Hydrolysis conditions of black bean and brown rice and application of the hydrolysate in trial production of plant-based milk

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ABSTRACT

Brown rice and black beans were known as good food sources. The main objective of this study was to evaluate the physico-chemical and antioxidative properties of brown rice and black bean extracts treated with different enzymes such as Bialfa-T, Glucozyme 2x, Bialfa-T-Glucozyme 2x, Biase, Biomatasa-L andBiase-Biomatasa-L. These extracts were initially used to produce plant-based milks. The results showed that the combination of Bialfa-T and Glucozyme enzymes expanded the recovery yield and total phenolic content. Combined Bialfa-T and Glucozyme enzymes also resulted in the highest antioxidant capacity in both brown rice and black beans extracts. The product made from the mixture of brown rice extract and black bean extract at a ratio of 1:1 (v/v) had an acceptableorganoleptic quality.

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1. Introduction

Nowadays, people increasingly tend to use plant-based food as an alternative source of animal products (Mäkinen et al., 2016) due to the fear of chronic diseases such as lactose intolerance, diabetes, cardiovascular, etc. Besides that, plant-based food are selected for dietary needs and religious issues. Plant milk is the water extracts of legumes, oilseeds, and cereals (Zandona et al., 2020). According to the information reviewed by Hayat et al. (2014), black beans and common beans are excellent sources of dietary nutrition such as protein, fiber, minerals (iron, zinc, copper, phosphorous, and aluminum), vitamin B complex. Moreover, the beans have a low glycemic index, low lipid content. The major bioactive compounds reported in beans are phenolic compounds (ferulic acid, p-coumaric acid, and gallic acid), flavonoids (kaempferol, quercetin, catechin, and proanthocyanidin), and anthocyanins (3-O-glucosides of malvidin, petunidin). Utilization bean help protect the consumer health by preventing disease such as cardiovascular, obesity, diabetes, etc. Rice (*Oryza sativa*) is a staple food and is widely cultivated all over the world. Brown rice is whole rice, which only the husk is removed. The brown rice was found to have a greater content of nutrients such as protein, lipids, minerals (calcium, sodium, potassium, and vitamins (B complex, E) than the refined rice. Besides, brown rice contains bioactive compounds such as phenolic compounds, anthocyanins, especially γ-oryzanols, GABA. Many scientific reports show brown have the health benefits such as antioxidant activity, antidiabetic activity, antiobesity and cholesterol-lowering activity, etc (Saleh et al., 2019). Mixing brown rice and black bean in the diet can provide a balance of the biological value of the two materials (Dos Santos et al., 1979).

In Vietnam, processed products from black beans or brown rice are still not diversified. They are mostly used as homemade milk or instant roasted powders. At present, combined milk from these two materials is not available. If the extraction relies only on the ability of water to play as a solvent for the solubility of the solutes in the raw material, the recovery yield of bioactive compounds is not high. In many cases, using enzymes such as amylase for hydrolyzing both the cotyledon and the aleuron layer of the grains could result in higher extraction performance for these essential nutrients in the final product (Xu et al., 2015; Zeng et al., 2018). This study aimed to evaluate the enzyme ability to improve the quality of the black bean and brown rice extract (recovery yield, bioactive compound), as well as produce a non-dairy milk product from the combination of brown rice and black bean.

2. Material and Methods

2.1. Material and chemical

2.1.1. Material

The black bean used in this study was supplied from Phu Minh Tam Company (Vietnam). The beans had black outer skin and green inner cotyledon, with no presence of termites and a moisture content of 17%. Brown rice with a moisture content of 16% and free of termites was supplied from Kim Thien Loc Company (Vietnam).

2.1.2. Chemical

In this study, the used chemicals and reagents included 1,1-Diphenyl-2-picrylhydrazyl (DPPH) with free radical > 97% (HPLC grade) from TCI (Japan); Folin-Ciocalteus reagent from Merck (Germany; gallic acid), sodium carbonate anhydrous (Na_2CO_3), L-ascorbic acid ($C_6H_8O_6$) and methanol (CH3OH), 3,5-Dinitrosalicylic acid (DNS acid), L-glucose ($C_6H_{12}O_6$), phenolphthalein ($C_{20}H_{14}O_4$), sodium sulfite (Na_2SO_3), sodium hydroxide (NaOH) and potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) all from Xilong (China).

2.1.3. Enzyme source

Enzymes used in this study included Bialfa-T, Glucozyme 2, and Biomatasa-L. These liquid form enzymes were produced by BIOCON company (India) and supplied by CTC Vietnam Nutrition Solution Limited Liability Company. These enzymes were stored at 5 - 7°C in the fridge. The attributes of the enzyme were displayed in Table 1.

