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A comparative study on nutritional and nutraceutical properties of finger millet (*Eleusine coracana*) and rice (*Oryza sativa*)

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Abstract

The composition and nutraceutical properties of finger millet were compared with rice to enhance future applications of finger millet in the functional food industry. The composition, *in vitro* fermentation ability, antioxidant activity, and α -amylase inhibitory activity of Ravana and Osadha finger millet varieties and Bg300 and Basmati rice varieties were tested. Osadha and Ravana reported lower ($P < 0.05$) crude protein contents compared to two rice varieties. Osadha had the lowest crude fat content. Total, soluble, and insoluble dietary fiber contents of two finger millet varieties were higher ($P < 0.05$) than the rice varieties. Osadha reported the highest dietary fiber content. Both finger millet varieties, especially Osadha had higher ($P < 0.05$) total flavonoid and phenolic contents, *in vitro* 2, 2-diphenyl-1-picrylhydrazyl scavenging and 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid scavenging activities than two rice varieties. Though, the alpha-amylase inhibitory activities of Osadha and Ravana were higher ($P < 0.05$) than that of Basmati and Bg300, they were very low compared to the acarbose. Hydrogen and carbon dioxide production during the microbial fermentation were higher ($P < 0.05$) in Ravana and Osadha than Basmati and Bg300. Therefore, the results of this study suggest that finger millet, especially the Osadha variety possesses more health-benefiting functional properties compared to Bg300 and Basmati rice varieties.

Keywords: Finger millet; Rice; Dietary fiber; Antioxidant; Nutraceutical

1. Introduction

Non-communicable diseases (NCD) cause for 71% of global deaths annually. Over 80% of world premature NCD deaths are due to cardiovascular diseases, cancers, respiratory diseases, and diabetes respectively. In Sri Lanka, NCD are a major cause of premature deaths. Unhealthy eating is among the main risk factors of NCD deaths [1].

Phenolics and flavonoids in foods are bioactive secondary plant metabolites that have antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anti-cholesterolemic, anti-cardiovascular and anti-carcinogenic properties [2, 3]. Dietary fiber in food is complex carbohydrates that resist enzymatic digestion in the human small intestine. Depending on their molecular weight, structure, and solubility, dietary fibers possess various health benefits [4]. Insoluble dietary fibers are cell wall constituents such as cellulose, hemicellulose, and lignin. Soluble dietary fiber includes non-cellulolytic polysaccharides including pectin, Inulin, gums, β -glucans. Dietary fiber is known to give protection against cardiovascular diseases, diabetes, colon cancer and obesity [5].

Cereals are the main source of energy in the meals of humans as well as livestock worldwide. Finger millet, which is also known as "Kurakkan" in Sri Lanka, is considered as a therapeutic food for diabetes. It is a rich source of minerals such as Ca, P, Fe, Zn, fiber and phytochemicals [2]. Rice is among the highest cultivated cereals in the world and staple food of many developing countries. Malnutrition and the incidence of non-communicable diseases are among the main causes

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which hinder the economic development of developing countries in the world. Therefore, the scope of this study was to determine the nutritional composition and nutraceutical potential of finger millet, compared to rice to encourage its applications in the functional food industry.

2. Material and methods

2.1. Collection of samples

Dried breeder seeds of Ravana and Osadha finger millet varieties were acquired from Field Crop Research and Development Institute (FCRDI), Maha-iluppallama, Sri Lanka. Bg300, a local white rice variety was purchased from Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka. Basmati rice, imported from Pakistan was purchased from a local market in Sri Lanka.

2.2. Sample preparation

Finger millet were cleaned to remove the loosely attached pericarp of the grain. Rice seeds were obtained by manually removing only the husk of the paddy. Seeds were dried in a drying oven (YAMATO IC600, Yamato Scientific Co. Ltd., Japan) at 60 °C until a constant weight was achieved. Then, the seeds were ground into a powder form using a grinder (MX-151SG1, Panasonic Co. Ltd, China). Samples were stored under airtight conditions until analysis [6].

