

Quantification of Riboflavin and Thiamine in GI (Geographical Indication) Branded Yogurts Collected from Bogura, Bangladesh Using HPLC Equipped with a Fluorescence Detector

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Abstract

Thiamine (vitamin B1) and riboflavin (vitamin B2) are both essential vitamins for human health. Thiamine (vitamin B1) is essential for energy metabolism, nervous system function, carbohydrate metabolism, cardiovascular health, digestion, muscle function, etc. On the other hand, Riboflavin (vitamin B2), which acts as an antioxidant, is essential for energy production, healthy skin, hair and nails, eye health, red blood cell production, nervous system function, etc. Due to its specialty, Bogura's famous yogurt was recognized as a Geographical Indication (GI) on June 26, 2023 by the Directorate of Patents, Designs, and Trademarks (DPDT) under the Ministry of Industry, the Government of the People's Republic of Bangladesh (Prothom Alo, 2023). The purpose of the study was the quantitative determination of two water-soluble vitamins, thiamine and riboflavin, in the GI branded yogurt and whey (Ghol), collected from Bogura, Bangladesh to ensure health benefits. The methods employed for the quantitative analysis of these vitamins were reversed-phase high performance liquid chromatography (RP-HPLC) equipped with a fluorescence detector. The extraction process involved acid hydrolysis followed by enzymatic hydrolysis with takadiastase enzyme. Chromatographic separation was achieved with a Shimadzu Prominence HPLC system using isocratic elution mode on a C18 column (4.6 mm × 250 mm, 5 μm). The correlation coefficients for thiamine and riboflavin were 0.9975 and 0.9974, respectively. The recovery rates for thiamine and riboflavin were found as 97.70% and 105.40%, respectively. Twenty-two samples were analyzed. Thiamine and riboflavin were found in the range of 0.0647 to 0.1396 mg/100 g and 0.0227 to 0.3749 mg/100 g, respectively. It can be concluded that these yogurts were rich in thiamine and riboflavin. Therefore, these yogurts are beneficial for health.

Key words: GI brand, yogurt, whey, thiamine, riboflavin, bogura.

Introduction

Yogurt is a food produced by the bacterial fermentation of milk (FDA, 2016). The bacteria used to make yogurt are known as yogurt cultures. Fermentation of sugars in the milk by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic tart flavour (FDA, 2016). The milk that is most frequently used to produce yogurt is cow's milk. Yogurt can also be made from the milk of buffalo, goats, ewes, mares,

camels and yaks. It can be raw or pasteurized. Every variety of milk yields notably distinct outcomes. Yogurt is made by microorganisms called *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Furthermore, extra lactobacilli and bifidobacteria can be added before to, during, or subsequent to yogurt culture. Certain countries have laws requiring yogurt to have a minimum of one million colony-forming units (CFU) of lactobacillus per millilitre (Lee *et al.*, 2012). The

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bacterial culture is added and fermentation which is allowed to take place for 4 to 12 hours at a warm temperature of 30-45°C (86–113°F). Higher temperatures operate more quickly but run the risk of a lumpy texture or whey separation (Clark, 2017).

The history of yogurt of Bogura, also known as Bogurar Doi, is extensive. This business was founded by the milkman families about 200 years ago. The yogurt of Bogura is credited to Ghetu Ghosh of Sherpur in the Bogura district. He used to walk 20 kilometres from Sherpur Upazilla to Bogura to sell his yogurt. However, in Bogura, Gaur Gopal Ghosh is credited with creating creamed yogurt (https://en.wikipedia.org/wiki/Curd_of_Bogra, Azad, 2016). Sir John Anderson, President Yahya Khan, Queen Victoria, Elizabeth II and many other well-known people consumed yogurt from Bogura. To influence them, Yahya Khan sent the meals to influential people (<https://www.boguratribune.com/?p=618>). Even though the history of Bogura yogurt is just around 2.5 years old, the Golden Age took place before India attained independence (Bashar, 2014). At the time, it was drafted in a very private manner. This degree of confidentiality could no longer be maintained. In Sherpur, yogurt manufacturing is currently quite popular. There are much fewer members of the Ghosh family among them (Shanto, 2021). Making yogurt of Bogura requires a clay pot, some old curd, sugar, and cow's milk. To make the yogurt, milk is cooked in a pan (Prothom Alo, 2015)

