SORGHUM FLOUR AND COCONUT WATER AS CULTURE MEDIUM FOR BIOMASS PRODUCTION AND SPRAY DRYING OF *Lactocaseibacillus paracasei*

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ABSTRACT

A study was carried out using commercial coconut water to formulate a culture medium free of dairy diluent for *Lactocaseibacillus paracasei* growth, added with commercial sorghum flour or extruded sorghum flour. The fermentation process was conducted at 37°C for 20 h and MRS synthetic standard medium was used as a reference for comparison. Changes in pH, reducing sugar concentration and biomass growth (CFU/mL) were measured at the beginning and end of fermentation. Results showed that both sorghum flour types increased total bacteria to above 8.5 log CFU/mL in 20 h. In order to enhance the viability of probiotics microorganisms during storage and gastrointestinal passage, the fermented medium containing *L.paracasei* was microencapsulated using spray drying and Maltodextrin MDE 10 as encapsulating agent. The powder product was packaged and kept under freezer at -8 °C was stable for six months. The *in vitro* gastrointestinal tract survival test, the final concentration was 5 log CFU/mL.

Keywords: *Lactobacillus*, sorghum, coconut water, probiotics, spray dry.

1 INTRODUCTION

Probiotic foods can contribute to the balance of the intestinal microbiota and offer relevant health benefits. With the growth of the market for products aimed to consumers with dietary restrictions, whether for health or lifestyle reasons, the demand for non-dairy products with probiotics is also growing.[1] Probiotic cultures commercially available in Brazil are imported and represent a high cost for the processing companies.

Embrapa has three native strains classified in the genus *Lactobacillus*, including the *Lactocaseibacillus paracasei*, which were isolated, selected and characterized in previous projects based on harmlessness and properties associated with probiotic functionality.[2] However, the need to adapt cultures to processing steps and to the intrinsic characteristics of plant matrices has limited their applications. The availability of national cultures with probiotic properties at a lower cost can favor the production and, therefore, the consumption of functional foods, beneficial to the health of the consumer.

Sorghum is a unique cereal crop because of its tolerance to drought and adaptation to dry tropical and subtropical ecosystems. The carbohydrate and protein contents of sorghum are considerably high compared to other cereals such as maize, wheat and rice.[3] The coconut palm is a fruit tree that can be easily found in regions with a tropical climate, and the coconut ends up being a fruit widely used in different types of foods and drinks. Coconut water is rich in sugars, vitamins, amino acids and minerals that perform different biofunctional functions.[4]

Probiotic microorganisms must be able to survive passage through the gastrointestinal tract (GIT), reaching the intestine and temporarily integrating into the local microbiota, where they will carry out activities associated with benefits to the health of the host. Encapsulation techniques have been used not only to maintain survival rates and viability higher during processing and over the shelf life and but also after consumption. A common method employed to encapsulate probiotics is spray drying with different polymers, such as polysaccharides and proteins, as encapsulating agents.[5]

This work aimed to use sorghum flour and coconut water as cultivation media for *L. paracasei*. The work also evaluate the microencapsulation of the microorganisms by spray drying technology using maltodextrin as wall material. The microencapsulated material was evaluated for stability during the shelf life and survival of probiotics during simulated using in vitro gastrointestinal digestion.

2 MATERIAL & METHODS

The strain *Lactocaseibacillus paracasei* was cultivated in standard MRS medium or in dairy-free medium with sorghum flour (commercial flour or type BRS 305 extruded flour) and commercial coconut water aiming at the production of biomass incubated at 37 °C, the optimum growth temperature for these bacteria, in a rotatory shaker at 100 rpm for 20 hours. The difference between the two sorghum flours is that the extruded flour is darker and more soluble than the commercial flour. The coconut water was commercially obtained from local market. The selected strain was activated in MRS broth at 37°C for 20 hours. An aliquot (5%, v/v) of the activated culture will be transferred to 250 mL Erlemeyer flasks containing 100 mL of each medium. The parameters

monitored were pH, reducing sugar concentration and cell growth. To obtain dry powdered biomass, a Lab Plant brand spray dryer was be used, with a nozzle 1 mm atomizer and due to the high operating temperatures of the equipment it was necessary to use the encapsulating agent such maltodextrin DE10 aiming to maintain the viability of the strain. The fermented broth brix was measured and maltodextrin MDE10 was added in 1:1 proportion. The powder product obtained was packaged in laminated metallized PET packages, vacuum-sealed and kept in freezer at -8 °C for 6 months. Both cell growth and viability of the *L. paracasei* strain were determined by plate enumeration after deep seeding on MRS agar and incubation at 37 °C for 72 h, with results expressed in CFU/mL and in CFU/g.

