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Research Article

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In vitro starch digestibility and glycemic response of formulated barnyard millet bread

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ABSTRACT

Barnyard millet is a grain crop of drought resistant and it is generally grown as a rainfed crop and consumed just like rice with potential benefits to human health. Its digestibility of protein is 40 per cent. However, processed barnyard millets digestion properties have not been reported. So in this study, the in vitro starch digestibility and in vivo glycemic indices (GI) of raw, boiled, roasted and germinated barnyard millet incorporated bread products were investigated at the percentage of 10,20,30,40, and 50 replacing the wheat flour. The results showed that the kinetic constant, k, which reflects the rate of hydrolysis in the early stage, ranged between 0.0295 and 0.1559. The k was the lowest in roasted barnyard millet bread, and high in raw barnyard millet bread. More interestingly, the trend of $C\infty$ and k were not fully consistent with each other. The highest incorporation of the barnyard millet flour (50%) incorporated bread showed the lower eGI. Among various processing technique, all variations of germinated barnyard millet breads showed low eGI followed by raw barnyard millet breads. Medium eGI was observed in all variations of both boiled and roasted barnyard millet breads. Therefore, barnyard millet, especially the processed millet, may serve as a potential source of nutraceutical and functional food that could delay the development of type 2 diabetes.

KeyWords: Barnyard millet, Bread, Starch digestibility, Glycemic Index

Introduction

India showed a sharp increase in the epidemic of diabetes, from 33 million people in the year 2000 to 72 million people in 2021. This is further set to reach a 125 million by 2045[1]. Recent studies indicate a consistent drop in the mean age of people with type 2 diabetes (T2DM), despite the fact that the main causes of the disease burden have decreased death rates and an aging population[2-4]. As is well known, postprandial blood glucose regulation is significantly influenced by the amount and quality of dietary carbohydrates[5]. Numerous studies have demonstrated that slowly absorbed and digested carbohydrates are independently linked to a lower risk of type 2 diabetes[6-8], and a number of official dietary guidelines have recommended making meal choices based on the glycemic index (GI) [9]. Many factors may decrease the rate and extent of starch digestion and subsequently GI values, including the enzyme resistance of amylose–lipid complexes[10]. Nowadays, it is possible to produce low-GI foods, such as millet, pasta and foods containing modified starch, by controlling the ingredients and processing conditions[11].

Millets are labelled as today's nutri-cereals" and "yesterday's coarse grains. They are considered to be "future crops" as they are resistant to most of the diseases and acclimate well to the harsh environment of the arid and semi-arid regions of Asia and Africa[12.] One of the pioneering and significant minor millets in the semiarid regions of Asia and Africa is barnyard millet (Echinochloa sp.)[13]. A crop that grows in unfavorable environments, such as dry regions, barnyard millet requires little input. It is the most abundant source of carbs and has a better nutritional value; wholegrains provide 71.87% carbohydrates[14], whereas polished grains have 89.06%[15]. Barnyard millet is composed of starch (66%), protein (15%), lipids (7%), fibers (12.6%), minerals, vitamins, essential amino acids[16], and numer-

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ous micronutrients[17]. The barnyard millet starch granules are typically a mixture of spherical and polygonal shapes (6.46–12.23 μm)[18]. Research has reported that in comparison with foxtail, proso, kodo, and little millet, barnyard millet starch has a lower potential to increase blood glucose, serum cholesterol, and triglyceride levels[19]. Millets are habitually processed before consumption to extend the shelf life, improve nutritional and sensory properties. Primary processing techniques such as dehulling, soaking, germination, roasting, drying, polishing and milling (size reduction) are followed to make millets fit for consumption. Modern processing methods such as fermenting, parboiling, cooking, puffing, popping, malting, baking, flaking, extrusion, etc., are also used to develop millet-based value-added processed food products[20] These processing techniques aim to enhance the digestibility and nutrient bioavailability of the developed products[21].

Bread is a food consumed all over the world by all age people. It is prepared from white flour, yeast, sugar, fat, salt, water, etc., by a series of operations like mixing, kneading, fermentation, proofing and baking[22]. Millets contain no gluten, so they are not suitable for raised bread. When combined with wheat, they can be used for raised bread[23]. However, there are still limited studies regarding the starch digestion characteristics and invitro glycemic responses of processed barnyard millet incorporated breads.Therefore, the objectives of this study were to determine the effects of different processing methods on the in vitro starch digestion characteristics, and the estimated glycemic index (eGI) of the processed barnyard millet incorporated breads.

