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- Ayan Dey
- Chanda Vilas Dhumal
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- Content type:Review Article
- Published: 10 November 2020
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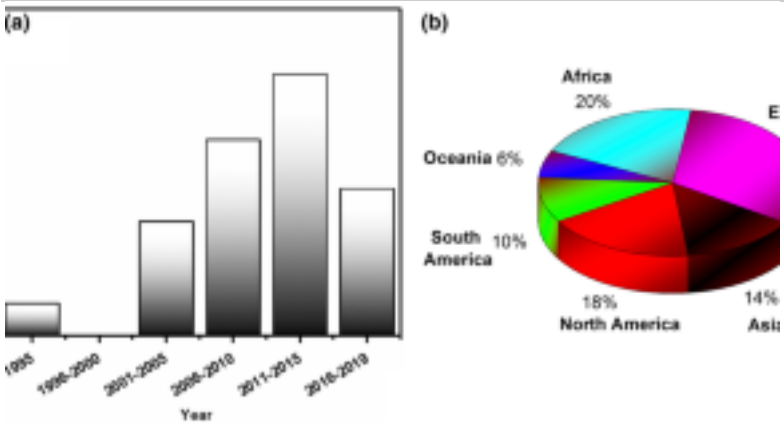

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- Sonal Patil
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- Content type:Review Article
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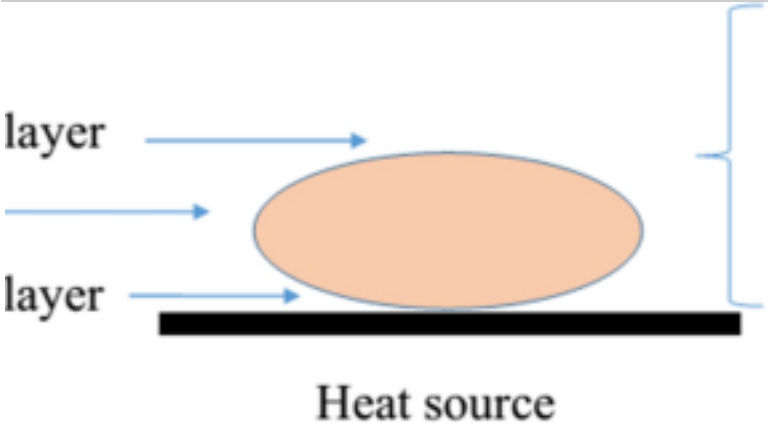

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- Nidhi Dangi
- Baljeet S. Yadav

- Content type:Original Article
- Published: 04 November 2020

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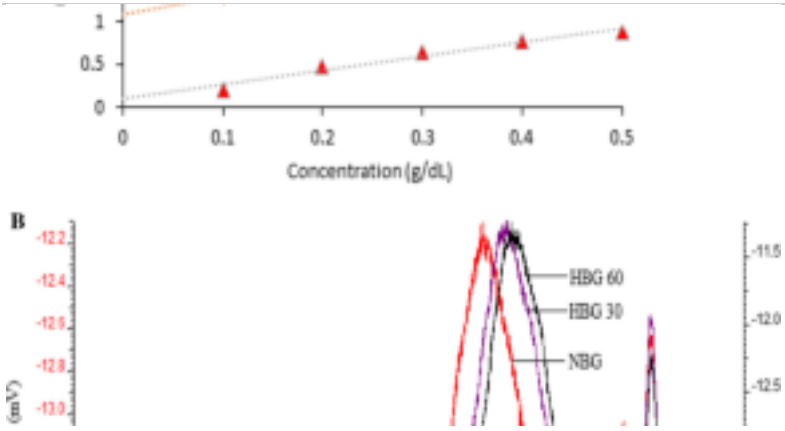

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- Zeynep Elbir
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- Deise Tramontin
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- Content type:Original Article
- Published: 04 January 2021

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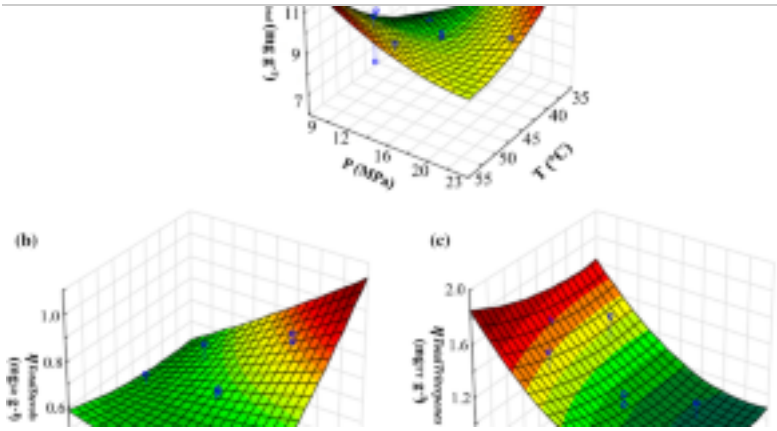

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- Samar Elshamy
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- Content type:Original Article
- Published: 22 January 2021

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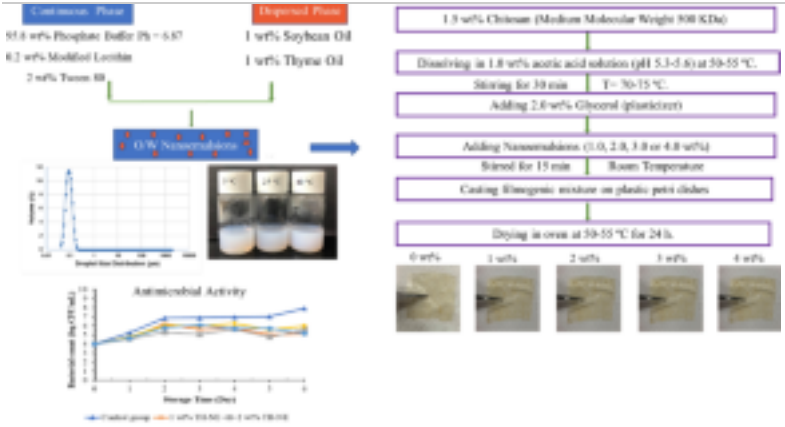

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- Aarti Bains
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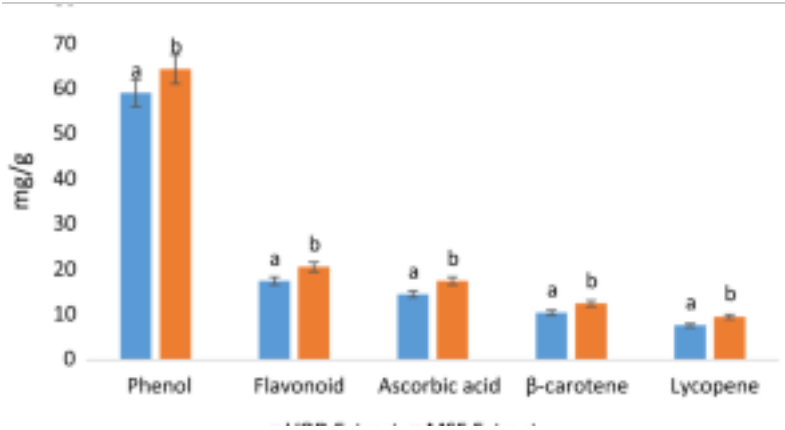

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- Published: 21 November 2020

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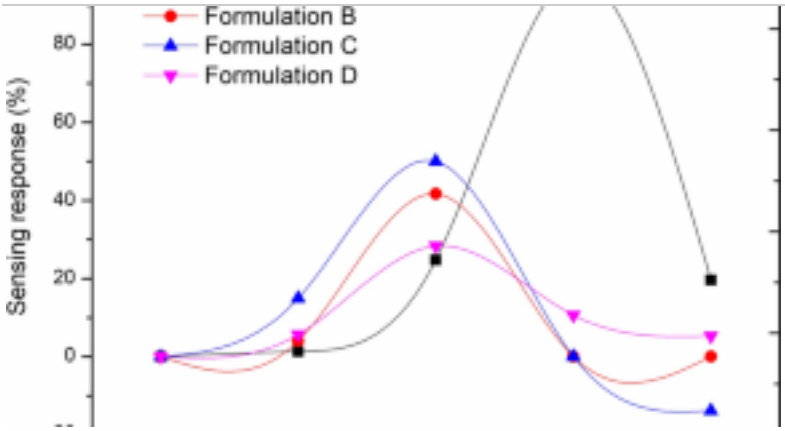

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- Feriel Ghribi
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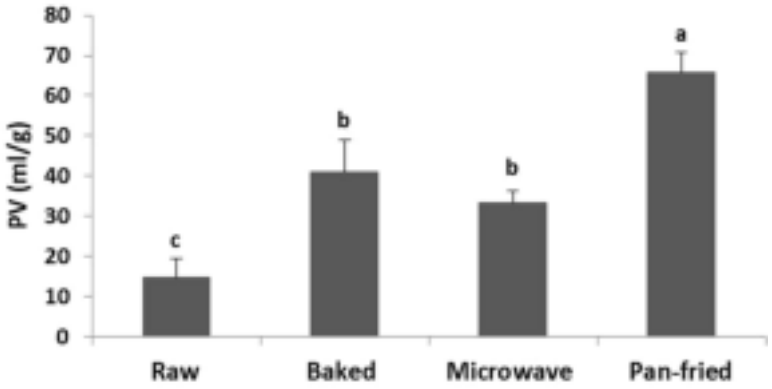