The survival of *L. paracasei* on in vitro simulated gastrointestinal conditions was determined according to Brodkorb et al. (2019).[6] The digestion assay was carried out in a metabolic bath at 37 °C under agitation. Gastric phase simulation lasted 2 hours, with pH adjustment to 3.0 and addition of pepsin. The simulation of the enteric phase include the addition of bile and pancreatin at pH 7.0. The mixture was incubates again for 2h. At the beginning of the test and after each phase of the in vitro simulated digestion assay, viable cells were counted using Pour plate enumeration after deep seeding on MRS agar and incubation at 37 °C for 72 h, with results expressed in CFU/mL (ISO 20128:2006).

3 RESULTS & DISCUSSION

The culture of *L.paracasei* was carried out in MRS broth (standard medium), medium containing only coconut water and coconut water plus 6% p/v of extruded or commercial sorghum flour. The coconut water has 50 g/L of reducing sugars while MRS medium has only 20 g/L of reducing sugars. The MRS medium is the standard medium for *Lactobacillus* growth and is intended to use a dairy-free medium with coconut water as a carbon source with or without sorghum flour as a complement to nitrogen supply. Several studies evaluated yeast extract as a nitrogen source but sorghum flour is 10 times cheaper compared to yeast extract. Several technological processes, such as thermoplastic extrusion, have been used to change the antinutritional factors and to promote the human consumption of sorghum products.[7] However, extruded sorghum is still not available in market. Thus, it was decided to evaluate the difference of extruded sorghum flour with the commercial sorghum flour in the production of *L. paracasei* biomass. The results obtained are shown in **Figure 1**. In the standard medium MRS, the biomass production was 8.56 ± 0.15 log CFU/mL and in the medium containing only coconut water the biomass production was inferior with 8.17 ± 0.16 log CFU/mL. When sorghum flour was added, the production was higher than MRS in both cases with 8.85 ± 0.17 and 8.91 ± 0.15 CFU/mL with extruded and commercial respectively.

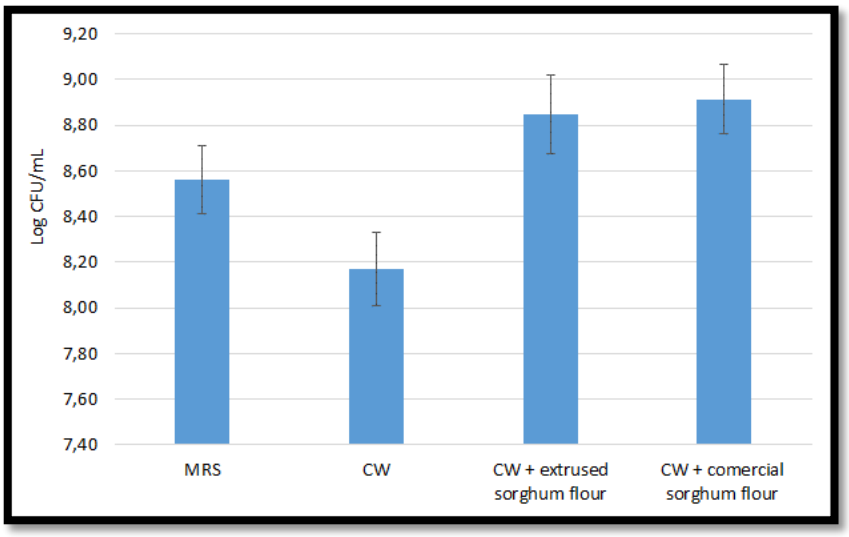

Figure 1: Biomass production of *L. paracasei* in different media.

