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FOOD TECHNOLOGY

Elaboration, characterization, and probiotic viability of synbiotic non-dairy drink based on coconut

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ABSTRACT: *Coconut is a fruit grown in more than 80 countries owing to its outstanding nutritional and biological value and it is an important crop for the food industry" por "Coconut is a fruit grown in more than 80 countries and owing to its outstanding nutritional and biological value and it is an important crop for the food industry. Thus, developing new coconut-based products is attractive to explore the benefits provided by microorganisms and improve the nutritional and bioactive composition of coconut products, such as by preparing fermented beverages. This study developed and characterize a drink based on dry coconut with the prebiotic fructooligosaccharide fermented by the probiotic Lactobacillus casei. The drink was formulated, filtered, fermented, matured, and stored under refrigeration (4 °C) for 28 days; it was evaluated for its physical, chemical, antioxidant, and microbiological characteristics. Compared to the standard non-fermented sample during storage, the fermented drink showed significant variations ($P < 0.05$) in instrumental color, acidity, and pH, while changes in soluble solids and stability index were observed after 7 days of storage. Regarding the chemical composition, all parameters varied significantly after fermentation. The total phenolic compound content and antioxidant capacity increased significantly after fermentation. Significant reductions were observed ($P < 0.05$) in the viability of Lactobacillus casei after exposure to gastrointestinal tract conditions, with the following counts (in log CFU mL^{-1}) after 0 and 28 days of storage: – initial: 9.23 ± 0.04 and 9.05 ± 0.12; after the gastric phase: 6.21 ± 0.09 and 5.90 ± 0.01; and after the intestinal phase: 4.59 ± 0.33 and 4.75 ± 0.23, respectively.*

Elaboração, caracterização e viabilidade probiótica de bebida simbiótica não-láctea a base de coco

RESUMO: *O coco é uma fruta cultivada em mais de 80 países e devido ao seu excelente valor nutricional e biológico é uma importante cultura para a indústria alimentícia. Assim, desenvolver novos produtos à base de coco é atrativo para explorar os benefícios proporcionados por microrganismos e melhorar a composição nutricional e bioativa de produtos de coco, como na preparação de bebidas fermentadas. Este estudo desenvolveu e caracterizou uma bebida à base de coco seco com o prebiótico frutooligossacarídeo, fermentada pelo probiótico Lactobacillus casei. A bebida foi formulada, filtrada, fermentada, maturada e armazenada sob refrigeração (4 °C) por 28 dias; além de avaliada em relação às suas características físicas, químicas, antioxidantes e microbiológicas. Quando comparada à amostra padrão não-fermentada ao longo do armazenamento, a bebida fermentada apresentou variações significativas ($P < 0.05$) para cor instrumental, acidez e pH, enquanto as alterações para sólidos solúveis e índice de estabilidade foram observadas a partir do 7° dia de armazenamento. Em relação à composição química, todos os parâmetros variaram significativamente após o processo fermentativo. O teor de compostos fenólicos totais e capacidade antioxidante aumentaram significativamente após a fermentação. Reduções significativas foram observadas ($P < 0,05$) na viabilidade do Lactobacillus casei após a exposição às condições do trato gastrointestinal, com as seguintes contagens (em UFC mL-1) após 0 e 28 dias de armazenamento: - inicial: 9,23 ± 0.04 e 9,05 ± 0,12; após a fase gástrica: 6,21 ± 0,09 e 5,90 ± 0,01; e após a fase intestinal: 4,59 ± 0,33 e 4,75 ± 0,23, respectivamente.*

Palavras-chave: *probióticos, prebióticos, Lactobacillus casei, novos produtos, bebida fermentada não-láctea.*

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INTRODUCTION

Consumers are increasingly looking for healthy options for food and their way of life. This behavior stimulates a search for products that are more natural, less processed, and free of components such as additives and allergens that can negatively impact human health. This scenario is encouraging the food industry to change the profile and design of the products offered (ASCHEMANN-WITZEL, 2015; GRANATO et al., 2017; ASCHEMANN-WITZEL et al., 2019). Functional foods play an important role in this context, providing benefits to physical and/or mental health in addition to nutritional support when consumed in recommended amounts associated with a balanced diet (KÜSTER-BOLUDA & VIDAL-CAPILLA, 2017).

The functional food market is significant to the food industry. It is estimated that the value of this niche reached US $304.5 billion in 2020, with an annual growth rate of 8.5% (BOGUE et al., 2016). To be considered a functional food, the product must meet two requirements: toxicological safety and health benefit effectiveness, assessed through clinical trials or by evidence obtained from experimental scientific studies.

Products formulated with prebiotics are classified as functional foods. According to Gibson et al. (2017), prebiotics are components present in food that are selectively metabolized by microorganisms and confer benefits to the host. Inulin and fructooligosaccharides (FOSs) are examples of prebiotics. Since 2014, the definition largely adopted for probiotics was the one proposed by HILL et al. (2014), as living microorganisms that confer some benefit to the health of the host when ingested in adequate amounts. Complementing this definition, ZENDEBOODI et al. (2020) proposed that probiotics could be defined as viable or non-viable microbial cells, potentially beneficial to the health of the host; among probiotics, *Lactobacillus casei* stands out. This microorganism belongs to the group of lactic acid bacteria (LAB) widely used to prepare fermented products. LAB are considered to have Qualified Presumption of Safety by the European Union and Generally Regarded as Safe by the United States Food and Drug Administration. The conservative effect attributed to LAB is related to the production of organic acids, hydrogen peroxide, competition for nutrients, production of bacteriocins, proteins, fatty acids, phenolic acids, and peptides (SALADINO et al., 2016). Mixtures of live microorganisms and substrates (used selectively by host microorganisms) that offer one or more health benefits to the host are defined as symbiotic. The synergistic symbiotic type stands out among the different types of symbiotics. In this situation, the substrate is directed co-administered with microorganisms, favoring their development and metabolism. Although the prebiotic substrate can benefit intestinal microbiota, ingested microorganisms are the main focus of synergistic symbiotics (SWANSON et al., 2020).

Most products that contain microorganisms with a known probiotic claim are produced from milk, making them impossible to be consumed by lactose intolerant people, who are allergic to milk proteins, and those with ideological dietary restrictions, such as vegetarians and vegans. According to LUCATTO et al. (2020), the use of probiotics in dairy matrices is favorable due to the chemical composition of milk, which satisfies the requirements of the metabolism of microorganisms. However, the use of proteins and plant-based compounds to develop new products that meet consumer demand is growing (KANDYLIS et al., 2016).

The market for non-dairy milk is growing considerably, as these products do not contain animal-origin ingredients and are a source of fibers, minerals, unsaturated fatty acids, vitamin B, and isoflavones. In the scientific field, many raw materials have been used to prepare these products, such as soybeans, oats, rice, nuts, and almonds. One alternative is the use of coconut, the fruit of coconut trees (*Cocos nucifera L.*), which grows in more than 80 countries (ARUNACHALAM, 2012). Coconuts can be used fresh or processed as whole fruits or their parts: mesocarp fibers, milk, kernel or flesh, and husk (AGYEMANG-YEBOAH, 2011). This fruit is a source of important amino acids, fatty acids, and vitamin B (DEBMANDAL & MANDAL, 2011); it also contains tryptophan, an important amino acid for human metabolism, associated with the production of serotonin, a hormone that regulates sleep and provides a sense of well-being (PATIL & BENJAKUL, 2018).

Based on this, the objective of this study was to develop and characterize a synbiotic drink based on dry coconut pulp, with FOS, fermented by *Lactobacillus casei*, and free of animal components. We also studied the viability of *Lactobacillus casei* and its survival through the gastrointestinal tract in vitro.

MATERIALS AND METHODS

Raw materials (basic ingredients)

Dried coconut, demerara sugar, and citrus pectin were obtained from local markets (Seropédica,

RJ, Brazil and Volta Redonda, RJ, Brazil). FOS was supplied by Sweetmix® (Sorocaba, SP, Brazil). FD-DVS nu-trish® LC 01, a lyophilized culture of a single strain (*Lactobacillus casei* 01®) from Christian Hansen® (Valinhos, SP, Brazil) was used as the probiotic.

Obtaining pulp and preparation of coconut extract

The coconuts were washed under tap water with a brush, sanitized by immersion in sodium hypochlorite solution (200 ppm for 15 min), and rinsed with tap water. Then, the coconuts were punctured to remove the water and placed in an oven at 180 °C for 15 min to facilitate its opening. The pulp was extracted manually, mixed with hot water (80 °C) at a 1:3 ratio (pulp:hot water), and processed in an industrial blender for 10 min. After homogenization, the obtained liquid was filtered and used as the coconut extract (CE).