2.2. Experimental design

2.2.1. The slurries preparation of brown rice and black bean

Brown rice (50 g) was roasted at 120°C in 45 min and 50 g of black beans were roasted at 150°C in 30 min in an air fryer (Paula Deen 9.5 Qt Family-Sized Air Fryer, USA). After that, the grain was added with 250 g of water and blended by a blender (Philips 600W blender, Netherlands) for 2 min. The slurry was used for subsequent enzyme hydrolysis treatments.

2.2.2. Effect of enzyme treatment on physicochemical properties of brown rice extract

For improving the recovery yield of the extracts of black bean and brown rice after hydrolyzing. The distribution company advises using enzyme combinations such as Bialfa-T - Glucozyme 2x or Biase - Biomatasa-L. Thus, the combined enzyme solutions were used in this study. Moreover, the effect of a single enzyme on the quality of the extracts of the black bean and brown rice was investigated to clarify the function of using mixed enzymes.

This experiment was also studied for the performance of physicochemical properties (i.e recovery yield, viscosity, and total soluble solids content) of the brown extracts under the hydrolysis activity, with fixed recommended conditions from the manufacturer and the result of the preliminary test. The experiment was triplicated.

Treatment 1. Bialfa-T enzyme treatment: The prepared slurry (prepare in section 2.2.1) was mixed well with 200 g water and heated to the temperature of 60°C using an electromagnetic

Table 1. The attribute of enzymes was used in this study*

Type	Activity	The hydrolysis conditions			Description
		pH	Temperature (°C)	Dose (g/100 g material)	
Bialfa-T	17500 IU/g	5.0 - 7.0	95 - 105	0.5 - 1.0	Bialfa-T is a liquid form and produced from *Bacillus Licheniformis*. It is an amylase enzyme (1.4-a-D-glucan-4-glucanohydrolase). The enzyme hydrolyses the glycoside alpha-d-1,4 bonds of the starch at random, producing soluble dextrins and oligosaccharides.
Glucozyme 2x	400 AGU/mL	4.0 - 5.5	65 - 70	0.25 - 0.5	Glucozyme 2x has a liquid form and produced from *Aspergillus Niger*. It is an exo-1,4-alpha-glucosidase (1,4D-Glucan glucanohydrolase). The enzyme can hydrolyze the alpha-D-1,6 branches as well as the alpha-D-1,4 polymeric bonds of the starch.
Biase	14000 IU/g	5.1 - 5.6	85 - 90	0.5 - 1	Biase has a liquid form and produced from non-GMO microorganisms. It has high beta-glucanase activity (endo-beta-1,3-1,4-glucanase). It acts on the amylose and amylopectin chains of the starch, turning them into short dextrin and maltose chains
Biomatasa-L	400 BioCon unit/mL	4.0 - 5.5	65 - 70	0.25 – 0.75	Biomatasa-L has a liquid form and produced from *Aspergillus Niger*. It has the ability to hydrolyse maltose in two glucoses. The final product is exclusively glucose

*: The information is supplied from the manufacturer.

stove (KGEB-1200, USA). The 0.1% Bialfa-T enzyme (g enyzme/g of grain) was added into the mixture (pH 6.2-6.5), stirred well, and incubated at 90°C for 30 min using a waterbath (WT-42, BioBase, China). Next, the mixture was then heated up to 100°C and kept for 10 min to inactivate the enzyme and filtered using cheesecloth to obtain the hydrolyzed extract. The extract was measured physicochemical properties in the same day.

Treatment 2. Glucozyme 2x enzyme treatment: The preparation for this treatment was done as previously described in section 2.2.1. The concentration of 0.05% Glucozyme enzyme and the incubation at 65°C for 60 min were applied for this treatment.

Treatment 3. The mixture of Bialfa-T and Glucozyme enzyme treatment: 100 g of water was added to the prepared slurry (section 2.2.1). The suspension was incubated at 90°C for 30 min to utilize the hydrolysis activity of the 0.1% Bialfa – T enzyme. The mixture was then added with 100 g of water and incubated at 65°C for 60 min for the hydrolysis of Glucozyme 2x, with a concentration of 0.05%. The activity of the enzymes in the mixture was inactivated at 100°C for 10 min before filtering using cheesecloth to obtain the hydrolyzed extract.

Treatment 4. Biase enzyme treatment: The preparation for this treatment was done as previously described in section 2.2.1. The concentration of 0.1% Biase enzyme and the incubation at 90°C for 30 min was applied for this treatment.

Treatment 5. Biomatasa-L enzyme treatment: The preparation for this treatment was done as previously described in section 2.2.1. Concentration of 0.05% Biomatasa enzyme and the incubation at 65°C for 60 min were applied for this treatment.