2.3. Composition analysis

The dry matter, ash, crude fat, crude protein, and crude fiber contents were analyzed according to the AOAC (1995) [6] procedures. Nitrogen free extract (NFE) was obtained by subtracting the ash, crude protein, crude fat and crude fiber contents from the dry matter content. The insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and total dietary fiber (TDF) contents of each sample were determined according to the enzymatic gravimetric method described by Prosky et al. (1992) [7]. One (1) g of duplicate samples was transferred into 400 mL tall form beakers and suspended in phosphate buffer (0.08 M, pH 6). The sample was digested with α -amylase (Sigma-A3306, EC 3.2.1.1) (pH 6, 100 °C, 30 min), protease (Sigma-P3910, EC 3.4.21.62) (pH 7.5, 60 °C, 30 min), and amyloglucosidase (Sigma- A9913, EC 3.2.1.3) (pH 4, 60 °C, 30 min) respectively. The enzyme digest was transferred through pre-weighed glass fritted crucible into a pre-weighed suction flask. The residue was washed with water and water washings in the suction flask were saved for the analysis of SDF. After washing the residue with 95% ethanol and acetone, the crucible with residue was dried overnight in a drying oven (YAMATO IC600, Yamato Scientific Co., Ltd., Japan) at 105 °C and weighed accurately. The protein content of the residue was analyzed from one set of duplicates and the residue of the other duplicate sample was incinerated in a muffle furnace (Carbolite Co., England) for 5 h at 520 °C for the determination of ash. Filtrate and water washings were mixed with four 100 mL portions of 95% ethanol and precipitated the SDF. The precipitate was filtered through fritted glass crucible and the crucible with residue was dried overnight and weighed. TDF content was calculated by calculating the summation of SDF and IDF values.

2.4. Analysis of antioxidant properties

2.4.1. Preparation of water extracts

Fifty (50) mg of each sample was diluted in 5 mL of distilled water to make the final concentration 10 mg/mL. Then, the mixture was homogenized for 15 min in a homogenizer (IKA® ULTRA TURRAX® Tube Drive, Hamburg, Germany). After that, the supernatant was taken for the assay after centrifuging for 15 min at 12000 $\times g$, at 20 °C using a microcentrifuge (5340R, Germany).

2.4.2. Determination of total phenolic content (TPC)

TPC was determined according to the Folin-Ciocalteu method described by Adom and Liu (2002) [8] with minor modifications. The water extract (50 μ L), deionized water (150 μ L) and 50 μ L of 1 N Folin-Ciocalteu's reagent were added into the wells of a microplate and kept for 5 min. Then 200 μ L of 10% (w/v) sodium carbonate (Na_2CO_3) was added. Absorbance was taken after 30 min of incubation period, at 760 nm using UV- visible microplate spectrophotometer (Multiskan®, 2011 Thermo Fisher Scientific Inc., Japan). The Standard curve for Gallic acid (GA) was prepared and results were expressed as mg of GA equivalent (GAE) mg/g of dry weight.

2.4.3. Determination of total flavonoid content (TFC)

TFC was determined according to Dewanto et al. (2002) [9] with minor modifications. The water extract (0.5 mL), 5% (w/v) sodium nitrite (NaNO_2 , 0.15 mL) and distilled water (2 mL) were added into a test tube and kept for 6 min. Then,

10% (w/v) aluminum trichloride (AlCl_3 , 0.15 mL) was added and incubated for 6 min at room temperature. Finally, 4% (w/v) sodium hydroxide (NaOH, 2 mL) was added and made the volume to 5 mL with distilled water. After incubating for 15 min, absorbance was taken at 510 nm. The standard curve for catechin was prepared and TFC was calculated as Catechin equivalent (CE) mg/g of dry sample.

2.4.4. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH assay described by Sanjeevkumar et al. (2016) [10] was followed with minor modifications. The concentration series was prepared by adding distilled water and 100 μL of DPPH solution to different volumes of water extract (0, 30, 60, 90, 120, and 150 μL) to make the final volume 250 μL . It was incubated for 30 min at room temperature in dark conditions and the absorbance was taken at 517 nm. The concentration of the sample at 50% of inhibition was taken as the IC_{50} (Half maximal inhibitory concentration) value.

2.4.5. 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) cation radical scavenging assay

The ABTS assay was conducted according to Loganayaki et al. (2013) [11] with slight modifications. ABTS solution (1.25 mM) was mixed with potassium persulfate (2.0 mM) in a 1:1 ratio and kept in a dark place for 12 h at room temperature to generate a bluish-green solution. After that, 50 μL of water extract and 150 μL of ABTS solution were added into the wells of the microplate. Absorbance was measured at 734 nm. Six readings were taken by measuring the absorbance at every min for 6 min. Results were expressed as μM of Trolox equivalents (TE) /g of dry weight.

