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Development and evaluation of a fermented coconut water beverage with potential health benefits

Flávera Camargo Prado [a], *Juliano De Dea Lindner* [b,*], *Juliana Inaba* [c], *Vanete Thomaz-Soccol* [a], *Satinder Kaur Brar* [d], *Carlos Ricardo Soccol* [a]

[a] *Bioprocess Engineering and Biotechnology Division, Chemical Engineering Department, Federal University of Paraná, 81531-991 Curitiba, Brazil*

[b] *Food Science and Technology Department, Federal University of Santa Catarina, 88034-001 Florianópolis, Brazil*

[c] *Chemistry Department, Ponta Grossa Estate University, 84030-900 Ponta Grossa, Brazil*

[d] *Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, Québec, Canada G1K 9A9*

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ABSTRACT

Coconut water is a liquid obtained from coconuts (*Cocos nucifera* L.) with natural hydrating qualities, functional health properties and nutritional benefits. The aim of this work was to develop an innovative, non-dairy, fermented functional beverage using coconut water as the main ingredient for providing the intrinsic health properties. Among seven autochthonous lactic acid bacteria strains isolated from natural fermentation of coconut water, *Lactobacillus plantarum* was selected due to its inhibitory activity against some pathogens and its characteristics that favor survival under some technological and gastrointestinal conditions. The fermentation was carried out for 8 h at 37 °C in the presence of yeast extract, soy protein hydrolysate and sucrose. Coconut water is a non-dairy substrate that can be introduced as a new vehicle for the consumption of functional cultured beverage, especially by vegans and/or vegetarians and lactose intolerant consumers.

1. Introduction

Coconut water (CW) is the clear, nutritive liquid obtained from the endosperm of coconuts (*Cocos nucifera* L.) and is largely consumed in tropical countries. Nowadays, CW consumption is increasing worldwide and represents one of the fastest growing beverage categories due to its natural hydrating qualities, enhanced taste, functional health properties and nutritional benefits (Campbell-Falck, Thomas, Falck, Tutuo, & Clem, 2000; DebMandal & Mandal, 2011). CW is low in calories and fat, thus, it could be effectively used as a natural alternative to replenish lost electrolytes during exercise in sports nutrition (Saat, Singh, Sirisinghe, & Nawawi, 2002). The nutritional composition of CW has been well documented. The wide applications of CW can be attributed to its unique chemical composition of sodium, potassium, phosphorus, chloride and magnesium, vitamins, sugars, proteins, free amino acids and growth promoting factors (Matsui, Gut, Vitoriano de Oliveira, & Tadini, 2008; United States Department of Agriculture (USDA), 2008; Yong, Ge, Ng, & Tan, 2009). The unique composition of CW makes it important in the treatment of human diarrhea in poor regions

* *Corresponding author.* Food Science and Technology Department, Federal University of Santa Catarina, 88034-001 Florianópolis, Brazil. Tel.: +55 48 37216183; fax: +55 48 37216183.
E-mail address: juliano.lindner@ufsc.br (J. De Dea Lindner).

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of the world, hydrating the individuals and protecting gastrointestinal tract against different infections.

Frequent consumption of CW could provide some effects to the consumers. CW had been analyzed by Chang and Wu (2011) and for the first time they reported the presence of (+)-catechin and (–)-epicatechin. Catechins possess antioxidant, antimicrobial and anticancer activities (Nurulain, 2006). Fresh CW is a rich source of kinetin (a cytokinin plant growth hormone) which can delay the onset of aging characteristics in human skin cells (Ge et al., 2005). Coconut is able to synthesize different antimicrobial peptides in water, with diverse properties and mechanisms of action. Mandal et al. (2009) described antimicrobial peptides from CW with activity against human pathogenic bacteria. Several research findings have confirmed many health benefits of the multiple coconut parts in various forms. DebMandal and Mandal (2011) reviewed numerous studies that described coconut properties and effects, such as electrolyte nature of CW and its antioxidant, cardioprotective, antithrombotic, antiatherosclerotic, hypolipidemic, anticholecystitic, antibacterial, antiviral, antifungal, antiprotozoal, anticancer, immunostimulatory, antidiabetic, hepatoprotective and hormone like properties.

