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Research Article
Coconut Bio-yoghurt Phytochemical-chemical and Antimicrobial-microbial Activities

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Abstract

Background and Objective: Coconut flour is known to be highly nutritious flour and contains high dietary fiber. The aim of this study was to make "bio-yoghurt beverage grafted with coconut flour and containing probiotic bacteria which is recommended as a functional milk product. **Materials and Methods:** Chemical, phytochemical studies and antimicrobial activity have been performed on coconut flour which was used to make the beverage. Bio-yoghurt beverages made with different ratios of coconut flour (0, 2, 4 and 6%) were chemical, microbiological and sensory tested when fresh and during storage for 15 days of cold storage. **Results:** Chemical composition of coconut flour showed reasonable contents of moisture, ash, fat, fatty acids, protein, crude fiber, total solids, total phenols and antioxidants. Coconut flour showed antibacterial activity against some foodborne pathogenic bacteria. The addition of coconut flour to make bio-yoghurt beverages increased its contents of total solids, protein, ash, fiber, acidity, antioxidant activity, total phenols, improved yoghurt sensory and rheological properties especially when probiotic bacteria were used. The results did not reveal any significant differences ($p>0.05$), between the bacterial counts of yoghurt starter culture, *Lactobacillus rhamnosus*, *Lactobacillus casei* in yoghurt control (without coconut) and bio-yoghurt treatments (with coconut), during cold storage. Moulds and yeasts were not detected in all bio-yoghurt treatments; meanwhile they appeared in yoghurt control after 15 days indicating coconut antifungal activity and subsequently a period of time to save more for this drink. All bio-yoghurt beverages with different ratios of coconut flour were sensory accepted and showed more favorable properties, particularly for the viscosity. **Conclusion:** A new functional dairy product of bio-yoghurt beverage contain coconut flour high fiber content was prepared with probiotic bacteria and showed good chemical, microbiological and sensory/rheological properties.

Key words: Phytochemical, chemical, antimicrobial, microbial, coconut flour, coconut bio-yoghurt

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coconut flour is white soft flour prepared from the pulp of coconut. It is really a by-product prepared during the coconut milk manufacture process. Coconut is naturally low in digestible carbohydrate, free gluten, more economical than most other nut flours, as loading with more health promoting fiber, significant nutrients and amazing tastes. Coconut flour is really high in fiber with almost twice the quantity established in wheat bran. It contains more calorie free fiber than other wheat replacements[1,2]. Coconut flour also delivers a good source of protein, as it contains more protein than improved flour, corn meal and also as much as wheat flour. It is believed to be as a "functional food" because it offers many health benefits besides its nutritional content[3]. Coconut flour can provide more income value to the industry, as adding a good nutritious, great healthy source of dietary fiber, free from trans fatty acids and low in carbohydrates[4]. Studies revealed that the consumption of high fiber coconut flour increases fecal bulk[5]. Fiber in coconut presented very effective in regulatory sugar and insulin levels in the blood, therefore, coconut is suitable for diabetes. Coconut Flour prevents the risk of colon cancer because of the metabolism of butyrate which is significant and enables better digestion and promotes digestive well-being[1].

Yoghurt is a well-known fermented milk product as it contributes to the mitigation of lactose intolerance, safety against gastrointestinal infection, anti-carcinogenic effect and immune system inspiration[6]. Probiotics play an important role in immunological, digestive and respiratory functions[7]. Additionally, Probiotic bacteria are becoming gradually important in the context of human nutrition, as scientific evidence continues to collect on the properties, functionality and benefits of probiotics for the promotion of human health[8].

Yoghurt produced from coconut milk and skimmed cow milk, using starter cultures[9]. Starter culture developed for the production of coconut milk yoghurt using endogenous isolates and evaluated the yoghurt quality[10], yoghurts produced from tiger nut, coconut, coconut composite and evaluated the biochemical composition, vitamin/mineral composition, pH, acidity and sensory qualities[11]. Consuming symbiotic foods that contain prebiotics like fibers[12] and probiotics (lactic acid bacteria)[13] would offer more additional nutritional benefits that can help and boost overall health and well-being.

From the knowledge, coconut flour has not been used before in the manufacture of dairy products, particularly in the local market. Therefore, this study aimed to evaluate coconut flour chemically, microbiologically and use it to produce new fermented dairy products like "bio-yoghurt high fiber content" as a symbiotic dairy product with special taste, properties, functions and as a source for many important ingredients especially dietary fibers and contain probiotic bacteria as well.

MATERIAL AND METHODS

Materials: This study was carried out in the Department of Dairy (laboratories) at the Egyptian National Research Centre, Dokki, Egypt, during August, 2018-February, 2019 (6 months). Coconut flour was purchased from the local market. Fresh skim milk was procured from Animal Production Research Institute, Agriculture Research Center Giza, Egypt.

Bacterial strain: Strains of lactic acid bacteria, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus* (as yoghurt starter culture) and *Lactobacillus casei* (NRC cultures), were obtained from stock cultures of Dairy Microbiology Lab., National Research Centre, Dokki-Cairo, Egypt. *Lactobacillus rhamnosus* Tistr 541 was brought from Thailand institute of Scientific and Technological Research, Bangkok, Thailand. *Lactobacillus plantarum* Dsaz 0174 and strains of pathogenic bacteria, *Escherichia coli* 0157:H7 ATCC 6933, *Klibsiella pneumonia*, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 2023, *Pseudomonas aeruginosa* ATCC 9027, as reference strains (ARC cultures) were obtained from the stock cultures of the Agricultural Research Centre in Giza. All strains were routinely maintained by sub-culturing once a week in tryptone soya broth/agar and stored at 4°C until use.