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- Ovais Shafiq Qadri
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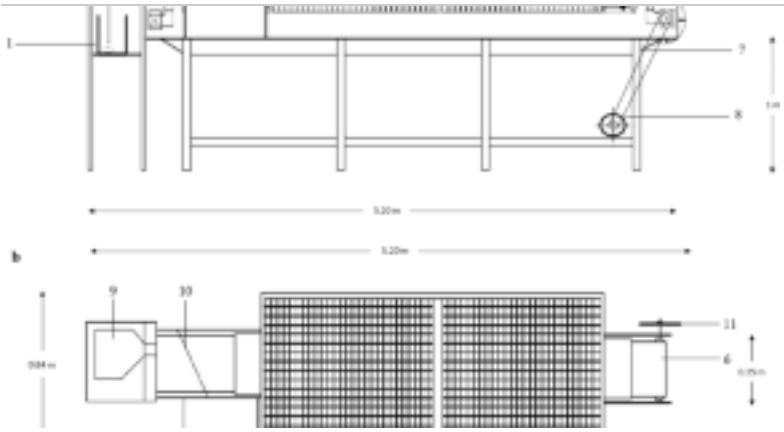

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- Pankaj Jha

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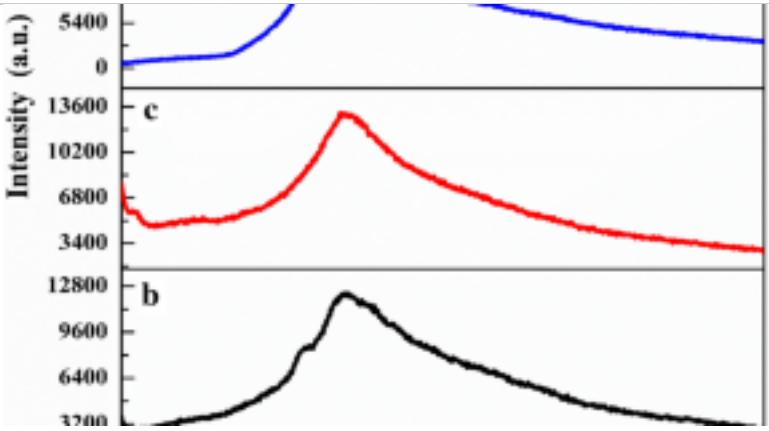

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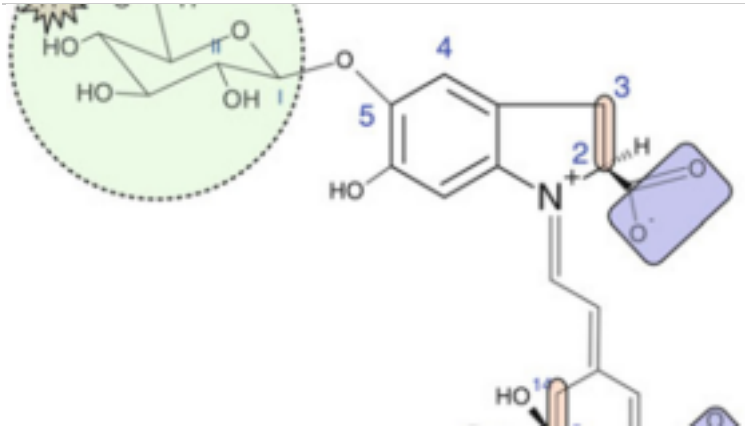

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- Shalini Arora
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- Published: 03 January 2021

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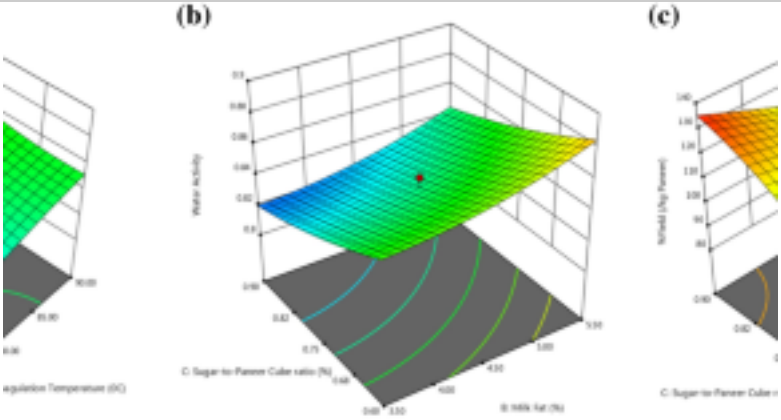

14. **Correlation between physical and sensorial properties of gummy confections with different formulations during storage**

- Suzan Tireki
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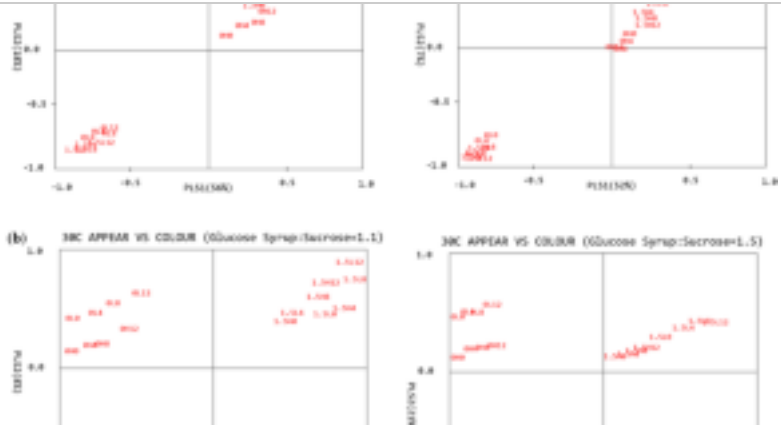

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- Ivelina Ivanova
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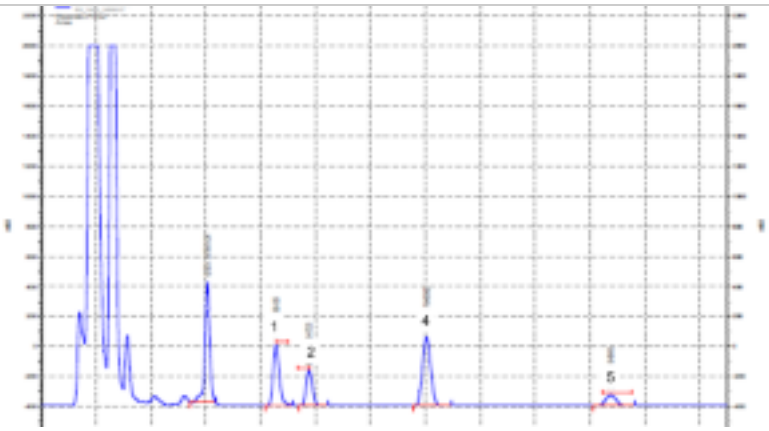

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- Mônia Stremel Azevedo
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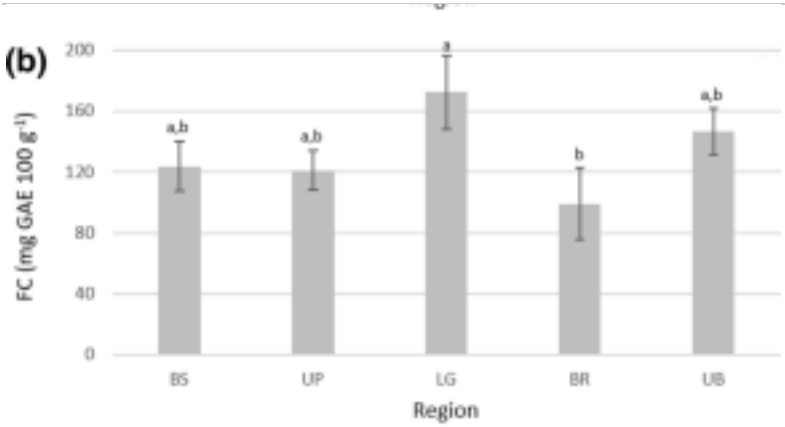

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- Iman Katouzian
- Ramezan Ali Taheri

- Content type:Original Article
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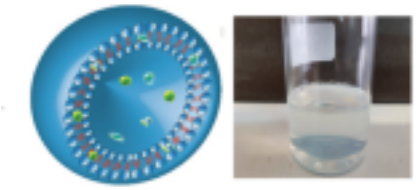

Encapsulation of olive leaf extract in chitosan-coated nanoliposome (chitosome)

Optimum sample choser central composite desi

18. **Probiotic fermented oat dairy beverage: viability of *Lactobacillus casei*, fatty acid profile, phenolic compound content and acceptability**

- Vera Maria Klajn
- Camila Waschburger Ames
- Ângela Maria Fiorentini

- Content type:Original Article
- Published: 24 January 2021
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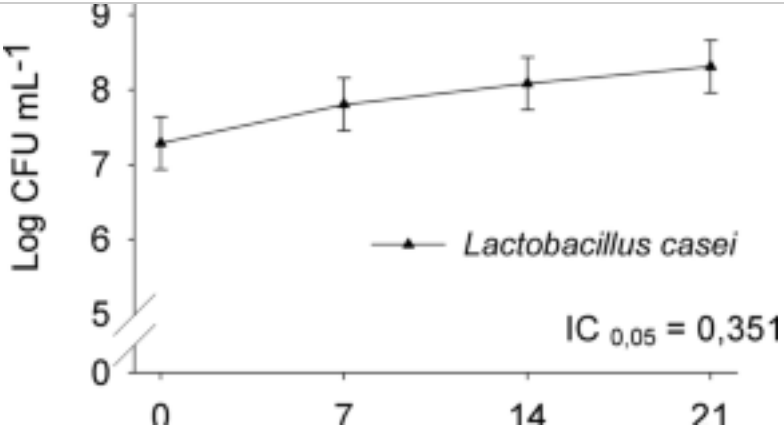