Treatment 6. The mixture of Biase and Biomatasa-L enzyme treatment: The preparation for this treatment was done as previously described in section 2.2.1. The suspension was incubated at 90°C for 30 min to utilize the hydrolysis activity of the 0.1% Bias enzyme. The mixture was then added with 100 g of water and incubated at 65°C for 60 min for the hydrolysis of 0.05% Biomatasa enzyme. The activity of the enzymes in the mixture was inactivated at 100°C for 10 min before filtering using cheesecloth to obtain the hydrolyzed extract.

Based on the results include recovery yield, viscosity, and total soluble solids of brown rice extract at each treatment. The best one was further investigated with the comparison on reducing sugar content, total phenolic content (TPC), and antioxidant capacity, to that of the control sample.

2.2.3. Effect of enzyme treatment on physicochemical of black bean extract

This experiment was done followed section 2.2.2; in which, the brown extract was replaced by black bean extract.

2.2.4. Effect of the ratio between brown rice extract to black bean extract on the sensory quality of product

The ratios between brown rice and black beans extracts investigated in this experiment were 1:2; 1:1; 2:1 (v/v). The grain extracts were prepared as expressed in sections 2.2.2 and 2.2.3. To make the solution, 1 L of the combined brown rice and black beans extracts were supplemented with sucrose ester of fatty acids (as emulsifier), pectin LMP, coconut milk powder, and sucrose with the concentrations of 0.003%, 0.7%, 2.0%, and 5% (w/v), respectively. The mixtures were homogenized at 15,000 rpm for 15 mins using a homogenizer (Ultra-Turrax® T 25, Germany) then heated to 80°C and hot poured into the glass bottle. The products were sterilized at 105°C for 10 min using a sterilizer (KT-40DP, Japan). The samples were stabilized for 24 h before sensorial evaluation.

2.3. Analytical method

2.3.1. Determination recovery yield

The recovery yield (%) of the extract was calculated as the following formula:

$$RY\ (\%) = \frac{A}{B} * 100$$

Where: RY is recovery yield (%). A is the mass of extract (g). B is the initial mass of sample (g).

2.3.2. Determination of viscosity

A viscometer (V-E Viscometer from AMETEK Brookfield, USA) was used to measure the viscosity of extracts at 30°C.

2.3.3. Determination of total soluble solids content

The total soluble solids content was measured by using a refractometer (Atago, 0-33%, Japan).

2.3.4. Extraction of bioactive compounds

The sample was extracted with 80% methanol at the rate of 1:9 (v/v). The mixture was stirred and left for 30 min at room temperature (29-31°C.). Next, the sample was filtered by filter paper (brand 102 qualitative) and diluted with 80% methanol (Xu et al., 2008).

2.3.5. Determination of total phenolic content

The method followed the procedure of Singleton & Rossi (1965) and Lim et al. (2007). The aliquot of 0.3 mL of the diluted extract was poured into test tubes, then 1.5 mL Folin-Ciocalteu reagent (diluted 10 times) and 1.2 mL of 7.5% sodium carbonate were added. The mixture was stirred well and left at room temperature for 30 minutes in dark. The absorbance was measured by using a spectrophotometer (Jasco V730, Japan) at 765 nm. The total phenolic content in this study were determined and calibrated with gallic acid ($y = 0.0133x + 0.0541$, $R^2 = 0.9982$). Its value was expressed as mg of gallic acid equivalent per 100 g dry matter (dm).

2.3.6. Determination antioxidant activity

The method followed the procedure of Thaipong et al. (2006). The aliquot of 0.2 mL of the diluted extract was mixed with 4 mL of 0.1 mM DPPH solution in a test tube. Then, the mixture was left 30 min at room temperature in the dark. The absorbance was measured by using a spectrophotometer (Jasco V730, Japan) at 517 nm. The antioxidant activity in this study were determined and calibrated with ascorbic acid ($y = -0.0098x + 1.1139$, $R^2 = 0{,}9985$). Its value was expressed as mg of ascorbic acid equivalent (AAE) per 100 g dry matter (dm).

2.3.7. Determination reducing sugar content

The method followed the procedure of Miller (1959). Preparation A solution: 10 g of 3,5-dinitrosalicylic acid (DNS acid), 2 g phenol, 0.5 g sodium sulfite; 10 g of sodium hydroxide, and 200 g sodium potassium tartrate were added into a 1000 mL volumetric flask and filled up to the mark by distilled water. The aliquot of 1 mL of the diluted extract was mixed with 3 mL of A solution in a test tube. Next, the test tube was heated in the boiling water for 5 min. After that, it was cooled quickly by an ice bath. The absorbance was measured by using a spectrophotometer (V-760 UV-Visible Spectrophotometer, USA) at 550 nm. The antioxidant activity in this study were determined and calibrated with glucose ($y = 0.0103x - 0.0983$, $R^2 = 0.9971$). Its value was expressed as mg of glucose equivalent (AAE) per 100 g dry matter (dm).

