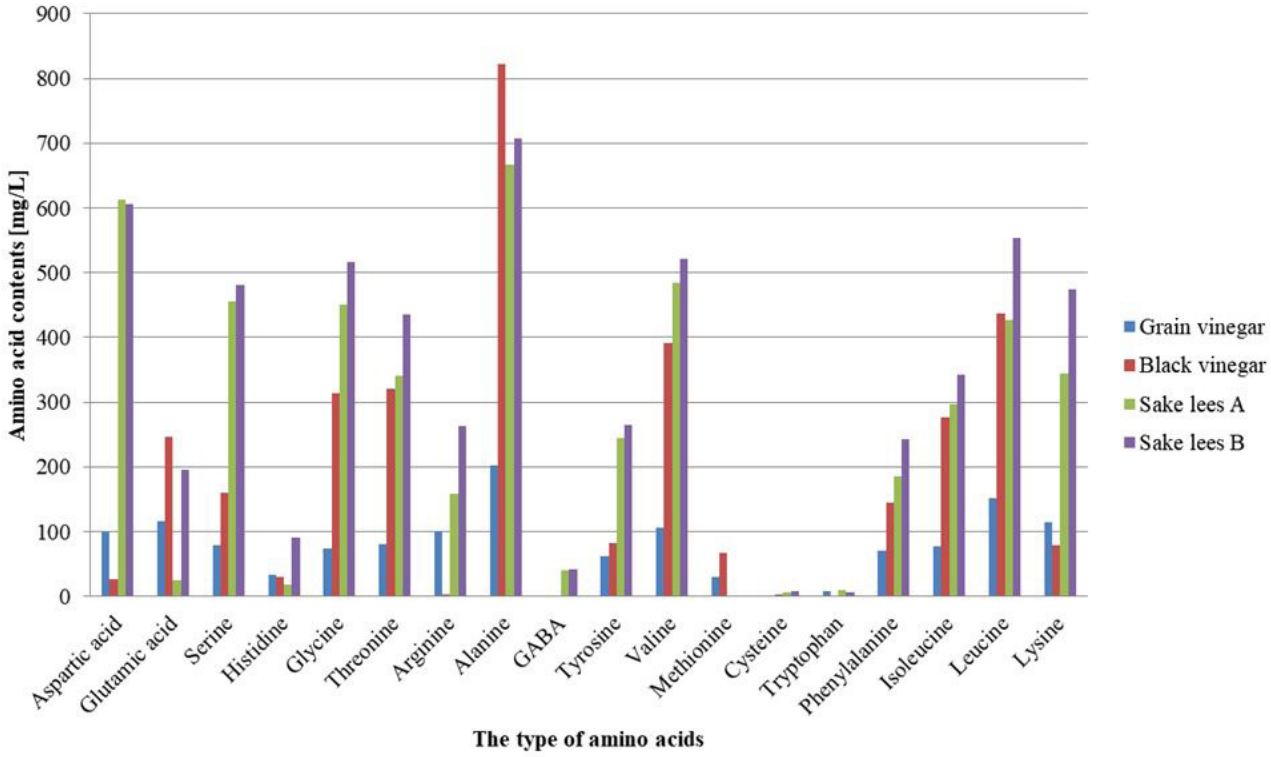


Figure 8: Comparison of the amino acid content from each vinegar

Next, Figure 9 shows a comparison of the total amino acid amount of each vinegar, and Figure 10 shows a comparison of the essential amino acid content in the total amino acid amount. The vinegar derived from sake lees contains a large amount of amino acids as shown before. Of particular note is that essential amino acids account for about 50% of the total amount of amino acids. As everybody knows, essential amino acids cannot be synthesized and produced in the body; it is obtained by ingesting a protein source in the daily diet, but if we try to get a protein source from food alone, the necessary amount will be excessive. Therefore, by using the vinegar obtained in this study as a seasoning, essential amino acids can be ingested efficiently on a daily basis. We will continue to look further into this research.

