CÓDIGO:

UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO

SELEÇÃO DE MESTRADO ACADÊMICO EM NUTRIÇÃO – 2020

LINHA DE QUALIDADE DE ALIMENTOS

CAPA DA PROVA ESCRITA

	INSTRUÇÕES
1	O mascaramento da prova escrita será realizado por meio do sorteio de um CÓDIGO. Posteriormente, o candidato deverá preencher o formulário que contém o CÓDIGO com o nome completo e assinatura, e deposita-lo em um envelope que será lacrado e assinado pelos Fiscais.
2	Preencher os campos com o CÓDIGO nos seguintes locais: **capa da Prova Escrita e páginas destinadas para responder as questões dissertativas.** A falta deste procedimento impedirá a correção da prova, culminando com a eliminação do candidato. A identificação do candidato em qualquer outro local da prova resultará na eliminação do Processo Seletivo
3	Essa Prova Escrita contém 02 (duas) questões dissertativas.
4	Quando o Fiscal autorizar, verifique se a prova está completa e sem imperfeições gráficas que impeçam a leitura. Detectado algum problema, comunique-o imediatamente ao Fiscal. Não destaque nenhuma folha da prova.
5	As questões dissertativas serão avaliadas considerando apenas o que estiver escrito nas páginas pautadas, utilizando caneta esferográfica azul ou preta. Se necessário, solicite folhas adicionais ao fiscal. Escreva de modo legível.
6	Os rascunhos não serão considerados para efeito de correção.
7	Os candidatos dispõem de, no máximo, **4 (quatro) horas** para responder toda prova escrita.
8	Antes de retirar-se definitivamente da sala, devolva ao Fiscal a prova, juntamente com os rascunhos destinados para responder as questões dissertativas.

QUESTÕES DISSERTATIVAS

Leia atentamente as sessões de “Materiais e Métodos” e “Resultados” do artigo intitulado “*Soybean residue (okara) fermentation with the yeast Kluyveromyces marxianus”* publicado, no periódico *Food Bioscience (2019)*, de autoria de Yang Hu, et al. (Food Bioscience 31 (2019) 100439. doi: 10.1016/j.fbio.2019.100439).

Com base nessas informações, responda as questões dissertativas que se seguem:

(1) Interprete e descreva os resultados apresentados nas Tabelas 1, 3 e 4 e na Figura 1.

(2) Elabore um resumo estruturado sobre o artigo, contendo os seguintes itens: Introdução, Objetivos, Métodos, Resultados e Conclusão.

Food Bioscience 31 (2019) 100439

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Soybean residue (okara) fermentation with the yeast *Kluyveromyces marxianus*

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2. Materials and methods

2.1. Materials and reagents

K. marxianus was isolated from Kefir grains (Su et al., 2018) that had been preserved by the Chinese Microbe Preservation Management Committee (CGMCC, No. 13907, Beijing, China). The soybeans (Heihe 43, grown in Heilongjiang, China) were supplied and vouched by the Shandong Shengfeng Seeds Co., Ltd. (Jining, Shandong, China) was used in this study. Petroleum ether (boiling range: 30–60 °C), phenol, concentrated sulfuric acid, mercuric iodide, glucose, sodium hydroxide, redistilled phenol, sodium hydrogen sulfite, and sodium tartrate were of analytical grade and were purchased from Beijing Chemical Works (Beijing, China). 2-Methyl-3-heptanone, benzaldehyde, and 1-octen-3-one were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). *n*-Hexanal and hexanol were purchased from Aladdin (Shanghai, China). 2-Pentylfuran, *trans*-2-nonenal, *trans*-2,4-decadienal, 1-octen-3-ol and *trans*-2,4-nonadienal were purchased from Fluka (Shanghai, China). Trypsin and phytic acid were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Beef extract, peptone and yeast extract were purchased from Beijing Aoboxing Bio-tech Co., Ltd. (Beijing, China). Tween 80 was purchased from Fuchen (Tianjin) Chemical Reagent Co., Ltd. (Tianjin, China). *N*-Benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA) and Tris base were obtained from the Beijing Huamaike Biotechnology Co., Ltd. (Beijing, China).

2.2. Solid-state fermentation of soybean residue (okara)

Raw soybeans preserved at room temperature (18–25 °C) were soaked in tap water at a ratio of 1:5 (w/v) for 12 h. The soybeans were ground into particles with 9 times (w/v) the amount of water (80 °C) at a low speed for 3 min using a soymilk machine (JYL-Y20, Joyang Co., Ltd., Jining, Shandong, China) and filtered through a 60-mesh sieve to obtain the soybean residue. The soybean residue (okara) was then sterilized at 121 °C for 20 min in a vertical pressure steam sterilization pot (YXQ-S-50A, Shanghai Boxun Enterprise Co., Ltd., Shanghai, China).

K. marxianus was inoculated into basal medium (0.8% beef extract, 1% peptone, 0.4% yeast extract, 2% glucose, 0.2% potassium hydrogen phosphate, 0.2% diammonium hydrogen citrate, 0.5% sodium acetate, 0.02% magnesium sulfate, 0.04% manganese sulfate and 1% Tween 80, all w/v, pH 5.7 ± 0.2). The cultures were incubated for 15 h at 30 °C and 120 rpm on an incubator shaker. The sterilized soybean residue was inoculated with *K. marxianus* inoculum solution at a ratio of 5% (v/w), which was ~10^7 cells/mL determined using serial dilution. Subsequently, the soybean residue was fermented at 28 °C for 44 h. After fermentation, samples were sterilized and freeze-dried (FDU-7006, Operon Co., Gimpo, Korea) to obtain a sample of *K. marxianus* fermented soybean residue (FSR). The same procedure without inoculation was followed for the control group of unfermented soybean residue (USR). All samples were stored at − 20 °C before use and storage not more than 6 months.

2.3. Determination of nutritional components

The ash content was determined using AOAC (2000) method number 942.05. The crude fat content was measured using AOAC (2006) method number 2003.05. The crude protein content was estimated using the Kjeldahl method according to AOAC (2000) method number 920.87, and the crude protein was estimated using 6.25 as the conversion factor. The insoluble and soluble dietary fiber contents were determined using AOAC (2000) method number 994.13.

The polysaccharide content was determined using a phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Sample or standard glucose (2 mL) was mixed with 1 mL of 6% phenol and 5 mL of concentrated sulfuric acid, and the absorbance was measured at

490 nm. (Infinite M200, Tecan (Shanghai) Trading Co., Ltd., Shanghai, China). The glucose standards were measured from 2 to 9 mg/mL to obtain the standard curve. The amino acid analyzer (A300, MembraPure GmbH, Berlin, Germany) was used to determine the content of amino acids according to AOAC (2000) method number 994.12. The soybean residue was hydrolyzed with 6 M HCl in nitrogen gas at 110 °C for 22 h and diluted with a sodium citrate buffer (pH 2.2). After pre-column derivatization with *o*-phthalaldehyde and fluorenylmethyl chloroformate, the amino acids were analysed, and the proportion of each amino acid in the total amino acid was calculated to find changes before and after fermentation. The results were expressed as the percentage of each amino acid in the total amino acids with the exception of tryptophan. This method only reflects the peaks measured and leads to results being close to 100% regardless of actual recovery.

2.4. Determination of anti-nutritional factors

2.4.1. Determination of trypsin inhibitor activity

The method of Li et al. (2017) was used. Two mL of soybean residue suspension (10 mg/mL) was mixed with 5 mL 6 mg/mL BAPNA (in 0.05 M Tris – HCl buffer, pH 8.2, 0.02 M $CaCl_2$) and 1 mL of 0.2 mg/mL trypsin solution (in 0.04 mM HCl) at 37 °C for 10 min as the reaction mixture using 1 mL of 30% (v/v) acetic acid solution to terminate the reaction and the mixture was measured at 410 nm. One unit of activity of trypsin was defined as an increase in absorbance of 0.01 at 37 °C. A trypsin inhibitor unit (TIU) has been defined in terms of trypsin units inhibited/g of sample.