Methodology

Collection of Barnyard millet samples

Barnyard millet (*Echinochloa esculenta*), along with wheat flour, yeast, sugar, salt, and fat, was sourced from local markets in Salem district, Tamil Nadu, India. The millet grains underwent a sedimentation process to remove sand, grits, and other heavy impurities. After cleaning, the grains were first dried in the shade for 24 hours, followed by sun drying, and then stored in airtight containers for later use.

Processing of Barnyard millet into flour

Barnyard millet was processed using three methods namely boiling, roasting and germination as per the given procedures. For boiling a batch of Barnyard millet was held in a pan of boiling water (1:3 grain to water, weight to volume basis) for 10 minutes. For roasting required amount of barnyard millet was roasted in an open pan for 10 minutes at 190°C. For germination, barnyard millet was soaked in water overnight. The water was drained and the grains will be tied in a moist muslin cloth and left to sprout at room temperature for 72hrs. Later both the boiled and germinated grains were laid out on steel trays in thin layers of less than 2 cm. The trays were placed in a hot air oven and dried at 50°C for 24hrs. The dried samples were milled to flour using a hammer mill and stored in an air tight polythene bag in a cool and dry place until further use.

Bread formulation and preparation

Five different proportions (10%, 20%, 30%, 40%, and 50%) of millet flour—processed in raw, boiled, roasted, and germinated forms—were used to partially substitute wheat flour in bread formulations. Each variation included 3 g of yeast, 9 g of sugar, 3 g of fat, 0.5 g of salt, and a sufficient amount of water. In total, 20 different formulations were developed along with a control sample for further evaluation. Bread was prepared using the straight dough method. All flours were combined with 60% (w/w) water, along with a sugar-yeast solution and other ingredients, and mixed in a planetary mixer until a uniform dough was achieved. Hydration levels were monitored during mixing. The dough was kneaded for 10–12 minutes, then manually punched and left to rest for 10–15 minutes—a step referred to as the bench rest, where the initial fermentation takes place. Following this, the dough was shaped and placed in pre-oiled molds to prevent sticking. These were then kept in a proofer at 35–36°C for 50–55 minutes. After proofing, the dough was baked in a preheated deck oven set to 210°C (top) and 200°C (bottom) for 45–50 minutes. Prior to consumption, the bread was reheated in a microwave for 20 seconds.

Proximate composition

All the formulated processed barnyard millet incorporated breads were evaluated for the protein and fat content using the Dumas method and a nitrogen-to-protein conversion factor of 6.25 (AOAC 992.23-1992, 1998; ISO 16634, 2005)[24] and Folch, Lees, and Sloane Stanley (1957)[25] respectively.

In vitro starch digestibility

The samples were analyzed for in vitro starch digestion based on Englyst et al.[26] with some modifications. The samples, which included roughly 0.5 g of starch, were distributed out in 50 mL centrifuge tubes with two glass balls in 25.0 mL of acetate buffer (0.1 M, pH 5.2). Following a vigorous vortex mix, the tubes were cooled to 37 °C and immersed in a boiling water bath for 30 minutes. Invertase