Yogurt is frequently associated with probiotics because it may contain live cultures, which have been theorized to have advantageous effects on immunological, cardiovascular, or metabolic health (Gijbsbers *et al.* 2016). High-quality clinical evidence was lacking as of the early 21st century to draw the conclusion that ingesting yogurt lowers the risk of illness or otherwise enhances health (Ong *et al.*, 2019). According to meta-analyses, postmenopausal women who consume 80 grams of low-fat yogurt daily have a decreased incidence of hip fractures and a lower risk of type 2 diabetes (Gijbsbers *et al.* 2016). According to an analysis published in 2021, eating

yogurt may improve bone health and lower risk of developing certain diseases, including cancer and the metabolic syndrome. Additionally, a link was found between yogurt consumption and better lactose tolerance and digestion (Savaiano *et al.*, 2020).

When milk has been strained and curdled, the liquid that is left over is called whey. It is a by-product of the production of cheese or casein and has a number of industrial applications. Sweet whey is a by-product produced during the production of rennet-based hard cheeses like Swiss and cheddar. Acid whey, commonly referred to as sour whey, is a by-product produced when acid dairy products, like strained yogurt, are produced. Whey proteins are made up of immunoglobulins, protease peptones, serum albumin, lacto globulin and lacto globulin (Farrell *et al.*, 2004). Whey should not be consumed by people who are lactose intolerant because it contains lactose.

In addition to traces of fat, liquid whey also contains lactose, vitamins, protein and minerals. Whey can help manage and lessen blood sugar rises in people with type 2 diabetes by enhancing insulin secretion, according to research from Lund University in Sweden (Frid *et al.*, 2005). Dairy products produce stronger insulin responses (Insulin index, II, 90–98) than expected from their comparatively low glycemic indices (GI 15–30). Insulinogenic effects from dairy products have been seen in healthy subjects, both when taken as a single meal and when added to a mixed meal. The insulin-releasing capacity of dairy products has been linked to the protein fraction, particularly the whey fraction and the subsequent release of amino acids after digestion has been hypothesized to underlie the insulinogenic features of milk (Nilsson *et al.*, 2005). Whey and other milk proteins can cause allergic reactions in certain people but this should not be confused with lactose intolerance. Whey-sensitive individuals may be able to tolerate evaporated, boiled or sterilized milk due to the fact that whey proteins are changed by high temperatures. Hard cheeses are the least allergenic for those who are allergic to whey proteins since they are high in casein but low in whey proteins. The most

significant allergens in cheese, however, are heat-stable casein proteins, and a person may be sensitive to one or both of these proteins.

One of the water-soluble B vitamins is thiamine which is also referred to as vitamin B1. This vitamin is essential for energy metabolism and consequently, for cell growth, development and function (Said *et al.*, 2010). Natural sources of thiamine include meat, fish and whole grains. Additionally, it is included in bread, cereals, infant formula, fortified breakfast cereals, pork, fish, beans, green peas, enriched cereals, bread, noodles, rice, sunflower seeds, yogurt, etc. Riboflavin, often known as vitamin B2, is a water-soluble vitamin that is frequently found in both plant-based and animal-based foods, including milk, meat, eggs, almonds, fortified grains and green vegetables (National Institutes of Health, 2018). It is an easily absorbed micronutrient that has an impact on many biological processes. Vitamin B2 is essential for numerous biological activities. Vitamin B2, which is important for energy metabolism as well as the metabolism of lipids, ketone bodies, carbohydrates and proteins, is required for these processes. It is necessary for the healthy growth of the skin, digestive system lining, blood cells and brain function.