2.4.2. Determination of phytic acid contents

Phytic acid was measured using a method that was previously described with slight modification (Liang, Han, Nout, & Hamer, 2008). Phytic acid standards were measured from 24 to 64 μg/mL to obtain the standard curve. Sample or standard (3 mL) was mixed with 1 mL of Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$, 0.3% 5-sulfosalicylic acid dihydrate), and the absorbance was measured at 500 nm using distilled water as a control.

2.5. Measurement of beany odor

The beany odor was determined using the method described by Yuan and Chang (2007a). Briefly, a 0.5 g sample was equilibrated at 37 °C for 10 min and then was extracted using an automatic headspace sampler (Turbo Matris HS 16, PerkinElmer, Wellesley, MA, USA) for 30 min at 60 °C. Then the samples were injected into the gas chromatograph (Clarus-580, PerkinElmer) with a polar resin of DB-Wax (Carbowax, 30 m × 0.25 mm, i.d. × 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA). The following conditions were used for gas chromatography: the initial oven temperature was 35 °C (2 min hold) and then was raised at 10 °C/min to 225 °C (5 min hold); flame ionization detector at 250 °C; the flow rate of carrier nitrogen gas, 1 mL/min; and inlet temperature, 150 °C. Quantification of the peak areas of *n*-hexanal, hexanol, benzaldehyde, 1-octen-3-ol, 1-octen-3-one, 2-pentylfuran, *trans*-2-nonenal, *trans*-2,4-decadienal, *trans*-2,4-nonadienal was done using 2-methyl-3-heptanone as the internal standards and assuming that the instrumental response was the same for all compounds (Gao, Zhang, Regenstein, Yin, & Zhou, 2018). The results were summed and expressed as mg beany odor compounds/g soybean residue (dry matter).

2.6. Changes in processing properties

2.6.1. Water absorption index (WAI) and water soluble index (WSI)

These procedures were done according to Du, Jiang, Yu, and Jane (2014). The WAI and WSI of soybean residue after fermentation were determined at several pH values (4, 5, 7, 9). Briefly, a 2.5 g sample of soybean residue was dispersed in 30 mL of deionized water (GWA-UN 4-C20, Beijing Persee General Instruments Co., Ltd., Beijing, China) and mixed evenly using a mixer (Ika Lab Dancer, Leibei (Shanghai) Scientific Instruments Co., Ltd., Shanghai, China), which was then heated in a water bath at 70 °C for 30 min. After being cooled to room temperature, the mixture was transferred to centrifuge tubes (50 mL) and was then centrifuged at 3,000 g for 20 min (3K15, Sigma, Berlin, Germany). The supernatant was decanted into a pre-weighed evaporating dish and dried overnight at 105 °C where the solid contents were then measured, while the remaining sediments in the centrifuge tube were weighed. WAI and WSI were calculated as:

$$WAI\ (water\ absorption\ index,\ g/g) = \frac{Weight\ of\ sediment}{Dry\ weight\ of\ sample}$$

$$WSI\ (water\ soluble\ index,\ \%) = \frac{Dry\ weight\ of\ dissolved\ solids\ in\ supernatant \times 100}{Dry\ weight\ of\ sample}$$

2.6.2. Emulsification activity index (EAI) and emulsification stability index (ESI)

Using the method of Kang et al. (2017), soybean residue samples of 0.5 g were dispersed in 25 mL of distilled water. After mixing, the pH of each mixture was adjusted to 4, 5, 7, and 9 using 1 M HCl or 1 M NaOH, at room temperature, shaken for 1 h, and subsequently centrifuged at 10,000 *g* for 10 min at 20 °C, where the supernatant was then collected for analysis of emulsification. Oil (5 mL) and 15 mL of supernatant were mixed at room temperature and then were emulsified using a high-speed homogenizer (FA25, Fluko, Shanghai, China) at 13,000 rpm for 2 min and then were poured into a beaker. Samples (25 μL) were taken at 0 and 30 min and mixed with 5 mL SDS solution, and the absorbance was measured at 500 nm, with 0.1% SDS used as a blank control. EAI and ESI were calculated as:

$$EAI\ (Emulsification\ activity\ index,\ m^2/g) = \frac{2\ T \times A_0 \times N}{(C \times Q \times 10000)}$$

$$ESI\ (Emulsification\ stability\ index,\ min) = \frac{A_0 \times 30}{(A_0 - A_{30})}$$

where, T = 2.303, N is the multiple dilutions (250), C is the protein concentration (g/mL) in an aqueous solution of protein before emulsion formation, Q is the volume fraction of the oil phase in the emulsion (assumed to be 0.25), A_0 is the absorbance of the diluted emulsion after homogenization, and A_{30} is the absorbance of the emulsion after remaining stationary for 30 min.

2.7. Determination of moisture states and distribution

A low-field NMR method (Li et al., 2014) was used to measure the moisture states and distribution. Samples were put into a 1.5 cm diameter tube and were then put into the RF coil center of the permanent magnetic field. The resonance frequency was 23.137 MHz, the magnet strength was 0.55 T, the cumulative scan was 8 times, the echo time was 105 ms, and the echo count was 8000 (MRI analyzer NMI20, Suzhou Niumai Analysis Instrument Co., Ltd., Suzhou, Jiangsu, China). Using PQ001 analysis software, which came with the instrument, and CPMG (Carr – Purcell – Meiboom – Gill)series samples (Carr & Purcell, 1954; Meiboom & Gill, 1958) with a spin-spin relaxation time T_2 signal and a T_2 attenuation curve was done using a multi-exponential fitting analysis with Niumai's Multi Exp Inv Analysis software program, T_2 relaxation information was obtained. Relaxation time was observed as T_{21}, T_{22}, and T_{23}, which represented the combined water, immobile water, and free water, respectively.

2.8. Microstructure determination

The USR and FSR samples were coated with gold (JS-1600M, Beijing HTCY Technology Co., Ltd., Beijing, China) and photographed using a SEM (TM 3030 Plus, Tianmei (China) Scientific Instrument Co., Ltd., Shanghai, China). The determination conditions were accelerating voltage of 15 kV, electron beam of 4.0 times, and the working distance of 17 mm, and the electronic probe was a secondary detector of electrons (Xia, Wang, Liu, Li, & Zhou, 2016). The samples were observed with a magnification of 1,000 and 5,000 times.

2.9. Statistical analysis

All experiments were done in triplicate and data are expressed as mean values ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) and the means were compared using the Fisher's least significant difference (LSD) test using the IBM Statistical Package for the Social Sciences (SPSS, IBM Inc., Chicago, IL, USA) software, version 19.0 and $p < 0.05$ was considered to be statistically significant.

3. Results

Table 1
Proximate composition of soybean residue (okara) before and after fermentation with *K. marxianus* (g/100 g dry matter).

	USR	FSR
Protein	26.0 ± 0.2[a]	24.7 ± 0.0[a]
Crude fat	10.0 ± 0.3[a]	12.4 ± 0.4[b]
Insoluble dietary fiber	53.8 ± 0.3[a]	50 ± 2[a]
Soluble dietary fiber	2.9 ± 0.1[a]	7.5 ± 0.2[b]
Polysaccharide	0.87 ± 0.03[a]	2.9 ± 0.1[b]
Ash	3.5 ± 0.4[a]	3.5 ± 0.5[a]

[a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

Table 2
The proportion of each amino acid in total amino acids before and after fermented soybean residue (okara) with *K. marxianus*.