The pH decreased in all media evaluated due to the lactic acid formed during the fermentation. When the pH was already lower than 4.0, the physiology of the microorganism is affected, reaching the stationary phase, causing a consumption of only half of the reducing sugar in 20 hours in the media containing coconut water. After the fermentation process, the broth was mixed with the Maltodextrin DE10 encapsulating agent and was dried in a Lab Plant Spray Dryer. The lactic ferment powder obtained under the optimized conditions remained stable for a period of six months both when stored under freezing (-15°C), maintaining about 100% of cell viability in a freezer (Table 1). The optimization of cultivation conditions and media free of dairy constituents followed by drying and microencapsulation, can help the probiotic culture to be resistant to application in vegetable products with low pH and/or subject to thermal processing.

Table 1: Cell viability in freezer (-15°C)

	initial	3 months	6 months
***L.paracasei* viability**	**8.95 ± 0,15**	**8.94 ± 0,14**	**9,05 ± 0,13**

The in vitro gastrointestinal tract survival test refers to the ability of microorganisms to resist the adverse conditions encountered in the stomach and intestine during digestion. This is particularly relevant for probiotics and other bacteria that may offer health benefits when consumed. In vitro survival studies simulated conditions in the gastrointestinal tract, including stomach acid,

digestive enzymes, and the gut environment. This way, the viability of microorganisms can be measured after exposure to these conditions.

The amount of CFU/mL required a microorganism to be considered beneficial after passing through the gastrointestinal tract may vary depending on the microorganism. It was observed that a significant number of lactobacillus survived the gastrointestinal tract, inducing them to exert beneficial effects in the intestine. *Lactocaseibacillus paracasei* is known for its ability to resist acidic conditions, in part due to its production of lactic acid, which can help neutralize the acidic environment. Some in vitro studies have demonstrated that *L. paracasei* can efficiently adhere to intestinal cells, which is a crucial step for its probiotic activity. Some models may be more rigorous than the human gastrointestinal environment and results may vary depending on this. In vitro gastrointestinal tract survival studies are essential to evaluate the probiotic potential of *L. paracasei* and understand how this strain can survive and potentially exert beneficial effects in the human intestine. *L. paracasei* viable cells has decrease 3 log CFU/mL after the gastric phase that lasted 2 hours, with pH adjustment to 2.5 and addition of pepsin and lipase. After this no decrease was observed during the enteric phase (Figure 2). This result showed that the power of *L. paracasei* produced in a dairy-free medium and encapsulating with maltodextrin can maintain the viability during storage and gastrointestinal digestion.

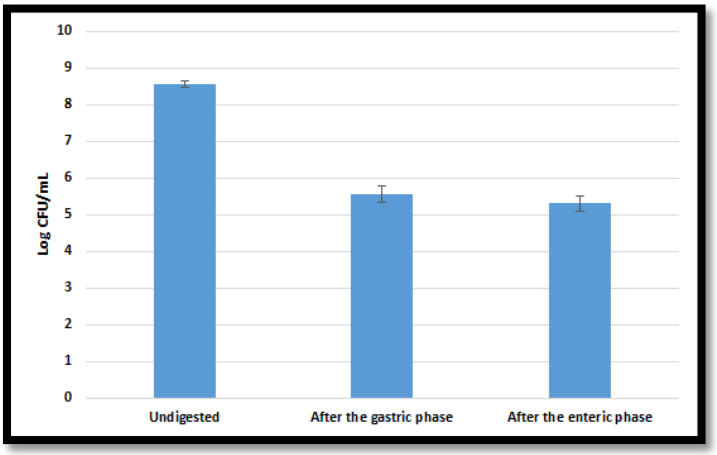

Figure 2: Survival of powder *L. paracasei* undigested, after the gastric phase and after the enteric phase of in vitro gastrointestinal test.

4 CONCLUSION

Once the technological suitability for non-dairy products and the probiotic effect in humans are confirmed, the culture produced with the native strain *L. paracasei* could be the first national probiotic culture for application in non-dairy foods, with the Embrapa brand, to be made available in Marketplace. The results showed that the culture medium with commercial coconut water plus sorghum flour increased total bacteria to above the standard synthetic MRS medium used as a reference. The powder product containing *L.paracasei* microencapsulated with Maltodextrin MDE 10 as encapsulating agent was able to survive the in vitro gastrointestinal tract survival test.

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