2.3.8. Determination sensory quality

The panelist panel includes 30 people who are students from 18 to 20 year olds. They did not use any food before 30 min of testing the sample. The participant received the 20 mL of the test sample was kept in a glass cup. The test samples were coded with a 3-digit number. The acceptability of consumers for the attribute of the sample such as color, odor, and the taste was evaluated based on the 7 points hedonic scale; in which 1: Strongly disliked and 7: Strongly liked. After testing each sample, the participant used water to clear the taste.

2.4. Statistical analysis

All experiments were done in triplicate. Results were expressed as mean ± standard deviation. The collected data were analyzed by using JMP 13.0 software and ANOVA One-way analysis of variance to determine the significant differences ($P < 0.05$). Diagrams were built by using the Microsoft Excel 2010 software.

3. Result and Discussion

3.1. Effect of enzyme treatment on physico-chemical properties of brown rice extract

3.1.1. Effect of enzyme treatment on recovery yield of brown rice extract

The different enzyme treatments significantly ($P < 0.05$) affected on the recovery yield, viscosity, and total soluble solids of the brown rice extract (Table 2). All of the samples treated with the mixed enzyme had a recovery yield (78 -

Table 2. Effect of enzyme treatment on physicochemical properties of brown rice extract

Treatments	Attribute		
	Recovery yield (%)	Viscosity (cP)	Total soluble solids (%)
Glucozyme 2x	$53.90^{e} \pm 3.05$	$63.67^{b} \pm 1.44$	$5.33^{d} \pm 0.29$
Bialfa-T	$64.16^{d} \pm 1.03$	$47.86^{d} \pm 0.79$	$8.17^{c} \pm 0.29$
Bialfa-T-Glucozyme 2x	$87.53^{a} \pm 1.52$	$27.70^{f} \pm 0.75$	$10.00^{a} \pm 0.50$
Biase	$67.83^{c} \pm 3.65$	$53.45^{c} \pm 0.45$	$7.83^{c} \pm 0.29$
Biomatasa-L	$51.24^{f} \pm 0.11$	$78.12^{a} \pm 0.14$	$3.83^{e} \pm 0.29$
Biase - Biomatasa	$78.33^{b} \pm 0.10$	$34.48^{e} \pm 0.38$	$9.00^{b} \pm 0.50$
P_{value}	< 0.0001	< 0.0001	< 0.0001

Values are expressed as mean ± standard deviation of three replications. The values have a different uppercase letter mean significant difference within the same column ($P < 0.05$) based on one-way ANOVA.

88%), total soluble solids (9-10%) higher than the single enzyme (51 - 71% and 3.8 - 8.0%, respectively). In contrast, brown rice extract's viscosity of the mixed enzyme was 27-34 cP and lower than the single enzyme (47 - 78 cP).

In between the four single enzyme treatments, the Biomatasa-L gave the hydrolysis extract had the highest viscosity (78.12 cP) as well as the lowest TSS (3.83%) and recovery yield (51.24%). While the brown rice extract treated with the Bialfa -T enzyme got the lowest viscosity (47.86 cP) and the highest TSS (8.17%) as well as the high recovery yield (64.16%). For the mix enzyme treatments, the Bialfa-T - Glucozyme 2x had greater activity than the Biase - Biomatasa in hydrolysis brown rice extract.

Moreover, the relation of the viscosity, recovery yield, and total soluble solid of the brown rice extract was found to fit the linear model (Figure 1). In detail, the viscosity of the extract decreased leading to the recovery yield and total soluble solids increased. The correlation coefficient of these attributes with the viscosity was $R^2 = 0.91$ and $R^2 = 0.93$, respectively.

The brown rice component has amylose and amylopectin (Abeysundara et al., 2017). It seems that during liquefaction and saccharification, the combination of Bialfa-T (cleaving the α - 1,4 glycosidic bonds present in the inner part of the amylose or amylopectin chains) and Glucozyme 2x (cleaving the α - 1,4 and 1,6 glycosidic bonds present in the inner part of the amylose or amylopectin chains) gave the hydrolysis activity higher than the single enzyme. On the other hand, using the endo enzyme (Bialfa-T) as the first enzyme in the hydrolysis period can help produce more amount of low molecular weight carbohydrates chain which promotes the hydrolysis ability of the exo-enzyme (Glucozyme 2x) as the second enzyme.

3.1.2. Effect of enzyme treatment on antioxidant activity of brown rice extract

The change of bioactive compound in brown rice extract under enzyme treatment also was found in this study (Table 3). As expected, the Biafla-T – Glucozyme 2x treatment improved the recovery yield, total phenolic content, and antioxidant activity of brown rice extract. These attributes increased approximately 2.4 times, 1.6 times, and 1.3 times, respectively. This phenomenon also occurred in previous studies such as the hydrolysis of brown rice with mesophilic α-amylase (Xu et al., 2015; Zeng et al., 2018). According to these authors, the starch in the cereal noticeably deteriorated which facilitated the extraction of the phenolic compounds from the food matrix during the hydrolysis period.