Riboflavin is an antioxidant that helps free radicals change into safe forms. Riboflavin is frequently taken by people to relieve migraines, lower blood homocysteine levels and avoid riboflavin deficiency. Rock lobsters, milk, cheese, fortified bread and cereal, spinach, salmon, yogurt, chicken breast, liver, kidneys, mature soybeans, yeast and other legumes are all excellent sources of vitamin B2 (Somer, 1992). Thiamine (vitamin B1) and riboflavin (vitamin B2) are both essential vitamins for human health. Both of these improve the immune system and are essential for energy metabolism, nervous system function, cardiovascular health, skin health, eye health, antioxidant activity, red blood cell production, brain function, digestive health, stress management, etc.

Therefore, the objective of the study is the quantitative determination of thiamine and riboflavin

in yogurt and whey (Ghol) to ensure the benefit of health.

Materials and Methods

Chemicals and reagents: All the chemicals and reagents used in the extraction and analyses were of analytical grade and purchased from local suppliers. The HPLC grade solvents and standard vitamins were collected from Sigma Aldrich (now Merck), Germany.

Sample collection: Twenty-two yogurt and whey samples were collected from Sathmatha, Bogura (Bogura main town), a well-known city in Bangladesh on 4 April, 2023 and stored at 4 °C until work (Figure 1). Among these, 20 samples were yogurt [5 special yogurt, 5 sugarless (tok) yogurt, 5 cup yogurt and 5 premium (shahi) yogurt] and 2 samples were whey.

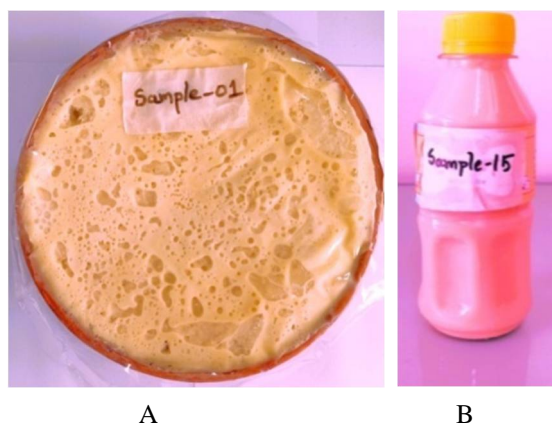


Figure 1. Samples of yogurt (A) and whey (B).

Extraction process: Accurately weighed 6 g of each sample was taken into a 125 ml Erlenmeyer flask. Next, about 60 ml of 0.1 M hydrochloric acid was added, followed by capping the flask with aluminum foil and mixing. Then the flasks were placed in a boiling water bath for 30 minutes, with further mixing at 10-minute intervals. The flasks were removed from the water bath and cooled to below 50°C. Then, 5 ml of a 10% takadiastase solution was added, and the flasks were capped and incubated in a 37°C water-bath for overnight. Afterward, the flasks were cooled to room temperature and quantitatively transferred into a 100 ml volumetric flask. It was

diluted to the volume with deionized water and then filtered through filter paper. Then the filtrate were collected in a 125 ml Erlenmeyer flask and was passed through a 0.45 µm membrane filter to collect an aliquot into a 2 ml amber glass vial for HPLC analysis (Puwastien *et al.*, 2011).

For the recovery study, the recovery sample was spiked with 2 ml of a 10 ppm standard solution.

Preparation of standard solutions for calibration curves: The standard vitamins solutions of 200 ppb, 400 ppb, 6000 ppb, 800 ppb and 1000 ppb were prepared in different Erlenmeyer flasks and referred as working standards. The working standards were also treated in the same manner as the samples, including being diluted to a final volume of 100 ml. An aliquot of each concentration was collected into a 2 ml amber glass vial, which had been prepared for HPLC analysis, by passing through a 0.45 µm filter unit.

Chromatographic conditions: The vitamins were analyzed according to previously published procedures in the ASEAN manual for nutrient analysis (Puwastien *et al.*, 2011). The analyses were carried out using the following conditions.