Amino acid	USR (%)	FSR (%)
Cystine	1.81	6.78
Aspartic acid/Asparagine	11.1	8.78
[a]Threonine	3.83	5.75
Serine	3.43	4.63
Glutamic acid/Glutamine	24.2	21.5
Glycine	6.25	8.78
Alanine	8.07	4.79
[a]Valine	4.03	5.59
[a]Methionine	0.50	0.00
[a]Isoleucine	3.12	7.58
[a]Leucine	14.0	10.0
Proline	nd	nd
[a]Phenylalanine	1.71	1.19
[a]Lysine	6.76	5.67
Histidine	3.03	1.59
Arginine	8.07	7.18
Tryptophan	nd	nd
Total essential amino acids	34.0	35.8
Total amino acids	100	138

[a] Essential amino acids; nd: not detected (n = 1).

Table 3
Activities of anti-nutritional factors in soybean residue (okara) after *K. marxianus* fermentation (dry matter).

Anti-nutritional factors	USR	FSR
Trypsin inhibitor activity (TIU/g)	1710 ± 2[b]	124 ± 2[a]
Phytic acid (mg/g)	16.8 ± 0.1[b]	6.4 ± 0.1[a]

[a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

Table 4
Changes observed in selected beany odors (μg/g dry matter) in fermented soybean residue (okara) with *K. marxianus*.

Compounds	USR	FSR
n-Hexanal	3.5 ± 0.1[c]	1.7 ± 0.03[a]
Hexanol	3.7 ± 0.2[b]	0.8 ± 0.1[a]
Benzaldehyde	0.09 ± 0.01[a]	0.06 ± 0.04[a]
1-Octen-3-ol	0.1 ± 0.01[a]	0.0 ± 0.0[a]
1-Octen-3-one	0.11 ± 0.01[a]	0.13 ± 0.00[a]
2-Pentylfuran	0.17 ± 0.01[a]	0.18 ± 0.01[a]
trans-2-Nonenal	0.19 ± 0.02[a]	0.19 ± 0.03[a]
trans-2,4-Decadienal	0.68 ± 0.01[a]	0.72 ± 0.11[a]
trans-2,4-Nonadienal	0.6 ± 0.1[a]	0.7 ± 0.1[a]
Total beany odors	9.2 ± 0.2[b]	4.4 ± 0.1[a]

[a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

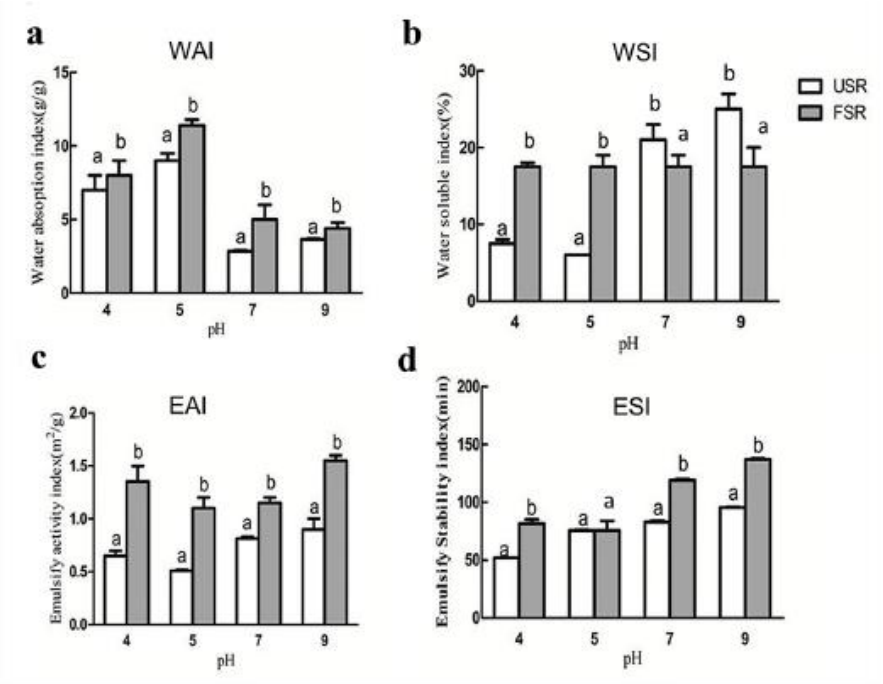

Fig. 1. Physicochemical properties of soybean residue (okara) fermented with *K. marxianus*. [a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

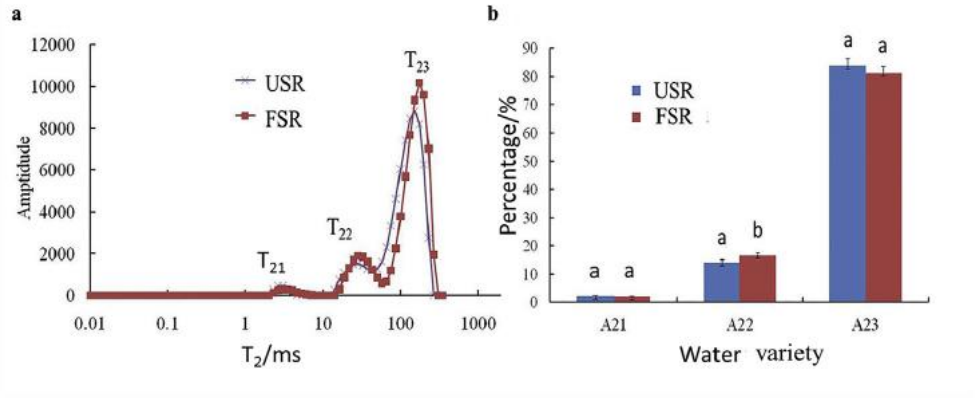

Fig. 2. LF-NMR T_2 relaxation curves of soybean residue (okara) before and after *K. marxianus* fermentation.

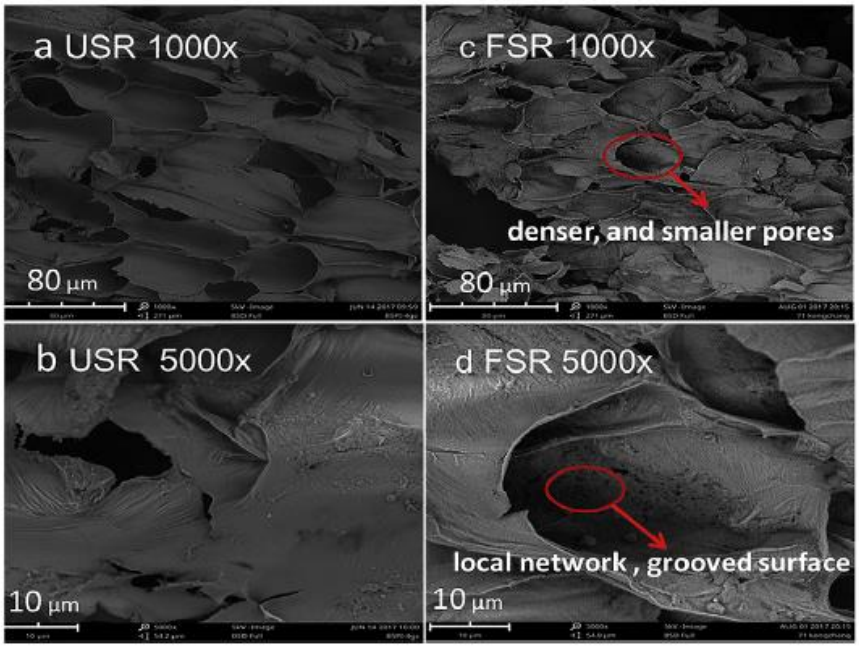

Fig. 3. SEM particle structure of soybean residue (okara) before and after *K. marxianus* fermentation.