2.5. Determination of α -amylase inhibitory activity

2.5.1. Extraction of α -amylase inhibitory compounds

Twenty (20) mg of each sample was diluted in 5 mL of dimethyl sulfoxide (DMSO) to make the final concentration of 4 mg/mL. Then, the mixture was homogenized for 15 min in a homogenizer. After that, the supernatant was taken for the assay after centrifuging for 15 min at $12000 \times g$, at 20°C .

2.5.2. α -Amylase inhibitory assay

α -Amylase inhibitory activity was determined according to the method described by Nickavar et al. (2008) [12] with minor modifications. Concentration series of the inhibitor (test compound) was made as 4, 2, 1, 0.5, 0.25 mg/mL. Also, control was maintained without the addition of the sample. Firstly, 50 μL of α -amylase from porcine pancreas (Sigma-A3176, EC 232-565-6) was mixed with 50 μL of test compound and incubated for 30 min at room temperature. A blank was maintained for each concentration without adding the enzyme. Then 100 μL of 0.5% (w/v) starch (Sigma-S2004) was added, except for blanks. Instead of that, 100 μL of DNSA (3,5-Dinitrosalicylic Acid) color reagent was added into the blank. Then sample replicates and blanks were incubated for 3 min at room temperature. After that 100 μL of color reagent was added into sample replicates only. Instead of that, 100 μL of starch was added into the blanks. Replicates and blanks were incubated for 15 min at 85°C . After cooling, 900 μL of deionized water was added and absorbance was taken at 540 nm. The same procedure was followed for the acarbose to obtain the standard curve. The inhibition % was calculated as IC_{50} values.

2.6. Microbial fermentation study

Microbial fermentation study was conducted according to the methods described by Mikkelsen et al. (2004) [13] and Guo et al. (2003) [14] with minor modifications. The unadaptive cecal microflora for the study was obtained from the cecal contents of swine. The swine cecum was obtained from the slaughterhouse of the Livestock Field Station, Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Antibiotics were not given for the swine during the fattening period. A 10 g/100mL suspension of cecal digesta was prepared in sodium phosphate buffer and homogenized for about two min. The suspension was pressed through a double layer of cheesecloth to remove the feed and other substances in the cecal content. The slurry was then centrifuged for 15 min at $20,000 \times g$ and bacterial pellet was obtained. It was dissolved in sodium phosphate buffer again to the original volume. The slurry was kept under a continuous flow of carbon dioxide (CO_2) gas.

Each sample (0.5 g) was inoculated with 8 mL of bacterial suspension in a 15 mL vacutainer tube. Vacutainers were closed with rubber caps and wrapped with parafilm and sealed the top with Vaseline. Then, samples were transferred to the incubator (YAMATO IC600, Yamato Scientific Company Limited, Japan) and allowed to incubate at 39°C . Vacutainers were taken out at 0, 2, 4, 8, 18, 20 and 24 h. A blank was maintained without adding the substrate. All the steps were carried out in aseptic conditions. Carbon dioxide and hydrogen composition (%) of the headspace of the

vacutainers were analyzed using gas chromatography techniques (GC-9 AM Shimadzu Gas Chromatograph with a capillary column at 130 °C temperature).

2.7. Statistical analysis

Results were expressed as mean±SD. Means were separated using the Least Significant Difference method at $p < 0.05$. SAS software package (SAS Institution Inc., 2003, Cary, USA) was used for the analysis.

3. Results and discussion

3.1. Nutritional composition

Table 1 Nutritional composition of Basmati, Bg300, Ravana, and Osadha

Nutrient (%)	Basmati	Bg300	Ravana	Osadha
Dry matter	95.97±0.24 ^a	95.75±0.34 ^a	98.18±0.63 ^b	97.68±0.54 ^b
Ash	0.40±0.14 ^a	2.31±0.44 ^b	1.61±0.14 ^c	2.01±0.22 ^b
Crude protein	9.19±0.35 ^a	9.98±1.08 ^a	4.63±0.95 ^b	6.48±0.21 ^c
Crude fat	2.51±0.24 ^a	2.38±0.34 ^a	3.14±0.41 ^b	1.30±0.10 ^c
Crude fiber	0.09±0.03 ^a	0.79±0.14 ^b	3.17±0.53 ^c	3.40±0.31 ^c
NFE	83.79±0.33 ^a	80.33±0.75 ^b	85.64±0.91 ^c	84.72±0.40 ^c

Values (g/100g) are expressed as Mean±Standard Deviation (n=4) on dry matter basis.