Methods

Chemical analysis of coconut flour: The chemical composition including crude fiber determinations in coconut flour was determined according to the method of AOAC[14]. The fat content in coconut flour was determined by using Soxhlet according to the methods of IDF[15].

Fatty acids contents in coconut flour: The fatty acid composition of the coconut flour samples were identified and measured using gas liquid chromatography on a Hewlett Packard Model 6890 with a flame ionization detector using capillary column 30.0 m×530 μm×1.0 μm. The carrier gas used was nitrogen set at a flow rate of 15 mL min^{-1} and split-ratio of 8:1. Esterification of fatty acid for methyl ester preparation was carried out according to Luddy *et al.*[16].

Table 1: Illustrate treatments of bio-yoghurt with and without different coconut flour concentrations "ratios" and microbial formulae

Treatments	Coconut flour concentrations and microbial formulae for bio-yoghurt treatments
C1	Yoghurt starter+*Lactobacillus rhamnosus*
C2	Yoghurt starter+*Lactobacillus casei*
C3	Yoghurt starter only
T1	2% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*
T2	2% coconut flour+yoghurt starter+*Lactobacillus casei*
T3	4% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*
T4	4% coconut flour+yoghurt starter+*Lactobacillus casei*
T5	6% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*
T6	6% coconut flour+yoghurt starter+*Lactobacillus casei*

All milk mixtures with and without different ratios of coconut flour (0, 2, 4 and 6%) were inoculated with 2% yoghurt starter (1:1) and 2% probiotic bacteria

Manufacture of bio-yoghurt fortified with different concentrations of coconut flour: Coconut flour mixed with fresh buffalo`s skim milk after heat treatment at 90°C/3 min, cooled and adjusted to 42°C. The milk mixtures with different ratios of coconut flour (0, 2, 4 and 6%) were inoculated with 2% yoghurt starter (1:1) and 2% probiotic bacteria (*Lactobacillus casei* and *Lactobacillus rhamnosus*, individually*)*. Bio-yoghurt beverage treatments were illustrated in Table 1 "(patent No.61/2019, the Patent Office at the Egyptian Academy of Scientific Research and Technology). All treatments were incubated at 42°C until complete coagulation then cooled and stirred. The bio-yoghurt packed in plastic cubs 50 mL and stored at refrigerator till the end of storage[17]. Samples of bio-yoghurt were analyzed for chemical, microbiological, sensory properties when fresh and during the storage period. Bio-yoghurt manufacturing was done 2 times during this study. The two probiotic strains used in preparing bio-yoghurt were the best bacterial strains that showed high growth occurred with coconut flour powder out of five tested probiotic bacterial strains (*L. casei*, *L. plantarum*, *L. lactis* subsp., *cremors*, *L. lactis* subsp., *lactis* and *L. rhamnosus*).

Chemical analysis of bio-yoghurt fortified with different concentrations "ratios" of coconut flour: The titratable acidity (calculated as lactic acid), ash, fiber contents and pH values of bio-yoghurt fortified with different concentrations of coconut flour were determined according to AOAC[14]. The protein and moisture contents were estimated from the crude nitrogen content of the samples determined by the Kjeldahl and oven-drying methods, respectively, IDF[18].

Determination of total phenolic compounds and antioxidants: Total phenolic content and antioxidant activity in coconut flour and bio-yoghurt samples were determined according to Moldovan *et al.*[19].

Apparent viscosity (cP.s, as rheological property) of stirred bio-yoghurt fortified with coconut flour: Apparent viscosity was measured at room temperature using a Brookfield digital viscometer (Middleboro, MA 02346, USA). The sample was subjected to shear rates ranging from 3-100 S^{-4} for an upward curve. Viscosity measurements were expressed as centipoise (cP.s) and were performed in triplicate[20,21].

Determination of coconut flour antibacterial activity: The antibacterial activity of the coconut flour was carried out using a disc diffusion method described by Nair and Chanda[22]. Coconut flour dissolved in dimethyl sulfoxide (DMSO). The pathogenic indicator bacteria strains *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 2023 and *Pseudomonas aeruginosa* ATCC 9027 as reference strains were activated in tryptone soy broth at 37°C for 24 h. Control negative was discs with Dimethyl sulfoxide (DMS). All tests were carried out in triplicates. The plates were incubated at 37°C for 24 h and hallow zones were measured in mm minus the disc diameter.

Microbiological analysis of bio-yoghurt fortified with different concentrations of coconut flour: Four samples of Bio-yoghurt were microbiologically examined for each treatment after 0, 7, 15 and 21 days of cold storage period. Samples were microbiologically examined for total aerobic colony count (TACC), mould and yeast, *Staphylococcus aureus* and *coliform* bacteria using the selective media and the conventional methods according to FDA[23], APHA[24] and Harrigan and McCance[25]. *Streptococcus thermophilus* was enumerated on M17 agar after aerobic incubation at 37°C for 48h El-Kholy *et al.*[26]. *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus* and *Lactobacillus casei* enumerated using modified MRS agar supplemented with 0.05% L-cysteine-HCl and the plates were incubated at 37°C for 48 h according to Harrigan and McCance[27] and Abbas *et al.*[28].

Sensory evaluation: Bio-yoghurt beverage was sensory evaluated when fresh and after 15 days of storage (as a common time for yoghurt shelf life in the market) by ten panelists of the staff member of Dairy Department at Food Industries and Nutrition Division, National Research Center, using the score sheet according to Badawi *et al.*[29].

