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The Potential of Cashew Apple Waste as a Slimming Agent

Asmawati Asmawati[1], Marianah Marianah[1], Manar Fayiz Mousa Atoum[2,3], Desy Ambar Sari[1,*], Irum Iqrar[4,5], Zahid Hussain[6], Roy Hendroko Setyobudi[7], and Nurhayati Nurhayati[1]

[1]Department Agricultural Product Technology, Faculty of Agriculture, University of Muhammadiyah Mataram, Jl. KH. Ahmad Dahlan No. 1 Pagesangan Mataram 83127, West Nusa Tenggara, Indonesia;[2]Molecular Biology and Genetics, The Hashemite University, PO Box 330127, 13133 Zarqa, Jordan;[3]Department of Medical Laboratory Sciences, The Hashemite University, Zarqa, Jordan; [4]Department of Biotechnology, Quaid-i-Azam University, NCB Building Islamabad 45320, Pakistan; [5]Pakistan Academy of Sciences, 3 Constitution Ave, G-5/2, Islamabad Capital Territory, Pakistan; [6]The University of Agriculture, Peshawar, 25130, Khyber Pakhtunkhwa, Pakistan; [7]Department of Agriculture Science, Postgraduate Program, University of Muhammadiyah Malang, Jl. Raya Tlogo Mas No. 246, Malang, 65145, East Java, Indonesia

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ABSTRACT

The cashew apple (*Anacardium occidental* L.) is a tropical fruit that is a byproduct of the cashew nut processing industry. Rich in vitamins, polyphenols, sugars, minerals, amino acids, and dietary fiber, it contains bioactive compounds and several active components (ascorbic acid, anacardic acid, carotenoids, condensed tannins, quercetin, and other phenolic) that serve as antioxidants. This study aims to determine the effect of giving cashew apple juice and its nutritional impact on Wistar rats (*Rattus norvegicus* – Berkenhout 1769). The study adopted 21 male Wistar rats for 7 d fed with AIN 76 (American Institute of Nutrition 1976) diet, divided into three groups of seven rats. The first group was administered with fresh cashew apple juice treatment 0.14 % (CAJT 0.14 %), the second group with cashew apple juice 0.12 % (CAJT 0.12 %), and the last group with aquadest (Placebo) per day orally for 28 d. Employing an experimental method, variables of protein using Kjeldahl method, starch using the direct acid hydrolysis method, and calcium levels in the feces of experimental rats were observed. The data was obtained through analysis of variance, and differences among samples were tested using Least Significant Different (LSD). The results showed that CAJT 0.14 % significantly reduced protein digestibility (11.49 %) and starch feed (0.69 %) as well as weight rate by 78.85 g (38.34 %) while increased calcium excretion (0.44 %). CAJT 0.12 % followed at 11.36 %, 0.68%, 38.64 % (88.74 g), and 0.44 % respectively. Placebo was not affective towards research variables. The effect of consuming cashew apple juice regarding nutrient digestibility shows its potential for a commercial process as a functional food and a slimming agent, which answers the environmental need for waste utilization.

Keywords: *Anacardium occidentale* (L.), Bioactive compound, Caju, Functional food, *In vivo* test, *Rattus norvegicus* (Berkenhout, 1769), Tannin, Waste utilization.

1. Introduction

A tropical plant commonly found at an altitude of about 1 000 m above sea level, a cashew tree or *caju* (*Anacardium occidental* L.) can grow well on various types of soil, even the dry ones with poor nutrients (Runjala and Kella, 2017). The fruit produced by this plant consists of two edible parts: cashew nut and cashew apple – while the first is its actual fruit, the latter is pseudo fruit formed from an enlarged fruit stalk (Balandrán-Quintana *et al.,* 2019). Oliveira *et al*. (2020) have highlighted that cashew nut, the main commodity of the plant, represents only 10 % of the total fruit weight. So far, cashew apple has only been used as animal feed if not disposed of as waste (Aidoo *et al*., 2022). Several researchers (Gadikar *et al*., 2021; Prabhudessai *et al*., 2013; Setyobudi *et al*., 2021a) suggest using this waste as feedstock for biogas. However, considering the low pH, a two-stage digester technology is recommended (Abdullah *et al*., 2020; Hendroko *et al*., 2013).