(3000 U mL−1, 0.3 mL) was then added, vortexed, and incubated for 30 minutes at 37 °C. In the end, to get the free glucose (FG) fraction, 0.2 mL of each sample was added to 4 mL of pure ethanol and thoroughly mixed. As previously mentioned, the remaining samples were mixed with 10.0 mL of freshly made pepsin solution (5.0 g L−1 pepsin and 5.0 g L−1 guar gum in 0.05 mol L−1 HCl, 5 glass balls), put in a water bath at 37 °C for 30 minutes, and then add 10 mL of acetate buffer (0.1 M, pH 5.5, 37 °C) came in. The enzyme mixture was made by dispersing 3.0 g of pancreatin in 20.0 mL of water using a magnetic stirrer for 10 minutes, centrifuging at 1500g for 10 minutes to obtain pancreatin supernatant (15.0 mL), and then adding 0.75 mL of amyloglucosidase (1200 U mL−1) and 1 mL of invertase (3000 U mL−1) to the mixture. A 5.0 mL of the enzyme mixture was added to begin the starch digestion process. The samples were shaken horizontally at 160 rpm for two hours while being digested at 37 °C. 0.2 mL of each sample was added to 4.0 mL of pure ethanol and well mixed to obtain the glucose portion for 20 min (G_{20}) and 120 min (G_{120}) following precisely 20 and 120 minutes of digestion. After collecting 0.2 ml of G120 samples, the tubes underwent a thorough vortex mix. Following 30 minutes of boiling water incubation, the contents were cooled to 0 °C and combined with 10.0 mL of potassium hydroxide (7.0 mol L−1). To determine the total glucose portion (TG), 0.2 mL of each sample was added to 1.0 mL of 1.0 mol L−1 acetic acid containing 40.0 µL of amyloglucosidase (100.0 U mL−1) after 30 minutes of ice-water incubation. The mixture was then placed in a water bath at 70 °C for 30 minutes and a boiling water bath for 10 minutes. Finally, it was cooled to room temperature and 20.0 mL of water was added. All the aforementioned samples (FG, G_{20}, G_{120}, and TG) were centrifuged for five minutes at 1500 g. A GOD-POD diagnostic kit was used to measure the amount of glucose in the supernatant using the glucose oxidase-peroxidase technique. Thermo Scientific Multiskan GO (Thermo Fisher Scientific, MA, USA) was used to measure the OD values (x-axis). To create a standard curve (y = 4526x − 21.7, R2 = 0.9998), standard glucose solutions with concentrations of 125.0, 250.0, 500.0, 1000.0, and 2000.0 µM L−1 each were simultaneously placed through the same tests.

According to Englyst et al.[27] from the data of in vitro starch digestion, the contents of different starch fractions such as Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS), as well as the contents of different available glucose fractions: Rapidly Available Glucose (RAG) and Slowly Available Glucose (SAG) on a dry basis were calculated. The results of this study were expressed as a percentage of total starch or total available glucose.[28]

Estimated Glycemic Index (eGI)

The estimated Glycemic Index (eGI) and in vitro starch digestion kinetics were determined based on glucose concentrations measured at different time intervals during the hydrolysis process—specifically at 20, 40, 60, 80, 100, 120, and 180 minutes. The percentage of starch hydrolyzed at any given time (t) was represented by **C**, with **C∞** indicating the maximum extent of hydrolysis and **K** as the rate constant. These parameters were applied to the first-order kinetic model: [C = C∞ (1 − e−kt) [28]. To compute the Hydrolysis Index (HI), the area under the hydrolysis curve (AUC) for each millet bread sample was compared to the AUC of a reference food—fresh white bread. The estimated Glycemic Index was then calculated using the equation: **eGI = 39.71 + 0.549 × HI**, with glucose serving as the reference standard (glucose GI = 100). The resulting value was then multiplied by 0.7 to yield the final eGI for the bread samples.

Statistical analysis

For every analysis, a minimum of three replicates were conducted. SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses. For in vitro digestibility, the mean ± standard deviation (SD) was used. Duncan's Post Hoc test was performed after a One-Way ANOVA, and a p value of less than 0.05 was deemed significant.

Results and Discussion

Effects of processing on protein and lipid content of the processed barnyard millet breads

Among various processing methods, the protein content of roasted barnyard millet flour incorporated breads was the lowest. By comparison, it can be seen that the content of protein increased slightly in both raw and boiled barnyard millet added bread but significantly in germinated barnyard millet incorporated breads. Ravi and Rana (2024)[29] from their study revealed that fermentation can improve the digestibility of millet and accelerate the body's absorption of it by partially predigesting complex proteins and carbohydrates. Including fermentation in the millet processing process can enhance its nutritional value, taste, and overall appeal as a nutritious food choice (Taylor and Kruger, 2019)[30]. Regarding the lipid content, among various processing methods, the lowest was observed in the breads incorporated boiled and roasted barnyard millet flour followed by the raw barnyard millet flour but significantly slightly more lipid content was observed in the germinated barnyard millet incorporated breads (Table 1). High-temperature processing o millets, such as roasting, puffing, and popping, can degrade fats, leading to reduced fat content and potential rancidity issues due to lipolysis and oxidation of fatty acids[31]. On the other hand, simple processing techniques like soaking, germination, and malting can enhance protein digestibility and mineral bioavailability while maintaining a lower fat content [32].