Preparation of the synbiotic coconut drink

The synbiotic coconut drink (SCND) was prepared according to the procedures described by Salmerón et al. (2015), with some modifications. First, a non-fermented drink (NFD) was produced and used as a control in this study. FOS (2.5% w/v), pectin (0.5% w/v), and demerara sugar (5% w/v) were dissolved in CE. The mixture was pasteurized at 80 °C for 10 min and cooled in an ice bath until the temperature reached 37 °C. To prepare the SCND, *Lactobacillus casei* culture was added to this mixture at an initial count of 6.50 log CFU g^{-1}, at a ratio of 0.10 g of culture to 1.00 L of the NFD and incubated under anaerobic conditions in an unventilated oven at 37 °C for 12 h. After preparation, both NFD and SCND were bottled in sterile plastic bottles and subjected to maturation for 24 h under refrigeration at 4 ± 1 °C. The drinks were refrigerated at 4 °C for 28 days for further analyses.

SCND and NFD (Figure 1) were analyzed for physicochemical and microbiological parameters after 0, 7, 14, and 28 days of refrigerated storage. All assays were performed in triplicate.

Physical and chemical analyses

Physical and chemical quality

Instrumental color was determined by reflectance using Color Quest XE equipment (Hunter Lab, Reston, USA). The soluble solids (SS) content was determined using a manual refractometer (Instrutherm, Brazil). Acidity (LA) was evaluated by the titration method, with phenolphthalein ethanolic solution as the indicator and 0.1 N sodium hydroxide solution as the titrant, and expressed in terms of lactic acid (AOAC, 2012). The pH was determined using a digital potentiometer (Oharus Starter 2100, Canada).

Chemical composition

The total carbohydrate content was determined using the phenol–sulfuric acid method (DUBOIS et al., 1956). Moisture, protein, lipid, dietary fiber, and ash contents were determined according to the AOAC (2012) methods.

Stability index

The physical stability of the drink was evaluated by investigating foam formation and/or phase separation using a test tube. The procedures followed the methodology proposed by spada et al. (2015), with modifications. Fifty milliliters of the drink was placed in graduated cylindrical tubes, and the initial height was measured (t = 0 days). The tubes were capped and stored under refrigeration conditions. After prefixed intervals (0, 7, 14, 21, and 28 days), the height of the upper phase (Hs) was measured and compared to the previous measure (Ht). The stability index (SI) was calculated using Equation 1:

$$SI = 100 \times \frac{Hs}{Ht} \qquad \text{Equation 1}$$

Determination of total phenolic content

The extracts were prepared as follows: The sample was dried in a water bath at 100 °C, and 2 g of the sample was solubilized in 15 mL of an ethanolic solution (80% v/v) in a conical falcon tube. The mixture was kept at 25 °C for 180 min under constant agitation (100 rpm) in the absence of light. The sample was then centrifuged at 1350 x g for 15 min. Finally, the supernatant was filtered and stored in an amber bottle to preserve photosensitive compounds.

The total phenolic content (TPC) was determined using the method described by SWAIN & HILLIS (1959). In a test tube, 1 ml of Folin Ciocalteu's reagent was added to 1 ml of the prepared extract and 10 ml of distilled water. The tube was left to stand for 3 min. Then, 1.5 ml of Na_2CO_3 (10% w/w) solution was added. The mixture was vortexed to ensure homogenization and maintained in the absence of light for 120 min. The absorbance at 725 nm was measured using a spectrophotometer with 80% ethanol as a blank. Results were expressed in µg eq. gallic acid/mL of the sample (with a conversion to wet basis from moisture), using a standard curve of gallic acid, constructed with concentrations in the range from 5.00 to 40 µg/mL.

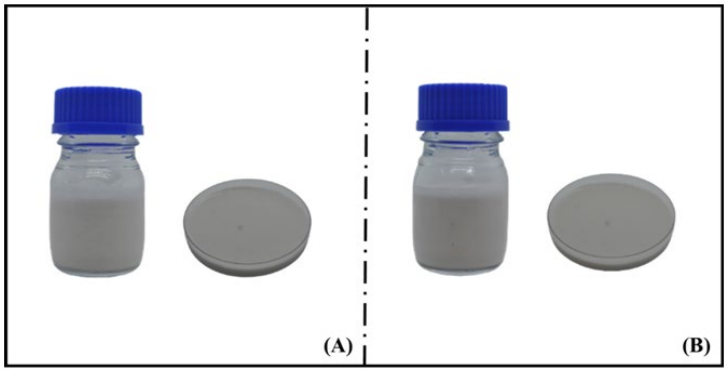

Figure 1 - (A) Synbiotic coconut drink (SCND); (B) a non-fermented drink (NFD).

Antioxidant capacity by the oxygen radical absorbance capacity method

The antioxidant capacity (AC) was determined using an extract. To prepare the extract, the sample was dried in a water bath at 100 °C. Two grams of the sample was then solubilized in 10 mL of an ethanolic solution (50% v/v) in a Falcon tube. The mixture was subjected to ultrasonication for 30 min and centrifuged at 900 x g for 20 min. The supernatant was transferred to an amber volumetric flask. Ten milliliters of 70% (v/v) acetone solution was added to the precipitate, ultrasonicated for 30 min, and centrifuged at 900 x g for 20 min. The obtained supernatant was transferred to the same volumetric flask, with the volume completed using an acetone solution (50% v/v).

The AC by the Oxygen Radical Absorbance Capacity (ORAC) method was determined following a methodology adapted from ZULUETA et al. (2009). The assay was performed using 96-well microplates. The extract (25 µL) was mixed with 150 µL of fluorescein (55.5 nM) and incubated for 15 min at 37 °C. Then, 25 µL of 2.2 azobis dihydrochloride solution (2-methylpropionamide) - AAPH (155 mM) was added. Using a fluorimeter, fluorescence was measured for 50 min with the following parameters: $\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 520 nm, using 50% acetone as the blank. Results were expressed in µmol eq. Trolox/mL of the sample (with a conversion to wet basis for moisture), using a standard Trolox curve, constructed with concentrations in the range from 20 to 100 µmol/mL.

Microbiological analyses

Microbiological quality

The microbiological quality of the drink was evaluated by the enumeration of molds and yeast, *Salmonella spp*. determination, enumeration of *Bacillus cereus,* and total and thermotolerant coliforms, following APHA (2001). The assays were performed after 0, 7, 14, 21, and 28 days of storage.

Enumeration of probiotic

The microdrop technique was used for enumeration. Aliquots (20 µL) were incubated in plates containing Man, Rogosa, and Sharpe agar (Merck, Darmstadt, Germany), containing a 2% w/v vancomycin sterile solution (0.5 mL of solution in 1000 mL of agar), using serial dilutions in sterile peptone (CHAVES et al., 1999). The plates were incubated at 37 ± 1 °C for 72 h in an unventilated oven under anaerobic conditions. *Lactobacillus casei* enumeration was performed after 0, 7, 14, 21, and 28 days of refrigerated storage (4 °C).

Survival of Lactobacillus casei in simulated gastrointestinal conditions

The in vitro tests followed the methodology described by FAVARIN et al. (2015), with some modifications. Tests were performed on days 0 and 28, divided into two phases: gastric and enteric; the enteric phase was further divided into two stages.

To simulate the gastric phase, the drink was exposed to a previously prepared solution comprising KCl (1.12 g L^{-1}), NaCl (2.00 g L^{-1}), and NaH_2PO_4 (0.40 g L^{-1}), to which 3.50 g L^{-1} of mucine III (Sigma-Aldrich, St. Louis, MO, EUA) and 0.26 g L^{-1} of purified pepsin (Sigma-Aldrich, St. Louis, MO, EUA) were added. Then, sufficiently concentrated HCl was added until a pH 1.4–1.9 was achieved. The first stage of the enteric phase was simulated by adding 10 g L^{-1} of bile solution (Sigma-Aldrich, St.

Louis, MO, USA), 1.95 g L^{-1} of pancreatin (Sigma-Aldrich, St. Louis, MO, EUA), and $NaHCO_3$ to adjust the pH to 4.30–5.20. To simulate the second stage, the same concentration of bile solution and pancreatin was used, but the pH was adjusted to 6.5–7.5.

The simulation was performed under constant stirring (50 rpm) at 37 °C in a temperature-controlled incubator (NT 715, Novatecnica, Rio de Janeiro, Brazil). The survival of *Lactobacillus casei* after exposure to the gastrointestinal tract conditions was evaluated using the microdrop counting technique described by CHAVES et al. (1999). Aliquots were removed at different intervals: four during the gastric phase (0, 30, 60, and 120 min), two during the first stage of the enteric phase (180 and 240 min), and two during the second stage of the enteric phase (300 and 360 min), resulting in a 6 h procedure to simulate the digestive process.