Cashew apple contains water (83.6 g 100 g^{-1}) and nutritional substances such as vitamin C (126 mg 100 g^{-1} to 372 mg 100 g^{-1}), which is 6 to 7 times higher than in citrus fruits, dietary fiber (312 mg), carbohydrates (11.1 g), and calcium (0.9 mg 100 g^{-1} to 21.4 mg 100 g^{-1}) (Damasceno *et al*., 2008; Bhakyaraj and Singaravad, 2012); cashew apple should be able to serve as a good source of energy (Cristina *et al*., 2012; Honorato *et al*., 2007). Rich in bioactive compounds of polyphenols (gallic acid, protocatechuic acid, cryptoxanthin, zeinoxanthin, and lutein 214.8 mg 100 mL^{-1} to 215.1 mg 100 mL^{-1}) and organic acids (malic, citric, and lactic acids 0.1 g 100 g^{-1} to 0.36 g 100 g^{-1} (Sucupira *et al*., 2020), tannins 0.22 g 100 g^{-1} to 0.58 g 100 g^{-1} (Sobhana and Mathew, 2015), carotene 0.03 mg 100 g^{-1} to 0.74 mg 100 g^{-1} (Lopes *et al*., 2012), anacardic acid 1.1 g (Nambelaa *et al.,* 2022), the fruit is therefore packed with antioxidants (Andayanie *et*

[*] Corresponding author. e-mail: desyambars@gmail.com.

al., 2019; Laddha *et al*., 2020). Specifically, chemically active components of ascorbic acid, anacardic acid, carotenoids, condensed tannins, quercetin, and other phenolic compounds are essential in anti-mutagenic mechanism (Onuh *et al.*, 2017; Setyobudi *et al*. 2019) with an ability to stimulate DNA repair or reverse DNA damage. The details above should prove that cashew apple is nutritious and healthy to consume (Tai *et al*., 2020).

The other positive impact of consuming cashew apple juice is that it can reduce the concentration of total cholesterol, LDL triglycerides T6, and increase the concentration of HDL (cholesterol in the blood) (Asmawati *et al*., 2021; Carvalho *et al*., 2018). Several previous researches also reported the presence of tannin, which is known for its capacity to form insoluble complexes with macromolecules (proteins, fats, and carbohydrates) as well as micro-components (vitamins and minerals) to decrease availability and bioavailability (Emmanuelle *et al*., 2016; Setyobudi *et al*., 2021b and 2022; Soltan *et al.*, 2013), Total tannin (hydrolysable) in cashew is about 0.64 mg 100 g^{-1} while condensed tannin is about 0.18 mg 100 g^{-1}. Tannins content are commonly known for protein binding and leather-forming activities. Apart from the ability to precipitate protein, tannin equally decreases digestibility and palatability (Aliyu and Hammed, 2008; Dabonne *et al*., 2015). High concentrations of these compounds were discovered in experimental animals' feces, consequently suppressing their growth and weight gain rates, which showed potential as slimming agents (Ebere *et al*., 2015; Menci *et al*., 2021). The above findings have become the bases of research on cashew apple juice's nutritional digestibility, aimed to see if it is possible to be a source of functional food serving as a slimming agent.

2. Materials and Methods

2.1. Materials

2.1.1. Cashew apple juice

The raw material of yellow-orange cashew apples was obtained from North Lombok, Indonesia. After sorted and washed, the fruit was blanched for 1 min to soften the texture and then extracted; the juice served as the first treatment with tannin content of 0.14 % (CAJT 0.14 %). As for the other treatment, the fruit was soaked in a calcium hydroxide solution [$Ca(OH_2)$ 3 %] for 15 min after washed and blanched, then extracted for its juice with tannin content of 0.12 % (CAJT 0.12 %).