Conditions for thiamine determination:

Column: Reversed phase C18 (4.6 mm × 250 mm, 5 µm)

Mobile phase: 0.005 M hexanesulphonic acid in 85:15 methanol: water, pH 6.0

Oxidant: 0.001M potassium ferricyanide in 0.375 M sodium hydroxide

Detector: Fluorescence, excitation 360 nm, emission 435 nm

Injection Volume: 10 µl

Flow Rate: 1.0 ml/min

Conditions for riboflavin determination:

Column: Reversed phase C18 (4.6 mm × 250 mm, 5 µm)

Mobile phase: 50% methanol in water

Detector: Fluorescence, excitation 440 nm, emission 530 nm

Injection Volume: 10 µl

Flow Rate: 1.0 ml/min

The oxidant was introduced via T-piece, and it was positioned between the column and the detector.

The system was equilibrated until a stable baseline was obtained, and consistent peak height and retention times were ensured. The injector was then passed and the calibrating standards were run as a test injection to check system performance.

Preparation of mobile phase for thiamine (Puwastien *et al.*, 2011): 1.5469 g of 1-hexanesulfonic acid sodium salt monohydrate was measured accurately by a measuring device. 1275 ml of methanol and 225 ml of water were measured accurately by a measuring cylinder. Both of them were mixed together in a beaker to make a solution of 85% methanol in water. 1.5469 g of 1-hexanesulfonic acid sodium salt monohydrate was dissolved in the 1500 ml of 85% methanol in water. Then, 1 ml of glacial acetic acid was added, and the pH of the solution was adjusted to 6.0 by the addition of 5% aqueous sodium bicarbonate.

Preparation of mobile phase for riboflavin: 750 ml of methanol and 750 ml of water were measured accurately by a measuring cylinder (Puwastien *et al.*, 2011). Both of them were mixed together in a beaker to make a solution of 50% methanol in water. This solution was sonicated for 10 minutes. Then mobile phase were filtered by vacuum pump.

Calibration curves of thiamine and riboflavin: Five different concentrations of 200, 400, 600, 800 and 1000 ppb were prepared from the extracted standard of thiamine and riboflavin. Then 10 µl of each solution was injected into the HPLC using an auto-sampler. In the case of thiamine, the analysis was monitored by a fluorescence detector at 360 nm as the excitation wavelength and 435 nm as the emission wavelength, and for riboflavin, the analysis was monitored by a fluorescence detector at 450 nm as the excitation wavelength and 530 nm as the emission wavelength.

Results and Discussion

To prepare the standard calibration curves of thiamine and riboflavin, the peak areas were plotted

against concentrations. The calibration curves were found with the correlation coefficients for thiamine and riboflavin as 0.9975 and 0.9974, respectively (Figures 2 and 3).

Recovery study for thiamine: The concentration of the recovery sample (calculated from the standard calibration curve) was 847.2184 ppb. The concen-

tration of the control sample (calculated from the standard calibration curve) was 17.63856 ppb. The concentration of the added standard was 800 ppb.

$$\% \text{ Recovery} = \frac{(847.2184 - 17.63856) \times 100}{800} = 97.70$$

Calibration curve of riboflavin

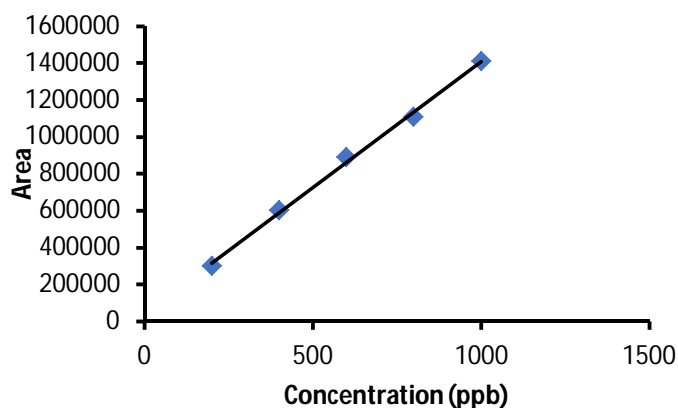


Figure 2. Calibration curve of riboflavin.