Table 1 Protein and lipid content of raw and processed barnyard millet bread samples

Samples	Variations	Protein (g)	Lipid (g)
Wheat bread	Control	6.24±0.35^{a}	0.256±0.02^{a}
Raw Barnyard millet breads (RBM)	RBM1 (10%)	8.56±0.010^{d}	1.16±0.015^{d}
	RBM2 (20%)	8.66±0.030^{de}	1.263±0.02^{de}
	RBM3 (30%)	8.77±0.026^{e}	1.33±0.015^{e}
	RBM4 (40%)	8.95±0.032^{e}	1.373±0.005^{e}
	RBM5 (50%)	9.05±0.036^{f}	1.423±0.02^{e}
Boiled Barnyard millet breads (BBM)	BBM1 (10%)	8.02±0.015^{c}	0.66±0.015^{c}
	BBM2 (20%)	8.24±0.010^{cd}	0.74±0.03^{c}
	BBM3 (30%)	8.35±0.025^{d}	0.84±0.01^{cd}
	BBM4 4(0%)	8.41±0.010^{d}	0.88±0.005^{d}
	BBM5 (50%)	8.56±0.020^{d}	0.92±0.015^{d}
Roasted Barnyard millet breads (RoBM)	RoBM1 (10%)	7.63±0.015^{b}	0.36±0.01^{b}
	RoBM2 (20%)	7.72±0.015^{b}	0.44±0.01^{bc}
	RoBM3 (30%)	7.85±0.036^{c}	0.58±0.01^{c}
	RoBM4 (40%)	7.97±0.026^{c}	0.62±0.015^{c}
	RoBM5 (50%)	8.02±0.015^{c}	0.67±0.015^{c}
Germinated Barnyard millet breads (GBM)	GBM1 (10%)	9.12±0.015^{f}	1.47±0.01^{e}
	GBM2 (20%)	9.24±0.010^{f}	1.53±0.01^{e}
	GBM3 (30%)	9.46±0.015^{fg}	1.58±0.011^{e}
	GBM4 (40%)	9.62±0.222^{g}	1.67±0.026^{ef}
	GBM5 (50%)	9.97±0.015^{h}	1.75±0.015^{f}

All values are in Mean ± SD, ($p<0.05$); different letters used in the same column as superscripts indicate significant difference between the means

Effects of different processing methods on the starch digestion of the processed barnyard millet breads

In the present study, the effects of different processing methods such as boiling, roasting and germination on starch digestion of barnyard millet incorporated breads were investigated (Table 2). The Rapidly Digestible Starch (RDS) content showed a mild variation from 34-35 % between the processed barnyard millet breads and the significantly lowest RDS was observed in the control bread sample. Regarding the results on Slowly Digestible Starch (SDS) content all the barnyard millet bread samples showed the variation between 42-44% of SDS and no significant difference was found between the SDS content including the control bread sample. No significant difference was observed within the raw, roasted and germinated barnyard millet bread samples regarding the total starch content but significant difference was observed between the processed barnyard millet breads compared to control bread except roasted barnyard millet breads. The Resistance Starch (RS) content showed the range between 4-6% and also significant difference between the processed barnyard millet breads. This phenomenon was the most obvious in control bread, in which the RS content was 8% which is significantly higher compared to the other processed barnyard millet breads. RS is considered as a source of dietary fibre and can provide a number of beneficial effects.

From the point of available glucose, the Rapidly Available Glucose (RAG) content ranged from 40.35 ± 0.01 to 42.55 ± 0.02 after various processing methods, and significant difference was observed between the various processed bread samples and also with the control bread. No significant difference was observed within and between the processed barnyard millet bread samples regarding the SAG content including the control bread. The FG content showed the range between 2-3% and also significant difference between the processed barnyard millet breads. This phenomenon was the most obvious in control bread, in which the FG content was 1.84% which is significantly lower compared to the other processed barnyard millet breads. Like FG, similar trend was observed for the total glucose also among processed barnyard millet breads. Various researches reported that, there were many factors contributing to the in vitro starch digestibility, such as amylose content, type of cultivar, partial size, processing and storage conditions [33-34].