19. **Quantification of aflatoxin and ochratoxin contamination in animal milk using UHPLC-MS/SRM method: a small-scale study**

- Rukshan Mehta
- Sweekruthi A. Shetty
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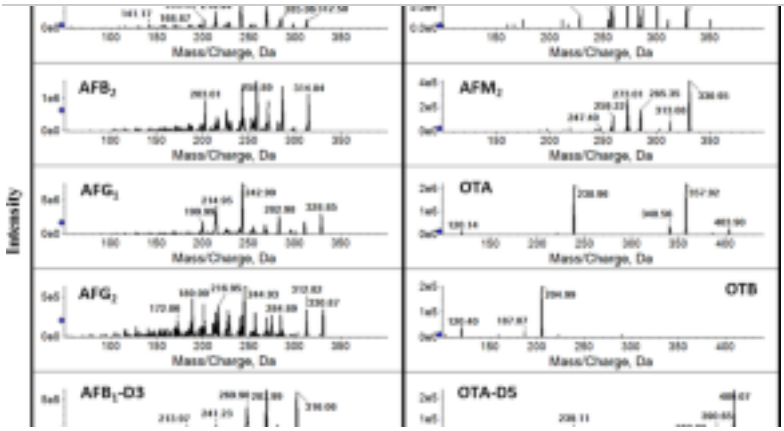

20. **Using BAMLET complex in a functional spreadable cheese elaborated with bovine colostrum**

- Karen Argelia Reyes-Portillo
- Aurora Quintero-Lira
- Sergio Soto-Simental

- Content type:Original Article
- Published: 04 February 2021

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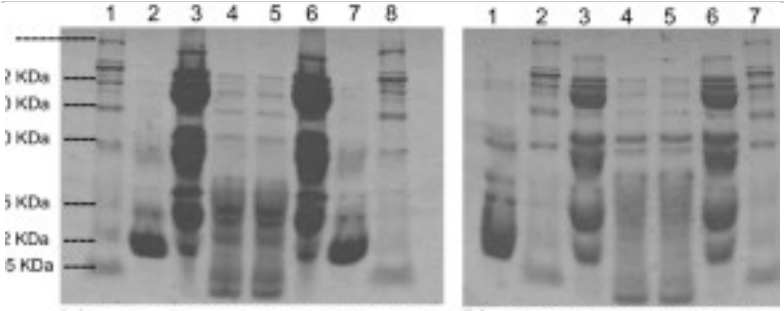

(a) Protein bands: lanes 1 and 8, MM markers; 2 and 7: α-LA; 3 and 6: whey of native bovine colostrum (NC); 4 and 5: whey of spreadable cheese with BAMLET complex (SC)

(b) Protein bands: lanes 1 and 8 c and 7: MM markers; 3 and 6: w native bovine colostrum (NC); 4 whey of spreadable cheese with BA complex (SC)

21. **Degradation of 5-methyltetrahydrofolate in model and egg yolk systems and strategies for its stabilization**

- Yan Yang
- Junhua Li
- Shijian Dong

- Content type:Original Article
- Published: 03 February 2021

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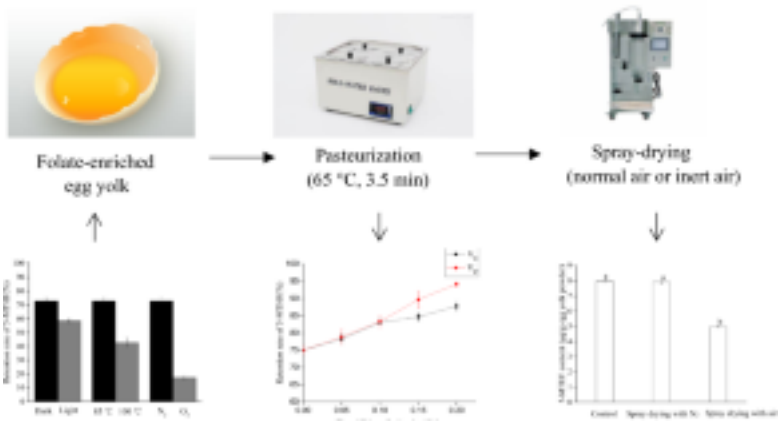

22. **Vacuum drying of Chilean papaya (*Vasconcellea pubescens*) fruit pulp: effect of drying temperature on kinetics and quality parameters**

- Antonio Vega-Gálvez
- Jacqueline Poblete
- María Gabriela Goñi

- Content type:Original Article
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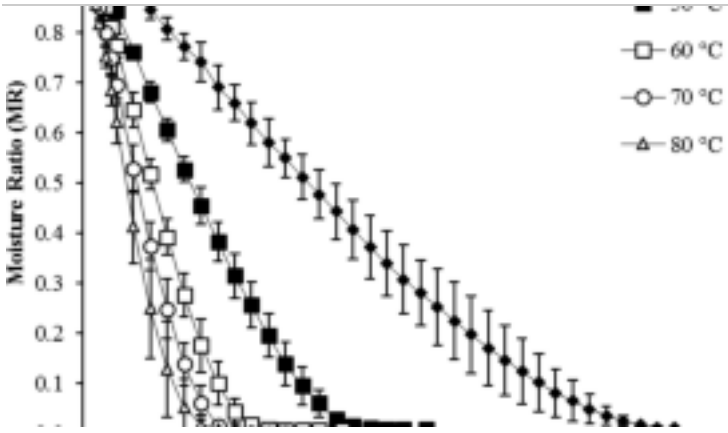

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- Safa Bejaoui
- Feriel Ghribi
- M'hamed El Cafsi

- Content type:Original Article
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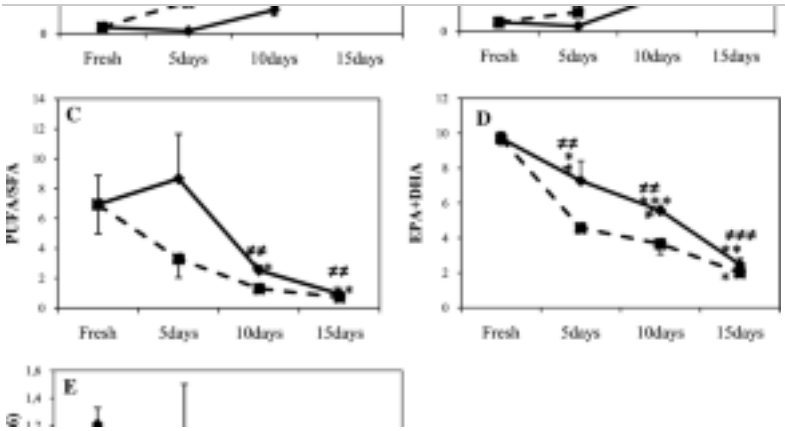

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- Wen-Ying Liu
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- Content type:Original Article
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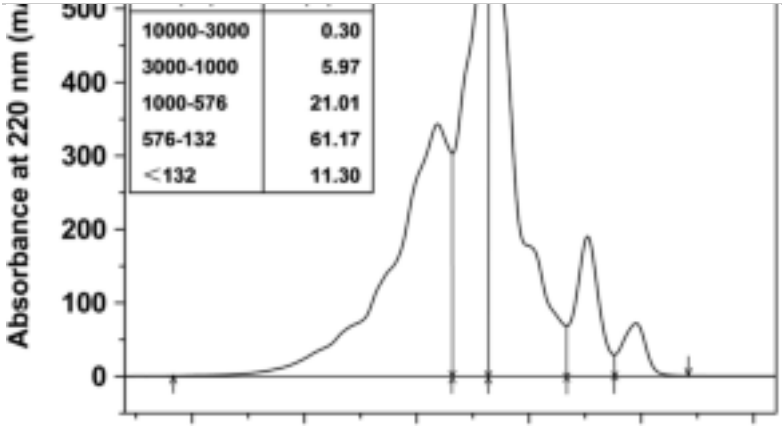

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- Muhammet İrfan Aksu
- Emre Turan

- Content type:Original Article
- Published: 27 February 2021

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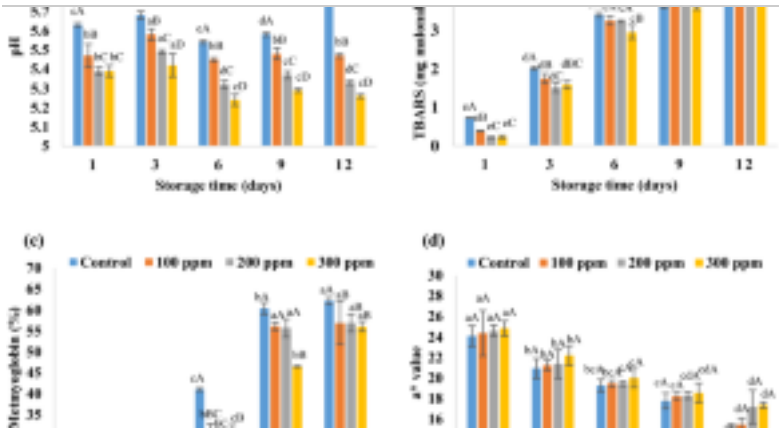