Statistical analysis: All experiments and analysis were done in triplicate. Data were statistically analyzed using the GLM procedure of SAS[30] software (Version 9.2). Level of significance between treatments was determined by the Duncan test. Probability of <0.05 was considered as significantly different.

RESULTS AND DISCUSSION

Chemical composition of coconut flour: As presented in Table 2, coconut flour was a high as a source of fiber content (50.50%), a good source of fat (18.99%) and protein (10.67%). Also, Table 2 showed the high antioxidant (79.99%) and total phenols (154.4 mg g^{-1}) contents in coconut flour powder. The main fatty acid content in coconut oil was lauric acid (45.91%), it was considered half of all triglycerides in coconut oil, as presented in Fig. 1. the coconut oil contained unsaturated fatty acids consisting of oleic (4.38%) and linoleic acids (1.34%) as triglycerides.

Chemical composition of the bio-yoghurt beverage fortified with coconut: The chemical composition of bio-yoghurt was presented in Table 3. The moisture content of bio-yoghurt beverage ranged between 83.41-88.91% and the moisture decreased with the increase of coconut flour addition from 2-6% compared with the control. Protein and ash contents in bio-yoghurt beverage were high in the treatments of T5 and T6, as contained more than 7% in bio-yoghurt with the highest ratio (6%) of coconut flour. The highest fiber content was also recorded with T5 and T6. Meanwhile, results in Table 4 showed that the pH value at zero time for bio-yoghurt beverage was significantly high in the control treatment and decrease significantly in the treatments with more addition of coconut flour and significantly decreased during the storage period and vice versa for the titratable acidity.

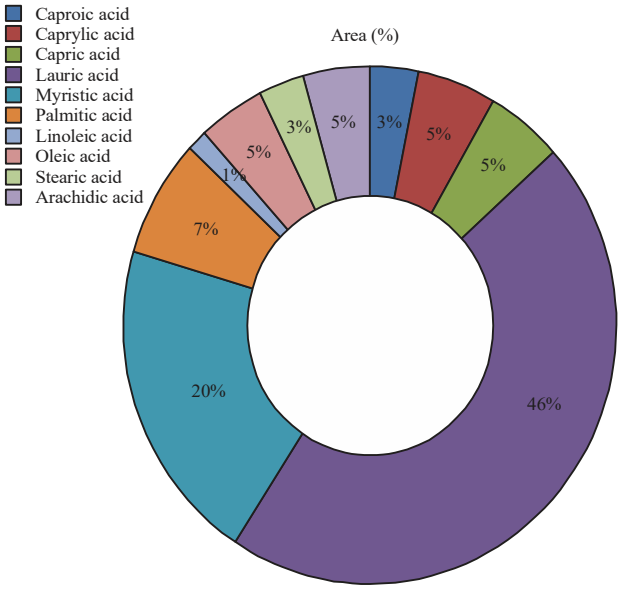

Fig. 1: Fatty acids content in coconut flour used in manufacture of bio-yoghurt fortified with coconut flour

Table 2: Chemical composition of coconut flour powder

Coconut flour	Fat (%)	Protein (%)	Ash (%)	Moisture (%)	Fiber (%)	Antioxidant (%)	Total phenols (mg g^{-1})
	18.99±0.71	10.67±0.23	4.06±0.16	3.28±0.06	50.50±0.5	79.99±0.01	154.4±0.51

Table 3: Chemical composition of bio-yoghurt fortified with coconut flour

Treatments	Moisture (%)	T.S. (%)	Fiber (%)	Protein (%)	Ash (%)
C1	88.65 ± 0.12^{B}	11.35 ± 0.04^{I}	-	4.20 ± 0.17^{H}	0.764 ± 0.012^{D}
C2	88.37 ± 0.36^{C}	11.63 ± 0.07^{G}	-	4.41 ± 0.052^{F}	0.762 ± 0.046^{D}
C3	88.91 ± 0.54^{A}	11.59 ± 0.06^{H}	-	4.32 ± 0.069^{G}	0.725 ± 0.012^{D}
T1	86.06 ± 0.03^{D}	13.94 ± 0.04^{F}	1.06 ± 0.023^{C}	5.69 ± 0.012^{D}	0.871 ± 0.017^{C}
T2	85.90 ± 0.06^{E}	14.10 ± 0.06^{E}	1.08 ± 0.012^{C}	5.53 ± 0.012^{E}	0.862 ± 0.012^{C}
T3	84.80 ± 0.17^{F}	15.20 ± 0.06^{D}	2.30 ± 0.029^{B}	6.43 ± 0.029^{C}	0.923 ± 0.049^{BC}
T4	84.30 ± 0.53^{G}	15.70 ± 0.069^{C}	2.28 ± 0.017^{B}	6.42 ± 0.017^{C}	0.931 ± 0.069^{B}
T5	83.41 ± 0.42^{I}	16.59 ± 0.012^{A}	3.21 ± 0.081^{A}	7.48 ± 0.035^{A}	1.162 ± 0.017^{A}
T6	83.60 ± 0.23^{H}	16.40 ± 0.156^{B}	3.22 ± 0.041^{A}	7.42 ± 0.017^{B}	1.173 ± 0.017^{A}

C1: Yoghurt starter+*Lactobacillus rhamnosus*, C2: Yoghurt starter+*Lactobacillus casei*, C3: Yoghurt starter only, T1: 2% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T2: 2% coconut flour+yoghurt starter+*Lactobacillus casei*, T3: 4% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T4: 4% coconut flour+yoghurt starter+*Lactobacillus casei*, T5: 6% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T6: 6% coconut flour+yoghurt starter+*Lactobacillus casei*, means with the different capital superscript letters (A,B,...) within the same column indicate significant ($p \leq 0.05$) differences between treatments