Table 2 Effect of different processing methods on total starch fractions and available glucose levels of barnyard millet breads

Samples	Variations	Total starch fraction				Available glucose			
		RDS%	**SDS%**	**TS %**	**RS%**	**RAG%**	**SAG%**	**FG%**	**TG%**
Wheat bread	Control	27.27 ± 0.015^{a}	46.97 ± 0.026^{b}	82.27 ± 0.023^{a}	8.026 ± 0.03^{j}	32.146 ± 0.011^{a}	52.19 ± 0.026^{b}	1.84 ± 0.01^{a}	93.25 ± 0.015^{a}
Raw Barnyard millet breads (RBM)	RBM1 (10%)	34.93 ± 0.040^{b}	43.81 ± 0.023^{b}	84.13 ± 0.025^{d}	5.39 ± 0.036^{g}	41.66 ± 0.015^{de}	48.68 ± 0.023^{b}	2.75 ± 0.025^{e}	96.24 ± 0.03^{de}
	RBM2 (20%)	34.98 ± 0.017^{b}	43.82 ± 0.020^{b}	84.21 ± 0.034^{d}	5.40 ± 0.04^{g}	41.65 ± 0.02^{de}	48.69 ± 0.02^{b}	2.78 ± 0.005^{ef}	96.35 ± 0.037^{e}
	RBM3 (30%)	34.99 ± 0.011^{b}	43.84 ± 0.032^{b}	84.22 ± 0.005^{d}	5.38 ± 0.023^{g}	41.73 ± 0.02^{e}	48.71 ± 0.037^{b}	2.85 ± 0.01^{ef}	96.43 ± 0.015^{e}
	RBM4 (40%)	35.03 ± 0.020^{b}	43.81 ± 0.020^{b}	84.25 ± 0.011^{d}	5.41 ± 0.015^{g}	41.85 ± 0.034^{e}	48.67 ± 0.025^{b}	2.93 ± 0.02^{f}	96.54 ± 0.03^{ef}
	RBM5 (50%)	35.03 ± 0.040^{b}	43.84 ± 0.035^{b}	84.27 ± 0.015^{d}	5.38 ± 0.015^{g}	41.95 ± 0.025^{e}	48.72 ± 0.04^{b}	3.02 ± 0.02^{fg}	96.66 ± 0.026^{f}
Boiled Barn-yard millet breads (BBM)	BBM1 (10%)	34.67 ± 0.015^{b}	43.72 ± 0.005^{b}	83.24 ± 0.017^{b}	4.84 ± 0.017^{b}	41.05 ± 0.005^{d}	48.58 ± 0.005^{b}	2.53 ± 0.01^{d}	95.02 ± 0.01^{c}
	BBM2 (20%)	34.71 ± 0.015^{b}	43.73 ± 0.017^{b}	83.31 ± 0.026^{bc}	4.86 ± 0.020^{bc}	41.16 ± 0.01^{d}	48.59 ± 0.017^{b}	2.58 ± 0.005^{d}	95.15 ± 0.03^{c}
	BBM3 (30%)	34.76 ± 0.017^{b}	43.72 ± 0.025^{b}	83.38 ± 0.011^{bc}	4.89 ± 0.020^{cde}	41.24 ± 0.01^{d}	48.58 ± 0.025^{b}	2.62 ± 0.01^{de}	95.26 ± 0.02^{c}
	BBM4 4(0%)	34.80 ± 0.030^{b}	43.68 ± 0.015^{b}	83.41 ± 0.017^{bc}	4.92 ± 0.023^{ef}	41.34 ± 0.02^{d}	48.53 ± 0.015^{b}	2.67 ± 0.015^{de}	95.35 ± 0.025^{c}
	BBM5 (50%)	34.85 ± 0.011^{b}	43.62 ± 0.011^{b}	83.43 ± 0.015^{c}	4.95 ± 0.005^{f}	41.46 ± 0.02^{de}	48.47 ± 0.011^{b}	2.74 ± 0.03^{e}	95.44 ± 0.015^{cd}
Roasted Barn-yard millet breads (RoBM)	RoBM1 (10%)	34.31 ± 0.017^{b}	43.10 ± 0.005^{b}	82.20 ± 0.026^{a}	4.783 ± 0.02^{a}	40.35 ± 0.015^{b}	47.89 ± 0.005^{b}	2.23 ± 0.032^{bc}	93.57 ± 0.01^{ab}
	RoBM2 (20%)	34.36 ± 0.020^{b}	43.06 ± 0005^{b}	82.27 ± 0.020^{a}	4.84 ± 0.030^{b}	40.44 ± 0.01^{bc}	47.84 ± 0.011^{b}	2.25 ± 0.015^{bc}	93.66 ± 0.032^{b}
	RoBM3 (30%)	34.42 ± 0.011^{b}	43.00 ± 0.026^{b}	82.28 ± 0.017^{a}	4.85 ± 0.025^{bc}	40.57 ± 0.005^{bc}	47.77 ± 0.03^{b}	2.32 ± 0.015^{c}	93.74 ± 0.030^{b}
	RoBM4 (40%)	34.45 ± 0.04^{c}	43.01 ± 15.53^{b}	82.32 ± 0.025^{a}	4.86 ± 0.015^{bcd}	40.65 ± 0.04^{c}	47.80 ± 0.06^{b}	2.38 ± 0.01^{c}	93.85 ± 0.025^{b}
	RoBM5 (50%)	34.47 ± 0.035^{b}	42.98 ± 0.005^{b}	82.37 ± 0.025^{a}	4.90 ± 0.005^{de}	40.75 ± 0.01^{c}	47.76 ± 0.011^{b}	2.44 ± 0.04^{cd}	93.97 ± 0.01^{b}
Germinated Barnyard millet breads (GBM)	GBM1 (10%)	35.09 ± 0.023^{b}	44.54 ± 0.020^{b}	85.61 ± 0.450^{e}	6.24 ± 0.010^{i}	42.146 ± 0.015^{f}	49.49 ± 0.02^{b}	3.15 ± 0.01^{g}	98.57 ± 0.011^{g}
	GBM2 (20%)	35.13 ± 0.020^{b}	44.56 ± 0.020^{b}	85.89 ± 0.030^{f}	6.206 ± 0.015^{hi}	42.25 ± 0.01^{f}	49.51 ± 0.02^{b}	3.22 ± 0.01^{gh}	98.65 ± 0.025^{g}
	GBM3 (30%)	35.17 ± 0.011^{b}	44.52 ± 0.011^{b}	85.92 ± 0.015^{f}	6.22 ± 0.020^{hi}	42.36 ± 0.02^{f}	49.47 ± 0.011^{b}	3.28 ± 0.01^{h}	98.75 ± 0.025^{g}
	GBM4 (40%)	35.21 ± 0.025^{b}	44.53 ± 0.047^{b}	85.93 ± 0.028^{f}	6.19 ± 0.055^{hi}	42.46 ± 0.015^{fg}	49.48 ± 0.052^{b}	3.34 ± 0.01^{h}	98.82 ± 0.02^{g}
	GBM5 (50%)	35.22 ± 0.025^{b}	44.53 ± 0.030^{b}	85.97 ± 0.015^{f}	6.12 ± 0.023^{hi}	42.55 ± 0.025^{fg}	49.48 ± 0.035^{b}	3.42 ± 0.02^{hi}	98.94 ± 0.015^{g}