In spite of several functional properties described for coconut, there are no reports on the state of art technological development of fermented beverages based on CW. Recently, we reported a closely related topic on the development of an innovative non-dairy functional fermented beverage using herbal mate extract as a natural ingredient which has hypocholesterolemic and hepatoprotective properties (Ferrari Pereira Lima, De Dea Lindner, Thomaz-Soccol, Parada, & Soccol, 2012). To commercialize CW as a ready-to-drink beverage, adequate preservative process is important as CW is susceptible to oxidative enzymatic or microbial spoilage. Heat treatment is the most widely used preservation method for CW as the temperature could lead to the inactivation of microbial and enzymatic activities (Tan, Cheng, Bhat, Rusul, & Easa, 2014). Currently, CW is processed by heat pasteurization that alters the product sensory quality and changes its nutritional content (Cappelletti et al., 2015).

According to De Dea Lindner et al. (2013), fermented products are gaining popularity and acceptance because of their functional benefits. The incorporation of lactic acid bacteria (LAB) in food and beverages is a global trend, and the functional properties of these products have been scientifically demonstrated. New products have been launched, particularly non-dairy beverages based on fruit and cereals (Soccol et al., 2012). Beverages containing probiotic strains, for example, represent a promising market. However, the development of this sector is confronted by challenges, such as the appropriate processing and subsequent storage methods necessary to guarantee the survival of these microorganisms (Cruz, Antunes, Sousa, Faria, & Saad, 2009).

Although the application of LAB in dairy foods has been widely explored, CW is an innovative matrix for the application of this microbial group. The aim of this work was to develop an innovative fermented beverage using CW as the main ingredient which would also be functional due to its intrinsic health properties and natural hydrating qualities. CW can be used as a new non-dairy functional beverage, especially by vegetarians and lactose intolerant consumers.

2. Materials and methods

2.1. Extraction and preparation of CW

Green coconuts (*Cocos nucifera* L. var. *nana*) between 6 and 8 months of age were purchased at the local market in Fortaleza, Brazil. The CW used in this work was extracted by perforation with a manual, stainless-steel punch after the primary epicarp had been brushed and washed with water containing 100 ppm of active chlorine. The total water from the 12 selected fruits was homogenized, filtered through a filter paper of 1:11 μm (Whatman, Maidstone, UK) and maintained at –20 °C to prevent any microbial or enzymatic activity.

2.2. Physico-chemical characterization of the CW

Three different coconuts were used to determine the physicochemical characteristics of the water. The pH was measured using a pH meter model HI9321 (Hanna Instruments, Woonsocket, RI, USA). Total soluble solids concentration was determined directly using a digital refractometer (PR-101, ATAGO, Tokyo, Japan) at 25 °C. The concentration of reducing sugars was measured using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959), and total sugar content was measured using acid hydrolysis (AOAC International, 2000). The protein content was determined according to Lowry, Rosebrough, Farr, and Randall (1951). Glucose and fructose contents were determined by HPLC as described by Ferrari Pereira Lima et al. (2012). CW was diluted 1:10 (v/v) in ultra-pure water (MilliQ®), centrifuged at 4500 *g* and filtered in a 0.22 μm pore size filter (Merck Millipore, Darmstadt, Germany). Chromatographic analyses were performed using a LC-10AD chromatographic system (Shimadzu, Kyoto, Japan) equipped with a model RID-10A detector (Shimadzu) and an Aminex HPX-87H cation exchange column (300 mm × 7.8 mm I.D., Bio-Rad Laboratories, Hercules, USA). Analyses were performed using a filtered and degassed 5 mmol/l reagent grade H_2SO_4 (Carlo Erba, Milan, Italy) as the mobile phase at a flow rate of 0.6 ml/min. Eluates were monitored at 215 nm. The calibration curves were obtained by preparing a standard mix of the sugars (Sigma, St. Louis, MD, USA). The resulting peak areas were calculated for duplicate 25 μl injections and plotted against concentration. Each assay was carried out in duplicate, and the average values, expressed in g/l, of the glucose and fructose concentrations were calculated.

2.3. Natural fermentation for isolation and screening of autochthonous LAB from CW

An aliquot of the extracted CW was used to isolate autochthonous LAB microflora from the coconut. A 200 ml aliquot of CW was incubated at 37 °C for 48 h in aerobic conditions. In order to isolate colony-forming units (CFU) of cultivable populations, 1 ml of naturally fermented CW was serially diluted tenfold in 0.05 mole/l sodium citrate (Sigma) buffer, pH 7.5, and 100 μl of each dilution were spread on plates of MRS (de Man Rogosa Sharpe) agar (Acumedia, Baltimore, MD, USA) pH 5.4 in triplicate and incubated at 37 °C for 48 h as described by De Dea Lindner et al. (2008). More than 100 CFUs were isolated and