Table 4: pH value and acidity (%) of bio-yoghurt beverage fortified with coconut flour fresh and during storage period

	Storage period (days)					
	pH vale			Acidity (%)		
Treatments	0	7	15	0	7	15
C1	5.12±0.017^{Aa}	4.98±0.115^{Bb}	4.87±0.017^{Ac}	1.10±0.017^{Ec}	1.52±0.017^{CDb}	1.63±0.035^{Da}
C2	5.14±0.012^{Aa}	5.05±0.023^{Ab}	4.97±0.023^{Ac}	1.16±0.012^{Dc}	1.47±0.115^{Db}	1.53±0.012^{Ea}
C3	5.03±0.017^{Ba}	4.90±0.006^{Cb}	4.81±0.023^{Ac}	1.14±0.017^{DEc}	1.40±0.115^{Eb}	1.49±0.034^{Ea}
T1	4.73±0.012^{Fa}	4.70±0.115^{EFa}	4.43±0.012^{Bc}	1.60±0.115^{Ab}	1.83±0.115^{Aa}	1.87±0.012^{Aa}
T2	4.79±0.017^{Ea}	4.67±0.017^{Fa}	4.70±0.029^{ABa}	1.60±0.117^{Ac}	1.67±0.115^{Bb}	1.75±0.029^{Ba}
T3	4.80±0.029^{Ea}	4.71±0.023^{EFa}	4.63±0.012^{ABa}	1.33±0.017^{Cc}	1.57±0.115^{Cb}	1.73±0.029^{BCa}
T4	4.76±0.017^{EFa}	4.74±0.023^{Ea}	4.67±0.012^{ABb}	1.30±0.115^{Cc}	1.40±0.029^{Eb}	1.68±0.023^{CDa}
T5	4.95±0.017^{Ca}	4.83±0.017^{Db}	4.78±0.012^{ABb}	1.53±0.023^{Bc}	1.67±0.115^{Bb}	1.87±0.029^{Aa}
T6	4.87±0.115^{Da}	4.88±0.115^{CDa}	4.72±0.017^{ABb}	1.55±0.017^{ABb}	1.69±0.029^{Ba}	1.70±0.012^{BCa}

C1: Yoghurt starter+*Lactobacillus rhamnosus*, C2: Yoghurt starter+*Lactobacillus casei*, C3: Yoghurt starter only, T1: 2% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T2: 2% coconut flour+yoghurt starter+*Lactobacillus casei*, T3: 4% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T4: 4% coconut flour+yoghurt starter+*Lactobacillus casei*, T5: 6% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T6: 6% coconut flour+yoghurt starter+*Lactobacillus casei*, means with the different capital superscript letters (A, B, C...) within the same column indicate significant ($p \leq 0.05$) differences between treatments, means with the different small superscript letters (a, b, c) within the same row are significantly ($p \leq 0.05$) different between treatments during storage period

Table 5: Antioxidant activity and phenols in bio-yoghurt beverage fortified with different ratios of coconut flour

	Storage period (days)			
	Antioxidant (%)		Total phenols (mg g^{-1})	
Treatments	0	15	0	15
C1	4.35±0.035^{Gb}	5.22±0.12^{Fa}	65.01±1.097^{Fb}	88.24±0.20^{Ha}
C2	4.21±0.023^{Ib}	5.21±0.12^{Fa}	63.45±0.32^{Gb}	85.32±0.40^{Ia}
C3	4.29±0.012^{Hb}	5.21±0.12^{Fa}	67.38±0.058^{Eb}	89.14±0.15^{Ga}
T1	4.57±0.012^{Eb}	5.24±0.023^{Fa}	145.60±0.23^{Db}	158.45±0.32^{Fa}
T2	4.48±0.023^{Fb}	5.35±0.17^{Ea}	145.70±0.173^{Db}	158.80±0.12^{Ea}
T3	5.13±0.012^{Cb}	5.66±0.17^{Da}	154.80±0.115^{Cb}	165.50±0.29^{Ca}
T4	4.89±0.0058^{Db}	5.87±0.12^{Ca}	154.90±0.95^{Cb}	162.35±0.38^{Da}
T5	6.17±0.017^{Ab}	6.48±0.17^{Aa}	164.80±0.115^{Ab}	173.25±0.43^{Ba}
T6	6.10±0.058^{Bb}	6.35±0.029^{Ba}	164.10±0.52^{Bb}	175.05±0.14^{Aa}

C1: Yoghurt starter+*Lactobacillus rhamnosus*, C2: Yoghurt starter+*Lactobacillus casei*, C3: Yoghurt starter only, T1: 2% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T2: 2% coconut flour+yoghurt starter+*Lactobacillus casei*, T3: 4% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T4: 4% coconut flour+yoghurt starter+*Lactobacillus casei*, T5: 6% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T6: 6% coconut flour+yoghurt starter+*Lactobacillus casei*, means with the different capital superscript letters (A, B, C...) within the same column indicate significant ($p \leq 0.05$) differences between treatments, means with the different small superscript letters (a, b, c) within the same row are significantly ($p \leq 0.05$) different between treatments during storage period

Antioxidant activity and total phenols: Data in Table 5 indicated that the antioxidant activity of bio-yoghurt beverage gradually increased with increasing addition of coconut flour and also increased after 15 days of cold storage, as the highest value recorded (6.17, 6.48 %) with 6% coconut flour. Also, total phenols significantly increased with increasing the ratio of coconut flour addition and storage period, as the highest value was 164.80 mg g^{-1} at T6 and175.05 mg g^{-1} after 15 days.