RDS-Rapidly Digestible starch, SDS-Slowly Digestible Starch, TS- Total Starch, RS-Resistant Starch, RAG-Rapidly Available Glucose, SAG-Slowly Digestible Glucose, FG-Free Glucose, TG-Total Glucose; All values are in Mean ± SD, ($p<0.05$); different letters used in the same column as superscripts indicate significant difference between the means

Effects of different processing methods on the in vitro starch digestibility and estimated glycemic index of the raw and processed barnyard millet breads

The kinetics of in vitro starch digestibility and eGI of raw and processed barnyard millet breads at 180 minutes are listed in Table 3. The maximum equilibrium concentration, C∞, ranged between 29.17 ± 0.02 and 43.34 ± 0.01. These results were obviously lower than those of gluten-free breads with an average of 96.5[35]. The kinetic constant, k, which reflects the rate of hydrolysis in the early stage, ranged between 0.0295 and 0.1559. The k was the lowest in roasted barnyard millet bread, and high in raw barnyard millet bread. More interestingly, the trend of C∞ and k were not fully consistent with each other. The highest incorporation of the barnyard millet flour (50%) incorporated bread showed the lower eGI. Among various processing technique, all variations of germinated barnyard millet breads showed low eGI followed by raw barnyard millet breads. Medium eGI was observed in all variations of both boiled and roasted barnyard millet breads.