Values with different superscripts within a row are significantly different at $P < 0.05$.

Table 1 shows the nutritional composition of four samples. Both rice varieties had significantly higher ($P < 0.05$) crude protein contents compared to two finger millet varieties. However, the crude protein content of Osadha variety was higher ($P < 0.05$) than the Ravana variety. The highest crude fat content was reported from the Ravana variety while the Osadha variety showed the lowest crude fat content. Protein and fat contents of Osadha variety in the current study are similar to the protein and fat contents of finger millet (5-8% and 1-2%, respectively) as reported by Mathanghi & Sudha (2012) [15]. In contrast to Osadha, protein and fat contents of Ravana are slightly different from the protein and fat contents of finger millet as reported by Mathanghi & Sudha (2012) [15]. Both finger millet varieties had higher ($P < 0.05$) crude fiber contents than two rice varieties. However, the crude fiber content of Bg300 rice variety was significantly greater ($P < 0.05$) than Basmati rice. The NFE mainly represents soluble carbohydrates. It was higher ($P < 0.05$) in both finger millet varieties than two rice varieties. However, the soluble carbohydrate contents of Ravana and Osadha were not significantly different while Basmati had a higher ($P < 0.05$) soluble carbohydrate content than Bg300. The ash contents of Osadha and Bg300 were not significantly different ($P < 0.05$). Basmati rice reported the lowest ash content.

3.2. Dietary fiber

Table 2 TDF, IDF, and SDF contents of Basmati, Bg300, Ravana, and Osadha

Component %	Basmati	Bg300	Ravana	Osadha
TDF	6.27±0.21 ^a	7.57±0.15 ^b	11.05±0.23 ^c	12.06±0.06 ^d
IDF	6.23±0.09 ^a	7.44±0.02 ^b	10.67±0.19 ^c	11.62±0.35 ^d
SDF	0.04±0.03 ^a	0.13±0.04 ^b	0.38±0.02 ^c	0.44±0.04 ^d

Values (g/100g) are expressed as Mean Standard Deviation (n=2) on dry matter basis.

Values with different superscripts within a row are significantly different at $P < 0.05$.

The TDF, IDF and SDF contents of four samples were significantly different ($P < 0.05$). Both finger millet varieties had significantly higher ($P < 0.05$) TDF, IDF and SDF contents compared to two rice varieties. Osadha variety had the highest TDF, IDF and SDF contents while Basmati rice had the lowest TDF, IDF and SDF contents (Table 2). Among the other millet types, only the finger millet is having a five-layered testa, which makes it a good source of dietary fiber [16]. Mathanghi and Sudha et al. (2012) [15] reported that TDF content of finger millet is around 15-20%. Soluble and insoluble dietary fiber contents of native Indian finger millet are 1.8% and 15.7% respectively. β -Glucan and arabinoxylan are the major components of SDF in finger millet while the IDF is mainly composed of lignin, cellulose, and

hemicellulose. In finger millet, dietary fiber content and composition are highly variable with the region [17]. Robin et al. (2012) [18] reported that TDF content of whole white rice flour is 1.3%, in which IDF and SDF contents are 0.9% and 0.4%, respectively.

3.3. Total phenolic and flavonoid contents

Table 3 TFC, TPC, antioxidant activity, and α -amylase inhibitory activity of Basmati, Bg300, Ravana, and Osadha

Characteristic	Basmati	Bg300	Ravana	Osadha
TFC (mg CE/g)	0.37±0.01 ^a	0.35±0.01 ^a	0.39±0.01 ^a	1.05±0.08 ^b
TPC (mg GAE/g)	3.85±0.18 ^a	4.43±0.17 ^b	6.40±0.09 ^c	8.08±0.17 ^d
ABTS (μ mol TE/g)	1.37±0.08 ^a	5.24±0.06 ^b	45.58±0.99 ^c	70.97±2.63 ^d
DPPH [IC ₅₀ (mg/mL)]	15.74±1.61 ^a	7.45±1.42 ^b	3.25±0.22 ^c	2.44±0.22 ^c
Alpha-amylase inhibition [IC ₅₀ (mg/mL)]	74.83±6.34 ^a	69.86±6.34 ^a	37.88±2.89 ^b	30.78±3.52 ^b

Values are expressed as Mean±Standard Deviation (n=3).