Calibration curve of thiamine

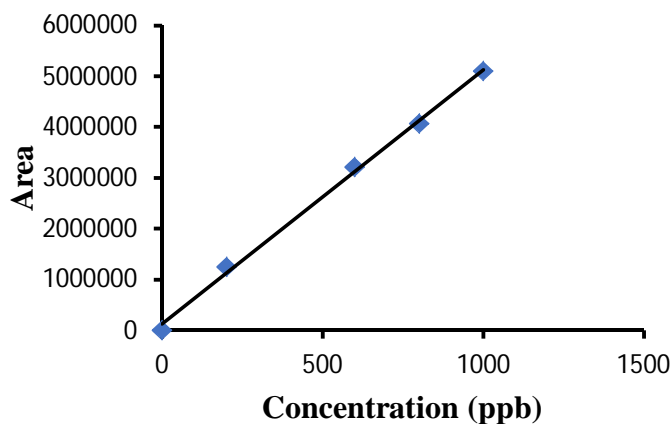


Figure 3. Calibration curve of thiamine.

Recovery study for riboflavin: The concentration of the recovery sample (calculated from the standard calibration curve) was 787.974 ppb. The concentration of the control sample (calculated from

the standard calibration curve) was 155.5788 ppb. The concentration of the added standard was 600 ppb.

$$\% \text{ Recovery} = \frac{(787.974 - 155.5788) \times 100}{600} = 105.3992$$

Table 1. % Recovery for thiamine and riboflavin.

Vitamin	% Recovery
Thiamine	97.70
Riboflavin	105.40

A total of 22 samples were run by HPLC to determine the concentration of thiamine and riboflavin. Among these, 20 samples were yogurt [5 special yogurt, 5 sugarless (tok) yogurt, 5 cup yogurt, and 5 premium (shahi) yogurt], and 2 samples were whey. The amount of thiamine and riboflavin obtained in samples are summarised in the Tables 2 and 3.

Table 2. Concentration of thiamine in mg/100 g.

Sample type	Sample ID	Concentration (ppb)	Concentration (mg/100 g)
Special Doi	01	65.6066	0.1093
	02	73.0391	0.1126
	03	80.5260	0.1341
	04	83.8520	0.1396
	22	72.1001	0.1202
Tok Doi	05	74.1425	0.1235
	06	38.9097	0.0647
	07	53.0768	0.0884
	08	44.7120	0.0744
	09	68.3877	0.1141
Cup Doi	10	72.3104	0.1205
	11	82.7351	0.1378
	12	77.3449	0.1289
	13	70.1262	0.1168
	14	91.1286	0.1519
Ghol	15	51.9617	0.0865
	16	77.3122	0.1288
Shahi Doi	17	71.0204	0.1183
	18	65.3081	0.1088
	19	68.5796	0.1147
	20	64.0002	0.1066
	21	68.9105	0.1147

Table 3. Concentration of riboflavin in mg/100 g.

Sample Type	Sample ID	Concentration (ppb)	Concentration (mg/100 g)
Special Doi	01	155.5788	0.2591
	02	120.3443	0.2004
	03	165.7374	0.2761
	04	117.2693	0.1952
	22	216.1220	0.3598
Tok Doi	05	72.4936	0.1207
	06	69.0589	0.1149
	07	77.9322	0.1298
	08	54.3331	0.0905
	09	80.2290	0.1338
Cup Doi	10	119.5251	0.1992
	11	120.3443	0.2005
	12	136.1609	0.2269
	13	103.8354	0.1730
	14	93.4721	0.1558
Ghol	15	13.6233	0.0227
	16	39.0619	0.0651
Shahi Doi	17	214.6084	0.3574
	18	136.1609	0.0651
	19	224.8866	0.3746
	20	213.8193	0.3562
	21	225.1024	0.3749