Statistical analysis

An analysis of variance test was applied to compare the effects of the different treatments, and the Tukey test was used to determine differences among treatments at a confidence interval of 5% (P). Statistical analyses were performed using STATISTICA software (version 7.0; StatSoft, Inc., Tulsa, Okla., USA). All tests were carried out in triplicate, and the results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Physical and chemical analyses during storage

Physical and chemical quality

The instrumental color tests indicated that all parameters analyzed significantly differed during storage ($P < 0.05$) (Table 1), demonstrating a change in the color of SCND. The parameters L^*, a^*, and b^* considerably increased, indicating that the drink became clearer and more yellowish. As a result, the $^*C$ parameter, which represents saturation, also increased, indicating an increase in the concentration of the coloring element that was responsible for the yellow color. The color change in fermented drinks depends on several factors, especially the matrix used, the microorganisms involved, and the process conditions applied. Color changes can occur due to the degradation of matrix pigments and proteolysis caused by the metabolism of probiotics (COSTA et al., 2107). In addition, the appearance and/or intensification of processes (oxidative or not) involving compounds such as lipids and polyphenols can lead to the formation of dark-colored compounds. Furthermore, coconut is a source of polyphenol oxidase, an enzyme involved in the enzymatic browning of foods. The color change may also be caused by the Maillard reaction or the generation of melanoidins (TAJCHAKAVIT et al., 2001). Several authors have reported color changes after fermentation and storage. BAÚ et al. (2014) observed a more yellowish color in samples of soy-based functional products fermented by kefir when supplemented with fibers. COSTA et al. (2017) reported the browning of a fermented drink made of a mixed extract of soy and rice byproducts during refrigerated storage.

Table 2 lists the changes in LA, pH, and SS observed during the refrigerated storage (4 ± 1 °C). The three parameters significantly varied at the 5% level immediately after the fermentation process. The LA and pH remained unstable throughout the 28 days of refrigerated storage, the first increasing over time and the second decreasing until pH 2.2. According to MATEJČEKOVÁ et al. (2017), this behavior is commonly observed in fermented products, whether

Table 1 - Color parameters of non-fermented drink (NFD) and synbiotic coconut drink (SCND).

Parameters	----------Days of storage*----------					
	Control (NFD)	----------SCND----------				
		0 days	7 days	14 days	21 days	28 days
L^*	$64.01^{e} \pm 0.38$	$67.39^{d} \pm 0.44$	$66.94^{d} \pm 0.67$	$69.67^{c} \pm 0.26$	$71.23^{b} \pm 0.69$	$83.37^{a} \pm 0.25$
a^*	$0.21^{b} \pm 0.04$	$-0.01^{d} \pm 0.02$	$-0.15^{e} \pm 0.11$	$-0.15^{e} \pm 0.04$	$0.08^{c} \pm 0.03$	$0.39^{a} \pm 0.46$
b^*	$1.82^{e} \pm 0.18$	$2.76^{c} \pm 0.05$	$2.76^{c} \pm 0.13$	$2.43^{b} \pm 0.03$	$3.11^{b} \pm 0.07$	$6.55^{a} \pm 0.76$
C^*	$1.83^{e} \pm 0.18$	$2.75^{c} \pm 0.06$	$2.76^{c} \pm 0.66$	$2.44^{d} \pm 0.04$	$3.12^{b} \pm 0.07$	$6.58^{a} \pm 0.74$
h°	$83.66^{c} \pm 1.03$	$90.20^{b} \pm 0.64$	$93.20^{a} \pm 2.22$	$93.56^{a} \pm 0.94$	$88.43^{c} \pm 0.49$	$86.15^{d} \pm 4.17$

*Storage under refrigeration (4 ± 1 °C). $^{a-c}$ Different letters in the same line indicate significant differences ($P < 0.05$).

Table 2 - Values of pH, soluble solids (SS), acidity in lactic acid (LA), and stability index (SI) of non-fermented drink (NFD) and synbiotic coconut drink (SCND).

Days of storage*	LA (g/100 mL)		pH		SS (°Brix)		SI (%)	
	NFD	*SCND*	*NFD*	*SCND*	*NFD*	*SCND*	*NFD*	*SCND*
0 days	$0.12^{B} \pm 0.50$	$0.24^{dA} \pm 0.00$	$5.78^{A} \pm 0.70$	$4.14^{aB} \pm 0.00$	$16.00^{A} \pm 0.50$	$14.50^{aB} \pm 0.00$	n/a**	$94.17^{a} \pm 0.50$**
7 days	n/a***	$0.27^{cd} \pm 0.04$	n/a***	$3.93^{b} \pm 0.02$	n/a***	$14.50^{a} \pm 0.50$	n/a**	$93.40^{a} \pm 2.36$
14 days	n/a***	$0.34^{bc} \pm 0.03$	n/a***	$3.76^{c} \pm 0.02$	n/a***	$13.33^{b} \pm 0.29$	n/a**	$84.00^{b} \pm 1.58$
21 days	n/a***	$0.44^{a} \pm 0.01$	n/a***	$3.66^{d} \pm 0.01$	n/a***	$13.17^{b} \pm 0.29$	n/a**	$81.16^{c} \pm 0.91$
28 days	n/a***	$0.38^{b} \pm 0.02$	n/a***	$2.20^{e} \pm 0.05$	n/a***	$13.67^{b} \pm 0.40$	n/a**	$66.33^{d} \pm 0.99$

*Storage under refrigeration (4 ± 1 °C). **Test performed in relation to the sample control (CND)***Tests not performed due to the growth of fungi visible to the naked eye. a–d Different letters in the same column for the same parameter indicate a significant difference ($P < 0.05$). A–BDifferent letters in the same line for the same parameter indicate a significant difference ($P < 0.05$).

probiotic or not, since microbial metabolism causes changes in the characteristics of the initial matrix, such as pH, LA, and SS. ANGELOV et al. (2006) confirmed this behavior when they studied an oat-based fermented drink: an increase in titratable LA, and reduction in pH occurred after 8 h of fermentation and during 21 days of refrigerated storage. The results of SANTOS et al. (2012) support these findings, as they presented a decrease in pH and changes in the acid concentration during fermentation for the production of caxiri. SUNNY-ROBERTS et al. (2004) observed the same behavior after the fermentation of "peanut milk," while KANTACHOTE et al. (2017) developed a coconut water-based drink fermented by *Lactobacillus plantarum* DW12.

BEDANI et al. (2013) also reported a reduction in the pH values of some formulations of a soy-based product fermented by two probiotic microorganisms (*Lactobacillus acidophilus* and *Bifidobacterium animalis* Bb-12) during 28 days of refrigerated storage (4 °C). LU et al. (2018) reported an increase in LA, associated with the production of organic acids (malic, citric, and acetic acids) during the production of a fermented vegetable drink made from durian pulp, LAB, and yeast. BERNAT et al. (2015a) reported partially similar results in their study, wherein they made an almond drink with inulin fermented by *Lactobacillus reuteri* and *Streptococcus thermophilus*, with stable pH values ($P < 0.05$) during the 28 days of storage. However, the titratable LA expressed as a percentage of lactic acid, varied significantly ($P < 0.05$). The same group of authors carried out another study using the same plant matrix (almond) but without inulin. Results indicated that the pH significantly differed between 1 and 7, 7 and 14, and 14 and 21 days of storage, but no significant differences were observed between the extremes (1 and 28 days of storage). Although the titratable LA increased, it only significantly differed among the 1st and 7th days of storage (BERNAT et al., 2015b). Results described by COSTA et al. (2017) partially agree with the studies mentioned above. According to the authors, the pH of a drink made with soy and rice decreased during 28 days of storage. However, the total LA of the drink also decreased.

Changes in pH and LA during product storage, a phenomenon known as post-acidification, occurs because of the presence of probiotic microorganisms; they can ferment the carbohydrates in the product, producing organic acids and other compounds, even if at low amounts. The produced compounds have a beneficial effect on the conservation of products, as they act as a barrier for pathogenic and food spoilage microorganisms (FARNWORTH et al., 2007; WANG et al., 2009).

The SS decreased ($P < 0.05$) after the 14th day of storage. COSTA et al. (2017) also observed the instability of SS in fermented drinks. They reported increases and decreases in this parameter over 28 days of storage. According to the authors, such changes can be justified by the ability of probiotic microorganisms to degrade previously insoluble compounds, generating molecules capable of being metabolized. In addition, the solubility of compounds

is directly affected by the pH and LA of the medium (DAMADORAN et al., 2010). Thus, the changes observed after the fermentation and post-acidification of SCND justify the variation in SS during storage.

SI

Table 2 shows the SI achieved after fermentation and during 28 days of storage. The SI values decreased by 34% ($P < 0.05$) after 28 days of storage. These changes can be attributed to the separation of the lipid fraction from the fermented drink. Phase separation can also be caused by protein coagulation induced by a decrease in pH. BERNAT et al. (2014) observed similar results when evaluating the stability of a fermented drink produced with "hazelnut milk." According to the authors, after 1 day of storage, a phase separation of approximately 11% was observed, and after 28 days, the phase separation was close to 25%. CODA et al. (2012) also observed changes in the water retention capacity of yogurt-like beverages produced with a mixture of cereals, soy, and grapes. According to the authors, these changes were discrete after fermentation. However, during the 30 days of storage, reductions of approximately 10% were observed for some formulations.