Viscosity measurements as rheological properties of bio-yoghurt beverage: Data in Fig. 2a and b for the viscosity of bio-yoghurt showed that the highest viscosity was recorded (10000 cP.s) with 6% coconut flour and increased (15000 cP.s) after 15 days of cold storage. Generally, results revealed significant increases in the viscosity of the bio-yoghurt with increasing the addition of coconut flour and storage period.

Antimicrobial activity of coconut flour: Result in Table 6 showed the antibacterial activity of coconut flour against food born pathogenic bacteria, where hallow of inhibition zones (mm) increased significantly due to the increase of coconut flour concentrations. *Klibsiella pneumonia* showed the highest sensitive (6.5 mm) to coconut flour followed by *Staphylococcus aureus* (6 mm), meanwhile, *Bacillus cereus* and *E. coli* O157:H7 showed more resistance (5 mm). Whereas, the results in Table 7 and 8 showed the presence of fungi in the control yoghurt samples (C1, C2 and C3), which did not appear in all the yoghurt (coconut) treatments, that indicated the antifungal activity of coconut.

Table 6: Antibacterial activity as inhibition zones (mm) shown by pathogenic bacteria due to different concentrations of coconut flour

Coconut flour (%)	*B. cereus* (mm)	*S. aureus* (mm)	*K. pneumonia* (mm)	*E. coli* O157:H7 (mm)	*P. aeruginosa* (mm)
Control	0.0	0.0	0.0	0.0	0.0
2	3.0 ± 0.11^{Cc}	4.0 ± 0.014^{Bb}	5.0 ± 0.09^{Ca}	3.0 ± 0.121^{Cc}	2.0 ± 0.019^{Bd}
4	4.0 ± 0.13^{Bb}	6.0 ± 0.013^{Aa}	6.0 ± 0.112^{Ba}	4.0 ± 0.014^{Bb}	6.0 ± 0.082^{Aa}
6	5.0 ± 0.01^{Ac}	6.0 ± 0.22^{Ab}	6.5 ± 0.032^{Aa}	5.0 ± 0.112^{Ac}	6.0 ± 0.17^{Ab}

Control is dimethyl sulfoxide (DMSO), 0.0: No inhibition zone, means with the different capital superscript letters (A,B,C) within the same column indicate significant ($p\leq0.05$) differences, means with the different small superscript letters (a,b,c,...) within the same row are significantly ($p\leq0.05$) different

Table 7: Microbiology of bio-yoghurt beverage fortified with *L. rhamnosus* and different ratios of coconut flour

Treatments	Storage time (days)	Total bacteria count (CFU g^{-1})	*Str. thermophilus* (CFU g^{-1})	*L. rhamnosus* (CFU g^{-1})	*L. bulgaricus* (CFU g^{-1})
C1	0	8.18 ± 0.21^{Hb}	9.20 ± 0.124^{Ca}	8.10 ± 0.05^{Hb}	9.140 ± 0.09^{Ca}
	7	8.98 ± 0.11^{Bc}	10.40 ± 0.19^{Aa}	9.15 ± 0.09^{Eb}	10.125 ± 0.024^{Ba}
	15	8.50 ± 0.14^{Ec}	10.30 ± 0.02^{Aa}	9.11 ± 0.01^{Eb}	10.150 ± 0.101^{ABa}
C3	0	7.30 ± 0.91^{Jb}	9.30 ± 0.082^{Ca}		9.180 ± 0.087^{Ca}
	7	8.12 ± 0.32^{Ib}	10.17 ± 0.25^{ABa}		10.211 ± 0.030^{Aa}
	15	8.50 ± 0.58^{Eb}	10.11 ± 0.023^{ABa}		10.154 ± 0.0124^{ABa}
T1	0	8.55 ± 0.41^{EDb}	9.10 ± 0.029^{Ca}	8.10 ± 0.12^{Hb}	9.150 ± 0.018^{Ca}
	7	9.10 ± 0.23^{Ab}	10.18 ± 0.078^{Aa}	9.78 ± 0.021^{Bb}	10.154 ± 0.029^{Aa}
	15	8.56 ± 0.012^{Dc}	10.11 ± 0.023^{Ba}	9.28 ± 0.029^{Cb}	10.113 ± 0.315^{Ba}
T3	0	8.26 ± 0.91^{Fb}	9.80 ± 0.039^{Ca}	8.25 ± 0.117^{Gb}	9.130 ± 0.251^{Ca}
	7	9.13 ± 0.23^{Ab}	10.12 ± 0.158^{ABa}	9.82 ± 0.212^{Bb}	10.170 ± 0.084^{ABa}
	15	8.25 ± 0.25^{GFc}	10.88 ± 0.018^{Aa}	9.80 ± 0.022^{Bb}	10.200 ± 0.065^{Aa}
T5	0	8.70 ± 0.58^{Cb}	9.45 ± 0.451^{Ca}	8.33 ± 0.032^{Fb}	9.114 ± 0.042^{Ca}
	7	9.13 ± 0.23^{Ab}	10.112 ± 0.036^{ABa}	9.95 ± 0.029^{Ab}	10.132 ± 0.121^{Ba}
	15	8.2 ± 0.02^{GHc}	10.88 ± 0.098^{Aa}	9.22 ± 0.039^{Db}	10.148 ± 0.091^{ABa}

C1: Yoghurt starter+*Lactobacillus rhamnosus*, C3: Yoghurt starter only, T1: 2% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T3: 4% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T5: 6% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, All treatment samples were free from moulds and yeasts, coliform and *S. aureus*, all the time, while the controls (C1 and C3) contained fungus after 15 days of cold storage, means with the different capital superscript letters (A,B,C,..) within the same column indicate significant ($p\leq0.05$) differences between treatments, means with the different small superscript letters (a,b,c) within the same row are significantly ($p\leq0.05$) different between treatments during storage period