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- I. A. Harahap
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- Content type:Original Article
- Published: 29 April 2021
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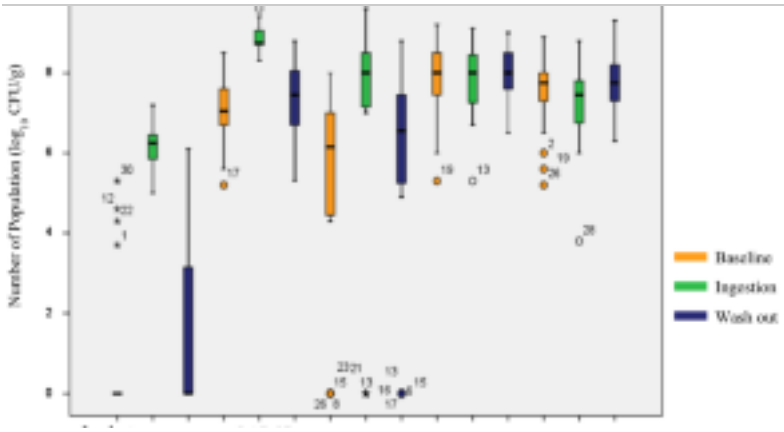

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- Pallawi Joshi
- Aparna Kumari
- Meenakshi Singh

- Content type:Original Article
- Published: 09 March 2021
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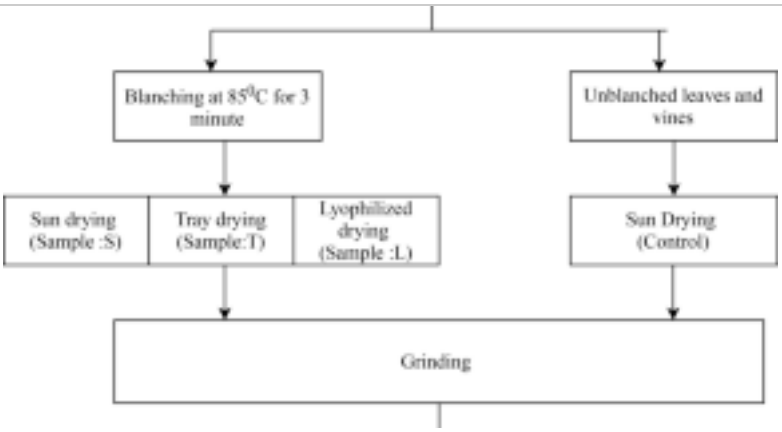

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- K. R. Jyothi
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- Iza F. Pérez-Ramírez
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- Silvia L. Amaya-Llano

- Content type:Original Article
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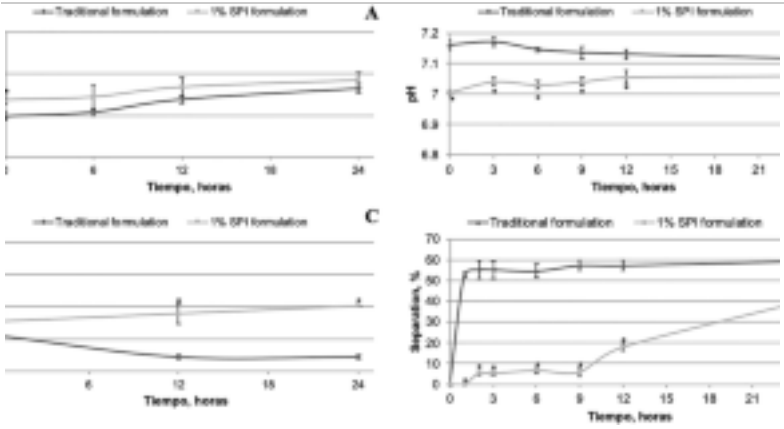

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- Keotshepile Precious Bojang
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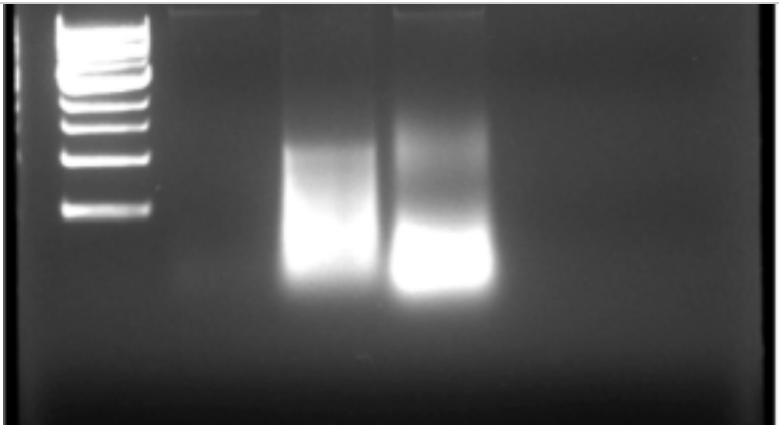

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- Gordana Rusak
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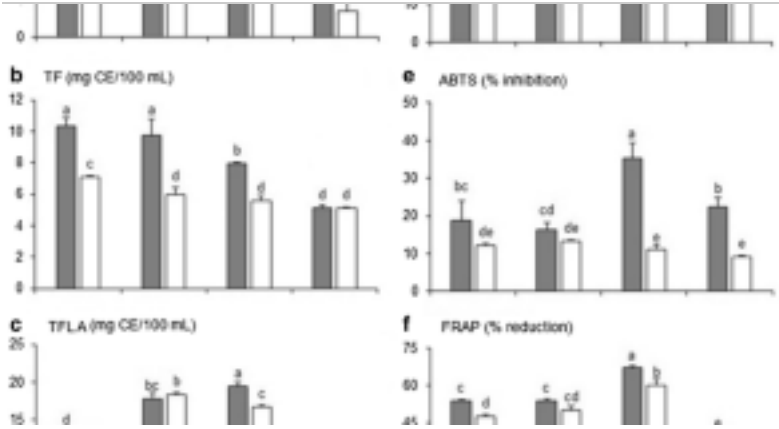

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- Mahfuzur Rahman
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- Content type:Original Article
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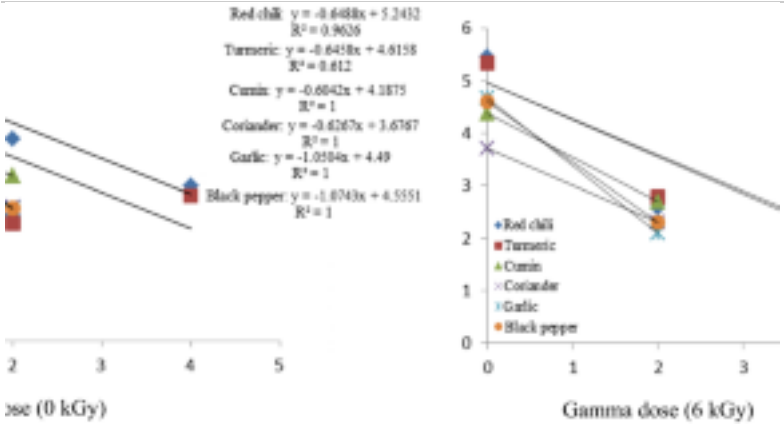

33. **[Reaction kinetics of physico-chemical attributes in coconut inflorescence sap during fermentation]**

- R. Pandiselvam
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- Sandip Shil

- Content type:Original Article
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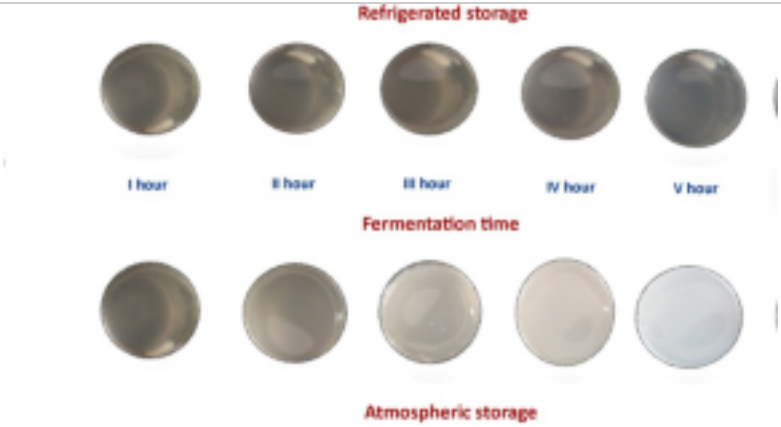

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- Narakorn Srisuk
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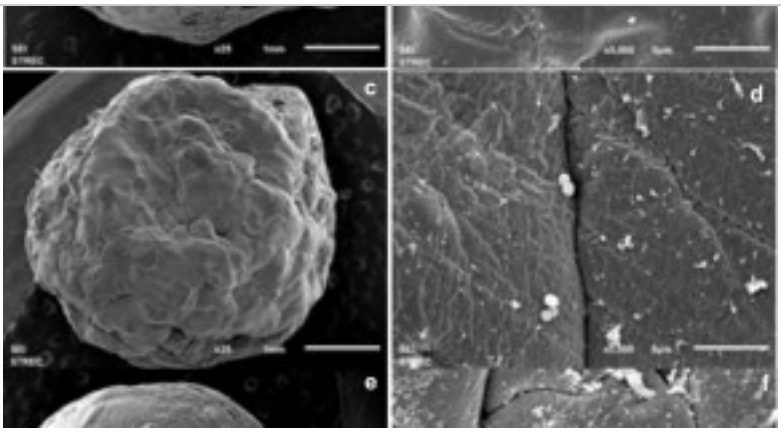