The phase separation caused by pH and LA changes in beverages can be avoided by the addition of hydrocolloids, such as gums and chemical stabilizers; they expand the hydrogen bonds and promote the formation of a gel (SONG et al., 2006). GRASSO et al. (2020) evaluated the composition and physicochemical and sensory properties of commercial products similar to yogurts made with different vegetable bases (soy, nuts, almonds, coconut, and hemp), and compared them to those of traditional yogurt. They observed that products without hydrocolloids had less physical stability, similar to traditional yogurt, to which this kind of additive is also not added. Thus, it is clear that the instability of fermented plant-based products is common and that the food industry commonly uses additives to mitigate it.

However, the developed product could be placed on the market without adding chemical additives, following the clean label trend, as the drink has only basic raw materials in its composition that are easily recognized by the consumer (ASIOLI et al., 2017). In addition, the use of non-transparent packaging and placing expressions such as "shake before drinking" or "shake before consuming" on the package could be solutions to minimize the impact of the variations in SI.

Chemical composition

Table 3 shows the chemical compositions of NFD and SCND, both on a wet basis. All analyzed parameters (moisture, lipids, proteins, total carbohydrates, fibers, and ashes) significantly differed ($P < 0.05$). After fermentation, the moisture content increased, and all other parameters decreased. Changes in the chemical composition of foods subjected to fermentation are already expected because microorganisms use these components for their metabolism. SENDRA et al. (2016) indicated that changes in the macronutrient profile of fermented foods manifest in different ways. These changes are dependent on factors such as operating conditions (time and temperature of fermentation, oxygen concentration, and absence or presence of oxygen, pH, and LA), microorganisms involved, and the concentration and availability of nutrients and fermentable compounds.

According to KAPRASOB et al. (2017), changes in the carbohydrate content are justified by the enzymatic hydrolysis process that occurs during fermentation. Through this process, complex carbohydrates are transformed into fermentable compounds, which can be easily metabolized by *Lactobacillus casei* to produce energy. SANTOS et al. (2012) also observed a reduction in the concentration of saccharides after the fermentation of sweet potatoes and cassava to produce caxiri, a typical indigenous Brazilian drink. SUNNY-ROBERTS et al. (2004)

Table 3 - Chemical composition of non-fermented drink (NFD) and synbiotic coconut drink (SCND).

	----------Components in wet base (%)----------					
	Moisture*	Lipid*	Protein*	Total carbohydrate*	Fiber*	Ash*
NFD	$74.10^{b} \pm 0.33$	$8.74^{a} \pm 1.15$	$1.28^{a} \pm 0.14$	$13.01^{a} \pm 0.94$	$2.77^{a} \pm 0.06$	$0.10^{a} \pm 0.02$
SCND	$81.39^{a} \pm 0.04$	$5.24^{b} \pm 0.17$	$0.72^{b} \pm 0.21$	$9.95^{b} \pm 0.35$	$2.55^{b} \pm 0.10$	$0.04^{b} \pm 0.02$

*Content in g/100 g of drink. $^{a–b}$ Different letters in the same column indicate significant differences ($P < 0.05$).

observed a similar behavior on producing fermented drinks with peanut extract. Similar results were also reported by ZHENG et al. (2014), who developed probiotic drinks by fermenting lychee juice treated with high hydrostatic pressure and by OJOKOH & OREKOYA (2016), during the fermentation of an agro-industrial residue (watermelon epicarp).

Dietary fibers are compounds that are selectively metabolized by *Lactobacillus casei* and promote their development, resulting in the growth of biomass (GIBSON et al., 2017). Fiber metabolism during NFD fermentation follows a principle analogous to the hydrolysis of total carbohydrates. During this process, the fibers are consumed as an alternative carbon source to produce energy. In addition, metabolites that confer benefits and therapeutic effects to human health are released (DAHL et al., 2017). Although, the content of fibers decreased during the fermentation process, the remaining amount was sufficient to characterize SCND as a prebiotic.

The ash content decreased by 41% during SCND production, changing from 0.39% to 0.23% after 12 h of fermentation. The value was already low in NFD, but even small amounts of minerals, the major components of ash, are very important for the metabolism of LAB, as they are directly involved in enzymatic processes that allow the multiplication and development of microorganisms, which explains their decrease after fermentation (WALKER, 2004).

The lipid fraction of NFD decreased by 11.7% after fermentation. This decrease can be explained by the fact that lipids can also be used as an energy source for microbial metabolism (SUNNY-ROBERTS et al., 2004). In addition, the lipid fraction may have been metabolized into compounds with lower molecular weights that were undetectable by the technique used. These short-chain fatty acids are extremely important for human health because they are an important carbon source for the maintenance of the host microbiome and to the intestinal balance (MARKOWIAK-KOPEĆ & ŚLIŻEWSKA, 2020). In contrast, some authors have reported the stability of this fraction during the fermentation of vegetables (PUERARI et al., 2015; OJOKOH & OREKOYA, 2016). Bacteria of the *Lactobacillus casei* group have metabolic versatility, which allows the use of several alternative substrates, such as lipids and their degradation products, to replace carbohydrates (MINERVINI & NEJATI, 2016).

Regarding the protein content, a reduction of 16.3% was observed because of the metabolism of *Lactobacillus casei*. Changes in the centesimal composition of vegetables after fermentation depend on several factors, and the amounts of compounds may increase or decrease. According to WANG & JI (2019), several probiotic microorganisms release exoenzymes that hydrolyze proteins into small peptides and amino acids, which can be easily transported and absorbed by the host. The detection and quantification of the transformation of proteins into small peptides and amino acids requires more sensitive techniques.

With the experimental techniques applied, we could evaluate the changes caused on the micronutrient content after the fermentation process. In further studies, more sensitive techniques could be applied, such as high-performance liquid chromatography and gas chromatography. With these techniques, it would be possible to evaluate the transformations caused by the metabolism of *Lactobacillus casei* on the matrix used and the determination of compounds formed during the production of SCND. This information could allow the elucidation of the possible benefits that these products can bring to the consumer, based on scientific literature.

Determination of TPC and AC by the ORAC method

The bioactive properties significantly increased (at the 5% level) after fermentation as per the two tests (Table 4). An increase of 16.76% in

Table 4 - Total phenolic content (TPC) and antioxidant capacity (AC) by the Oxygen Radical Absorbance Capacity (ORAC) method for the non-fermented drink (NFD) and synbiotic coconut drink (SCND).

	TPC (mg AGE* g^{-1} of sample)	AC – ORAC**
NFD	$3.40^{b} \pm 0.18$	$21.42^{b} \pm 1.93$
SCND	$3.97^{a} \pm 0.09$	$24.53^{a} \pm 1.18$

*Content in mg AGE g^{-1} of the sample. ** a–b Different letters in same column indicate significant differences ($P < 0.05$).

the TPC was observed after fermentation, while the antioxidant activity (determined by ORAC) increased by 14.52%, indicating that fermentation induced the release of bioactive compounds. According to CURIEL et al. (2015), the fermentation of vegetables by LAB is a viable option to improve and increase the AC of vegetables. Probiotic microorganisms can synthesize enzymes that cleave bonds between antioxidant compounds and sugars, leading to their release and detection in assays (LEE et al., 2008). Lactic acid production by LAB metabolism can stimulate the conversion and depolymerization of high-molecular-weight phenolic compounds into smaller compounds (MANTZOURANI et al., 2018).

In addition, microorganisms can produce metabolites that have a direct impact on AC (KIM et al., 2011). This perspective is reaffirmed by the concept of probiotics proposed by ZENDEBOODI et al. (2020), who claimed that the benefits provided by these microorganisms are not only associated with their viability.

Some authors have reported changes in the AC and TPC after the fermentation of plant matrices. MANTZOURANI et al. (2018) reported an increase in the content of phenolic compounds in cherry juice fermented by *Lactobacillus plantarum* after fermentation, during 4 weeks of storage. SABOKBAR & KHODAIYAN (2016) also observed similar behavior during the production of a drink based on pomegranate juice and whey fermented by kefir grains. MOUSAVI et al. (2013) evaluated the effects of fermentation on pomegranate juice properties and found a significant increase in AC.

