

Cashew Apple Juice *Anacardium Occidentale L* Probiotic Fermented from *Lactobacillus acidophilus*

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Abstract

The development of these probiotic beverages is important to diversify the market and to attend benefits to human health of large intestine. The aim of this study is using fruit juice from cashew apple as a substrate for the growth of *Lactobacillus acidophilus*. In order to create a new good probiotic beverages for human health and decrease environmental impurity from ripe cashew apples. The result shows that juice from ripe cashew apples after remove tannin and pasteurization at 90°C on 10 minutes and additional 11% (w/v) sucrose can be suitable substance for growth of *Lactobacillus acidophilus*. The optimum conditions of *Lactobacillus acidophilus* growth in cashew apple juice are: the initial pH 4.0-4.5, the temperature value 37°C. Cell biomass of *L. acidophilus* after being fermented at 37°C/48 hours is greater 10⁹ cfu/mL.

Key words: cashew apple, fermentation, *Lactobacillus acidophilus*, probiotic.

1. Introduction

Cashew tree (*Anacardium Occidentale L*) is a perennial plant originated from Northeastern Brazil but now widely grown in India, Vietnam, Tanzania and Mozambique. Especially in Vietnam, it is mostly planted in the south such as Binh Duong - Binh Phuoc (82,000 ha), Dong Nai (35,000 ha), Ba Ria - Vung Tau (20,000 ha), Tay Ninh (10,000 ha), but not in the north.

Cashew apple fruit includes two parts: true and false fruit. The true fruit is surrounded by shell and nut, false fruit is developed from pedicel. False fruit contains 90% whole fruit weight. The pulp of the cashew apple is very juicy with 85-90% water; 7-13% carbohydrate; 0.7 - 0.9% protein; 0.2% mineral; 0.1 lipid; vitamin C at high content (261.5 mg per edible part), five or six fold compared to orange, eight fold compared to mandarin orange; other vitamin B1, B2 and mineral calcium, phosphorous and ferrous. However, pulp of the cashew apple is usually discarded during harvest, so wasteful and harmful to environment.

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There are many research concerned to benefit of probiotic to human health such as bacteria resistance, lactose metabolized improvement, cholesterol reduction, *Helicobacter pylori* immune [4]. Probiotic is supplemented into milk and fermented dairy products. However, demand of non-milk probiotic products is increasing so that consumption of fruit and vegetable can be seen as an substrate for growing and accumulating of probiotic bacteria owing to high content of mineral, vitamin, fiber and anti-oxidizer.

Ana Lúcia Fernandes Pereira et al. optimized the growing condition of *L. casei* in cashew apple extract, evaluated the living ratio of this bacteria after being preserved in 4°C within 42 days. [1]. Kyung Young Yoon et al. used potato extract as substrate for fermentation from *L. acidophilus* LA9, *L. plantarum* C3, *L. casei* A4 and *L. delbrueckii* D7. This extract was incubated with bacteria within 24 hours at 30°C. Results were shown that lactic bacteria reduced pH to 4.1 and increased acidity to 0,65%, biomass $1 - 9 \times 10^9$ cfu/mL after 72 hours fermented. After 4 week preservation at 4°C, living cells were in range $10^6 - 10^8$ cfu/ml [2].

Main purpose of this research surveys the growth of *Lactobacillus acidophilus* in cashew apple extract to diversify probiotic products in the market based on available by-product from cashew apple cultivation.

2. Material and Method

2.1 Cashew apple extract

Cashew apple fruits were collected in Dong Nai province, utilized the pulp of cashew apple to get the extract. Tannin was eliminated by heating at 55°C, 60°C, 65°C, 70°C within 3, 5, 7, 9 minutes then cooling at 5°C in 10, 15, 20, 25 minutes to condensed tannin. Filtrate was then heated at 90°C in 10 minutes.

Recovery of tannin separation

$$H_1 = \frac{a_1 - a_2}{a_1} \times 100 \quad H_2 = \frac{a_2 - a_3}{a_2} \times 100$$

In there:

H₁: recovery of tannin after being heated, %.

H₂: recovery of tannin after being cooled, %.

a₁: content of tannin in raw extract, g.

a₂: content of tannin after being heated, g.

a₃: content of tannin after being cooled, g.