Reducing tannin level from 0.14 % to 0.12 % was due to findings of a few researchers. Emmanuelle *et al*. (2016), Aliyu and Hammed (2008), Osagie and Eka (1998) reported that tannin equally decreased digestibility and palatability. Orak *et al*., (2012) and Setyobudi *et al*. (2022) stated that tannin bound protein, suppressed digestion by inhibiting key enzymes, and rendered iron and vitamin B12 unavailable.

2.1.2. Experimental animal

The animals involved in the experiment were Wistar male rats (*Rattus* norvegicus – Berkenhout 1769), 6 wk old with an average body weight of 104 g ± 8 g, obtained from the Experimental Animal Development Unit (UPHP) of Universitas Gadjah Mada, Yogyakarta, Indonesia. The rats were fed under the standard feed set by the American Institute of Nutrition 1976 (AIN 76) made in the nutritional laboratory of the Faculty of Agricultural Technology of Universitas Gadjah Mada, Yogyakarta, Indonesia.

2.2. Research Procedure

The research procedure has been approved by the Ethical Commission of the Faculty of Medicine of Al-Azhar Islamic University, West Nusa Tenggara, Indonesia (Number 29/EC/FK-06/UNIZAR/VIII/2020) and carried out consistent with the steps in Figure 1. First, the AIN 76 standard feed was allotted to determine the levels of protein, carbohydrates, calcium, and initial body weight of experimental rats before treatment administering. Then, 21 male Wistar rats were led to 7 d of adaptation, fed with AIN 76 and distilled drinking water. Once the period was over, the rats were divided into three equal groups:

- **Group 1** was given Cashew Apple Juice Treatment 0.14 % (CAJT 0.14 %) with tannin content.
- **Group 2** was assigned to drinking Cashew Apple Juice Treatment 0.12 % (CAJT 0.12 %) with reduced tannin content.
- **Group 3** was given aquadest (placebo group).

The aforementioned rats received cashew apple juice (0.14 % and 0.12 %) and aquadest of 2 mL d^{-1} two times a day (morning and evening) by force-feeding while still fed with AIN 76 standard meal for 28 d (4 wk). In addition, all rat feces collected during the study (28 d) were analyzed for their protein, carbohydrate, and calcium levels to determine digestibility.

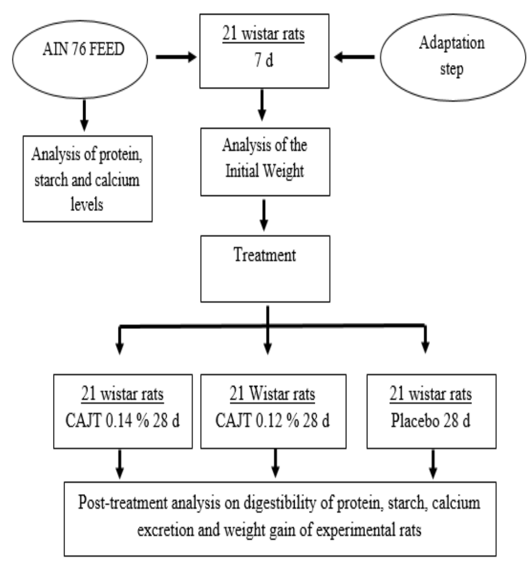

Figure 1. Scheme of the research

2.3. Measurement of research variables

2.3.1. Feed protein digestibility

Feed intake was weighed, and feces samples from the last 3 d (26 d to 28 d) were collected. To determine the effect of tannins on protein digestibility, 1 g of diet and feces samples were analyzed for nitrogen content using the micro Kjeldahl method (% N × 6.25). The 1 g of diet or feces sample was mixed with a digestion flask with 1.9 g ± 0.1 g potassium sulfate, 80 mg ± 10 mg mercuric oxide, and 2 mL H_2SO_4. Boiling chips were added to digest the sample and turn the solution colorless. Once cooled, the digest was diluted with distilled ammonia-free water before being transferred to the distillation apparatus. The tip of a 10 mL conical flask containing 5 mL boric acid solution and drops of an indicator variant was dipped into the solution, and 10 mL of sodium hydroxide-sodium thiosulphate solution was inserted. The ammonia produced by the boric acid was distilled and collected. The emulsion was then titrated until a violet color appeared at the tip of the condenser and rinsed before the titration process. Next, the reagent blank was run with an equal volume of aqua dest to one of the titration results. Finally, the titration result volume was subtracted from the sample's original volume. The results were calculated per Equation (1) (Kara *et al*., 2018; Mæhre *et al*., 2018).