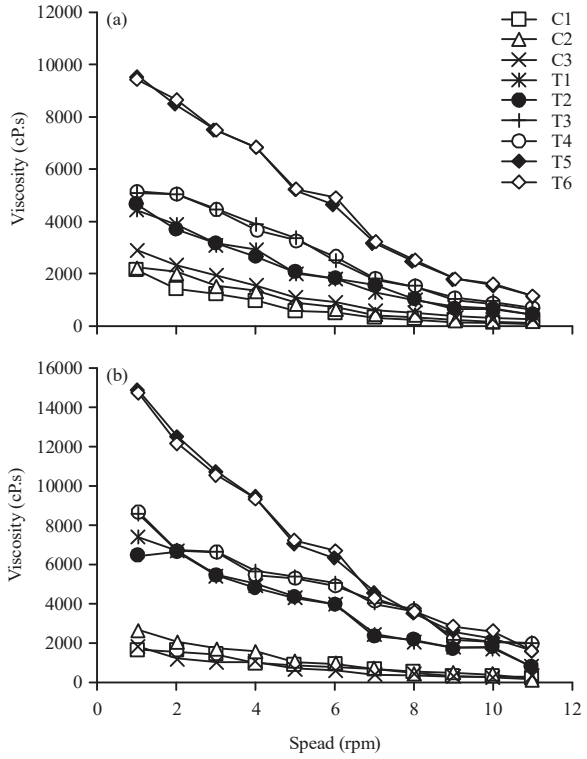

Fig (2a-b): Viscosity of bio-yoghurt beverage fortified with (a) Different ratios of coconut flour when fresh and (b) After 15 days storage

Microbiology examination of coconut bio-yoghurt beverage: In a preliminary study for activity and vitality of lactic acid bacteria in bio-yoghurt (with coconut), the results are shown in Table 9 revealed the significant superiority ($p\leq0.05$) of *L. rhamnosus* and *L. casei*, *L. plantarum*, *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremores* and therefore, such bacteria were chosen for the preparation of bio-yoghurt under study.

Again, Table 7 and 8 showed the support of adding the coconut for the activity and vitality of the lactic acid bacteria, despite the significant differences ($p\leq0.05$) between them, both in bio-yoghurt with and without coconut flour, whether it contained *L. rhamnosus* or *L. casei*, respectively. On the other hand, the duration of refrigerant storage did not affect the vitality and activity of lactic acid bacteria, were increased in counts after 1st week then decreased at the end of the storage period, despite the significant differences ($p\leq0.05$) between them. On the health aspect of the product, the results showed no moulds and yeasts, coliform bacteria or *Staph. aureus* was found in all treatment samples either fresh or during storage indicating the good hygienic conditions of the product.

Sensory evaluation: Results in Table 10 showed that the sensory overall acceptability was significantly higher and more favorable for bio-yoghurt beverages with different ratios of

Table 8: Microbiology of bio-yoghurt beverage fortified with *L. casei* and different ratios of coconut flour

Treatments	Storage time (days)	Total count bacteria (CFU g^{-1})	*Str. thermophiles* (CFU g^{-1})	*L. casei* (CFU g^{-1})	*L. bulgaricus* (CFU g^{-1})
C2	0	8.10 ± 0.36^{Gb}	9.20 ± 0.079^{DEa}	9.16 ± 0.191^{Fa}	9.12 ± 0.381^{Ca}
	7	9.22 ± 0.58^{Eb}	9.70 ± 0.181^{Eb}	9.60 ± 0.182^{Cb}	10.15 ± 0.0291^{Ca}
	15	9.20 ± 0.02^{Eb}	9.81 ± 0.085^{Cb}	9.16 ± 0.171^{Fb}	10.12 ± 0.188^{Ca}
C3	0	7.30 ± 0.054^{Hb}	9.30 ± 0.058^{Fa}		9.180 ± 0.18^{Ca}
	7	8.12 ± 0.019^{Gb}	10.165 ± 0.01^{Aa}		10.211 ± 0.25^{Aa}
	15	$8.50\pm0.58^{b\,F}$	10.11 ± 0.012^{Ba}		10.154 ± 0.321^{Ca}
T2	0	9.50 ± 0.021^{Da}	9.80 ± 0.018^{DEa}	9.31 ± 0.145^{Eb}	9.13 ± 0.180^{Eb}
	7	9.82 ± 0.58^{Cb}	10.15 ± 0.118^{Ba}	9.64 ± 0.018^{Cb}	10.21 ± 0.121^{Aa}
	15	9.30 ± 0.51^{Ec}	9.85 ± 0.079^{Db}	9.45 ± 0.191^{Dc}	10.14 ± 0.135^{Ca}
T4	0	9.50 ± 0.62^{Da}	9.38 ± 0.58^{Fb}	9.12 ± 0.141^{Fb}	9.10 ± 0.183^{Cb}
	7	10.21 ± 0.01^{Aa}	10.10 ± 0.57^{Ba}	9.64 ± 0.081^{Cb}	10.15 ± 0.281^{BCa}
	15	9.25 ± 0.1^{Ec}	9.75 ± 0.12^{Eb}	9.41 ± 0.111^{Dc}	10.14 ± 0.151^{Ca}
T6	0	9.33 ± 0.01^{Db}	9.36 ± 0.098^{Fb}	8.90 ± 0.091^{Gc}	10.13 ± 0.183^{Ca}
	7	10.15 ± 0.01^{Ba}	10.10 ± 0.51^{Ba}	10.20 ± 0.281^{Aa}	10.14 ± 0.142^{Ca}
	15	8.10 ± 0.09^{Gc}	9.76 ± 0.048^{Eb}	10.12 ± 0.018^{Ba}	10.20 ± 0.081^{ABa}