2.2 Testing methods

Using quantitative testing methods for chemical and physicochemical criteria: moisture content [TCVN 5613 – 91], total sugar [TCVN 4594 – 88], tannin [ISO 9648:1988], vitamin C [TCVN 4715 – 89]. polyphenol [Folin – Ciocalteu]. Experiments were conducted with three replications and then statistically summarized by StarGraphics 3.0 at reliability 0.05.

2.3 Culture medium preparation

Lactobacillus acidophilus was provided from biological laboratory of HCMC University of Technology. Inoculate this bacteria on agar medium and then accumulate level 1 and level 2. Biomass in MRS medium after 24 hours at 30°C attained at 7.86×10^9 cfu/ml. Inoculate 1ml of MRS broth into Erlen containing 100ml cashew apple extract to monitor the growth curve. Biomass of cashew apple extract attained 10^8 cfu/ml after 16 – 18 hours at 30°C. This bacteria in this culture was used to further fermentation.

2.4 Fermentation condition

Selected fermentation condition was based on growing characteristics of *Lactobacillus acidophilus* at four levels of temperature: 25, 32, 37 and 42 °C. pH initial was adjusted from 3.5 – 5.5 with difference 0.5. Sugar supplemented into cashew apple extract % (w/v) 5, 7, 9, 11, 13, 15%. Fermentation was executed at 30°C in 48 hours with above parameters. Output criteria would be living cells, pH, acid lactic, total sugar. Acid lactic content was defined [3]. Total acidity (g/l) converted to acid lactic in fermented culture.

$$(V - V_0) \times 0,1 \times \frac{90}{10} = (V - V_0) \times 0,9$$

In there:

V: volume (ml) of NaOH used to neutralize 10 ml of fermented sample.

V₀: volume (ml) of NaOH used to neutralize 10 ml of blank sample.

2.5 Determine living cells

Living cells were determined by diluting with distill water to 10^{-6} . Take 0.1 ml of diluted solution and then spread on MRS agar dishes. After that these dishes were incubated at 37°C in 72 hours. Dishes having 25 – 250 cfu would be selected to number. Numbers of bacteria were expressed by log cfu/ml [3].

Counting method

- Dilute sample to appropriate concentration.
- Take 0.1 ml sample and spread on Petri dishes.
- Incubate 30°C in 24 – 48 hours.
- Count colony present on agar dish.
- Number of bacteria in 1 ml sample were calculated as formula:

$$N = \frac{\sum \text{colonies}}{n_1 \times v_1 \times f_1 + \dots + n_i \times v_i \times f_i}$$

In there:

N: number of Petri dishes at an appropriate diluted level.

v: volume of sample inoculated.

f: ratio of dilute.

3. Results & Discussion

3.1 Tannin separation

Fig. 1 and 2 expressed tannin separation recovery after being heated at different temperatures in various intervals. Increasing temperature to 55°C, we got low reparation recovery and acrid product. Contrary, increasing temperature to 70°C, we got high

reparation recovery but low quality product, especially vitamin C reduction, non-specific nature of cashew and cook flavor. So this temperature was not selected. Meanwhile, heating at 60°C and 65°C the difference of tannin separation recovery at these experiments was not statistically significant ($P < 0.05$) so temperature 60°C with tannin separation recovery 37.13 ± 1.6 % was appropriate to select for further researches. There were statistically significant differences of tannin separation recovery at various intervals. However, at higher interval,

high tannin separation recovery but low quality product flavor. So, 5 minutes was very suitable to tannin denaturation and high quality product flavor. Fig. 3 showed the effect of cooling time to tannin separation recovery. There were statistically significant differences of cooling time intervals to tannin separation recovery. We got the highest tannin separation recovery at 20 minutes with 92.35 % tannin separated and lowest at 10 minutes with 85.56 %. However between 20 minutes and 25 minutes there were not statistically significant differences so cooling time at 20 minutes in 5°C was very ideal for tannin precipitation.