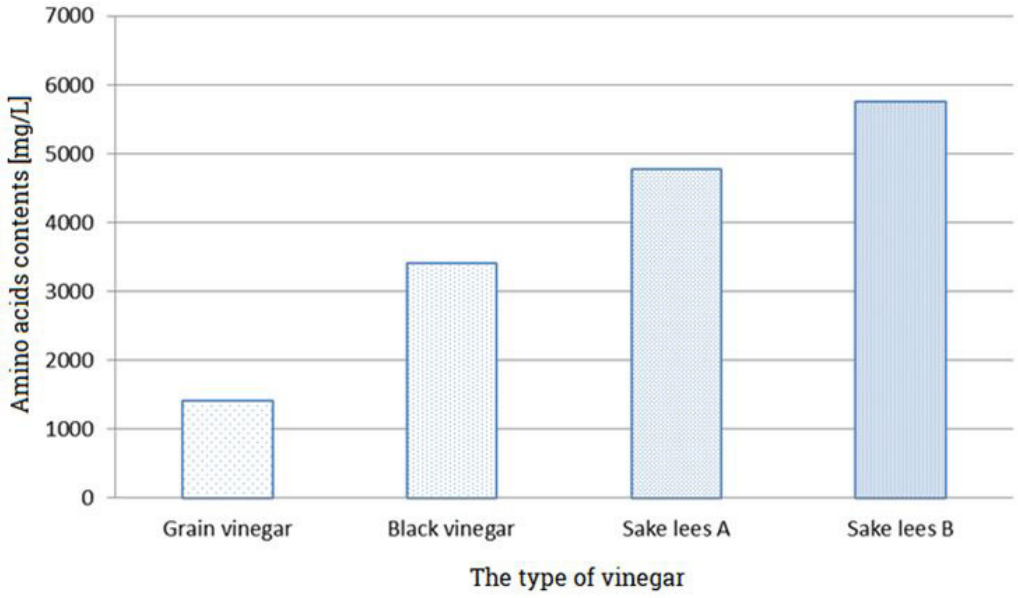


Figure 9: Total amino acid amount of each vinegar

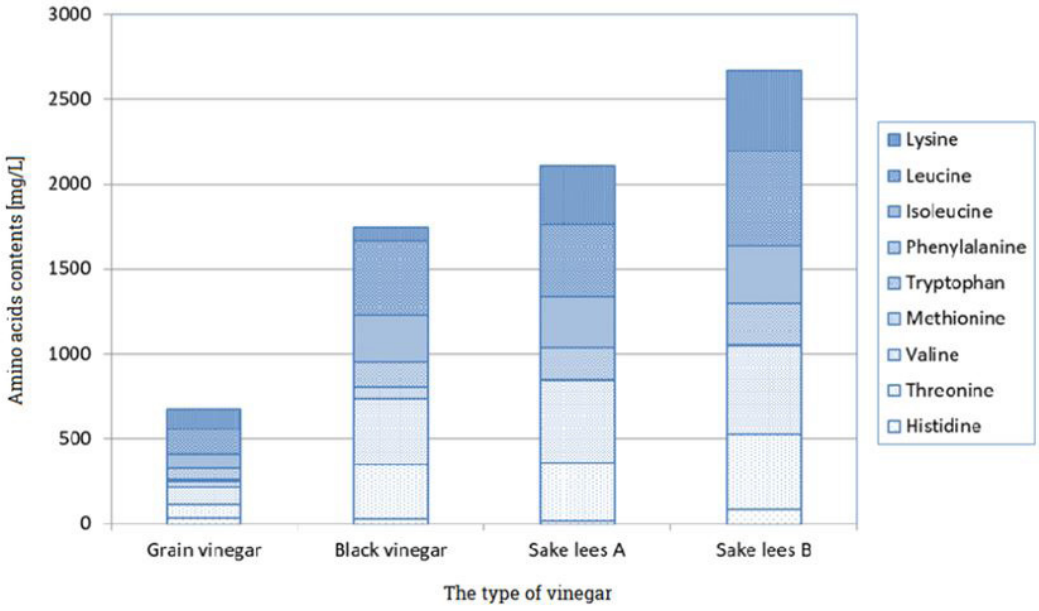


Figure 10: Essential amino acids of each vinegar

Comparison of mineral content

Figure 11 shows the mineral content of grain vinegar, black vinegar, sake lees A, and sake lees B.

This Figure shows that the vinegar derived from the subcritical water extract had almost double the amount of total nitrogen (TN) composition compared to existing vinegars. As in the case of the results described in section 4.2, this is most likely due to the large amount of amino acids and peptides that were not broken down into amino acids during the treatment of extracts from sake lees A and sake lees B. Thus, as a food-grade vinegar, vinegar produced from sake lees extracts could potentially be used as a beneficial food product that contains more amino acids and minerals than existing vinegars.