C2: Yoghurt starter+*Lactobacillus casei*, C3: Yoghurt starter only, T2: 2% coconut flour+yoghurt starter+*Lactobacillus casei*, T4: 4% coconut flour+yoghurt starter+*Lactobacillus casei*, T6: 6% coconut flour+yoghurt starter+*Lactobacillus casei*, All treatment samples were free from moulds and yeasts, coliform and *S. aureus* all the time, while the controls (C2 and C3) contained fungus after 15 days of cold storage, Means with the different capital superscript letters (A,B,C,...) within the same column indicate significant ($p\leq0.05$) differences between treatments, means with the different small superscript letters (a,b,c) within the same row are significantly ($p\leq0.05$) different between treatments during storage period

Table 9: Growth of different lactic acid bacteria in bio-yoghurt beverage fortified with different ratios of coconut flour

Coconut flour (%)	*L. lactis* subsp., *cremors* (CFU g^{-1})	*L. lactis* subsp., *lactis* (CFU g^{-1})	*L. rhamnosus* (CFU g^{-1})	*L. casei* (CFU g^{-1})	*L. plantarum* (CFU g^{-1})
Control	6.40 ± 0.917^{Db}	6.91 ± 0.016^{Da}	6.89 ± 0.097^{Da}	6.25 ± 0.016^{Dc}	6.11 ± 0.11^{Dd}
2	7.15 ± 0.013^{Ce}	7.30 ± 1.011^{Cd}	7.90 ± 0.18^{Cb}	8.66 ± 0.027^{Aa}	7.61 ± 0.017^{Cc}
4	7.22 ± 0.272^{Ad}	7.41 ± 0.019^{Bb}	8.00 ± 0.117^{Ba}	8.12 ± 0.114^{Ca}	7.81 ± 0.118^{Bb}
6	7.36 ± 0.471^{Ae}	7.80 ± 0.110^{Ad}	8.28 ± 0.013^{Ab}	8.4 ± 0.013^{Ba}	8.10 ± 0.12^{Ac}

Control (Yoghurt contained the bacterial strains but free from coconut, 0% concentration), means with the different capital superscript letters (A,B,C) within the same column indicate significant ($p\leq0.05$) differences, means with the different small superscript letters (a,b,c,...) within the same row are significantly ($p\leq0.05$) different

Table 10: Sensory evaluation of bio-yoghurt beverage fortified with different ratios of coconut flour

Parameters	Storage (days)	C1	C2	C3	T1	T2	T3	T4	T5	T6
Colour	Fresh	6 ± 1.15^{Da}	6 ± 0.58^{Da}	6 ± 0.58^{Da}	7.0 ± 1.15^{Ca}	7.0 ± 0.58^{Ca}	7.5 ± 0.58^{Ba}	7.5 ± 0.87^{Ba}	8.0 ± 0.58^{Aa}	8.0 ± 0.58^{Aa}
	15	6 ± 1.15^{Da}	6 ± 0.58^{Da}	6 ± 1.73^{Da}	7.0 ± 1.15^{Ca}	7.0 ± 1.15^{Ca}	7.5 ± 0.64^{Ba}	7.5 ± 0.87^{Ba}	8.0 ± 1.15^{Aa}	8.0 ± 0.58^{Aa}
Taste	Fresh	5 ± 1.15^{Da}	5 ± 1.73^{Da}	5 ± 1.15^{Da}	7.5 ± 0.29^{Cb}	7.5 ± 0.58^{Cb}	8.0 ± 0.58^{Ba}	8.0 ± 1.15^{Ba}	8.5 ± 0.87^{Ab}	8.5 ± 0.87^{Ab}
	15	5 ± 0.58^{Da}	5 ± 1.15^{Da}	5 ± 1.15^{Da}	8.0 ± 0.58^{Ca}	8.0 ± 1.15^{Ca}	8.5 ± 0.50^{Ba}	8.5 ± 0.29^{Bb}	9.0 ± 0.58^{Aa}	9.0 ± 0.058^{Aa}
Aroma	Fresh	4 ± 1.15^{Da}	4 ± 0.58^{Da}	4 ± 1.15^{Da}	6.0 ± 1.15^{Cb}	6.0 ± 1.15^{Cb}	7.0 ± 1.15^{Bb}	7.0 ± 1.15^{Bb}	8.0 ± 1.15^{Ab}	8.0 ± 1.15^{Ab}
	15	4 ± 0.58^{Da}	4 ± 0.64^{Da}	4 ± 0.87^{Da}	7.5 ± 1.15^{Ca}	7.5 ± 0.87^{Ca}	8.0 ± 0.58^{Ba}	8.0 ± 1.15^{Ba}	9.0 ± 0.00^{Aa}	9.0 ± 0.00^{Aa}
Mouth-feel	Fresh	3 ± 1.15^{Db}	3 ± 0.58^{Db}	3 ± 1.15^{Db}	6.0 ± 1.73^{Cb}	6.0 ± 0.58^{Cb}	7.5 ± 0.87^{Bb}	7.5 ± 0.29^{Ba}	8.5 ± 0.29^{Ab}	8.5 ± 0.29^{Ab}
	15	4 ± 1.15^{Da}	4 ± 1.09^{Da}	4 ± 0.58^{Da}	7.5 ± 0.87^{Ca}	7.5 ± 1.15^{Ca}	8.5 ± 0.29^{Ba}	8.5 ± 0.58^{Ba}	9.0 ± 0.29^{Aa}	9.0 ± 0.00^{Aa}
Overall acceptability	Fresh	5 ± 1.15^{Da}	5 ± 1.15^{Da}	5 ± 0.58^{Da}	7.0 ± 1.15^{Ca}	7.0 ± 1.15^{Cb}	8.0 ± 0.58^{Bb}	8.0 ± 0.58^{Bb}	9.0 ± 0.58^{Aa}	9.0 ± 0.58^{Aa}
	15	5 ± 0.58^{Da}	5 ± 0.58^{Da}	5 ± 1.15^{Da}	8.0 ± 0.58^{Ba}	8.0 ± 0.58^{Ba}	9.0 ± 0.58^{Aa}	9.0 ± 0.159^{Aa}	7.0 ± 0.58^{Cb}	7.0 ± 1.12^{Cb}