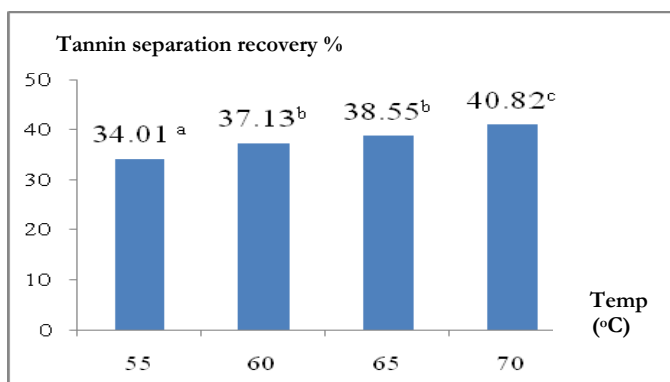


Fig. 1. Effect of heating temperature to tannin separation recovery

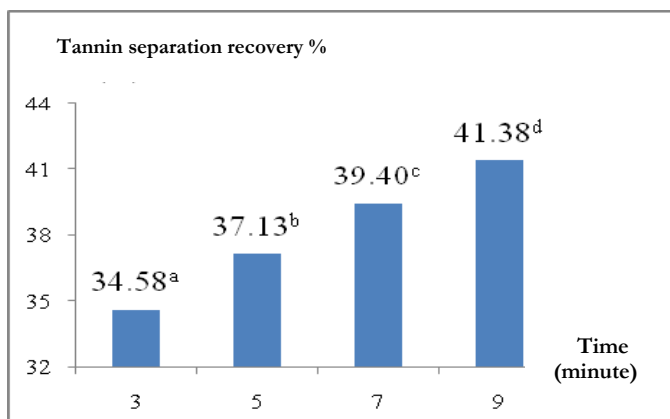


Fig. 2. Effect of heating time to tannin separation recovery

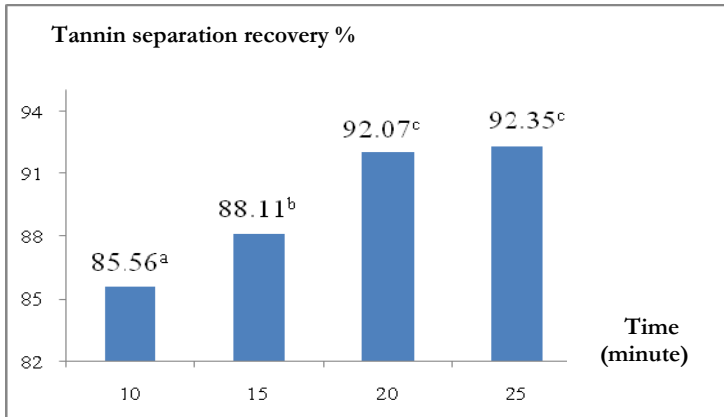


Fig. 3. Effect of cooling time to tannin separation recovery

3.2. Temperature and time for pasteurization

We conducted the research at three pasteurized intervals: 10 minutes, 15 minutes, 20 minutes and three pasteurized temperatures: 70°C, 80°C, 90°C with the aim to define the optimum temperature and time for pasteurization.

Table1. Temperature and time for pasteurization

Interval	Vitamin C content (mg/L)		
	70°C	80°C	90°C
10 minutes	131	123	102
15 minutes	114	104	91
20 minutes	98	85	78

Basing on vitamin C research, we can see temperature and interval increased, vitamin C decreased. At 70°C in 10 minutes, we got the highest C and gradually decreased at higher temperature. Sample would then be pasteurized 70°C in 20 minutes, TPC got at $8,9 \times 10^4$ cfu/ml, so we didn't choose 70°C. Checking 80°C in 15 minutes, TPC showed at 3×10^6 cfu/ml so we also didn't choose 80°C and 10, 15 minutes. At 80°C and 20 minutes, vitamin C content was very low so we didn't choose either. We selected 90°C to further experiments.