Microbiological analyses

Microbiological quality

The fermented beverage (SCND) proved to be microbiologically stable for 28 days of storage under refrigeration at 4 °C, as the development of quality indicator microorganisms was not observed—molds and yeasts, *Salmonella spp*., coagulase-positive *staphylococci*, total and thermotolerant coliforms, and *Bacillus cereus* (Table 5). Conversely, NFD showed the presence of molds and yeasts from the 14th day of storage under refrigeration (4 °C) (Table 5), showing visible-to-the-naked-eye fungi after the 7th day of storage; this indicates that the technique applied was not sufficiently sensitive for the early detection of molds and yeasts. Although, other microorganisms did not develop, the growth of molds and yeasts, as well as the occurrence of fungi visible to the naked eye, make the consumption of the non-fermented beverage (NFD) unfeasible.

Similar results were observed by COSTA et al. (2017), who investigated microbiological stability during the cold storage of a probiotic drink made with soy and rice byproducts. The development of *Salmonella* and *Bacillus cereus* was also not observed, and there was a reduction in coliform counts. Luana et al. (2014) observed the microbiological stability of enterobacteria, molds, and yeasts in a drink similar to yogurt produced

Table 5 - Microbiological quality and enumeration of probiotic abundance in non-fermented drink (NFD) and synbiotic coconut drink (SCND).

		Days of storage*				
		0 days	7 days	14 days	21 days	28 days
Molds and yeasts (CFU mL^{-1})	NFD	<10	<10**	2.13^{b} ± 0.35**	4.69^{a} ± 0.03**	4.77^{a} ± 0.83**
	SCND	<10				
Salmonella spp.	NFD	Absence in 25 mL of drink				
	SCND	Absence in 25 mL of drink				
Bacillus cereus (CFU mL^{-1})	NFD	<10				
	SCND	<10				
Total coliforms (MPN mL^{-1})	NFD	<3.00				
	SCND	<3.00				
Thermotolerant coliforms (MPN mL^{-1})	NFD	<3.00				
	SCND	<3.00				
Lactobacillus casei (log CFU mL^{-1})	NFD	<10				
	SCND	9.27^{a} ± 0.41	9.20^{a} ± 0.05	9.20^{a} ± 0.79	9.19^{a} ± 0.93	9.06^{a} ± 0.97

*Storage under refrigeration (4 ± 1 °C). **Growth of fungi visible to the naked eye. $^{a-c}$Different letters in the same line indicate significant differences (P < 0.05).

from oat flakes. According to the researchers, pasteurization can guarantee the safety of this type of product. In a study carried out using a drink based on cassava and rice (inspired by a drink typical of Brazilian indigenous tribes), fermented by LAB and yeasts, FREIRE et al. (2017) observed that the development of enterobacteria was inhibited by pasteurization and the growth of beneficial microorganisms. The authors pointed out that the application of heat treatment in to vegetable drinks associated with the fermentation process can inhibit the development of pathogenic and deteriorating microorganisms.

The result obtained for the fermented beverage (SCND) are significant, as they indicate the development of safe product free from microbiological contaminants. Moreover, the non-development of the pathogenic microorganisms analyzed suggested that the combination of pasteurization, fermentation, and refrigeration processes, as well as the bioconservative effect of *Lactobacillus casei* metabolism, was sufficient to guarantee the safety of the product. The microbiological stability of SCND is an important factor for possible market insertion.

The safety of the product was also achieved owing to changes in the profile of the drink. *Lactobacillus casei*, like other LAB, tends to be dominant in fermentation processes of vegetable matrices. This behavior is justified by the characteristics of this group of microorganisms, such as the metabolization of several carbohydrates, resistance, tolerance, and development capacity in acidic environments (MCDONALD et al., 1990). Changes in the pH and LA of the medium have beneficial effects on food product safety. According to PUERARI et al. (2015), the synthesis of organic acids by the metabolism of probiotics naturally creates an unfavorable environment for the development of spoilage and pathogenic microorganisms. Among the several factors that cause this inhibitory effect, we highlighted the changes in the cell membranes of microorganisms that affect the nutrient transport processes, causing changes in the cell pH, and leading to the inactivation of several structures and processes (CAPLICE & FITZGERALD, 1999).

Enumeration of probiotic counts and survival of Lactobacillus casei after exposure to gastrointestinal conditions in vitro

The *Lactobacillus casei* count was stable throughout storage, with only a minor significant variation between 21 and 28 days of storage (Table 5). A reduction from 9.27 to 9.06 log CFU mL^{-1} was observed during the storage period. Vegetable matrices (oilseeds, nuts, fruits, and cereals) have been shown to be suitable for the development of probiotics (CHAVAN et al., 2018). İçier et al. (2015) also obtained stable ($P < 0.05$) *L. acidophilus* counts for soy drinks made with apple juice during 21 days of storage and reported more than 8.00 CFU mL^{-1} of viable cells. MANTZOURANI et al. (2018), working with probiotic cherry juices, observed a reduction in the viability of free and immobilized *Lactobacillus plantarum* during 28 days of storage. However, some authors have reported a significant reduction (approximately 3.79 log) of free microorganisms, varying from 11.15 to 7.36 log CFU mL^{-1}. COSTA et al. (2017) described more notable reductions during the storage of soy-based probiotic drinks and by-products of rice processing. *Lactobacillus acidophilus*, *Bifidobacterium spp.*, and *Streptococcus thermophilus* counts were close to 10^8, 10^6, and 10^9 CFU mL^{-1}; respectively, after fermentation. However, after 14 days of storage, the counts decreased to values below 10^5, 10^4, and 10^8 CFU mL^{-1}, respectively.

Based on these results, the conditions of the fermentation process for CE were favorable for the development of *Lactobacillus casei*, which initially had a concentration of 6.50 log CFU mL^{-1} and ended at 9.27 log CFU mL^{-1} after 12 h of fermentation. Thus, the matrix proved to be a potential alternative for the production of probiotic fermented drinks with a functional claim. The combination of pasteurization, fermentation, and refrigeration ensured the product's stability with regard to the microbiological quality and probiotic viability.

After in vitro exposure to gastrointestinal tract conditions at 0 and 28 days of storage, the abundance of *Lactobacillus casei* decreased (Figure 2). After 28 days of storage, a statistically significant variation ($P < 0.05$) was observed for the abundance of *Lactobacillus casei* after the gastric phase (close to 3.02 log CFU), reaching the first stage of the intestinal phase with 6.21 log CFU mL^{-1}. After the intestinal phase, the amount of *Lactobacillus casei* decreased dramatically, reaching 4.59 log CFU mL^{-1} at the end of the simulation. A reduction of 4.64 log CFU was observed in the initial and final abundances. After 28 days of storage, a reduction of 34.8% in the initial abundance was observed after the gastric phase, at 5.90 log CFU mL^{-1} of *Lactobacillus casei*. Regarding the intestinal phase, the survival of probiotic microorganisms was slightly greater than that of the sample stored for 0 days, at 4.75 log CFU mL^{-1} of *Lactobacillus casei*. The abundance after in vitro exposure to gastrointestinal tract conditions during storage differed statistically ($P < 0.05$) only

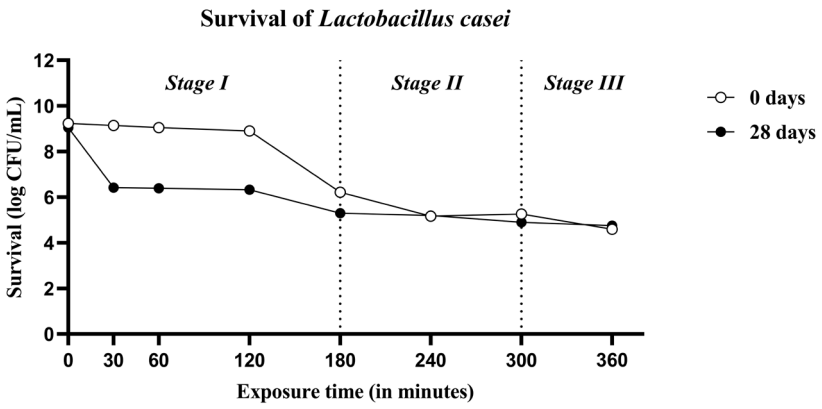

Figure 2 - Survival of *Lactobacillus casei* exposed to simulated in vitro gastrointestinal conditions.

after the gastric phase, with no significant difference ($P < 0.05$) after the intestinal phase, comparing the abundances at 0 and 28 days of storage.

BEDANI et al. (2013) reported similar results when studying a soy-based probiotic product supplemented with inulin and okara. Significant reductions were observed both at the end of the gastric phase and after the intestinal phase for the two microorganisms studied (*Lactobacillus acidophilus* and *Bifidobacterium animalis*). Comparing the initial and final abundances, the reductions were approximately 57.7% and 47.8%, respectively, higher than those found in the present study. When assessing the performance and survival of LAB in commercial non-dairy drinks, CÉSPEDES et al. (2013) also reported a reduction after the in vitro exposure of microorganisms to the gastrointestinal tract. The LAB abundance started close to 8.00 log CFU mL^{-1} and reduced to 2.00 log CFU mL^{-1} after the in vitro exposure of drinks stored at 5 °C to gastric and intestinal conditions.