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Utilize o espaço pautado a seguir para responder a questão 2. Não será considerada para correção nenhuma letra ou palavra escrita fora deste espaço.

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Food Bioscience 31 (2019) 100439

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ARTICLE INFO

Keywords:
Okara
Kluyveromyces marxianus
Beany odor
Fermentation

ABSTRACT

The soybean residue (okara) with high moisture content has generally been discarded, which has led to economic loss and socio-environmental problems. The effects of solid state fermentation using *Kluyveromyces marxianus* on the nutritional components, beany odor and processing properties of soybean residue were studied. The results showed that *K. marxianus* fermented soybean residue (FSR) had an increase in crude fat (24.5%), soluble dietary fibers (158%) and polysaccharides (262%), while phytic acid (61.7%) and the activity of a trypsin inhibitor (92.7%) decreased. A significant reduction in the beany odor was also observed. FSR showed higher water absorption and better emulsifying properties. Overall, the study indicated that fermentation with *K. marxianus* resulted in improved nutritional values and processing characteristics of the soybean residue.

1. Introduction

Soybean residue (okara) is a byproduct that mainly results from the manufacture of tofu, soymilk and other soy products. Large quantities of soybean residue are produced, especially in Asian countries. China is the leading producer and the largest consumer of soybean and produces approximately 70 million tonnes of soybean residue every year (Li, Qiao, & Lu, 2012a). Due to extra production costs, soybean residue is used as animal feed or discarded as waste, where it then creates an environmental burden. Soybean residue contains many nutritional components, such as 20–30% protein and 40–60% carbohydrates (Li, Lu, Nan, & Liu, 2012b). Therefore, it may be beneficial to convert soybean residue, a food waste, into value-added products.

Efforts have been made to better use soybean residue and this has led to the development of functional healthy food products such as dietary fiber and polysaccharides (Li et al., 2012b; Lu, Liu, & Li, 2013). The key to extending the use of soybean residue is to improve its poor textural quality and poor processing properties. Treatment methods, such as physical, chemical or biological methods, are available to improve the nutritional status of soybean residue (Chen, Ye, Yin, & Zhang, 2014; Huang, He, Zou, & Liu, 2015). Processes involving fermentation are one of the most feasible methods to affect nutritional status. Yao, Pan, Wang, and Xu (2010) showed that nutrients and microstructure changed markedly in soybean residue that were fermented with *Mucor racemosus* Fresenius. Zhu et al. (2010) showed that the antioxidant capacity of soybean residue was significantly improved using solid-state fermentation using *Bacillus subtilis* B2. Studies have shown that yeasts are better able to change soybean residue due to their strong metabolic activity and diversity. The soybean residue fermented with the dairy yeast *Yarrowia lipolytica* had a cheese-like odor and improved digestibility with increased antioxidant activity (Vong, Au, & Liu, 2016). The fermentation using *Saccharomyces cerevisiae* contributed to an increase in total phenolics and antioxidant activity of soybean residue (Vidiany et al., 2018).

Many studies have shown that *Kluyveromyces marxianus* can alter and improve the aroma profile of bread (Plessas et al., 2008), sugar beet molasses (Martínez, Sánchez, Font, & Barrena, 2017), and be used in waste disposal (Guneser, Karagul-Yuceer, Wilkowska, & Kregiel, 2016), which suggests that *K. marxianus* has the potential for improving odor. In this study, major nutrients, beany odor and processing properties of soybean residue fermented using *K. marxianus* were investigated.

Abbreviations: EAI, emulsification activity index; ESI, emulsification stability index; FSR, fermented soybean residue; LF-NMR, low field nuclear magnetic resonance; SEM, scanning electron microscopy; TIU, trypsin inhibition unit; USR, unfermented soybean residue; WAI, water absorption index; WSI, water soluble index

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https://doi.org/10.1016/j.fbio.2019.100439
Received 12 September 2018; Received in revised form 19 July 2019; Accepted 20 July 2019
Available online 22 July 2019

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2.2. Solid-state fermentation of soybean residue (okara)

Raw soybeans preserved at room temperature (18–25 °C) were soaked in tap water at a ratio of 1:5 (w/v) for 12 h. The soybeans were ground into particles with 9 times (w/v) the amount of water (80 °C) at a low speed for 3 min using a soymilk machine (JYL-Y20, Joyang Co., Ltd., Jining, Shandong, China) and filtered through a 60-mesh sieve to obtain the soybean residue. The soybean residue (okara) was then sterilized at 121 °C for 20 min in a vertical pressure steam sterilization pot (YXQ-S-50A, Shanghai Boxun Enterprise Co., Ltd., Shanghai, China).

K. marxianus was inoculated into basal medium (0.8% beef extract, 1% peptone, 0.4% yeast extract, 2% glucose, 0.2% potassium hydrogen phosphate, 0.2% diammonium hydrogen citrate, 0.5% sodium acetate, 0.02% magnesium sulfate, 0.04% manganese sulfate and 1% Tween 80, all w/v, pH 5.7 ± 0.2). The cultures were incubated for 15 h at 30 °C and 120 rpm on an incubator shaker. The sterilized soybean residue was inoculated with *K. marxianus* inoculum solution at a ratio of 5% (v/w), which was $\sim 10^7$ cells/mL determined using serial dilution. Subsequently, the soybean residue was fermented at 28 °C for 44 h. After fermentation, samples were sterilized and freeze-dried (FDU-7006, Operon Co., Gimpo, Korea) to obtain a sample of *K. marxianus* fermented soybean residue (FSR). The same procedure without inoculation was followed for the control group of unfermented soybean residue (USR). All samples were stored at −20 °C before use and storage not more than 6 months.

2.3. Determination of nutritional components

The ash content was determined using AOAC (2000) method number 942.05. The crude fat content was measured using AOAC (2006) method number 2003.05. The crude protein content was estimated using the Kjeldahl method according to AOAC (2000) method number 920.87, and the crude protein was estimated using 6.25 as the conversion factor. The insoluble and soluble dietary fiber contents were determined using AOAC (2000) method number 994.13.

The polysaccharide content was determined using a phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Sample or standard glucose (2 mL) was mixed with 1 mL of 6% phenol and 5 mL of concentrated sulfuric acid, and the absorbance was measured at 490 nm. (Infinite M200, Tecan (Shanghai) Trading Co., Ltd., Shanghai, China). The glucose standards were measured from 2 to 9 mg/mL to obtain the standard curve. The amino acid analyzer (A300, MembraPure GmbH, Berlin, Germany) was used to determine the content of amino acids according to AOAC (2000) method number 994.12. The soybean residue was hydrolyzed with 6 M HCl in nitrogen gas at 110 °C for 22 h and diluted with a sodium citrate buffer (pH 2.2). After pre-column derivatization with *o*-phthalaldehyde and fluorenylmethyl chloroformate, the amino acids were analysed, and the proportion of each amino acid in the total amino acid was calculated to find changes before and after fermentation. The results were expressed as the percentage of each amino acid in the total amino acids with the exception of tryptophan. This method only reflects the peaks measured and leads to results being close to 100% regardless of actual recovery.

2.4. Determination of anti-nutritional factors

2.4.1. Determination of trypsin inhibitor activity

The method of Li et al. (2017) was used. Two mL of soybean residue suspension (10 mg/mL) was mixed with 5 mL 6 mg/mL BAPNA (in 0.05 M Tris – HCl buffer, pH 8.2, 0.02 M $CaCl_2$) and 1 mL of 0.2 mg/mL trypsin solution (in 0.04 mM HCl) at 37 °C for 10 min as the reaction mixture using 1 mL of 30% (v/v) acetic acid solution to terminate the reaction and the mixture was measured at 410 nm. One unit of activity of trypsin was defined as an increase in absorbance of 0.01 at 37 °C. A trypsin inhibitor unit (TIU) has been defined in terms of trypsin units inhibited/g of sample.