35. **Fermentation of red pitahaya extracts using *Lactobacillus* spp. and *Saccharomyces cerevisiae* for reduction of sugar content and concentration of betacyanin content**

- Ashwini Gengatharan
- Garys A. Dykes
- Wee Sim Choo

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- Published: 26 April 2021
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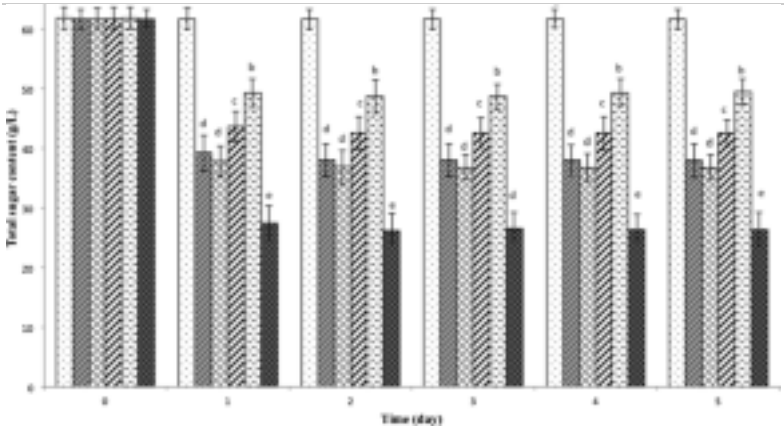

36. **Effects of drying on the structural characteristics and antioxidant activities of polysaccharides from *Stropharia rugosoannulata***

- Qi Wang
- Yalin Zhao
- Ying Liu

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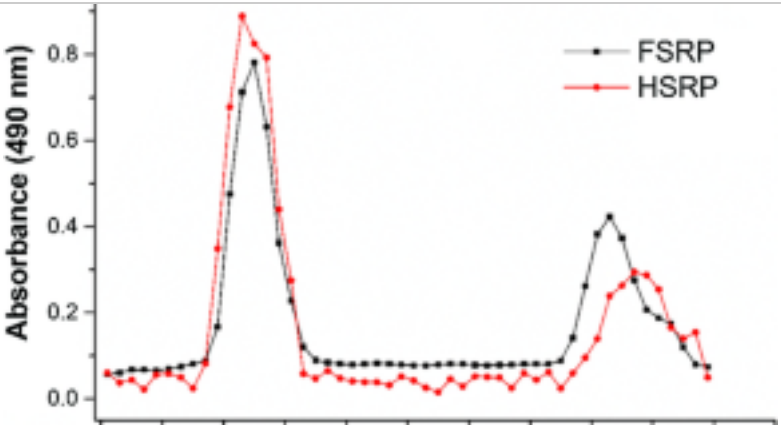

37. **Custard apple puree, fructooligosaccharide and soy protein hydrolysate as alternative ingredients in low carb pound cake**

- Adrielle Reis de Souza
- Marcio Schmiele

- Content type:Original Article
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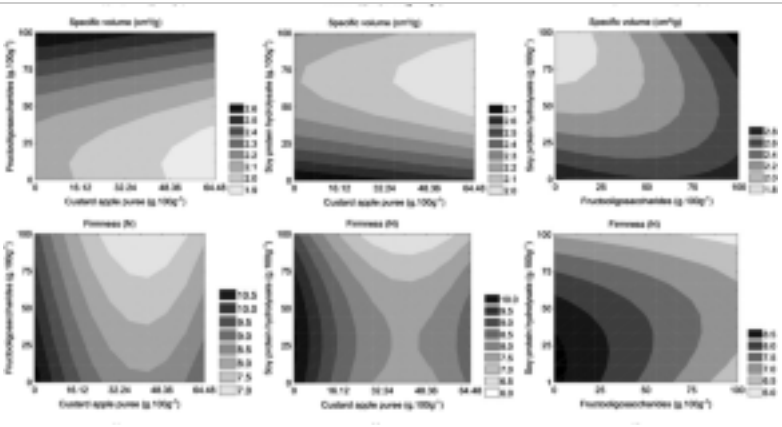

38. **Development of a fast and simple method to identify pure Arabica coffee and blended coffee by Infrared Spectroscopy**

- Alexandre Cestari

- Content type:Original Article
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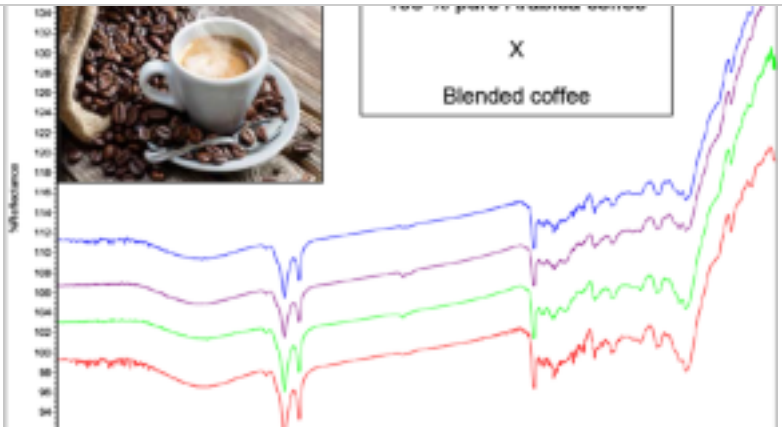

39. **Preservation of sweet pepper purees: effect on chemical, bioactive and microbial quality**

- Ramandeep Kaur
- Kamaljit Kaur

- Content type:Short Communication
- Published: 23 March 2021
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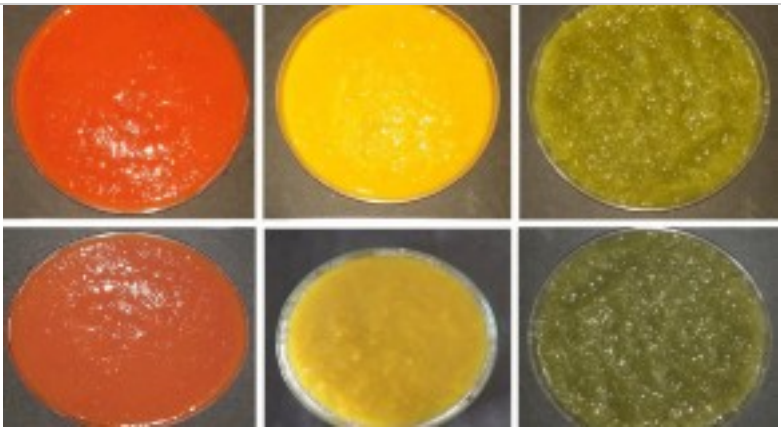

40. **Nix Pro Color Sensor provides comparable color measurements to HunterLab colorimeter for fresh beef**

- Conrad S. Schelkopf
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ORIGINAL ARTICLE

Recovery of Indigenous probiotic *Lactobacillus plantarum* Mut-7 on healthy Indonesian adults after consumption of fermented milk containing these bacteria

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Abstract Probiotics are live microorganisms that confer health benefits on the host when administered in adequate amounts, such as to support the balance of gut microbiota. In this study, the selected indigenous probiotic strain, *Lactobacillus plantarum* Mut-7, was used as a starter culture to produce fermented milk. A total of 28 healthy Indonesian youngsters and adults (male/female: 16/12; age 19.4–22.9 years old; normal BMI range 18.6–22.7 kg/m^2) were supplemented with the fermented milk. This study aimed to determine the recovery of *L. plantarum* Mut-7 through molecular analysis from the subjects feces after ingestion of 140 mL fermented milk containing 7.0 $\log_{10}$ CFU/ml of *L. plantarum* Mut-7 for 15 days. Molecular detection was performed using the rep-PCR technique and sequencing of DNA 16S rRNA. Consumption of fermented milk containing *L. plantarum* Mut-7 enabled reduction of total *E. coli* and *Coliform* non-*E. coli* in several subjects. It was able to increase the total LAB and total *L. plantarum* in subjects' feces. The number of *L. plantarum* and mesophilic LAB increased by 5.5 ± 1.6 $\log_{10}$ CFU/g, 1.8 ± 0.8 $\log_{10}$. On the other side, thermophilic LAB increased by 2.8 ± 3.0 $\log_{10}$ CFU/g in 23 out of 28 subjects. These findings proved that *L. plantarum* survived in the human gastrointestinal tract. Based on the molecular identification technique using rep-PCR technique and sequencing of gene 16S rRNA, two isolates had similarity to *L. plantarum* Mut-7 by a coefficient value of 100%.

Keywords Fermented milk · *Lactobacillus plantarum* · Indigenous probiotic · Feces

Introduction

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts (FAO 2001). The primary requirements of probiotics are to

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survive the passage through the stomach and colonize inside the colon. *Bifidobacteria* and *Lactobacilli* are two bacteria strains capable of residing in the human digestive tract and widely used as probiotics. Numerous studies reported the ability of some *Lactobacilli* to live in the human digestive tract after consumption of fermented milk for a certain period (del Campo et al. 2005; Goossens et al. 2006; Mai et al. 2017; Rahayu et al. 2016; Takeda et al. 2013; Utami et al. 2015; Yamano et al. 2006).