The results of viscosity, total soluble solids, and reducing sugar content as the evidence confirmed for the hydrolysis capacity of the enzyme Bialfa-T - Gluoczyme 2x in this experiment (Table 3). Specifically, the viscosity of brown rice extract decreased approximately 4.4 times, meanwhile, the total soluble solids and reducing sugar increased approximately 4 times and 21 times, respectively. This behavior agreed with the result of Konsula et al. (2004) and Rocha et al. (2010), who found the various starch such as rice, potato, corn, cassava after treating with α - amylase had the high reducing sugar content and low viscosity.

This phenomenon could be explained when being heated with water, the starch component in the extracted suspension absorbed water and swelled that making the extract became viscous. The extracted suspension of the control sample was with the absence of hydrolysis en-

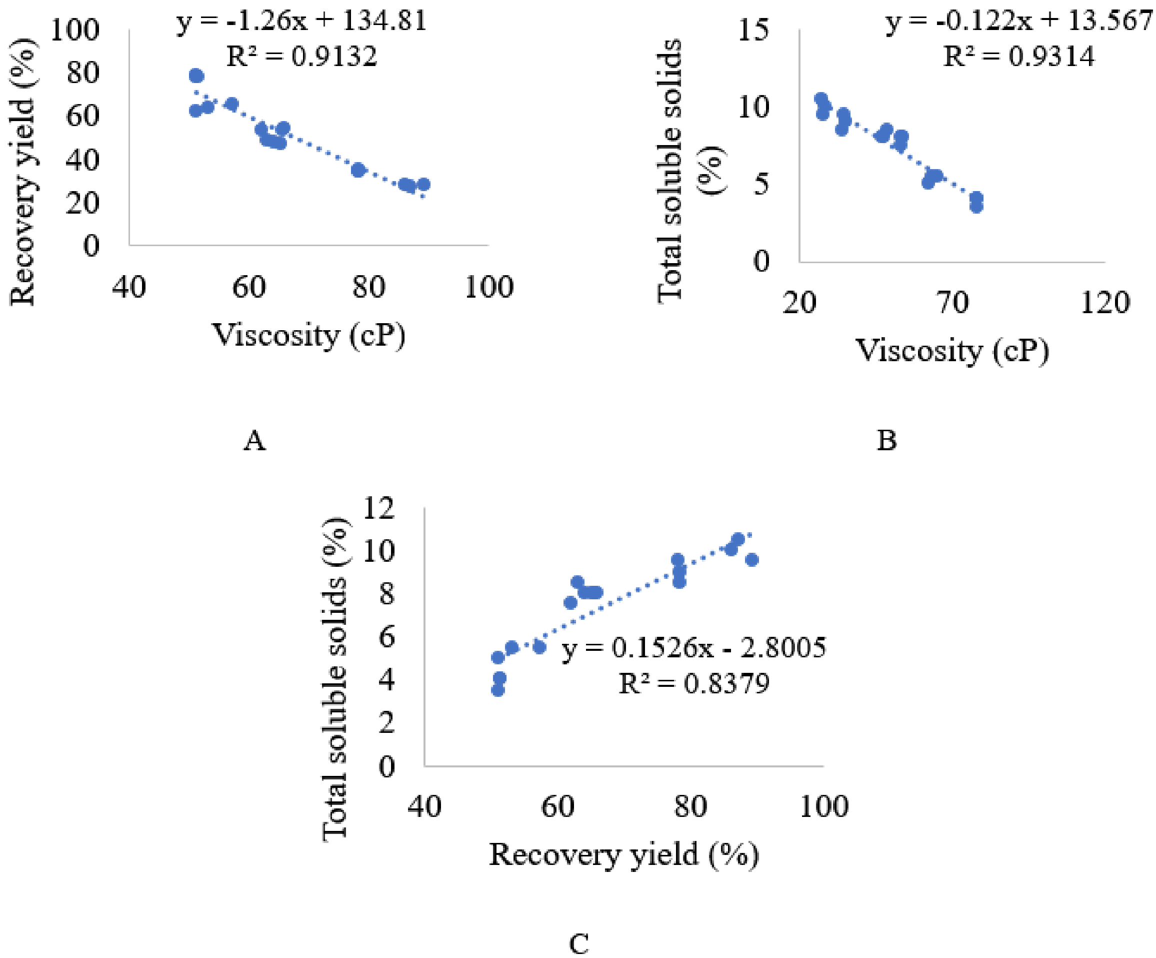

Figure 1. The correlation coefficient for the relationship between physicochemical properties of the brown rice extract (more specific what A, B, C)

zymes, the starch molecules had not been broken down into shorter polysaccharide chains and sugar molecules. That was the reason that caused the highest viscosity, hence the lowest recovery yield, compared to that of the other enzyme-treated samples.