2.4.2. Determination of phytic acid contents

Phytic acid was measured using a method that was previously described with slight modification (Liang, Han, Nout, & Hamer, 2008). Phytic acid standards were measured from 24 to 64 μg/mL to obtain the standard curve. Sample or standard (3 mL) was mixed with 1 mL of Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$, 0.3% 5-sulfosalicylic acid dihydrate), and the absorbance was measured at 500 nm using distilled water as a control.

2.5. Measurement of beany odor

The beany odor was determined using the method described by Yuan and Chang (2007a). Briefly, a 0.5 g sample was equilibrated at 37 °C for 10 min and then was extracted using an automatic headspace sampler (Turbo Matris HS 16, PerkinElmer, Wellesley, MA, USA) for 30 min at 60 °C. Then the samples were injected into the gas chromatograph (Clarus-580, PerkinElmer) with a polar resin of DB-Wax (Carbowax, 30 m × 0.25 mm, i.d. × 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA). The following conditions were used for gas chromatography: the initial oven temperature was 35 °C (2 min hold) and then was raised at 10 °C/min to 225 °C (5 min hold); flame ionization detector at 250 °C; the flow rate of carrier nitrogen gas, 1 mL/min; and inlet temperature, 150 °C. Quantification of the peak areas of *n*-hexanal, hexanol, benzaldehyde, 1-octen-3-ol, 1-octen-3-one, 2-pentylfuran, *trans*-2-nonenal, *trans*-2,4-decadienal, *trans*-2,4-nonadienal was done using 2-methyl-3-heptanone as the internal standards and assuming that the instrumental response was the same for all compounds (Gao, Zhang, Regenstein, Yin, & Zhou, 2018). The results were summed and expressed as mg beany odor compounds/g soybean residue (dry matter).

2.6. Changes in processing properties

2.6.1. Water absorption index (WAI) and water soluble index (WSI)

These procedures were done according to Du, Jiang, Yu, and Jane (2014). The WAI and WSI of soybean residue after fermentation were determined at several pH values (4, 5, 7, 9). Briefly, a 2.5 g sample of soybean residue was dispersed in 30 mL of deionized water (GWA-UN

4-C20, Beijing Persee General Instruments Co., Ltd., Beijing, China) and mixed evenly using a mixer (Ika Lab Dancer, Leibei (Shanghai) Scientific Instruments Co., Ltd., Shanghai, China), which was then heated in a water bath at 70 °C for 30 min. After being cooled to room temperature, the mixture was transferred to centrifuge tubes (50 mL) and was then centrifuged at 3,000 g for 20 min (3K15, Sigma, Berlin, Germany). The supernatant was decanted into a pre-weighed evaporating dish and dried overnight at 105 °C where the solid contents were then measured, while the remaining sediments in the centrifuge tube were weighed. WAI and WSI were calculated as:

$$WAI\ (water\ absorption\ index,\ \ g/g) = \frac{Weight\ of\ sediment}{Dry\ weight\ of\ sample}$$

$$WSI\ (water\ soluble\ index,\ \ \%) = \frac{Dry\ weight\ of\ dissolved\ solids\ in\ supernatant \times 100}{Dry\ weight\ of\ sample}$$

2.6.2. Emulsification activity index (EAI) and emulsification stability index (ESI)

Using the method of Kang et al. (2017), soybean residue samples of 0.5 g were dispersed in 25 mL of distilled water. After mixing, the pH of each mixture was adjusted to 4, 5, 7, and 9 using 1 M HCl or 1 M NaOH, at room temperature, shaken for 1 h, and subsequently centrifuged at 10,000 *g* for 10 min at 20 °C, where the supernatant was then collected for analysis of emulsification. Oil (5 mL) and 15 mL of supernatant were mixed at room temperature and then were emulsified using a high-speed homogenizer (FA25, Fluko, Shanghai, China) at 13,000 rpm for 2 min and then were poured into a beaker. Samples (25 μL) were taken at 0 and 30 min and mixed with 5 mL SDS solution, and the absorbance was measured at 500 nm, with 0.1% SDS used as a blank control. EAI and ESI were calculated as:

$$\text{EAI (Emulsification activity index, } m^2/g) = \frac{2\ T \times A_0 \times N}{(\ C \times Q \times 10000)}$$

$$\text{ESI (Emulsification stability index, min)} = \frac{A_0 \times 30}{(A_0 - A_{30})}$$

where, T = 2.303, N is the multiple dilutions (250), C is the protein concentration (g/mL) in an aqueous solution of protein before emulsion formation, Q is the volume fraction of the oil phase in the emulsion (assumed to be 0.25), A_0 is the absorbance of the diluted emulsion after homogenization, and A_{30} is the absorbance of the emulsion after remaining stationary for 30 min.

2.7. Determination of moisture states and distribution

A low-field NMR method (Li et al., 2014) was used to measure the moisture states and distribution. Samples were put into a 1.5 cm diameter tube and were then put into the RF coil center of the permanent magnetic field. The resonance frequency was 23.137 MHz, the magnet strength was 0.55 T, the cumulative scan was 8 times, the echo time was 105 ms, and the echo count was 8000 (MRI analyzer NMI20, Suzhou Niumai Analysis Instrument Co., Ltd., Suzhou, Jiangsu, China). Using PQ001 analysis software, which came with the instrument, and CPMG (Carr－Purcell－Meiboom－Gill)series samples (Carr & Purcell, 1954; Meiboom & Gill, 1958) with a spin-spin relaxation time T_2 signal and a T_2 attenuation curve was done using a multi-exponential fitting analysis with Niumai's Multi Exp Inv Analysis software program, T_2 relaxation information was obtained. Relaxation time was observed as T_{21}, T_{22}, and T_{23}, which represented the combined water, immobile water, and free water, respectively.

2.8. Microstructure determination

The USR and FSR samples were coated with gold (JS-1600M, Beijing HTCY Technology Co., Ltd., Beijing, China) and photographed using a SEM (TM 3030 Plus, Tianmei (China) Scientific Instrument Co., Ltd., Shanghai, China). The determination conditions were accelerating voltage of 15 kV, electron beam of 4.0 times, and the working distance of 17 mm, and the electronic probe was a secondary detector of electrons (Xia, Wang, Liu, Li, & Zhou, 2016). The samples were observed with a magnification of 1,000 and 5,000 times.

2.9. Statistical analysis

All experiments were done in triplicate and data are expressed as mean values ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) and the means were compared using the Fisher's least significant difference (LSD) test using the IBM Statistical Package for the Social Sciences (SPSS, IBM Inc., Chicago, IL, USA) software, version 19.0 and $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Nutritional components analysis

To evaluate the effect of the fermentation of soybean residue (okara) by *K. marxianus*, the proximate composition, and amino acid contents were determined. The changes in nutritional components in soybean residue (before and after *K. marxianus* fermentation) were determined (Table 1), and FSR's components were changed significantly. Compared with USR, crude fat and polysaccharide contents in FSR were increased by 24.5 and 262% ($p < 0.05$), respectively. The content of insoluble dietary fiber in soybean residue was more than 50%, but the soluble dietary fiber content was relatively lower, at only 2.9% dry matter. After fermentation, the insoluble dietary fiber content in FSR decreased, while the soluble dietary fiber in FSR had a 158% increase ($p < 0.05$). As shown in Table 2, the proportion of essential amino acids in FSR had increased, such as threonine, valine, and isoleucine.