Values with different superscripts within a row are significantly different at P<0.05.

As shown in Table 3, the TPC of the four samples were significantly different at P<0.05. Osadha variety showed the highest TPC and the Basmati rice showed the lowest TPC. In contrast, TFC of Ravana, Bg300, and Basmati were not significantly different (P>0.05) when Osadha finger millet variety showed the highest TFC. Phenolic compounds are highly efficient scavengers of free radicals and considered as the principle components that contribute to the antioxidant activity [19]. Siwela et al. (2007) [20] reported that finger millet varieties contain a considerable level of phenolic compounds and tannins. Moreover, polyphenols in brown color finger millet varieties are higher than the white varieties [21]. Both finger millet varieties used for the present study were brown color varieties. The color of the Ravana variety was slightly darker than the Osadha variety. Saldivar, (2003) [22] revealed that the phenolic content of finger millet was much greater than rice. The type and concentration of phenolic compounds in rice are also influenced by the color of the pericarp. Rice varieties with red and black pericarp are greatly associated with phenolic compounds compared to rice with light brown pericarp [23]. Siwela et al. (2007) [20] reported that flavonoids and condensed tannins contribute to the color of the finger millet grain. According to Chethan and Malleshi (2007) [21] finger millet is a rich source of the flavonoid, quercetin.

3.4. Antioxidant activity

The DPPH radical scavenging activities of both finger millet varieties were higher (P<0.05) than two rice varieties. Although, the TPC and TFC of Ravana variety were lower (P<0.05) than that of Osadha variety, any significant difference in DPPH radical scavenging activity was not observed between them. DPPH radical scavenging activity of Bg300 rice variety was higher (P<0.05) than Basmati rice (Table 3). Mathanghi and Sudha (2012) [15] reported that brown or red varieties of finger millet have a higher DPPH radical scavenging activity than white varieties. The DPPH radical scavenging activity in rice differs with bran composition, variety, and climatic conditions [24]. ABTS cation radical scavenging capacities of samples were significantly different (P<0.05). It was highest in Osadha finger millet variety while the Basmati rice showed the lowest (P<0.05). The results of the ABTS cation radical scavenging assay follow the same pattern concerning the results of the total phenolic content. Sharma et al. (2018) [3] reported that phytochemicals in finger millet exert higher antioxidant activity compared to maize and wheat. According to Banerjee et al. (2012) [2], high molecular weight phenolics are more efficient in scavenging ABTS•+ free radicals than low molecular weight phenolics.

3.5. α -Amylase inhibitory activity

The α -amylase inhibitory activities of two finger millet varieties were significantly higher (P<0.05) than the two rice varieties. IC₅₀ values between Ravana and Osadha as well as Basmati and Bg300 were not significantly different (Table 3). Dietary fiber and phenolic compounds in food act in a similar way to acarbose by inhibiting α -amylase and α -glucosidase enzymes during the digestion of carbohydrates in the digestive system of human. This action delays the absorption of glucose into the bloodstream and retards the postprandial hyperglycemia which is a therapeutic approach for the control of type II diabetes [12, 17]. Acarbose was used as the positive control for the experiment which had a very lower IC₅₀ value of 0.08±0.01 mg/mL. However, the IC₅₀ values of Osadha and Ravana finger millet varieties were greater (P<0.05) compared to acarbose.

Okoyomoh et al. (2013) [25] reported that finger millet exerts antioxidant and antidiabetic properties, as a result of a study where finger millet seed coat matter has fed to streptozotocin induced diabetic rats. Similarly, Rajasekaran et al. (2004) [26] identified the wound healing properties of finger millet on diabetic rats. Moreover, a significant decrease in postprandial blood glucose levels had been identified when finger millet preparations were fed to type II diabetes mellitus patients over a month [16]. Therefore, according to the results of the present study, antidiabetic property of finger millet may not be associated with the inhibition of the α -amylase enzyme.