Nakayama et al. (2015) reported the diversity of gut microbiota in school children from five Asian countries, namely China, Japan, Taiwan, Thailand, and Indonesia, which were classified into enterotype-like clusters, namely, *Prevotella* (P-type) or *Bifidobacterium/Bacteroides* (BB-type). The findings showed that most gut microbiota from China, Japan, and Taiwan was of BB-type, while Indonesia and Thailand were P-type, reflecting their high intake of resistant starch.

Currently, Rahayu et al. (2019) reported that the most abundant microbiota in the gut of healthy adult and elderly Indonesian were *Clostridium*, followed by *Prevotella, Atopobium, Bifidobacterium,* and *Bacteroides*. Meanwhile, Nakayama et al. (2015) described that *Prevotella* enterotype was dominant in the Indonesian population. Rahayu et al. (2019) also reported that in the elderly group, an increase of Enterobacteriaceae, *E. coli,* and *Coliform* were found. Meanwhile, *L. plantarum* has the highest prevalence ($> 85\%$) within the *Lactobacillus* group. A similar finding was also reported by Rahayu et al. (2016) that *L. plantarum* was found in Indonesian subjects.

The types of bacteria used as starter cultures for milk products' fermentation are *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* or other appropriate lactic acid bacteria (Indonesian National Food and Drug Control 2015). This regulation is an enabling factor for the development of fermented milk products with the application of other lactic acid bacteria as a starter culture, for example, by using an indigenous strain of probiotic agents such as *L. plantarum* Mut-7, *L. plantarum* Dad-13, *L. plantarum* T-3, *L. plantarum* Mut-13, and *L. paracasei* SNP-2. *L. plantarum* Mut-7 is an indigenous strain isolated from *gatot* (fermented raw cassava), while *L. paracasei* SNP-2 is isolated from healthy infants' feces. These five strains are potential candidates as probiotics based on their resistance to bile salt and simulated gastric juice, as well as their antagonistic capabilities against pathogens (Rahayu et al. 2015).

In this study, *Lactobacillus plantarum* Mut-7 was used as a starter culture to produce fermented milk and subsequently studied for its potential functionality as probiotics. It is necessary to do a viability test in the participant's feces after consuming the fermented milk to ensure that *L. plantarum* Mut-7 can grow and colonize the intestine.

A previous study by Rahayu et al. (2015, 2019) reported that since *L. plantarum* was a commensal bacterium in Indonesian subjects' intestine, a confirmation test for the probiotic strain using the molecular method is necessary. The molecular techniques performed to identify the *Lactobacillus* species' strain level were Rep-PCR (Antonio and Hillier 2003). Therefore, this study aims to prove that *L. plantarum* Mut-7 can survive in the digestive tract of healthy Indonesians who consumed fermented milk containing these bacteria. Molecular confirmation was performed to determine *L. plantarum* Mut-7 in the feces of subjects.

Material and methods

Study design

This study was an experimental study with pre and post-design (Utami et al. 2015, with slight modification). The treatment took 35 days and was divided into three phases, i.e., the baseline period (10 days), the ingestion period (15 days), and the washout period (10 days). During the ingestion period, subjects were supplemented with fermented milk containing *L. plantarum* Mut-7.

Fecal samples were collected at the end of each period, i.e., day 10 (end of the baseline period), day 25 (end of ingestion period), and day 35 (end of washout period). Fecal samples were collected in sterile tubes with a small spoon. The subjects were required to put fecal matter onto a soft paper to avoid urine exposure and quickly place the sample into the tube. Subsequently, the samples were taken to the laboratory in a cooler box ($< 10\ ^{\circ}C$) with travel time less than 1 h.

During the 35 days of treatment, subjects were required to record their food intake and drug consumption (if any). Subjects were asked to make entries of their daily consumptions and defecation during the treatment in a diary. Bristol Stool Form Scale was used to help the subjects to identify their feces' quality. The diary contains information, such as food intake, a checklist of fermented milk consumption (during ingestion period), frequency of defecation, feces quality (consistency and color), medical treatment, and complaints such as diarrhea, constipation, flatulence, vomiting or pain. Furthermore, all feces samples were analyzed to determine the content of *L. plantarum,* lactic acid bacteria, *E. coli,* and *coliform* non-*E. coli*.

Subjects

Subjects were recruited from the local community of Yogyakarta, Indonesia. Interested individuals were initially screened against the inclusion criteria, such as normal body

mass index and no previous illnesses history. All participants were fully informed of the study procedures and provided written informed consent before participation.

This study followed Good Clinical Practices, as defined by the International Conference of Harmonization (Dixon 1998). The Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University—DR Sardjito General Hospital, Yogyakarta, Indonesia, provided ethics approval for this study with approval certificate No. KE/FK/0321/EC/2017. Furthermore, informed consent was obtained from all subjects.

Study product

The fermented milk used in this study was produced by PT Yummy Food Utama (Jakarta, Indonesia). It contained 7.0 $\log_{10}$ CFU/ml of *L. plantarum* Mut-7 per 180 ml/bottle. This product was stored in a refrigerator (< 4 °C) before being consumed. *L. plantarum* Mut-7 was deposited in ampoules at the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Enumeration of microorganisms on fecal samples

Fecal samples were divided into three different categories of microbiological enumeration. First, *L. plantarum* was prepared by diluting the feces and cultivating LPSM (*Lactobacillus plantarum* Selective Media) by spread plate technique (Bujalance et al. 2006). Second, total lactic acid bacteria were prepared by diluting the feces and cultivating them into de Man, Rogosa, and Sharpe (MRS) agar by pour plate technique before being incubated at 37 °C and 42°C. Third, total *E. coli* and *Coliform* non-*E. coli* were prepared by diluting the feces and cultivating them in selective media by spread plate technique. The population of *E. coli* and *coliform* non-*E. coli* was estimated using Brilliance Agar. Each fecal sample was homogenized in nine volumes Phosphate-Buffered Saline (PBS) of the weight. The serial dilution of feces suspension was done using PBS. Each feces suspension was inoculated into all media and incubated at 37 °C for 48 h. Subsequently, all samples were enumerated with a colony counter.

DNA extraction

A total of Nine isolates from feces samples were selected from the baseline period to the post-consumption period. During this study, there were three feces samples in each period containing *L. plantarum* continuously. Therefore, the selected Nine isolates from feces samples were performed for DNA extracting process. The genomic DNA was extracted by the DNA Gram-Positive Bacteria extraction method (Geneaid PrestoTM Mini gDNA Bacteria Kit). Besides, there were nine different strains of *Lactobacillus plantarum* for comparison in this study, i.e., *Lactobacillus plantarum* Mut-7 (isolated from *gatot,* fermented raw cassava), *Lactobacillus plantarum* Dad-13 (isolated from *dadih*, fermented milk), *Lactobacillus plantarum* FNCC-0027 (CCRC 12251), *Lactobacillus plantarum* FNCC-0026 (CCRC 10069 type strain), *Lactobacillus plantarum* FNCC 0020 (IFO 3074/ as *Lactobacillus cucumeris*), *Lactobacillus plantarum* FNCC-0250, (isolated from *gatot,* fermented raw cassava), *Lactobacillus plantarum* FNCC0-137 (JCM 8347), *Lactobacillus plantarum* FNCC-0127 (JCM 1551), *Lactobacillus plantarum* FNCC 0123 (JCM 1149 type strain). These strains were preserved and deposited at Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta.

repetitive-polymerase chain reaction (Rep-PCR) amplification

Rep-PCR aimed to determine the genetic diversity of selected isolates. At this stage, there were two solutions, namely Gotaq and Ready To Go (RTG). The activity was carried out in cold conditions, namely by placing ice gel under the samples used. The composition of the solution in 1 ampoule of RTG was 1 µl of DNA, 1 µl of BOX A1R primer, and 23 µl of nuclease-free water, whereas the composition in 1 ampoule of Gotaq was 1 µl of DNA, 1 µl of Primer BOX WATER, and 23 µL Gotaq. The volume of all reactions was 25 µl. The PCR cycles took place under the following conditions: 94 °C (4 min) for 1 cycle; 92 °C (1 min), 50 °C (1.5 min), 68 °C (8 min) for 30 cycles; and 65 °C (10 min) for 1 cycle. The process of DNA sequencing was done by the 1st BASE DNA Sequencing Service, Malaysia.

Statistical analysis and phylogenetic tree

Wilcoxon Signed Rank Test with a significance level of 95% was performed to evaluate fecal quality differences (stool consistency, stool color, defecation frequency, and the number of days with bowel movement). The same statistical analysis was also conducted to compare the population of *L. plantarum*, lactic acid bacteria, *E. coli*, and coliform non-*E. coli* in feces between baseline, ingestion, and washout period. Meanwhile, DNA sequencing analysis results in nucleotide bases were utilized to identify the types of bacteria on GenBank throughout the Basic Local Alignment Search Tool (BLAST) site. NTSYS 2.2 was performed to construct the contig sequence data, while MEGA 6.0 was performed to construct a phylogenetic tree.

Results

Subjects characteristics

A total of 30 subjects were recruited at the beginning of the study. All of the subjects were healthy, with no history of previous illness or any drugs consumed during the study. Two subjects withdrew from the study due to personal reasons. At the end of the study, 28 subjects completed the treatment.