During the 28 days of storage, the abundance of *Lactobacillus casei* remained greater than 9.00 log CFU mL^{-1}. However, when exposed to the conditions of the gastrointestinal tract in vitro, the abundance of *Lactobacillus casei* decreased considerably. The resistance of probiotic microorganisms to the gastrointestinal tract depends on several factors, such as the food matrix used, the presence of inhibitors, pH of the medium, concentration and accumulation of organic acids, and processing and storage conditions (KARU & SUMERI, 2016; NEMATOLLAHI et al., 2016).

Traditionally, the therapeutic effects of probiotics have been associated with the presence of microorganisms in the intestine at a concentration of approximately 6.00 log CFU mL^{-1} (CASAROTTI et al., 2014; ESPITIA et al., 2016). However, it is now known that the benefits of probiotic microorganisms are not only associated with viability (ZENDEBOODI et al., 2020). According to the authors, dead or inactive probiotic microorganisms, as well as their metabolites and cellular components, can benefit the health of the host. Future studies are necessary to evaluate the occurrence of beneficial effects, even with a reduction in microorganism viability during gastrointestinal transit. Overall, the developed drink is a functional food due to the presence of fibers and probiotics.

CONCLUSION

The elaboration of coconut-based products using *Lactobacillus casei* and FOS proved to be viable for developing products with functional claims. These products can be consumed by individuals with dietary and/or ideological restrictions. In addition, the developed beverage can be classified as synergistic symbiotic that meets the demand of "clean labels," as it was developed using a combination of multiple conservation methods (pasteurization, fermentation, and refrigeration), and chemical preservatives were not used. The applied conservation methods resulted in microbiological stability for 28 days.

Significant changes were observed in physical and chemical parameters. The beverage presented a remarkable composition of macronutrients, mainly due to the lipid and fiber contents, and can be presented as a biological source of active constituents.

Improvements can still be made to increase the viability of probiotic microorganisms after exposure to the gastrointestinal tract. Microencapsulation, optimization of the fermentation process, and immobilization of microorganisms are possible alternatives. While the results are promising, more testing is required to accurately assess the changes that occur after fermentation. Sensory tests to determine beverage acceptance and the perception of changes after fermentation and storage are also suggested as aspects to further study.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. Sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of this manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

AGYEMANG-YEBOAH, F. Chap. 43**.** Health benefits of coconut (Cocos nucifera linn.) seeds and coconut consumption**.** In: PREEDY, V. R. et al. **Nuts and Seeds in Health and Disease Prevention**. Cambridge: Academic Press, 2011, p.361–367. Available from: <http://dx.doi.org/10.1016/B978-0-12-375688-6.10043-X>. Accessed: Apr. 08, 2020. doi: 10.1016/B978-0-12-375688-6.10043-X.

ANGELOV, A. et al. Development of a new oat-based probiotic drink. **International Journal of Food Microbiology**, v.112, n.1, p.75–80, 2006. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0168160506003230>. Accessed: Dec. 03, 2020. doi: 10.1016/j.ijfoodmicro.2006.05.015.

AOAC. **Official Methods of Analysis of AOAC International**: Agricultural Chemicals, Contaminants, Drugs, 2012. Gaithersburg: AOAC International.

APHA. **Compendium of Methods for the Microbiological Examination of Foods**, 2001. Washington: APHA International.

ARUNACHALAM, V. Coconut. In: _____. **Genomics of Cultivated Palms**. Amsterdam: Elsevier, 2012. Chap.2, p.13–27. Available from: <https://www.researchgate.net/publication/285180017_Coconut>. Accessed: Nov. 10, 2020. doi: 10.1016/b978-0-12-387736-9.00002-9.

ASCHEMANN-WITZEL, J. Consumer perception and trends about health and sustainability: Trade-offs and synergies of two pivotal issues. **Current Opinion in Food Science**, v.3, p.6–10, 2015. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2214799314000046>. Accessed: Oct. 24, 2020. doi: 10.1016/j.cofs.2014.08.002.

ASCHEMANN-WITZEL, J. et al. Consumers' categorization of food ingredients: Do consumers perceive them as "clean label" producers expect? An exploration with projective mapping. **Food Quality and Preference**, v.71, p.117–128, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0950329318300405>. Accessed: Oct. 21, 2020. doi: 10.1016/j.foodqual.2018.06.003.

ASIOLI, D. et al. Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. **Food Research International**, v.99, n.1, p.58–71, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0963996917303435>. Accessed: Oct. 21, 2020. doi: 10.1016/j.foodres.2017.07.022.

BAÚ, T. R. et al. Evaluation of a functional soy product with addition of soy fiber and fermented with probiotic kefir culture. **Brazilian Archives of Biology and Technology**, v.57, n.3, p.402–409, 2014. Available from: <http://dx.doi.org/10.1590/S1516-89132014005000005>. Accessed: Dec. 03, 2020. doi: 10.1590/S1516-89132014005000005.

BEDANI, R. et al. Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. **Food Microbiology**, v.34, n.2, p.382–389, 2013. Available from: <https://pubmed.ncbi.nlm.nih.gov/23541206/>. Accessed: Dec. 03, 2020. doi: 10.1016/j.fm.2013.01.012.

BERNAT, N. et al. Hazelnut milk fermentation using probiotic Lactobacillus rhamnosus GG and inulin. **International Journal of Food Science and Technology**, v.49, n.12, p.2553–2562, 2014. Available from: <https://ifst.onlinelibrary.wiley.com/doi/abs/10.1111/ijfs.12585>. Accessed: Dec. 03, 2020. doi: 10.1111/ijfs.12585.

BERNAT, N. et al. Development of a non-dairy probiotic fermented product based on almond milk and inulin. **Food Science and Technology International**, v.21, n.6, p.440–453, 2015a. Available from: <https://journals.sagepub.com/doi/10.1177/1082013214543705>. Accessed: Oct. 31, 2020. doi: 10.1177/1082013214543705.

BERNAT, N. et al. Probiotic fermented almond "milk" as an alternative to cow-milk yoghurt. **International Journal of Food Studies**, v.4, n.2, p.201–211, 2015b. Available from: <https://www.researchgate.net/publication/325793860_Probiotic_fermented_almond_milk_as_an_alternative_to_cow-milk_yoghurt>. Accessed: Oct. 31, 2020. doi: 10.7455/ijfs/4.2.2015.a8.

BOGUE, J. et al. Market analysis and concept development of functional foods. In: BAGCHI, D.; NAIR, S. **Developing New**

Functional Food and Nutraceutical Products. Cambridge: Academic Press, 2016. Chap. 2, p.29–45. Available from: <http://dx.doi.org/10.1016/B978-0-12-802780-6/00002-X>. Accessed: Oct. 24, 2020. doi: 10.1016/B978-0-12-802780-6.00002-X.

CAPLICE, E.; FITZGERALD, G. F. Food fermentations: Role of microorganisms in food production and preservation. **International Journal of Food Microbiology**, v.50, n.1–2, p.131–149, 1999. Available from: <https://doi.org/10.1016/S0168-1605(99)00082-3>. Accessed: Nov. 05, 2020. doi: 10.1016/S0168-1605(99)00082-3.

CASAROTTI, S. N. et al. Evaluation of the effect of supplementing fermented milk with quinoa flour on probiotic activity. **Journal of Dairy Science**, v.97, n.10, p.6027–6035, 2014. Available from: <http://dx.doi.org/10.3168/jds.2014-8197>. Accessed: Nov. 05, 2020. doi: 10.3168/jds.2014-8197.

CÉSPEDES, M. et al. Performance in nondairy drinks of probiotic L. Casei strains usually employed in dairy products. **Journal of Food Science**, v.78, n.5, p.M756–M762, 2013. Available from: <https://pubmed.ncbi.nlm.nih.gov/23527588/>. Accessed: Sep. 22, 2020. doi: 10.1111/1750-3841.12092.

CHAVAN, M. et al. Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume. **LWT**, v.91, p.339–344, 2018. Available from: <https://doi.org/10.1016/j.lwt.2018.01.070>. Accessed: Dec. 03, 2020. doi: 10.1016/j.lwt.2018.01.070.

CHAVES, A. H. et al. Effect of one strain of Lactobacillus acidophilus (LT 516) as probiotic for calves. **Revista Brasileira de Zootecnia**, v.28, n.5, p.1075–1085, 1999. Available from: <https://doi.org/10.1590/S1516-35981999000500025>. Accessed: Sep. 22, 2020. doi: 10.1590/S1516-35981999000500025.