Thus, the combination enzymes of Biafla-T and Glucozyme 2x was especially useful to improve the antioxidant activity, as compared to control sample.

3.2. Effect of enzyme treatment on physico-chemical of black bean extract

3.2.1. Effect of enzyme treatment on recovery yield of black bean extract

According to the results in Table 4, the effect of different enzyme treatments on the recovery yield, viscosity, and total soluble solids of black beans extract was akin to what was shown on the brown rice extract. The black bean extract achieved the recovery yield (78.63 - 78.42%) and TSS (6.00%) under the mix enzyme treatment higher than the single enzyme treatment (43.12 - 70.10%, 3.67 - 4.83%, repestively). Correspondingly these treatments, the black bean extract got the lowest viscosity of range 46.10 - 52.50 cP and the highest viscosity of range 52.50 - 81.99 cP; respectively.

In between the Bialfa-T - Glucozyme and Bias - Biomassa treatments, there is no statistically significant difference to recovery yield and TSS. However, the recovery yield's mean value of the Bialfa-T - Glucozyme treatment was still higher than Bias - Biomassa treatment. In addition, both the recovery yield and total soluble solids in black bean extract had a negative relative with

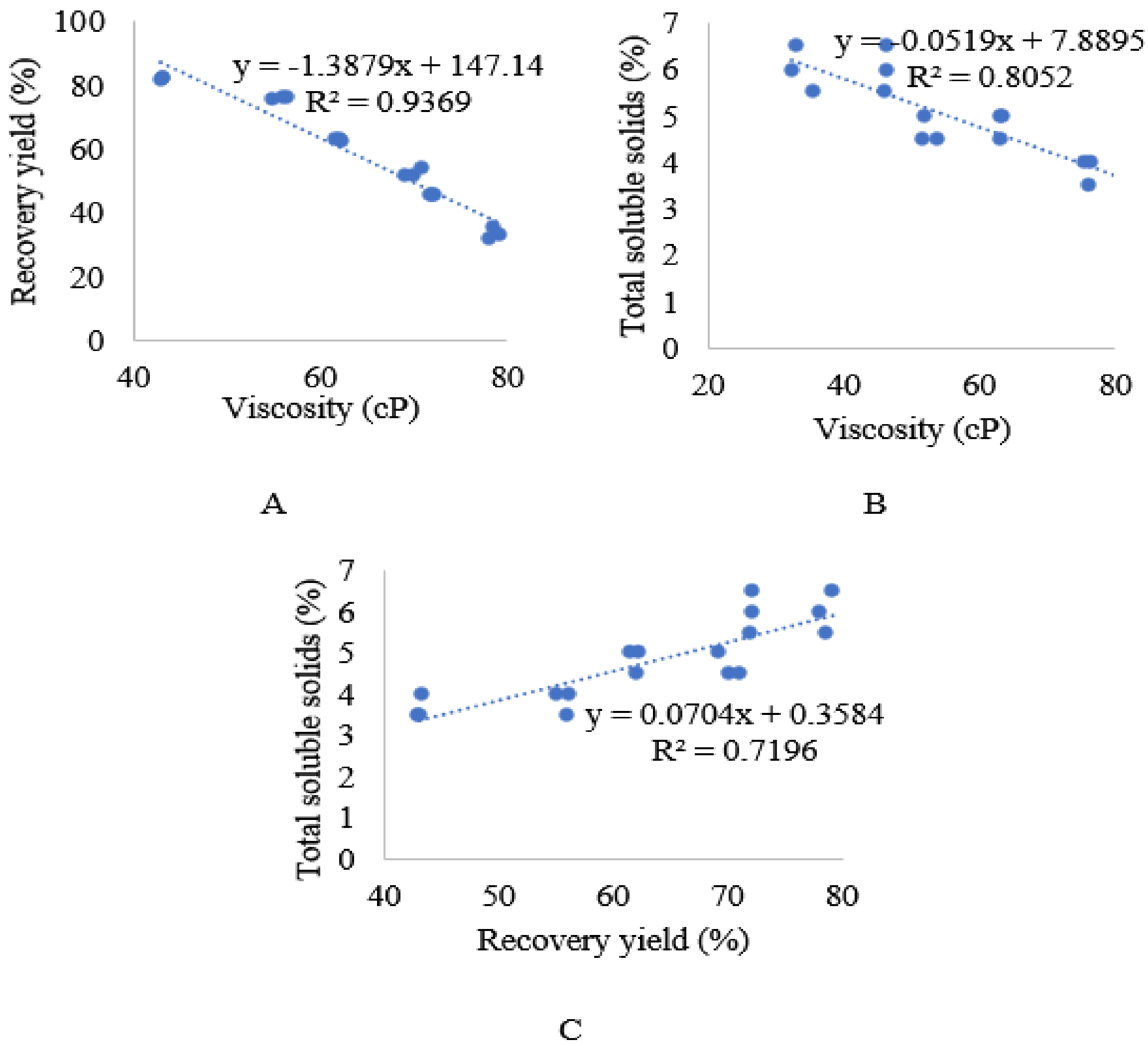

Figure 2. The correlation coefficient for the relationship between physicochemical properties of the black bean extract.