The subjects comprised of young adults (16 male (57%) and 12 female (43%)), with ages between 19.4 and 22.9 years old. All subjects had a normal BMI range between 18.6 and 22.7 kg/m^2, weight range between 45.0 and 72.0 kg, and height range between 153 and 172.8 cm.

Fecal quality and bowel movement

Fecal quality (measured based on the Bristol Stool Form Scale) and the number of bowel movements are presented in Table 1. Statistical analysis was performed using the Wilcoxon Signed Rank Test with $p < 0.05$. During the ingestion and washout period, the data showed no significant differences to the baseline period in all experimental parameters, i.e., stool consistency, stool color, defecation frequency, and the number of days with bowel movements.

In all periods, the stool consistency was type 4 (like a smooth, soft sausage or snake). Stool color remained constant with type 2, yellowish-brown. On average, the defecation frequency in all periods was ± 5–6 times/5 days. Meanwhile, the average number of days with bowel movements in all periods remained constant at 4.8 days/5 days or almost every day.

Effect of fermented milk containing *L. plantarum* Mut-7 consumption on the number of *L. plantarum* and lactic acid bacteria

Table 2 and Fig. 1 show some information on feces' microbiological analysis, including total *L. plantarum* in the gastrointestinal tract. Two different incubation temperatures were used to determine the total LAB. The temperature of 37 °C was for the mesophilic LAB, whereas the 42 °C was for the thermophilic LAB.

The number of *L. plantarum*, mesophilic LAB, and thermophilic LAB results from baseline and ingestion periods was statistically significant. The three LABs above have experienced a significant increase from baseline to ingestion period, with *L. plantarum* increased most dramatically by 5.5 log cycles followed by mesophilic LAB by 1.8 log cycles in all subjects. Meanwhile, the number of thermophilic LAB during the ingestion period improved in 23 subjects by 2.8 log cycles and decreased in 5 subjects by 4.9 log cycles.

Additionally, the number of *L. plantarum*, mesophilic LAB, and thermophilic LAB from the ingestion and washout period was not statistically significant. However, these bacteria have experienced a significant decrease from ingestion to wash out period. The vast majority of the subjects experienced a reduction of *L. plantarum* (27 subjects) and mesophilic bacteria (26 subjects) by 5.0 and 1.6 log cycles. On the contrary, *L. plantarum* and mesophilic LAB population slightly increased in one and two subjects by 0.3 and 0.2 log cycles, respectively. Furthermore, total thermophilic LAB decreased by 2.8 log cycles in 20 subjects and increased by 3.5 log cycles in 8 subjects. The populations of the bacteria mentioned above during the washout period were not lower than during the baseline period.

Effect of fermented milk containing *L. plantarum* Mut-7 consumption on total *E. coli* and coliform non-*E. coli*

Table 2 and Fig. 1 show the number of *E. coli* and *Coliform* non-*E. coli* during the treatment. The population of *E. coli* and *Coliform* non-*E. coli* from baseline and washout period was compared against the ingestion period. None of those comparisons were statistically significant. The population of *E. coli* and *Coliform* non-*E. coli* remained almost constant through all phases, with both a slight decrease and an increase within the subjects.

The coding in all isolates was given based on ingestion period, namely: Baseline period subject 1 (F1L1), Baseline

Table 1 Statistical analysis using Wilcoxon Signed Rank Test with $p < 0.05$ for fecal quality and number of bowel movements based on Bristol Stool Form Scale

Fecal quality	Baseline period	Ingestion period	*p* value	Washout period	*p* value
Stool consistency	4.13 ± 0.34	4.22 ± 0.35	0.097	4.96 ± 0.50	0.483
Stool color	2.34 ± 0.51	2.14 ± 0.55	0.179	2.25 ± 0.58	0.172
Defecation frequency (times/5, days mean ± SD)	5.84 ± 1.37	5.96 ± 1.45	0.119	5.61 ± 0.97	0.929
Number of days with bowel movements (days/5 days, mean ± SD)	4.84 ± 0.36	4.85 ± 0.31	0.666	4.82 ± 0.31	0.796

Table 2 Statistical analysis using Wilcoxon Signed Rank Test with $p < 0.05$ for the number of population of *L. plantarum*, lactic acid bacteria, *E. coli*, and coliform non-*E. coli* in feces

Microorganism	Number of population (log_{10} CFU/g) (mean ± SD)				
	Baseline period	Ingestion period	*p* value	Washout period	*p* value
L. plantarum	0.6 ± 1.6	6.2 ± 0.6	0.000*	1.3 ± 2.2	0.075
LAB 37 °C	7.1 ± 0.7	8.9 ± 0.3	0.032*	7.4 ± 0.9	0.269
LAB 42 °C	5.0 ± 2.8	6.5 ± 3.5	0.000*	5.4 ± 3.0	0.097
E. coli	7.8 ± 0.9	7.8 ± 0.8	0.354	8.0 ± 0.6	0.427
Coliform non-*E. coli*	7.5 ± 0.9	7.3 ± 1.0	0.624	7.7 ± 0.8	0.330

*Indicates a statistically significant result

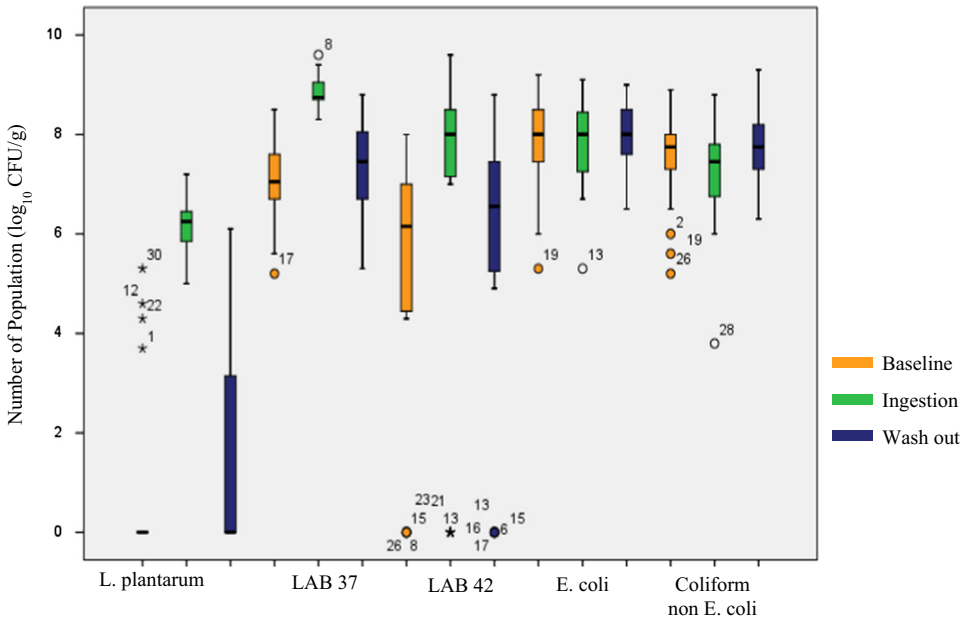

Fig. 1 The population of *E. coli* and Coliform non-*E. coli* in baseline, ingestion, and washout period

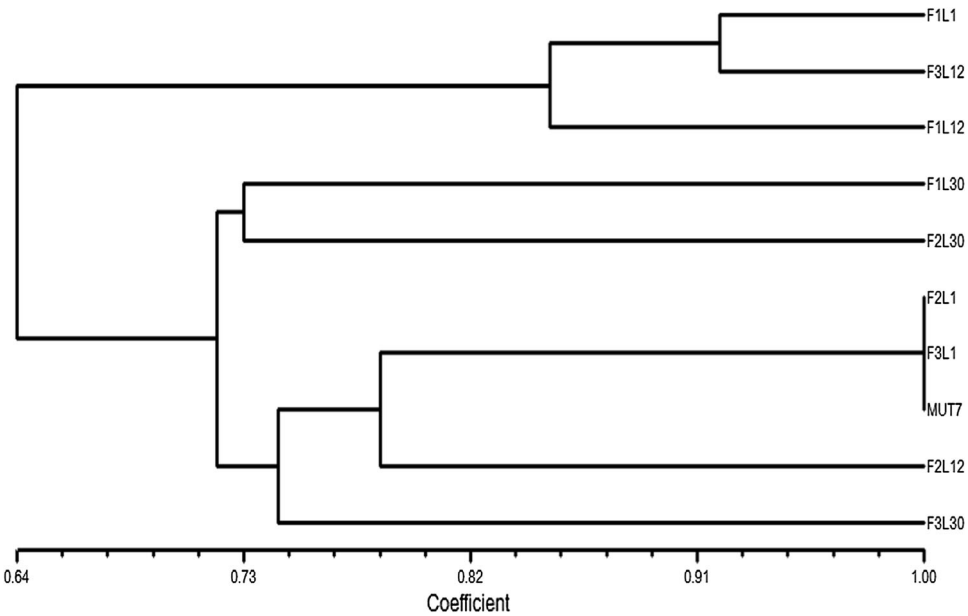

Fig. 2 Combined dendrogram for nine selected feces isolates from all phases and *L. plantarum* Mut-7 based on 16S rRNA gene sequencing using rep-PCR technique with the BOX A1R primer generated by combining the NTSYS and MEGA

period subject 12 (F1L12), Baseline period subject 30 (F1L30), Ingestion period subject 1 (F2L1), Ingestion period subject 12 (F2L12), Ingestion period subject 30 (F2L30), Washout period subject 1 (F3L1), washout period subject 12 (F3L12) and washout period subject 30 (F3L30). The phylogenetic tree from the nine selected isolates in the feces of subjects is shown in Fig. 2. It demonstrates two selected feces isolates that had similarities with *L. plantarum* Mut-7 with a coefficient of 100%, namely Ingestion period subject 1 (F2L1) and washout period subject 1 (F3L1). Based on these results, it was evident that *L. plantarum* Mut-7 was able to survive in the gastrointestinal tracts of subjects who consumed fermented milk containing *L. plantarum* Mut-7 for 15 days during consumption and ten days after consumption. Further analysis included 16S rRNA gene sequencing to identify strain types. Figure 3 shows the results of three isolates selected from the subjects. Based on the 16S rRNA gene sequence, all three isolates had 99% similarities.