CODA, R. et al. Yogurt-like beverages made of a mixture of cereals, soy and grape must: Microbiology, texture, nutritional and sensory properties. **International Journal of Food Microbiology**, v.155, n.3, p.120–127, 2012. Available from: <http://dx.doi.org/10.1016/j.ijfoodmicro.2012.01.016>. Accessed: Nov. 04, 2020. doi: 10.1016/j.ijfoodmicro.2012.01.016.

COSTA, K. K. F. D. et al. Changes of probiotic fermented drink obtained from soy and rice byproducts during cold storage. **LWT**, v.78, p.23–30, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0023643816307927>. Accessed: Dec. 03, 2020. doi: 10.1016/j.lwt.2016.12.017.

CURIEL, J. A. et al. Lactic acid fermentation as a tool to enhance the antioxidant properties of Myrtus communis berries. **Microbial Cell Factories**, v.14, no.1, p.67, 2015. Available from: <https://pubmed.ncbi.nlm.nih.gov/25947251/>. Accessed: Nov. 04, 2020. doi: 10.1186/s12934-015-0250-4.

DAHL, W. J. et al. Health benefits of fiber fermentation. **Journal of the American College of Nutrition**, v.36, n.2, p.127–136, 2017. Available from: <https://doi.org/10.1080/07315724.2016.1188737>. Accessed: Sep. 22, 2020. doi: 10.1080/07315724.2016.1188737.

DAMADORAN, S. et al. **Fennema's Food Chemistry**. 4° ed, 2010. Porto Alegre: Artmed. 900 p.

DEBMANDAL, M.; MANDAL, S. Coconut (Cocos nucifera L.: Arecaceae): In health promotion and disease prevention. **Asian Pacific Journal of Tropical Medicine**, v.4, n.3, p.241–247, 2011. Available from: <http://dx.doi.org/10.1016/S1995-7645(11)60078-3>. Accessed: Nov. 12, 2020. doi: 10.1016/S1995-7645(11)60078-3.

DUBOIS, M. et al. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v.28, n.3, p.350–356, 1956. Available from: <https://pubs.acs.org/doi/abs/10.1021/ac60111a017>. Accessed: Sep. 10, 2020. doi: 10.1021/ac60111a017.

ESPITIA, P. J. P. et al. Probiotics and their potential applications in active edible films and coatings. **Food Research International**, v.90, p.42–52, 2016. Available from: <http://dx.doi.org/10.1016/j.foodres.2016.10.026>. Accessed: Nov. 05, 2020. doi: 10.1016/j.foodres.2016.10.026.

FARNWORTH, E. R. et al. Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. **International Journal of Food Microbiology**, v.116, n.1, p.174–181, 2007. Available from: <https://doi.org/10.1016/j.ijfoodmicro.2006.12.015>. Accessed: Dec. 03, 2020. doi: 10.1016/j.ijfoodmicro.2006.12.015.

FAVARIN, L. et al. Survival of free and microencapsulated Bifidobacterium: Effect of honey addition. **Journal of Microencapsulation**, v.32, n.4, p.329–335, 2015. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25775038>. Accessed: Feb. 02, 2020. doi: 10.3109/02652048.2015.1017620.

FREIRE, A. L. et al. Nondairy beverage produced by controlled fermentation with potential probiotic starter cultures of lactic acid bacteria and yeast. **International Journal of Food Microbiology**, v.248, p.39–46, 2017. Available from: <http://dx.doi.org/10.1016/j.ijfoodmicro.2017.02.011>. Accessed: Dec. 03, 2020. doi: 10.1016/j.ijfoodmicro.2017.02.011.

GIBSON, G. R. et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Reviews. Gastroenterology and Hepatology**, v.14, n.8, p.491–502, 2017. Available from: <https://www.nature.com/articles/s41575-020-0344-2>. Accessed: Nov. 04, 2020. doi: 10.1038/nrgastro.2017.75.

GRANATO, D. et al. An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. **Trends in Food Science and Technology**, v.62, p.13–22, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0924224416303284>. Accessed: Dec. 04, 2020. doi: 10.1016/j.tifs.2016.12.010.

GRASSO, N. et al. Composition, physicochemical and sensorial properties of commercial plant-based yogurts. **Foods**, v.9, n.3, 2020. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7142433/>. Accessed: Oct. 31, 2020. doi: 10.3390/foods9030252.

HILL, C. et al. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. **Nature Reviews. Gastroenterology and Hepatology**, v.11, n.8, p.506–514, 2014. Available from: <https://pubmed.ncbi.nlm.nih.gov/24912386/>. Accessed: Dec. 03, 2020. doi: 10.1038/nrgastro.2014.66.

İÇIER, F. et al. Changes on some quality characteristics of fermented soy milk beverage with added apple juice. **LWT – Food Science and Technology**, v.63, n.1, p.57–64, 2015. Available

from: <https://www.sciencedirect.com/science/article/abs/pii/S0023643815002558>. Accessed: Dec. 03, 2020. doi: 10.1016/j.lwt.2015.03.102.

KANDYLIS, P. et al. Dairy and non-dairy probiotic beverages. **Current Opinion in Food Science**, v.7, p.58–63, 2016. Available from: <http://dx.doi.org/10.1016/j.cofs.2015.11.012>. Accessed: Dec. 03, 2020. doi: 10.1016/j.cofs.2015.11.012.

KANTACHOTE, D. et al. The use of potential probiotic Lactobacillus plantarum DW12 for producing a novel functional beverage from mature coconut water. **Journal of Functional Foods**, v.32, p.401–408, 2017. Available from: <http://dx.doi.org/10.1016/j.jff.2017.03.018>. Accessed: Dec. 03, 2020. doi: 10.1016/J.JFF.2017.03.018.

KAPRASOB, R. et al. Fermentation-based biotransformation of bioactive phenolics and volatile compounds from cashew apple juice by select lactic acid bacteria. **Process Biochemistry**, v.59, p.141–149, 2017. Available from: <http://dx.doi.org/10.1016/j.procbio.2017.05.019>. Accessed: Nov. 04, 2020. doi: 10.1016/j.procbio.2017.05.019.

KARU, R.; SUMERI, I. Survival of Lactobacillus rhamnosus GG during simulated gastrointestinal conditions depending on food matrix. **Journal of Food Research**, v.5, n.5, p.57-66, 2016. Available from: <http://www.ccsenet.org/journal/index.php/jfr/article/view/60862>. Accessed: Dec. 03, 2020. doi: 10.5539/jfr.v5n5p56.

KIM, J. et al. Correlation between antioxidative activities and metabolite changes during cheonggukjang fermentation. **Bioscience, Biotechnology, and Biochemistry**, v.75, n.4, p.732–739, 2011. Available from: <https://pubmed.ncbi.nlm.nih.gov/21512250/>. Accessed: Nov. 04, 2020. doi: 10.1271/bbb.100858.

KÜSTER-BOLUDA, I.; VIDAL-CAPILLA, I. Consumer attitudes in the election of functional foods. **Spanish Journal of Marketing – ESIC**, v.21, n.S1, p.65–79, 2017. Available from: <http://dx.doi.org/10.1016/j.sjme.2017.05.002>. Accessed: Oct. 24, 2020. doi: 10.1016/j.sjme.2017.05.002.

LEE, I. H. et al. Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. **International Journal of Food Microbiology**, v.121, n.2, p.150–156, 2008. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0168160507005260>. Accessed: Nov. 04, 2020. doi: 10.1016/j.ijfoodmicro.2007.09.008.

LU, Y. et al. A novel non-dairy beverage from durian pulp fermented with selected probiotics and yeast. **International Journal of Food Microbiology**, v.265, p.1–8, 2018. Available from: <http://dx.doi.org/10.1016/j.ijfoodmicro.2017.10.030>. Accessed: Sep. 22, 2020. doi: 10.1016/j.ijfoodmicro.2017.10.030.

LUANA, N. et al. Manufacture and characterization of a yogurt-like beverage made with oat flakes fermented by selected lactic acid bacteria. **International Journal of Food Microbiology**, v.185, p.17–26, 2014. Available from: <http://dx.doi.org/10.1016/j.ijfoodmicro.2014.05.004>. Accessed: Dec. 03, 2020. doi: 10.1016/j.ijfoodmicro.2014.05.004.

LUCATTO, J. N. et al. Performance of different microbial cultures in potentially probiotic and prebiotic yoghurts from cow and goat milks. **International Journal of Dairy Technology**, v.73, n.1, p.144–156, 2020. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/1471-0307.12655>. Accessed: Apr. 08, 2020. doi: 10.1111/1471-0307.12655.

MANTZOURANI, I. et al. Production of a novel functional fruit beverage consisting of cornelian cherry juice and probiotic bacteria. **Antioxidants**, v.7, n.11, 2018. Available from: <https://pubmed.ncbi.nlm.nih.gov/30424527/>. Accessed: Dec. 03, 2020. doi: 10.3390/antiox7110163.