The development of an HPLC process for precise and trustworthy analysis depends on the selection of suitable chromatographic conditions, notably the mobile phase. A mobile phase with a low salt concentration of 5.0 mM hexane-sulfonic acid was used in this technique. This option has a number of benefits, including a longer column lifespan due to less salt build-up and decreased backpressure, which boost the HPLC system's overall effectiveness and stability. The obtained correlation coefficients of 0.9975 for thiamine and 0.9974 for riboflavin show that the vitamin concentrations and their corresponding peak areas have a strong linear relationship. These excellent correlation coefficients suggest that the suggested HPLC approach may accurately and precisely measure thiamine and

riboflavin in yogurt and whey samples. Thiamine and riboflavin recovery rates are adequate at 97.70% and 105.40%, respectively. These recovery rates show that the approach can accurately measure the vitamin amounts in the samples. The range of thiamine concentrations in the various yogurt samples varied, as shown in table 2. Thiamine concentrations were consistently higher in the special yogurt samples (ID 01-04, 22), ranging from 0.1093 mg/100 g to 0.1396 mg/100 g. Thiamine levels in sugarless yogurt (Tok Doi) ranged from 0.0647 mg/100 g to 0.1235 mg/100 g, which was slightly lower. The values of 0.1168 mg/100 g to 0.1519 mg/100 g were found in cup yogurt samples (ID 10–14). The range of thiamine concentrations in whey (Ghol) was found between 0.1288 mg/100 g to 0.0865 mg/100 g, which were the lowest values. The riboflavin contents in the special yogurt samples (ID 01-04, 22) ranged from 0.1952 mg/100 g to 0.3598 mg/100 g, and they were consistently higher (Table 3). Sugarless yogurt (Tok Doi) concentrations ranged from 0.0905 mg/100 g to 0.1298 mg/100 g. Samples of cup yogurt (ID 10–14) revealed values between 0.1730 mg/100 g and 0.2269 mg/100 g. The lowest riboflavin contents were found in whey (Ghol) (0.0227 mg/100 g to 0.0651 mg/100 g). Overall, the suggested HPLC approach with a low salt mobile phase concentration shows good performance in measuring the amounts of thiamine and riboflavin in yogurt and whey samples. The measured concentration ranges, combined with the high correlation coefficients and respectable recovery rates, offer important information about the vitamin content of various dairy products.

Conclusion

This study provides important details about thiamine and riboflavin concentrations in several yogurt samples and whey. The results show that the special yogurt samples regularly had higher levels of both vitamins than ordinary varieties. While whey (Ghol) consistently has the lowest quantities, samples of cup yogurt and sugarless yogurt (Tok Doi). The percentage recovery values of 105.40% for riboflavin and 97.70% for thiamine show the analytical method's

accuracy and dependability. These results contribute to the understanding of the nutritional composition of yogurt and whey products and can be useful for quality control and nutritional labeling purposes. Moreover, yogurt intake has cultural importance due to Bengalis' love for yogurt. It frequently serves as a centerpiece of festivals and other gatherings, signifying the culmination of a delectable culinary journey. Bengalis have the opinion that entertainment is not complete without yogurt at the end, regardless of how many delicacies are offered.

Embracing the Bengali's love for yogurt has several health advantages in addition to giving meals taste and enjoyment. Probiotics included in yogurt, a fermented dairy product, help to maintain a healthy digestive system and boost the immune system. In addition, yogurt is a nutrient-rich addition to one's diet because it is a high source of thiamine and riboflavin. Enjoy the custom of eating yogurt throughout meals and special occasions while respecting its cultural significance and the health advantages it offers. Therefore, these yogurts are beneficial for your health and rich in thiamine and riboflavin.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- FDA 2016. Yogurt: from Part 131-Milk and Cream. Subpart B- Requirements for Specific Standardized Milk and Cream, Sec. 131.200. Code of Federal Regulations, Title 21, US *Food and Drug Administration*. 1 April 2016.
- Lee, Y.K., Shao, W., Jin, S., Wen, Y., Ganguly, B. and Rahayu, E.S. 2011. Probiotics regulation in Asian countries. In *lactic acid bacteria: microbiological and functional aspects*, 4th edn. CRC Press, United State p, 745. Clark M. 2017. Creamy Homemade Yogurt Recipe". *NYT Cooking*. Retrieved 19 March 2017.
- Azad, A.K. 2016. গৌর গোপালের বগুড়ার দই. *risingbd.com* (in Bengali). 13 April 2016. <https://www.risingbd.com/feature/news/156115>. Accessed on December 2023.