3.2. Anti-nutritional factors

Anti-nutritional factors not only have inhibitory effects on some digestive enzymes in organisms but can also react with some minerals in the food itself that then forms an insoluble complex that leads to a decreased rate of biological use of minerals and trace elements in the body, resulting in deficiencies in human nutrition and other diseases. In addition, phytic acid might affect the body's absorption of proteins, which might cause dysfunctions or disorders in digestion and absorption functions. Soybean residue has a variety of anti-nutritional factors, such as trypsin inhibitor, phytic acid, and other similar compounds. Compared with USR, the trypsin inhibitor and phytic acid contents in FSR were significantly reduced after *K. marxianus* fermentation ($p < 0.05$) (Table 3). This showed that *K. marxianus* fermentation

Table 1
Proximate composition of soybean residue (okara) before and after fermentation with *K. marxianus* (g/100 g dry matter).

	USR	FSR
Protein	26.0 ± 0.2[a]	24.7 ± 0.0[a]
Crude fat	10.0 ± 0.3[a]	12.4 ± 0.4[b]
Insoluble dietary fiber	53.8 ± 0.3[a]	50 ± 2[a]
Soluble dietary fiber	2.9 ± 0.1[a]	7.5 ± 0.2[b]
Polysaccharide	0.87 ± 0.03[a]	2.9 ± 0.1[b]
Ash	3.5 ± 0.4[a]	3.5 ± 0.5[a]

[a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

Table 2
The proportion of each amino acid in total amino acids before and after fermented soybean residue (okara) with *K. marxianus*.

Amino acid	USR (%)	FSR (%)
Cystine	1.81	6.78
Aspartic acid/Asparagine	11.1	8.78
[a]Threonine	3.83	5.75
Serine	3.43	4.63
Glutamic acid/Glutamine	24.2	21.5
Glycine	6.25	8.78
Alanine	8.07	4.79
[a]Valine	4.03	5.59
[a]Methionine	0.50	0.00
[a]Isoleucine	3.12	7.58
[a]Leucine	14.0	10.0
Proline	nd	nd
[a]Phenylalanine	1.71	1.19
[a]Lysine	6.76	5.67
Histidine	3.03	1.59
Arginine	8.07	7.18
Tryptophan	nd	nd
Total essential amino acids	34.0	35.8
Total amino acids	100	138

[a] Essential amino acids; nd: not detected (n = 1).

Table 3
Activities of anti-nutritional factors in soybean residue (okara) after *K. marxianus* fermentation (dry matter).

Anti-nutritional factors	USR	FSR
Trypsin inhibitor activity (TIU/g)	1710 ± 2[b]	124 ± 2[a]
Phytic acid (mg/g)	16.8 ± 0.1[b]	6.4 ± 0.1[a]

[a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

contributed to the degradation of the anti-nutritional factors in soybean residue, which showed that the FSR was suitable as a food resource.

3.3. Beany odor

The compounds of *n*-hexanal and hexanol are the most likely components in soybean milk or soybean residue to cause beany odor (Sun et al., 2010; Yuan & Chang, 2007b). As shown in Table 4, the total beany odor in soybean residue fermented with *K. marxianus* dropped by 52% ($p < 0.05$). The contents of *n*-hexanal and hexanol accounted for the highest proportions in USR, which were 3.5 and 3.7 μg/g. Compared with USR, FSR was effectively reduced in its *n*-hexanal and hexanol contents by 51 and 78%, respectively ($p < 0.05$). It is worth noting that 1-octen-3-ol at a low threshold (Zhang, Guo, Liu, & Chang, 2012) completely disappeared in FSR.

Table 4
Changes observed in selected beany odors (μg/g dry matter) in fermented soybean residue (okara) with *K. marxianus*.

Compounds	USR	FSR
n-Hexanal	3.5 ± 0.1[c]	1.7 ± 0.03[a]
Hexanol	3.7 ± 0.2[b]	0.8 ± 0.1[a]
Benzaldehyde	0.09 ± 0.01[a]	0.06 ± 0.04[a]
1-Octen-3-ol	0.1 ± 0.01[a]	0.0 ± 0.0[a]
1-Octen-3-one	0.11 ± 0.01[a]	0.13 ± 0.00[a]
2-Pentylfuran	0.17 ± 0.01[a]	0.18 ± 0.01[a]
trans-2-Nonenal	0.19 ± 0.02[a]	0.19 ± 0.03[a]
trans-2,4-Decadienal	0.68 ± 0.01[a]	0.72 ± 0.11[a]
trans-2,4-Nonadienal	0.6 ± 0.1[a]	0.7 ± 0.1[a]
Total beany odors	9.2 ± 0.2[b]	4.4 ± 0.1[a]

[a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

3.4. Processing characteristics

As the pH is an important factor in food processing, some physicochemical changes may be reversed by pH readjustment upon redispersion, thus improving solubility and stability (Elizalde, Bartholomai, & Pilosof, 1996; Luo, Vasiljevic, & Ramchandran, 2016). The solubility and emulsification characteristics are very important to the application of soybean residue in food processing, especially under various pH conditions. Generally speaking, 4 indicators including water absorption index (WAI) and water soluble index (WSI), emulsification activity index (EAI) and emulsification stability index (ESI) are key points to evaluate the stability of food. The pH of FSR was in the range of 6.8–7.0, which was not significantly different from that before fermentation. Fig. 1a and b shows significant differences of WAI and WSI between FSR and USR. In the range of pH 4–9, the WAI of USR was different, and the WAI in acidic solution was higher than that in neutral or alkaline solutions. The WAI of FSR was higher than that observed in USR ($p < 0.05$), indicating that the water absorption of soybean residue was greatly enhanced after fermentation. FSR was not affected by pH, while the WSI of USR was higher in non-acidic solutions. As shown in Fig. 1c and d, compared with the USR, both the EAI and ESI of FSR were improved, with the exception of the ESI shown at pH 5.

3.5. Effect of fermentation on water distribution of soybean residue (okara)

Fig. 2a shows the transverse relaxation time, proton density and moisture distribution of USR and FSR. USR or FSR in the LF-NMR relaxation map has 3 peaks, indicating the moisture in the soybean residue includes combination water, immobilized water, and free water. The highest proportion of free water is more than 90%. While the total water content is constant, the T_{23} peak of FSR shifts to the right, and kurtosis increases indicating that FSR's free water flows more easily, while the proportion of immobilized water increased after fermentation (Fig. 2b). Therefore, part of the mobilized water has changed to immobilized water.

3.6. Apparent structure of soybean residue (okara)

Microstructural characterization of the USR and FSR was done using SEM with the results shown in Fig. 3. Generally, USR was relatively smooth and complete, while the FSR structure was destroyed, and granular substances had increased to form an irregular state characterized as flaky, rough and uneven. As shown in Fig. 3c, the FSR structure became denser, and smaller pores formed, where the fiber block of soybean residue was decreased after fermentation as many small particles had appeared. More specific structural characteristics of FSR can be seen in Fig. 3d; some fiber surface spalling debris formed a structure of a local network, and an obvious grooved surface appeared.