MARKOWIAK-KOPEĆ, P.; ŚLIŻEWSKA, K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. **Nutrients**, v.12, n.4, 2020. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7230973/>. Accessed: Oct. 29, 2020. doi: 10.3390/nu12041107.

MATEJČEKOVÁ, Z. et al. Functional probiotic products based on fermented buckwheat with Lactobacillus rhamnosus. **LWT – Food Science and Technology**, v.81, p.35–41, 2017. Available from: <http://dx.doi.org/10.1016/j.lwt.2017.03.018>. Accessed: Dec. 04, 2020. doi: 10.1016/j.lwt.2017.03.018.

MCDONALD, L. C. et al. Acid tolerance of Leuconostoc mesenteroides and Lactobacillus plantarum. **Applied and Environmental Microbiology**, v.56, n.7, p.2120–2124, 1990. Available from: <https://pubmed.ncbi.nlm.nih.gov/16348238/>. Accessed: Nov. 05, 2020. doi: 10.1128/AEM.56.7.2120-2124.1990.

MINERVINI, F.; NEJATI, F. Lactobacillus casei Group. **Encyclopedia of Dairy Sciences**, n.3, p.275–286, 2016. Available from: <https://www.sciencedirect.com/science/article/pii/B9780081005965008532>. Accessed: Jan. 04, 2022. doi: 10.1016/B978-0-08-100596-5.00853-2.

MOUSAVI, Z. E. et al. Effect of fermentation of pomegranate juice by Lactobacillus plantarum and Lactobacillus acidophilus on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. **Food Biotechnology**, v.27, n.1, p.1–13, 2013. Available from: <https://www.tandfonline.com/doi/abs/10.1080/08905436.2012.724037>. Accessed: Nov. 04, 2020. doi: 10.1080/08905436.2012.724037.

NEMATOLLAHI, A. et al. Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. **Electronic Journal of Biotechnology**, v.21, p.49–53, 2016. Available from: <http://dx.doi.org/10.1016/j.ejbt.2016.03.001>. Accessed: Nov. 04, 2020. doi: 10.1016/j.ejbt.2016.03.001.

OJOKOH, A. O.; OREKOYA, E. Effect of fermentation on the proximate composition of the epicarp of watermelon (Citrullus lanatus). **International Journal of Swarm Intelligence and Evolutionary Computation**, v.05, n.3, 2016. Available from: <https://www.walshmedicalmedia.com/open-access/effect-of-fermentation-on-the-proximate-composition-of-the-epicarp-ofwatermelon-citrullus-lanatus-2090-4908-1000143.pdf>. Accessed: Apr. 08, 2020. doi: 10.4172/2090-4908.1000143.

PATIL, U.; Benjakul, S. Coconut milk and coconut oil: Their manufacture associated with protein functionality. **Journal of Food Science**, v.83, n.8, p.2019–2027, 2018. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/1750-3841.14223>. Accessed: Nov. 12, 2020. doi: 10.1111/1750-3841.14223.

PUERARI, C. et al. Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. **Food Microbiology**,

v.46, p.210–217, 2015. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0740002014002081>. Accessed: Oct. 29, 2020. doi: 10.1016/j.fm.2014.08.009.

SABOKBAR, N.; KHODAIYAN, F. Total phenolic content and antioxidant activities of pomegranate juice and whey based novel beverage fermented by kefir grains. **Journal of Food Science and Technology**, v.53, n.1, p.739–747, 2016. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4711455/>. Accessed: Nov. 04, 2020. doi: 10.1007/s13197-015-2029-3.

SALADINO, F. et al. In vitro antifungal activity of lactic acid bacteria against mycotoxigenic fungi and their application in loaf bread shelf life improvement. **Food Control**, v.67, p.273–277, 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0956713516301177>. Accessed: Dec. 03, 2020. doi: 10.1016/j.foodcont.2016.03.012.

SALMERÓN, I. et al. Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. **Journal of Functional Foods**, v.15, p.106–115, 2015. Available from: <http://dx.doi.org/10.1016/j.jff.2015.03.012>. Accessed: Feb. 20, 2020. doi: 10.1016/j.jff.2015.03.012.

SANTOS, C. C. A. do A. et al. Microbiological and physicochemical characterisation of caxiri, an alcoholic beverage produced by the indigenous Juruna people of Brazil. **International Journal of Food Microbiology**, v.156, n.2, p.112–121, 2012. Available from: <http://dx.doi.org/10.1016/j.ijfoodmicro.2012.03.010>. Accessed: Oct. 29, 2020. doi: 10.1016/j.ijfoodmicro.2012.03.010.

SENDRA, E. et al. Effect of food composition on probiotic bacteria viability. In: WATSON, R. R.; PREEDY, V. R. **Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion**. Amsterdam: Elsevier, 2016. Chap.17, p.257–269. Available from: <https://www.sciencedirect.com/science/article/pii/B9780128021897000174?via%3Dihub>. Accessed: Sep. 22, 2020. doi: 10.1016/B978-0-12-802189-7.00017-4.

SONG, K.-W. et al. Rheology of concentrated xanthan gum solutions: Steady shear flow behavior. **Fibers and Polymers**, v.7, n.2, p.129–138, 2006. Available from: <https://link.springer.com/article/10.1007/BF02908257>. Accessed: Sep. 22, 2020. doi: 10.1007/BF02908257.

SPADA, J. C. et al. Rheological modelling, microstructure and physical stability of custard-like soy-based desserts enriched with guava pulp. **CyTA - Journal of Food**, v.13, n.3, p.373–384, 2015. Available from: <https://www.tandfonline.com/doi/full/10.1080/19476337.2014.987698>. Accessed: Nov. 04, 2020. doi: 10.1080/19476337.2014.987698.

SUNNY-ROBERTS, E. O. et al. An evaluation of some quality parameters of a laboratory-prepared fermented groundnut milk. **European Food Research and Technology**, v.218, n.5, p.452–455, 2004. Available from: <https://link.springer.com/article/10.1007/s00217-004-0880-y>. Accessed: Oct. 29, 2020. doi: 10.1007/s00217-004-0880-y.

SWAIN, T.; HILLIS, W. E. The phenolic constituents of Prunus domestica. I.—The quantitative analysis of phenolic constituents. **Journal of the Science of Food and Agriculture**, v.10, n.1, p.63–68, 1959. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740100110>. Accessed: Feb. 20, 2020. doi: 10.1002/jsfa.2740100110.

SWANSON, K. S. et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of Synbiotics. **Nature Reviews. Gastroenterology and Hepatology**, v.17, n.11, p.687–701, 2020. Available from: <https://www.nature.com/articles/s41575-020-0344-2>. Accessed: Jan. 04, 2022. doi: 10.1038/s41575-020-0344-2.

TAJCHAKAVIT, S. et al. Kinetics of haze formation and factors influencing the development of haze in clarified apple juice. **Food Research International**, v.34, n.5, p.431–440, 2001. Available from: <https://doi.org/10.1016/S0963-9969(00)00188-5>. Accessed: Sep. 22, 2020. doi: 10.1016/S0963-9969(00)00188-5.

WALKER, G. M. Metals in yeast fermentation processes. **Advances in Applied Microbiology**, v.54, p.197–229, 2004. Available from: <https://pubmed.ncbi.nlm.nih.gov/15251282/>. Accessed: Dec. 03, 2020. doi: 10.1016/S0065-2164(04)54008-X.

WANG, J. et al. Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage. **Journal of Dairy Science**, v.92, n.6, p.2468–2476, 2009. Available from: <http://dx.doi.org/10.3168/jds.2008-1849>. Accessed: Dec. 03, 2020. doi: 10.3168/jds.2008-1849.

WANG, J.; JI, H. Influence of probiotics on dietary protein digestion and utilization in the gastrointestinal tract. **Current Protein and Peptide Science**, v.20, n.2, p.125–131, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/29769003/>. Accessed: Oct. 29, 2020. doi: 10.2174/1389203719666180517100339.

ZENDEBOODI, F. et al. Probiotic: Conceptualization from a new approach. **Current Opinion in Food Science**, v.32, p.103–123, 2020. Available from: <https://doi.org/10.1016/j.cofs.2020.03.009>. Accessed: Nov. 04, 2020. doi: 10.1016/j.cofs.2020.03.009.

ZHENG, X. et al. Comparing product stability of probiotic beverages using litchi juice treated by high hydrostatic pressure and heat as substrates. **Innovative Food Science and Emerging Technologies**, v.23, p.61–67, 2014. Available from: <http://dx.doi.org/10.1016/j.ifset.2014.01.013>. Accessed: Oct. 29, 2020. doi: 10.1016/j.ifset.2014.01.013.

ZULUETA, A. et al. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. **Food Chemistry**, v.114, n.1, p.310–316, 2009. Available from: <http://dx.doi.org/10.1016/j.foodchem.2008.09.033>. Accessed: Feb. 20, 2020. doi: 10.1016/j.foodchem.2008.09.033.