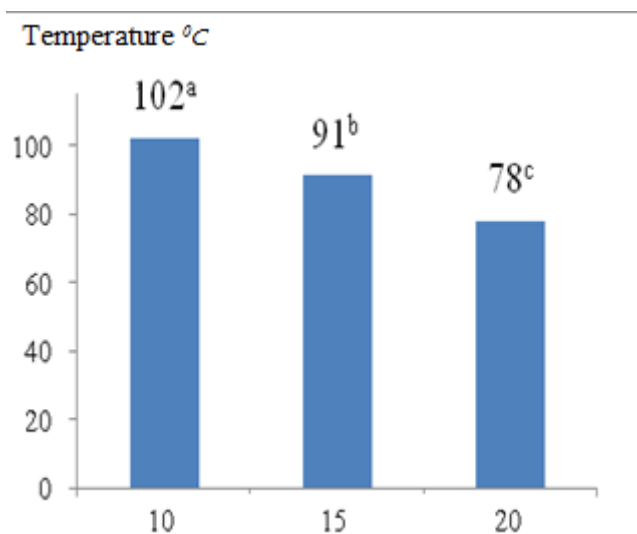


Fig. 4. Affect of pasteurization at 90°C

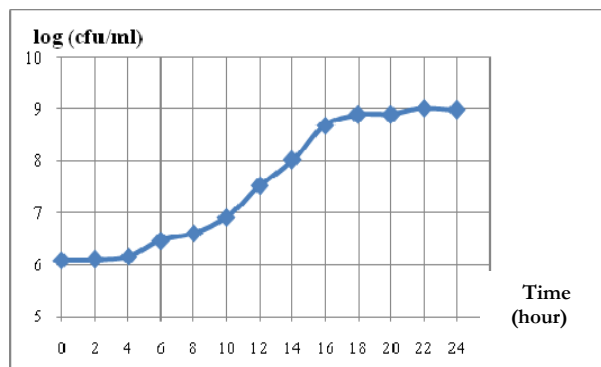


Fig. 5. Growth curve of *Lactobacillus acidophilus* in cashew extract culture

Cashew apple extract was pasteurized at 90°C in 10 minutes and then cooled before adding bacteria $7,86 \times 10^8$ cfu/ml. After that, we constantly monitored the growth curve during 24 hours. After 2 hours we took 0.1 ml of culture medium spread on Petri dishes to count living bacteria. The growth curve could be seen in Fig. 5.

3.2 Fermentation condition

In order to see clearly the fermentation, we built the growth curve of *L. acidophilus* in cashew apple extract. Fig. 5 obviously showed the high growth during 6 – 18 hours. Saccharose supplementation would not only create substrate for bacteria but also enhance the sensory characteristics. However, if there was too much sugar, bacteria would be prohibited. In contrast, there was too little sugar, acid lactic formation would be

low, sugar residue would also be scarce and affected to product quality. Fig. 6 showed acid lactic content and *Lactobacillus acidophilus* increasing 5 – 11%; the highest value at sugar concentration 11% with acid lactic 2,31g/L and bacteria 8.97 log cfu/ml (There were statistically significant differences among experiments with liability < 0.05). However, when sugar concentration increased to 13 – 15%, acid lactic content and bacteria also gradually decreased.