- "বগুড়ার প্রসিদ্ধ দই ও মিষ্টান্ন". *Bogura Tribune (in Bengali)*. 10 July 2019. <https://www.boguratribune.com/?p=618>. Accessed on December 2023.
- Wikipedia. https://en.wikipedia.org/wiki/Curd_of_Bogra. Accessed on December 2023.
- Bashar, L. 2014. রপ্তানি সম্ভাবনা থাকলেও নেই পৃষ্ঠপোষকতা. *Kaler Kantha (in Bengali)*. [https://www.kalerkantho.com/print-edition/industry-Accessed on December 2023.business/2014/07/15/106997](https://www.kalerkantho.com/print-edition/industry-Accessed%20on%20December%202023.business/2014/07/15/106997) Accessed on December 2023.
- Shanto, S. A. 2021. যেভাবে এলো বগুড়ার দই *Daily Ittefaq (in Bengali)*. <https://www.ittfaq.com.bd/305583/%E0%A6%AF%E0%A7%87%E0%A6%AD%E0%A6%BE%E0%A6%AC%E0%A7%87%E0%A6%8F%E0%A6%B2%E0%A7%8B%E0%A6%AC%E0%A6%97%E0%A7%81%E0%A6%A1%E0%A6%BC%E0%A6%BE%E0%A6%B0%E0%A6%A6%E0%A6%87%C2%AO> Accessed on December 2023.
- Clark M. and quote; Creamy Homemade Yogurt Recipe and quote;. NYT Cooking. Retrieved 19 March 2017.
- Gijsbers, L., Ding, E. L., Malik, V. S., De Goede, J., Geleijnse, J. M. and Soedamah-Muthu, S. S. 2016. Consumption of dairy foods and diabetes incidence: a dose-response meta-analysis of observational studies. *Am. J. Clin.* **103**, 1111-1124.
- Ong, A. M., Kang, K., Weiler, H. A. and Morin, S. N. 2020. Fermented milk products and bone health in postmenopausal women: a systematic review of randomized controlled trials, prospective cohorts, and case-control studies. *Adv. Nutr.* **11**, 251-265.
- Savaiano, D. A. and Hutkins, R. W. 2021. Yogurt, cultured fermented milk, and health: A systematic review. *Nutr. Rev.* **79**, 599-614.
- Farrell Jr, H. M., Jimenez-Flores, R., Bleck, G. T., Brown, E. M., Butler, J. E. and Creamer, L. K. 2004. Nomenclature of the proteins of cows' milk- sixth revision. *J. Dairy Sci.* **87**, 1641- 1674.
- Frid, A. H., Nilsson, M., Holst, J. J. and Björck, I. M. 2005. Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. *Am. J. Clin.* **82**, 69-75.
- Nilsson, M., Elmståhl, H. and Björck, I. 2005. Glucose and insulin responses to porridge and gruel meals intended for infants. *Eur. J. Clin. Nutr.* **59**, 646- 650.
- National Institutes of Health. 2018. Riboflavin: Fact Sheet for Health Professionals. Last modified August, 2020.
- Somer, E. 1996. The essential guide to vitamins and minerals. Harper Collins, USA, Second Edition, Revised and Updated
- Coates, P.M., Betz, J.M., Blackman, M.R., Cragg, G.M., Levine, M., Moss, J. and White, J.D. (Eds.). 2015. Encyclopedia of Dietary Supplements (2nd ed.). CRC Press. <https://doi.org/10.1201/b14669>
- Prothom Alo, 5 July 2023. বগুড়ার দই জিআই পণ্যের মর্যাদা পেলে, <https://www.prothomalo.com/bangladesh/3j8b8gmrvc>. Accessed on 06/12/2023.
- Puwastien, P., Siong, T.E., Kantasubrata, J., Craven, G., Feliciano, R.R. and Judprasong, K. 2011. ASEAN Manual for nutrient analysis, 1st edition, Institute of Nutrition, Mahidol University, Putthamonthon 4, Salaya, Nakhon Pathom 73170, Thailand.