C1: Yoghurt starter+*Lactobacillus rhamnosus*, C2: Yoghurt starter+*Lactobacillus casei*, C3: Yoghurt starter only, T1: 2% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T2: 2% coconut flour+yoghurt starter+*Lactobacillus casei*, T3: 4% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T4: 4% coconut flour+yoghurt starter+*Lactobacillus casei*, T5: 6% coconut flour+yoghurt starter+*Lactobacillus rhamnosus*, T6: 6% coconut flour+yoghurt starter+*Lactobacillus casei*, means with the different capital superscript letters (A,B,C,...) within the same column indicate significant ($p\leq0.05$) differences between treatments, means with the different small superscript letters (a,b,c) within the same row are significantly ($p\leq0.05$) different between treatments during storage period

coconut flour (scoring ~9 for T6, fresh), as a treatment than the bio-yoghurt without coconut (scoring ~5 for C1, fresh), as a control. Furthermore, the overall acceptability increased with increasing of coconut flour addition in the bio-yoghurt beverage (scoring ~7 and ~9 for T1 and T6, respectively).

Chemical, phytochemical and rheological examination of coconut flour and coconut bio-yoghurt beverage: Coconut powder contained high percentages of fiber content, fat, protein, antioxidant and total phenols, which were in consistent with Smith *et al.*[31]. As for fatty acid contents in coconut oil, results were in agreement with Dayrit[32] and Ghani *et al.*[33]. On the other hand, the chemical composition of bio-yoghurt samples was in agreement with Ndife *et al.*[34], as the potential of producing acceptable symbiotic yoghurt enriched with coconut. While, the changes in pH values and acidity in the current study could be due to lactic acid bacteria and coconut effects, that were inconsistent with Eke *et al.*[35], Estevez *et al.*[36] and Salama *et al.*[21]. The increase in antioxidant activity and total phenolic compounds may be clarified by the degradation of milk proteins by the proteolysis activity of yoghurt starter cultures as *Lactobacilli*, resultant in the release

of some degradation products capable of responding with Folin-Ciocalteu reagent[37-39]. The viscosity, as a rheological property, has been significantly improved by the excessive addition of coconut flour, which may be due to high fiber content, changes in acidity and protein matches and this is consistent with Salama *et al.*[21], Salama *et al.*[40] and Burkus and Temelli[41].

Antimicrobial activity of coconut flour and the microbiological quality of coconut bio-yoghurt: The high content of lauric acid, as the major fatty acid constituent in coconut, as shown in the current study, pronounced the coconut antimicrobial activity had been shown to possess wide-spectrum activity against bacteria, fungi and viruses[42-43]. This is in line with the results of the current study of coconut activity as an antifungal and antibacterial agent. The microbial changes of yoghurt starter culture and probiotic bacteria in coconut bio-yoghurt may be due to the presence of some growth promoter, such as, lactic acid production, volatile compound formation, free amino acids or vitamins present in coconut flour as reported by Ndife *et al.*[34], Lee *et al.*[44], Hartono *et al.*[45], Dharmasena[46] and Dave and Shah[47]. Furthermore, the presence of probiotic bacteria, as *Lactobacillus rhamnosus* and *Lactobacillus casei*, in the prepared coconut bio-yoghurt met the requirements of probiotic food to have health promoting effects due to JBA[48-50] and agreed with Zhao *et al.*[51]. Generally, the antibacterial and antifungal effects of the used yoghurt starter culture, probiotic bacteria and coconut addition to coconut bio-yoghurt have been clearly demonstrated in this study and agree with what has been reported by Attala[52].

Sensory evaluation of the prepared coconut bio-yoghurt: All of the panelists for the prepared coconut bio-yoghurt acknowledged the sensory quality of the product, which may be due to high oil content and sweetness imparted by the high carbohydrate content of the coconut flour, which had a significant effect on the sensory evaluation[53,54]. Overall, the study showed the good sensory quality of the coconut bio-yoghurt, which is consistent with many other studies[9,54,55].

CONCLUSION

In conclusion, the good phytochemical, chemical and antimicrobial properties of coconut flour helped to produce high-quality coconut bio-yoghurt, using *Lactobacillus rhamnosus* and *Lactobacillus casei*, in chemical, microbial and sensory properties, which may be released to produce more functional dairy products from skimmed milk for consumers with special needs.

SIGNIFICANCE STATEMENT

This study discovered the special addition of coconut flour with different ratios and probiotic bacteria to prepare a new product "bio-yoghurt" that can be beneficial for the dairy industry and consumers with special needs. This study will help the researchers to uncover the critical areas of functional dairy products that many researchers were not able to explore. Thus a new theory on coconut flour function and properties may be arrived at.

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