3.6. Microbial fermentation study

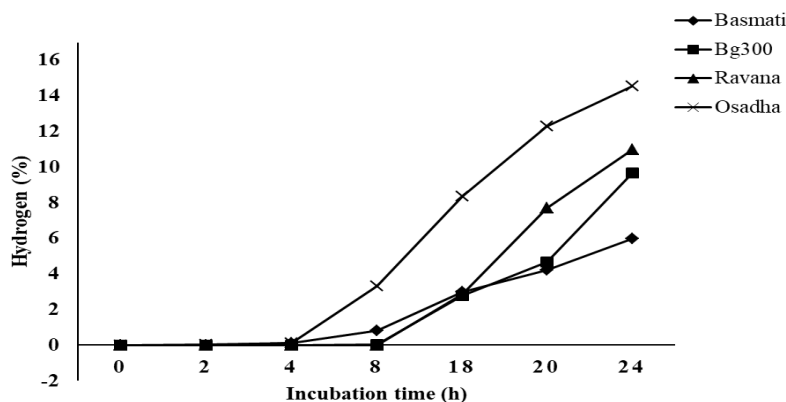


Figure 1 Hydrogen gas production by Basmati, Bg300, Ravana, and Osadha samples during the *in vitro* microbial fermentation at 39 °C.

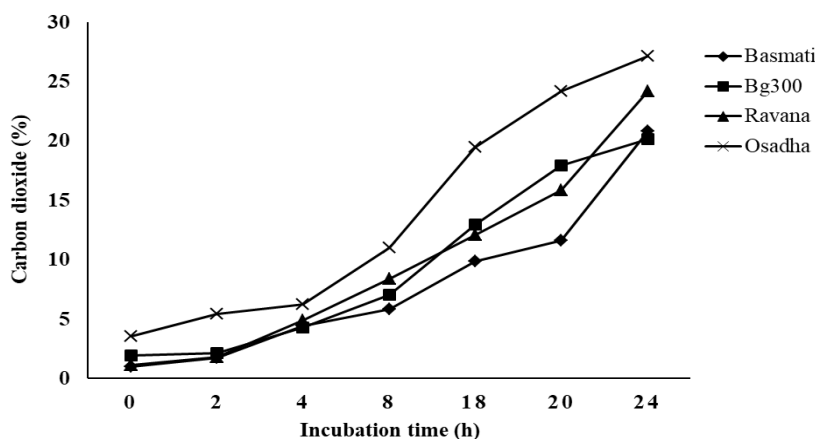


Figure 2 Carbon dioxide gas production by Basmati, Bg300, Ravana, and Osadha samples during the *in vitro* microbial fermentation at 39 °C.

The amount of gas produced during the incubation time with cecal microflora indicates the fermentability of dietary fiber in the samples. The production of hydrogen gas has increased with the incubation time in all four varieties. Osadha variety showed the highest hydrogen production. At the initial stage, hydrogen production of Basmati rice was higher than that of Ravana and Bg300, and then gradually decreased at the latter part of the incubation period. For Ravana and Bg300, the same trend of hydrogen production could be identified up to 18th h of incubation. After that, the hydrogen production of Ravana was higher than that of Bg300 (Figure 1). Production of hydrogen gas by the four samples during the incubation time followed the expected trend concerning the dietary fiber content. Similar to hydrogen, carbon dioxide production has increased with the incubation time in all four varieties and Osadha had the highest carbon dioxide production. However, the lowest carbon dioxide production was from Bg300 (Figure 2).

The soluble fiber in food undergoes microbial fermentation in the colon of humans and produces short-chain fatty acids (acetate, butyrate, and propionate). Poorly fermented insoluble fiber holds water and accelerates the intestinal transit time [27]. Butyrate is an energy source to the colonic epithelium and propionate aids in inhibiting the cholesterol synthesis [28]. In the present study, food samples were fermented using unadapted cecal microflora of swine. The pH

and incubation temperature were adjusted to simulate the real physiological conditions inside the body. Since the colon of swine is rather similar to humans, swine is the most appropriate animal model for fermentation studies of humans [29].

4. Conclusion

The nutritional composition of two finger millet varieties and two rice varieties differ widely. Osadha finger millet variety is comparatively high in protein and low in fat. Finger millet varieties used for the present study are rich sources of dietary fiber, phenolic, and flavonoid compounds compared to rice. Especially, the Osadha finger millet variety has a higher potential to use as an ingredient in the functional food industry due to its nutraceutical properties. However, further investigations should be done to evaluate the changes in nutritional composition and nutraceutical properties of finger millet with processing.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors, Priyanwada Lansakara, Ruvini Liyanage, Barana Jayawardana and Janak Vidanarachchi declare that there is no conflict of interest.

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