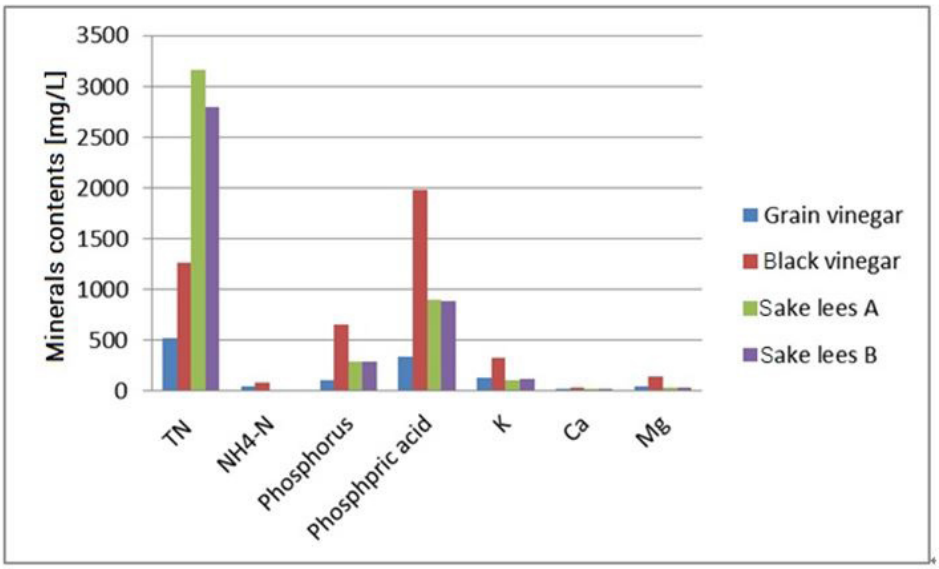


Figure 11: Comparison of the mineral content from each vinegar

Conclusion

In the present study, we aimed to develop a method for optimally utilizing brewing residues generated as by-products during food processing; sake lees, which are a vinegar residue, were subjected to subcritical water treatment, and vinegar was then produced by static fermentation. Quantitative analysis of the main components and its potential application as food-grade vinegar were assessed.

In terms of general composition, vinegars derived from subcritical water treatment of sake lees A and sake lees B had higher protein contents compared to existing vinegars; as for amino acids, the same or higher amounts were observed. Furthermore, the amount of TN in the critical water extract was almost twice as that of existing vinegars. In addition, the Sodium content of each vinegar was different. For product grain vinegar, the value was higher due to the addition of salt to adjust the taste, in the case of other vinegars, in which this process is not carried out, the value was small. However, note that the high sodium content of grain vinegar is not harmful to the human body.

In addition, the amount of essential amino acids in the vinegar derived from sake lees is about 50% in total amount of amino acids; we consider this to be a great finding since it will be easier to ingest essential amino acids that need to be consciously obtained from diets.

The findings of the present study suggested that the subcritical water extract of sake lees may be used not only to produce food-grade vinegar but also as an additive of *umami* components to brewed condiments such as soy sauce, and a nutritional supplement during fermentation. Moreover, besides its application in the food industry, we considered that it can be used as a liquid fertilizer for plants.

In the future, we intend to conduct further research on applications at laboratory and production scales.

Note

- The following methods were used to analyze the composition of sake lees

Moisture content: Atmospheric heating drying method

Total nitrogen: Kjeldahl method

Water-soluble amino acid content: Analyzed by HPLC

Total organic carbon: TOC measuring apparatus (TOC-VCSN manufactured by SHIMADZU)

Ammonic nitrogen content: the Indophenol method

Mineral content: Atomic absorption spectrophotometer

- The following methods were used to analyze the macro-nutrient content of the vinegars.

Energy: Modified Atwater method (calculated value)

Moisture: Heat-drying method under normal pressure

Protein: Kjeldahl method

Lipid: Liquid–liquid extraction method

Carbohydrate: Carbohydrate by difference method (calculated value)

Ash: Direct ash method

Sodium: Atomic absorption spectrophotometry

Salt equivalent: Sodium conversion (calculated value)

Acidity: Titration method

Conflict of interest

The authors declare that they have no conflict of interest.

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