4. Discussion

There has been a growing number of interesting studies on alternative methods to add value to soybean residue (okara) by microbial fermentation (Vong & Liu, 2016). *K. marxianus* is characterized by fast growth, thermotolerance, broad substrate spectrum (Pentjuss et al., 2017). Also, *K. marxianus* is generally regarded as a safe food or pharmaceutical processing material (Gombert, Madeira, Cerdan, & Gonzalez-Siso, 2016; Lane & Morrissey, 2010) and is also as biological agents added to food for the production of aroma compounds in the list of Qualified Presumption of Safety (QPS) used by the European Food Safety Authority (EFSA) (Fadda, Mossa, Deplano, Pisano, & Cosentino, 2017). In this study, yeast *K. marxianus* screened from Kefir grains was

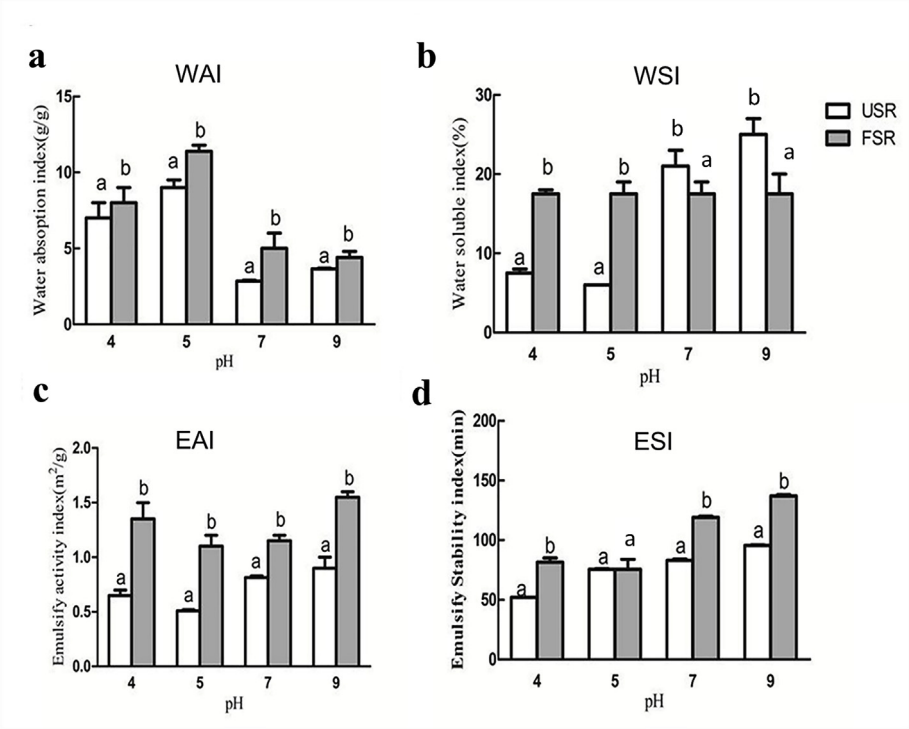

Fig. 1. Physicochemical properties of soybean residue (okara) fermented with *K. marxianus*. [a, b] Different letters in the same row indicate significant differences at $p < 0.05$. Values are the mean ± standard deviation of three independent replicates (n = 3).

used to the improvement of the nutritional and processing characteristics of soybean residue. Compared with no obvious changes in contents of crude protein and ash, the fat was observed with a significant increase by 24.5% ($p < 0.05$) after fermentation (Table 1). Such results were similar to those observed in the fermented soybean residue by the yeast *Yarrowia lipolytica* (Vong et al., 2016) and *Saccharomyces cerevisiae* (Vidiany et al., 2018). These results seem to have a close relation with a few extracellular and cell wall-bound lipases (Fickers, Marty, & Nicaud, 2011; Gupta, Kumari, Syal, & Singh, 2015). Furthermore, the contents of soluble dietary fiber and polysaccharides were also increased greatly in *K. marxianus*-fermented soybean residue than original which indicated it owning higher functional activity such as antiobesity (Matsumoto, Watanabe, & Yokoyama, 2007) and antioxidant (Ma, Sun, Yang, & Zhang, 2016). *K. marxianus* is reported to have the ability to secrete cellulases such as *β*-glucosidase (Yoshida et al., 2010) and to possibly transform insoluble dietary fiber into soluble dietary fiber (Wen, Niu, Zhang, Zhao, & Xiong, 2017). Anti-nutritional factors, such as trypsin inhibitors and phytic acid (Liener, 1994) can resist nutrition absorption. The content of trypsin inhibitors and phytic acid in FSR was significantly decreased (Table 3), which improved digestibility and strongly confirmed the favorable advantages of microbial fermentation. In addition to the improvement of nutrition, many important changes have been found in the processing characteristics of soybean residue using *K. marxianus* fermentation (Fig. 1). Lim et al. (2010) reported that the emulsification and emulsion stability of fermentation have the highest capability at pH 9 with *Monascus* fermentation. Compared with this finding, a wide range of EAI and ESI have been observed with soybean residue making it possible to use it as a raw material, especially in acidic food. There is also a significant change in the water state of FSR. Fig. 2 shows better mobility of free water and an increasing proportion of combined water in FSR. Electrostatic repulsion prevents oil droplet polymerization, thus improving protein adsorption at the oil-water interface and showing a higher capacity for emulsification (Elizalde et al., 1996). The SEM results showed (Fig. 3) obvious grooves on the surface of FSR fibers, and some fiber surface spalling can form a local network structure, which provides further explanation for the increased FSR absorbency.

The undesirable grassy smell, often called beany odor, still needs to

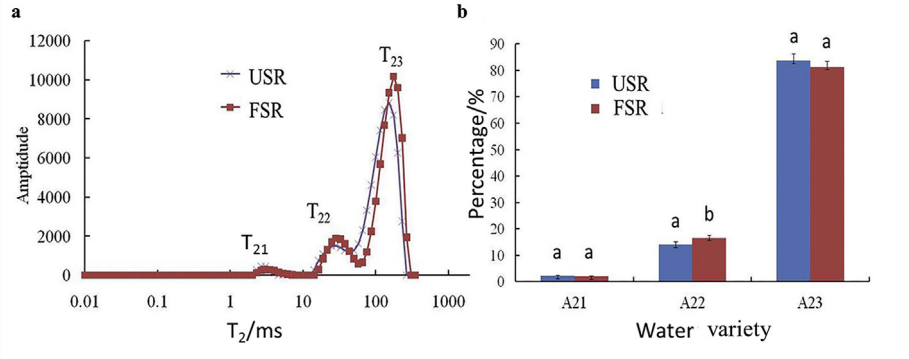

Fig. 2. LF-NMR T_2 relaxation curves of soybean residue (okara) before and after *K. marxianus* fermentation.

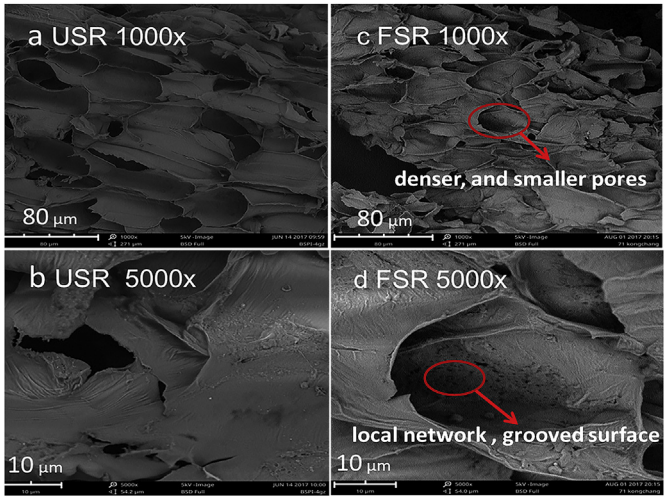

Fig. 3. SEM particle structure of soybean residue (okara) before and after *K. marxianus* fermentation.

be solved in the application of soybean residue in the food industry. These beany odors were mainly described with high contents of 9 substances including *n*-hexanal, hexanol, benzaldehyde, 1-octen-3-ol, 1-octen-3-one, 2-pentylfuran, *trans*-2-nonenal, *trans*-2,4-decadienal and *trans*-2,4-nonadienal (Yuan & Chang. 2007a; Yu, Liu, Hu, & Xu, 2018). The substances are generally formed from the lipoxygenases catalyzed oxidation of linoleic acid and linolenic acid. Even with heating or cooking, these beany odor substances cannot be completely removed. Vong and Liu (2017) used 10 kinds of yeast to ferment soybean residue and they found that *n*-hexanal decreased with these yeast fermentations. Similarly, the contents of *n*-hexanal and hexanol in the soybean residue fermented using *K. marxianus* decreased significantly, and even 1-octen-3-ol nearly disappeared (Table 4). *n*-Hexanal, hexanol and 1-octen-3-ol are generally considered to be very important substances that are responsible for beany odors. Fermentative microorganisms may metabolize these fatty acids and their derivatives to produce more desirable aroma compounds (Vong & Liu, 2016). A very sweet, creamy odor of FSR was noted that needs further investigation. These favorable odors are due to the *β*-glucosidase secreted by *K. marxianus* (Su et al., 2018; Tian et al., 2017; Yoshida et al., 2010) and the different type of amino acids such as valine and serine (Xu, Liu, & Zhou, 2012). With the growing number of studies on health function of soybean residue (Lemes et al., 2014; Villanueva-Suarez, Perez-Cozar, Mateos-Aparicio, & Redondo-Cuenca, 2016), this study provided evidence of the potential to improve okara with fermentation using *K. marxianus* for consumer goods.