the viscosity; which reached the correlation coefficients were $R^2 = 0.93$ and $R^2 = 0.80$; however, the total soluble and recovery yield had a positive relative ($R^2 = 0.72$) (Figure 2).

3.2.2. Effect of enzyme treatment on antioxidant activity of brown rice extract

The enzyme treatment was useful to increase the recovery yield of black bean extract. For example, this parameter of a sample with enzyme and without enzyme treatments is 79.62% and 21.41%, respectively (Table 5). The same behaviour as what shown on brown rice extracts, the combined Bialfa-T and Glucozyme 2x enzymes treatment released more bioactive compound from the black bean into the extract. In detail, the solution had higher total phenolic content (10.93 mg GAE/100 g dm), antioxidant activity (7.15 mg AAE/100 g dm), and the reducing sugar content (81.94 mg GE/100 g dm) than the enzyme untreated samples (8.94 mg GAE/100 g dm, 4.01 mg AAE/100 g dm, and 81.94 mg GE/100 g dm, respectively). The change of physicochemical of the black bean extract under the enzyme treatment could be explained similarly the brown rice.

3.3. Effect of ratio between brown rice extract to black bean extract on the sensory quality of product

Table 6 presents the sensorial quality of the product with different mixing ratios between

Table 4. Effect of enzyme treatment on physicochemical properties of black bean extract

Treatments	Attribute		
	Recovery yield (%)	Viscosity (cP)	Total soluble solids (%)
Glucozyme 2x	$43.12^{e} \pm 0.15$	$75.99^{b} \pm 0.37$	$3.83c \pm 0.29$
Bialfa-T	$61.97^{c} \pm 0.39$	$52.50^{d} \pm 1.20$	$4.67^{b} \pm 0.29$
Bialfa-T - Glucozyme	$78.63^{a} \pm 0.54$	$33.55f \pm 1.72$	$6.00^{a} \pm 0.50$
Biase	$70.10^{b} \pm 0.90$	$63.12^{c} \pm 0.14$	$4.83^{b} \pm 0.29$
Biomatasa-L	$55.76^{d} \pm 0.68$	$81.99^{a} \pm 0.14$	$3.67^{c} \pm 0.29$
Biase - Biomatasa	$78.42^{a} \pm 0.63$	$46.10^{e} \pm 0.15$	$6.00^{a} \pm 0.50$
P_{value}	< 0.0001	< 0.0001	< 0.0001

Values are expressed as mean ± standard deviation of three replications. The values have a different uppercase letter mean significant difference within the same column ($P < 0.05$) based on one-way ANOVA.

Table 3. Physicochemical properties of the brown rice extract untreated and treated with enzyme

Treatments	Recovery yield (%)	Viscosity (cP)	Total soluble solids (%)	TPC (mgGAE/100g dm)	Antioxidant activity DPPH (mg AAE/100 g dm)	Reducing sugar (mg GE/100 g dm)
The control sample	$36.45^{b} \pm 3.09$	$118.61^{ba} \pm 1.68$	$2.50^{b} \pm 0.50$	$1.09^{b} \pm 0.03$	$1.33^{b} \pm 0.03$	$8.81^{b} \pm 0.15$
Bialfa-T - Glucozyme 2x	$88.54^{a} \pm 1.57$	$27.70^{b} \pm 0.75$	$10.33^{a} \pm 0.76$	$1.69^{a} \pm 0.12$	$1.74^{a} \pm 0.07$	$193.37^{a} \pm 1.08$
P_{value}	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values are expressed as mean ± standard deviation of three replications. The values have a different uppercase letter mean significant difference within the same column ($P > 0.05$) based on one-way ANOVA.