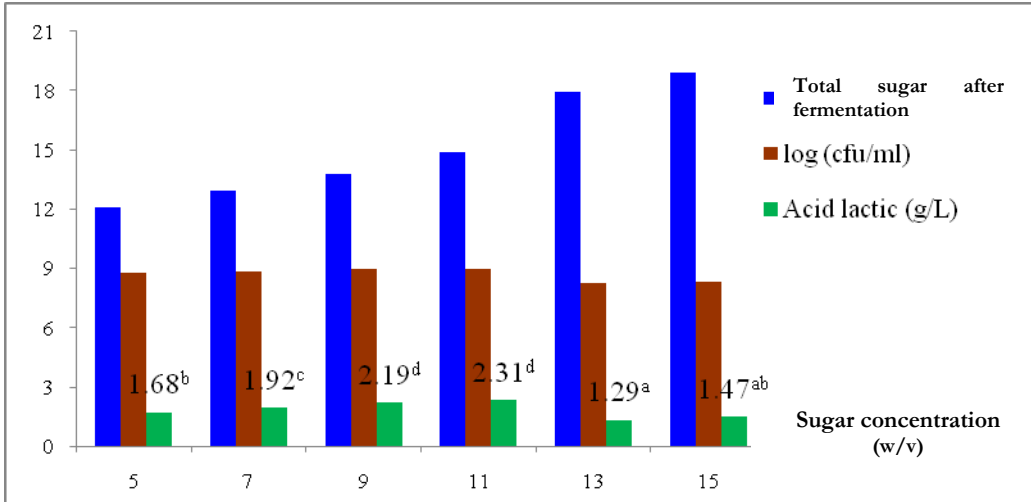


Fig. 6: Affect of sugar concentration to fermentation

pH initial and temperature had affected to the growth and stability of bacteria. Each species had an optimum temperature and pH appropriate for growing. Fig. 6 showed *L. acidophilus* well growing at pH 4.0 – 4.5 with the highest colony (8.95×10^8 cfu/ml for pH 4 and 9.47×10^8 cfu/ml for pH 4.5). When pH over 5.0 and 3.5 colonies significantly downward 7.53×10^8 cfu/ml with pH 5.5 and 6.9×10^8 with pH 3.5. This was very important because bacteria used for probiotic must be endured acidity in stomach to survive and trap on small intestine. Content of acid lactic was also be affected by pH initial because optimum pH would create favorable condition for strong growth of bacteria so substrate could be powerfully metabolized. Content of acid lactic received highest at pH 4.5 and lowest at pH 3.5 (2.91g/l and 0.84 g/l consequently). There were statistically significant differences among experiments with liability < 0.05. This result could be compared to one of Ana Lúcia Fernandes Pereira et al. (2010) with the optimum fermentation condition: pH = 6.4; temperature 30°C, living bacteria 7.48 log cfu/mL after 16 hours.

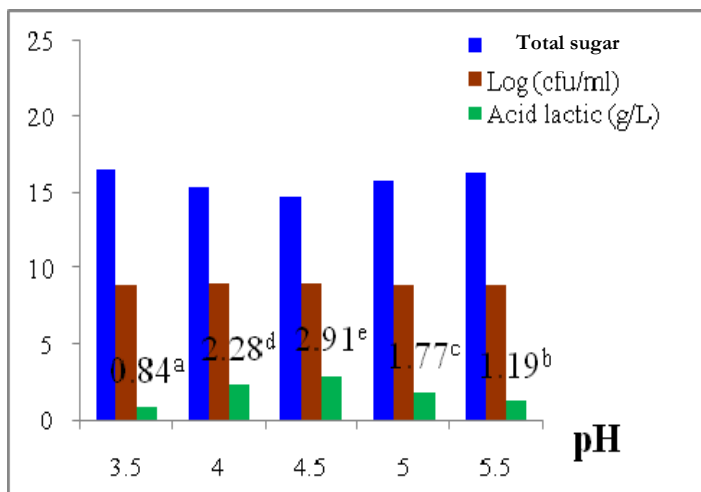


Fig. 7: Effect of pH to fermentation.

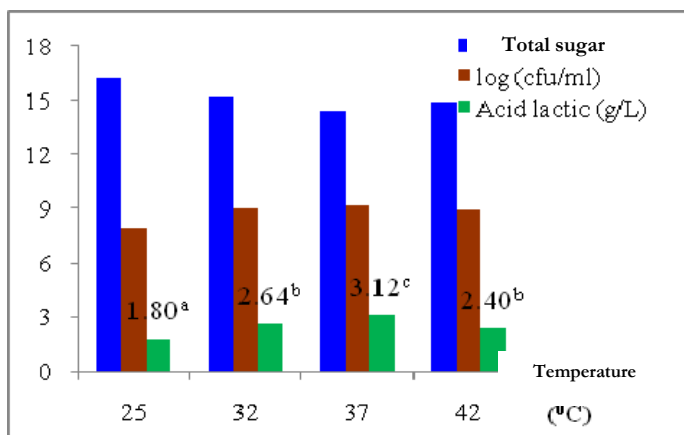


Fig. 8: Effect of temperature to fermentation

Compare to pH, temperature obviously affected to *Lactobacillus acidophilus* growth. Fig. 8 showed that at 37°C bacteria accumulated after fermentation 1.57×10^9 cfu/ml with the highest content of acid lactic 3.12g/l and at 25°C bacteria was very weak in four temperature regimes 8.35×10^7 cfu/ml with the highest content of acid lactic 1.8g/l. There were statistically significant differences among experiments with liability < 0.05. This result proved evidence that *Lactobacillus acidophilus* completely immobilized inside human body.

Conclusion

This research removed a large amount of tannin out of cashew apple juice with recovery 92.07%. By heating at 60°C in 5 minutes and cooling 50°C in 20 minutes, cashew apple extract could be used as beverage and fermented with *Lactobacillus acidophilus* to create probiotic product. Biomass of *L. acidophilus* was in range 16 – 18 hours. Other optimum

parameters of fermentation process: saccharose 11%, pH 4.0 – 4.5, temperature 37°C. Cashew apple juice could be evaluated as an appropriated substrate for *Lactobacillus acidophilus* fermentation. With vitamin C and mineral available inside juice, cashew apple juice fermented probiotic *Lactobacillus acidophilus* was highly value for human health.

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