Conclusions

Results of this study confirmed that the processed barnyard millet incorporated breads had a low and moderate estimated glycemic index. Furthermore, different processing methods of barnyard millet had a great influence on the *in vitro* starch digestibility and glycemic response of barnyard millet, which suggested that in daily life, it is necessary for humans to select appropriate processing methods according to their own health conditions. This research study will be beneficial to promote the development of millet industry and to popularize the value-added millet-based foods.

Table 3 In vitro glycemic responses of raw and processed barnyard millet incorporated bread at 180 minutes

Samples	Variations	C∞ (%)	K (min)	HI (%)	GI (%)
Wheat bread	Control	49.55 ± 0.02	0.0305 ± 0.0000	46.73 ± 0.02	65.37 ± 0.01
Raw Barnyard millet breads	RBM1 (10%)	35.84 ± 0.03	0.0682 ± 0.0000	29.33 ± 0.02	55.81 ± 0.01
	RBM2 (20%)	35.35 ± 0.02	0.0746 ± 0.0000	28.62 ± 0.02	55.42 ± 0.01
	RBM3 (30%)	34.74 ± 0.03	0.0898 ± 0.0000	27.58 ± 0.03	54.85 ± 0.02
	RBM4 (40%)	33.75 ± 0.04	0.1155 ± 0.0000	26.59 ± 0.03	54.31 ± 0.01
	RBM5 (50%)	33.64 ± 0.04	0.1559 ± 0.0000	25.56 ± 0.03	53.74 ± 0.02
Boiled Barnyard millet breads	BBM1 (10%)	39.54 ± 0.03	0.0413 ± 0.0000	34.96 ± 0.02	58.90 ± 0.01
	BBM2 (20%)	39.03 ± 0.03	0.0523 ± 0.0000	33.15 ± 0.02	57.91 ± 0.01
	BBM3 (30%)	38.35 ± 0.02	0.0581 ± 0.0000	32.06 ± 0.02	57.31 ± 0.01
	BBM4 4(0%)	37.56 ± 0.03	0.0592 ± 0.0000	31.32 ± 0.02	56.90 ± 0.02
	BBM5 (50%)	36.66 ± 0.04	0.0637 ± 0.0000	30.26 ± 0.04	56.32 ± 0.02
Roasted Barnyard millet breads	RoBM1 (10%)	43.34 ± 0.01	0.0295 ± 0.0000	41.20 ± 0.01	62.33 ± 0.01
	RoBM2 (20%)	42.56 ± 0.03	0.0302 ± 0.0000	40.23 ± 0.03	61.80 ± 0.02
	RoBM3 (30%)	41.96 ± 0.03	0.0339 ± 0.0000	38.63 ± 0.03	60.92 ± 0.02
	RoBM4 (40%)	41.37 ± 0.01	0.0375 ± 0.0000	37.28 ± 0.01	60.18 ± 0.01
	RoBM5 (50%)	40.66 ± 0.04	0.0425 ± 0.0000	35.76 ± 0.04	59.34 ± 0.02
Germinated Barnyard millet breads	GBM1 (10%)	33.28 ± 0.01	0.0808 ± 0.0000	26.70 ± 0.01	54.37 ± 0.00
	GBM2 (20%)	31.96 ± 0.02	0.0808 ± 0.0000	25.64 ± 0.02	53.79 ± 0.01
	GBM3 (30%)	30.35 ± 0.03	0.0789 ± 0.0000	24.41 ± 0.03	53.11 ± 0.02
	GBM4 (40%)	29.75 ± 0.04	0.0852 ± 0.0000	23.73 ± 0.03	52.74 ± 0.02
	GBM5 (50%)	29.17 ± 0.02	0.0996 ± 0.0000	22.92 ± 0.02	52.29 ± 0.01

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Conflicts of Interest/Competing Interests

The authors declare that they have no conflicts of interest or competing interests related to this research. There are no financial, personal, or professional relationships that could influence the work reported in this study.

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