Table 5. Physicochemical properties of the black bean extract untreated and treated with enzyme

Treatments	Recovery yield (%)	Viscosity (cP)	Total soluble solids (%)	TPC (mgGAE/100g dm)	Antioxidant activity DPPH (mg AAE/100 g dm)	Reducing sugar (mg GE/100 g dm)
The control sample	$21.41^{b} \pm 0.55$	$88.05^{a} \pm 0.13$	$1.83^{b} \pm 0.24$	$8.94^{b} \pm 0.07$	$4.01^{b} \pm 0.20$	$4.17^{b} \pm 0.03$
Bialfa-T - Glucozyme	$79.62^{a} \pm 1.54$	$33.55^{b} \pm 0.17$	$6.50^{a} \pm 0.24$	$10.93^{a} \pm 0.07$	$7.15^{a} \pm 0.17$	$81.94^{a} \pm 1.48$
P_{value}	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values are expressed as mean ± standard deviation of three replications.
The value have a different uppercase letter mean significant difference within the same column ($P > 0.05$) based on one-way ANOVA.

Table 6. Sensory quality of the product under the different mixing ratio between brown rice and black bean extracts

Brown rice and black bean extracts ratio (v/v)	Attribute		
	Color	Odor	Taste
1 : 2	$4.27^{c} \pm 0.58$	$4.37^{c} \pm 0.49$	$3.83^{c} \pm 0.79$
1 : 1	$5.27^{b} \pm 0.52$	$6.43^{a} \pm 0.86$	$6.23^{a} \pm 0.94$
2 : 1	$6.03^{a} \pm 0.61$	$4.90^{b} \pm 0.96$	$5.47^{b} \pm 1.01$

1: Strongly disliked and 7: Strongly liked brown rice and black beans extracts. The perceptions of panelists on the flavor of the product fluctuated, the total highest value for these attributes (5.27 - 6.43) was obtained on sample 1:1, and the lowest (3.83 - 4.27) was with the sample 1:2. The reason could be explained based on the balanced and harmonious flavor between the two ingredients.

4. Conclusions

The combination of Bialfa-T and Glucozyme enzyme resulted in the best effect on physicochemical properties and bioactive compounds for both brown rice and black bean extracts. In particular, the enzyme treatment gives the recovery yield and the antioxidant capacity of the brown rice extract higher 2.4 times and 1.3 times, respectively, than the control sample. Similarly, the hydrolyzed black bean extract has the recovery yield and the antioxidant capacity higher 3.7 times and 1.8 times, respectively than the control sample. The plant milk recipe has the ratio between brown rice extract and black bean extract 1:1 (v/v) to get the best sensory.

Conflict of interest

The authors declare no conflict of interest.

References

Abeysundara, A., Seneviratne, N., Wickramasinghe, I., & Ekanayake, D. (2017). Determination of changes of amylose and amylopectin content of paddy during early storage. *International Journal of Science and Research* 6(1), 2094-2097.

Dos Santos, J. E., Howe, J. M., Moura Duarte, F. A., & Dutra de Oliveira, J. E. (1979). Relationship between the nutritional efficacy of a rice and bean diet and energy intake in preschool children. *The American Journal of Clinical Nutrition* 32(7), 1541-1544.

Hayat, I., Ahmad, A., Masud, T., Ahmed, A., & Bashir, S. (2014). Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): an overview. *Critical Reviews in Food Science and Nutrition* 54(5), 580-592.

Konsula, Z., & Liakopoulou-Kyriakides, M. (2004). Hydrolysis of starches by the action of an α-amylase from *Bacillus subtilis*. *Process Biochemistry* 39(11), 1745-1749.

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry* 103(3), 1003-1008.

Mäkinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition* 56(3), 339-349.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31(3), 426-428.

Rocha, T. D. S., Carneiro, A. P. D. A., & Franco, C. M. L. (2010). Effect of enzymatic hydrolysis on some physicochemical properties of root and tuber granular starches. *Food Science and Technology* 30(2), 544-551.

Saleh, A. S., Wang, P., Wang, N., Yang, L., & Xiao, Z. (2019). Brown rice versus white rice: Nutritional quality, potential health benefits, development of food products, and preservation technologies. *Comprehensive Reviews in Food Science and Food Safety* 18(4), 1070-1096.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3), 144-158.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19(6-7), 669-675.

Xu, E., Wu, Z., Long, J., Wang, F., Pan, X., Xu, X., Jin, Z., & Jiao, A. (2015). Effect of thermostable α-amylase addition on the physicochemical properties, free/bound phenolics and antioxidant capacities of extruded hulled and whole rice. *Food and Bioprocess Technology* 8(9), 1958-1973.

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y., & Shi, J. (2008). Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chemistry* 106(2), 545-551.

Zandona, L., Lima, C., & Lannes, S. (2020). Plant-based milk substitutes: factors to lead to its use and benefits to human health. In Ziarno, M. (Ed.). *Milk Substitutes-Selected Aspects*. London, UK: IntechOpen.

Zeng, Z., Luo, S., Liu, C., Hu, X., Gong, E., & Miao, J. (2018). Phenolic retention of brown rice after extrusion with mesophilic α-amylase. *Food Bioscience* 21, 8-13.