5. Conclusion

The nutritional characteristics of soybean residue (okara) can be improved after *K. marxianus* fermentation, especially with the increase in the soluble materials and the decrease in the anti-nutritional factors. In addition, improving processing characteristics and the reduction in the beany odors in soybean residue using *K. marxianus* fermentation can be more easily accepted by customers, which may extend the utilization of soybean residue in applications relating to food processing. The *K. marxianus* may be used as a potential microorganism to process soybean residue products.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors are grateful to the Jilin Province Innovation Center for Biological Food and Manufacturing Technology for the excellent technical assistance. This work was supported by Earmarked Funds for the Modern Agro-industry Technology Research System under Grant CARS-04 (CN), the Department of Science and Technology of Jilin Province with Grant 20160519013JH (CN) and the Department of Science and Technology of Jilin Province with Grant 20170312022ZX (CN).

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UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO

Seleção de Mestrado Acadêmico em Nutrição – 2020

CHAVE DE RESPOSTA - QUESTÃO DISSERTATIVA – LINHA DE QUALIDADE DE ALIMENTOS

	Sugestão de pontuação	**Chave de resposta**
Tabela 1 **Vale 0,5**	**0,5**	(1) Descreva e interprete os resultados apresentados nas Tabelas 1, 3 e Fig. 1. **As alterações na composição nutricional do resíduo de soja (antes e após a fermentação por *K. marxianus*) foram determinadas e são apresentadas na Tabela 1.** Observou-se um **aumento significativo (p<0,05) dos componentes gordura bruta** (10 para 12,4)**, fibra dietética solúvel** (2,9 para 7,5) **e polissacarídeos** (0,87 para 2,9) no resíduo de soja (okara) após a fermentação por *K. marxianus*. Não houve alteração significativa na concentração de proteína, fibra dietética insolúvel e cinzas. Assim, a fermentação foi capaz de melhorar as características nutricionais do resíduo de soja.
Tabela 3 **Vale 0,5**	**0,5**	**A determinação dos fatores antinutricionais (Inibidor de tripsina e ácido fítico) no resíduo de soja antes e após a fermentação por *K. marxianus* foi realizada e os resultados são apresentados na Tabela 3.** Observou-se diminuição significativa na atividade do inibidor de tripsina e na concentração de ácido fítico (p<0,05), indicando que a fermentação por *K. marxianus* **contribuiu para a degradação dos fatores antinutricionais do resíduo de soja**. Assim, sugere-se que o resíduo de soja fermentado seja adequado para alimentação humana.
Tabela 4 **Vale 1,0**	**1,0**	**O odor "beany" total (cheiro de feijão) foi avaliado no resíduo de soja antes e após a fermentação por *K. marxianus* e os resultados são apresentados na Tabela 4.** Os conteúdos de n-hexanal e hexanol apresentaram percentuais significativamente menores (p<0,05) no RSF, passando de 3,5 µg/g de n-hexanol no resíduo não fermentado para 1,7 no RSF e 3,7 µg/g de hexanol para 0,8 no RSF. **O odor "beany" total (cheiro de feijão) diminuiu 52% no resíduo fermentado**, passando de 9,2 a 4,4 (p<0,05).
Fig. 1 **Vale 2,0**	**0,5**	As alterações nas características tecnológicas (de processamento) do resíduo de soja após a fermentação são apresentadas na Figura 1. A **Figura 1a** mostra diferenças significativas no índice de absorção de água (WAI) entre resíduo de soja antes e após a fermentação. **Na faixa de pH entre 4 e 9, o WAI aumentou significativamente**, indicando que a absorção de água do resíduo de soja foi elevada após a fermentação. Além disso, o WAI em solução ácida foi maior do que em soluções neutras ou alcalinas.

	0,5	A **Figura 1b** mostra que o índice de solubilidade de água (WSI) no resíduo de soja antes da fermentação foi maior em soluções neutra e alcalina. **Após a fermentação, o WSI não foi afetado pelo pH**.
	0,5	A **Figura 1c** mostra que o **índice de atividade emulsificante no resíduo fermentado aumentou significativamente em toda a faixa de pH** avaliada.
	0,5	A **Figura 1d** mostra que o **índice de estabilidade emulsificante no resíduo fermentado aumentou significativamente em toda a faixa de pH avaliada, com exceção no pH 5**, em que não houve diferença significativa entre o índice antes e após a fermentação.
Resumo **Vale 6,0**		**Introdução (0,5): Contextualizar a temática citando palavras chaves do título.** O resíduo de soja (okara) é um subproduto do processamento da soja que geralmente é descartado, ocasionando grandes perdas econômicas e problemas socioambientais. OU O resíduo de soja é um subproduto rico em nutrientes. Assim, o seu aproveitamento para alimentação humana é desejado, visando reduzir o desperdício de alimentos. OU A fermentação é um processo empregado para aumentar as propriedades nutricionais, melhorar as características tecnológicas e reduzir fatores antinutricionais de um alimento. **Objetivos (1,5):** O objetivo deste estudo foi avaliar os efeitos da fermentação por *Kluyveromyces marxianus* na composição nutricional, fatores antinutricionais e características tecnológicas (de processamento) do resíduo de soja (okara). **Métodos (1,0):** O resíduo de soja (okara) foi submetido a fermentação em estado sólido através da inoculação de *K. marxianus* na proporção de 5%. A mistura foi fermentada a 28 °C por 44 h. Após a fermentação, as amostras foram esterilizadas e liofilizadas para obter o resíduo de soja fermentado. O mesmo procedimento sem inoculação foi seguido para o grupo controle de resíduo de soja não fermentado. Em seguida, o resíduo de soja fermentado e não fermentado foi avaliado quanto a composição nutricional, fatores antinutricionais, características tecnológicas e odor “beany” total (cheiro de feijão). **Resultados (1,5):** Os resultados mostraram que a fermentação do resíduo de soja por *K. marxianus* aumentou significativamente ($p<0,05$) os componentes gordura bruta (24,5%), fibra dietética solúvel (158%) e polissacarídeos (262%). Já os fatores antinutricionais ácido fítico (61,7%) e inibidor de tripsina (92,7%) foram reduzidos significativamente. Além disso, o resíduo fermentado apresentou índice de absorção de água e propriedades emulsificantes maiores. O odor “beany” total (cheiro de feijão) diminuiu 52% no resíduo fermentado, passando de 9,2 a 4,4 ($p<0,05$). **Conclusões (1,5):** As características nutricionais do resíduo de soja (okara) podem ser aumentadas após a fermentação por *K. marxianus*, principalmente com a elevação na concentração de macronutrientes e na diminuição dos fatores antinutricionais. Além disso, a melhoria das características tecnológicas do resíduo de soja usando fermentação por *K. marxianus* pode possibilitar a utilização deste resíduo em aplicações relacionadas ao processamento de alimentos.