Discussion

Our findings indicate that the supplementation of fermented milk containing *L. plantarum* Mut-7 did not significantly affect the fecal quality and bowel movement of healthy Indonesian subjects having normal fecal quality (stool consistency type 4). These findings are in line with the previous studies by Utami et al. (2015) and Rahayu et al. (2016), where no significant differences were observed in the fecal quality of healthy Indonesian adults after the intake of fermented milk containing *L. casei* Shirota strain (LcS). Furthermore, Matsumoto et al. (2006) found that fecal quality and defecation frequency in subjects with a lower tendency of constipation problems were not significantly different after consuming fermented milk containing LcS. This study's findings supported previous studies. The effect of supplementation of fermented milk containing *L. plantarum* Mut-7 to healthy individuals keeps their digestive tract healthy, rather than improving it, indicated by no significant differences of fecal quality and bowel movement before and after fermented milk consumption.

In general, the population of *E. coli* remained constant throughout the treatment. Meanwhile, *Coliform* non-*E. coli* slightly decreased during the ingestion period. Within the subjects group, variations occurred on the decrease/increase of these bacteria during ingestion and washout periods. This finding is in line with the conclusion of the study done by Rahayu et al. (2016). Although the total of *Enterobacteriaceae*, *E. coli,* and *coliform* non-*E. coli* in the fecal matter was not significantly different. It decreased by more than 60% of subjects after consuming fermented milk. Another study (Herías et al. 1999) investigated the effect of the administration of a combination of *L. plantarum* and *E. coli* in a group of mice. The finding showed that one week after colonization, the total *E. coli* was lower than that in a group colonized with only *E. coli* in the small intestine and caecum. However, the levels became similar after five weeks. Consequently, these findings show that colonies of *L. plantarum* compete with *E. coli* for intestinal settlement and influence intestinal and systemic immunity.

The total LAB after 15 days increased in both mesophilic LAB and thermophilic LAB. According to Tabasco et al. (2007), the selective enumeration of *S. thermophiles* was aerobic incubation at 45 °C for 24 h. Such conditions

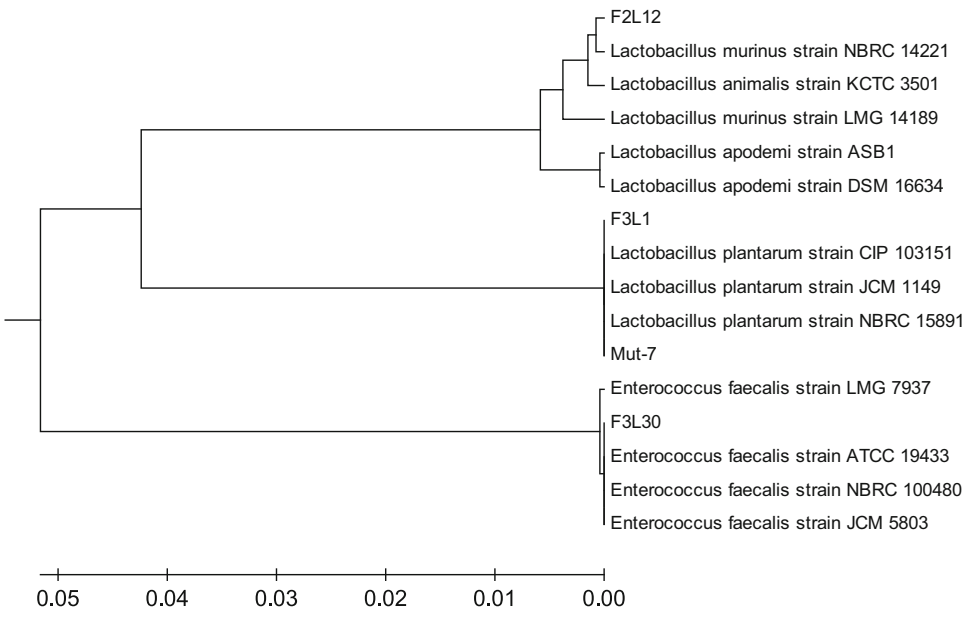

Fig. 3 Phylogenetic tree of three selected isolates based on 16S rRNA gene sequencing using rep-PCR technique with the BOX A1R primer and generated by combining the NTSYS and MEGA

disturbed the growth of *L. paracasei* subsp. *paracasei*, *L. delbrueckii* subsp. *bulgaricus*, and *B. lactis* found at 37 °C (Tabasco et al. 2007). Hence, the LAB total could be found at two different temperatures.

Lactobacillus plantarum Mut-7 is a lactic acid bacterium isolated from a type of fermented, dried cassava called *gatot*. This strain is a potential probiotic candidate since it was found to resist simulated gastric juice, bile salt, and anti-microbial activities against pathogens (Rahayu et al. 2015). In this study, the total population of *L. plantarum,* mesophilic LAB, and thermophilic LAB showed a significant increase compared to the baseline period. This finding is in line with several previous studies by Goossens et al. (2006) and Johansson et al. (2002). Consumption of fermented milk containing *L. plantarum* Mut-7 enhances the capacity to increase the number of LAB significantly. Similarly, Zago et al. (2011) stated that strains of *L. plantarum* had survived gastric transit and colonized the intestinal tract of humans and other mammals.

Antonio and Hillier (2003) stated that the rep-PCR technique showed good discrimination results on DNA fingerprinting, mainly in the genus *Lactobacillus* at the species level (Antonio & Hillier, 2003). Therefore, rep-PCR band patterns were able to differentiate *L. plantarum* Mut-7 from other strains of *L. plantarum* with distinctive patterns and different bands.

Based on 16S rRNA gene sequencing, the three selected isolates bore a similarity of 99%. Anderson et al. (2014) stated that the same species with the highest similarity and score bits were reflected by sequences with a $\geq$ 98% match to a database sequence (Anderson et al. 2014). The isolate from the ingestion period for subject 12 (F2L12) had a similarity to *Lactobacillus murinus* strain NBRC 14221 (NR_112689.1), while the isolate from the washout period for subject 1 (F3L1) had a similarity to *L. plantarum* JCM 1149 (NR_115605.1). The isolates from the washout period for subject 30 (F3L30) were similar to *Enterococcus fecalis* ATCC 19433 (NR_115765.1).

According to Nardi et al. (2005), *Lactobacillus murinus* is one of the major components of the digestive tract microbiota in rats and dogs (Nardi et al. 2005). The strain *Lactobacillus murinus* L1 has antagonistic activity against spoilage and pathogenic bacteria such as *Bacillus cereus* and *Shigella sonnei*. In a previous study, Rahayu et al. (2015) reported in the identification results, based on sequence similarity gene 16S rRNA, that *Lactobacillus plantarum* Mut-7 had a similarity to *L. plantarum ss. plantarum* JCM 1149 (Rahayu et al. 2015). Meanwhile, Nueno-Palop and Narbad (2011) reported that *E. fecalis* survived in the human digestive system and had the potential to colonize (Nueno-palop and Narbad 2011).

Conclusion

Consumption of fermented milk containing *L. plantarum* Mut-7 enabled reduction of the total *E. coli* and *Coliform* non-*E. coli* in several subjects. It was able to increase the total LAB and total *L. plantarum* in subjects' feces. Furthermore, the increase in total *L. plantarum* proved that *L. plantarum* survived in the human gastrointestinal tract. Based on the molecular identification results by rep-PCR technique and sequencing of gene 16S rRNA, two isolates were similar to *L. plantarum* Mut-7, namely F2L1 and F3L1 by a coefficient value of 100%.

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Author contributions IAH: carried out the experiment, performed the lab activities and data analysis, and wrote the manuscript. M: managed the participants and research needs. PNH: assisted in journal submission and manuscript revision. FHP: assisted in manuscript revision and language editing. MJ: supervised the ethical approval and acted as consultant for the clinical trial. JW, AD and MNC supervised the activities and findings of the molecular technique. ESR, INS and TU: supervised the findings of all of this work. SN and EZ: supervised the fermented milk production.

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Declarations

Conflict of interests The authors declare no competing interests.

Ethics approval The Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University – DR Sardjito General Hospital, Yogyakarta, Indonesia, provided ethics approval for this study with approval certificate No. KE/FK/0321/EC/2017.

Consent to participate Informed consent was obtained from all subjects. The subjects voluntarily agreed to participate in this research study. They understood they could withdraw at any time without any consequences of any kind. The subjects have had the purpose and nature of the study explained, and they understood that all information for this study were treated confidentially.

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