$$\textbf{Protein digestibility (\%)} = \frac{\textbf{N intake} - \textbf{N feces} \times \textbf{100}}{\textbf{N}\ \text{intake}} \qquad \textbf{(1)}$$

2.3.2. Feed starch digestibility

Feed intake was weighed, and feces samples from the last 3 d (26 d to 28 d) were collected. To determine the effect of tannins on starch digestibility, diet and feces samples were analyzed for starch content using the direct acid hydrolysis method (Kim *et al*., 2012; Kumar *et al*., 2022). Amount of 15 g dry basis of starch was added in a mixture of sulphuric acid and water (100 mL, 3.16 M), stirred, and left at a temperature of 35 °C for different lengths of time (0 d to 15 d). The solution was then cooled to 5 °C to recover non-hydrolyzed materials and centrifuged (6 000 *x g*) for 15 min. The precipitates formed during the process were rinsed with aqua dest to reach pH 7 (neutral), and the solid products were air-dried at 35 °C for 24 h before being stored in a sealed glass container at 4 °C. The hydrolysis recorded in the form of percentages were of suspended solids and dissolved non-hydrolyzed starch relative to the original starch solids, of which results were calculated as per Equation (2):

$$\textbf{Digestibility of starch (\%)} = \frac{\textbf{Starch intake} - \textbf{starch stool} \times \textbf{100}}{\textbf{Starch intake}} \qquad \textbf{(2)}$$

2.3.3. Calcium content

Calcium contained in diet and feces was determined quantitatively. 0.5 g of sample and 25 mL of 6 M HCl were mixed in a 250 mL beaker and boiled for approximately 30 men to make a 5 mL reduction. An amount of 5 mL of hot deionized water was added and boiled further, then filtered in a 50 mL volumetric flask. Deionized water was then mixed in to reach a 50 mL end solution. Ca content was analyzed using Buck Scientific 210VGP – Atomic Absorption Spectrophotometric (USA) at a wavelength of 422.2 nm (Nehad *et al*., 2018). This study's chemicals and reagents are classified as of analytical grade, purchased from Sigma Aldrich Chemical Co. (St Louis, Mo, USA).

2.4. Body Weight and Feed Intake

All rats were feed one time a day (morning), Each rat was provided with approximately 15 g fresh feed AIN 76 standard meal for 28 d (4 wk) using feeder jar to prevent it from being tilted or dislodged. Approximately 1 g to 2 g food remained when the feeder jar was removed and another jar provided at the same time on the subsequent day, providing the same amount for each day. During this period, body weight was measured every other day (Wolden *et al*., 2000; Serrano *et al*., 2017).

2.5. Data analysis

This research was conducted in proportion to the experimental method, and the plan was of completely randomized design. The data was obtained through analysis of variance, and differences among samples were tested using Least Significant Different (LSD) with the significance level set at $P < 0.05$ (Adinurani, 2016, 2022).

3. Results and Discussion

The overall results came out positive, meaning that cashew apple juice significantly affected the digestibility of protein and carbohydrates, absorption of calcium, and weight gain of experimental rats.

3.1. Protein digestibility

Table 1 shows how protein digestibility in experimental rats has decreased significantly after drinking cashew apple juice for 28 d.

Table 1. Protein digestibility of experimental rat with cashew apple juice

Treatment	Protein Digestibility Feed (%)	Protein Content Feces (%)
CAJT 0.14 %	33.05 b	11.49 a
CAJT 0.12 %	33.80 b	11.36 a
Placebo	52.39 a	8.17 b

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %
CAJT 0.14 %: Pure Cashew Apple Juice
CAJT 0.12 %: Cashew Apple Juice with tannin reduction
Placebo: aqua dest

Feed's low protein digestibility rates were associated with high fecal protein contents. Cashew apple juice consumption has significantly increased the protein (nitrogen) contents in rat feces per trial due to the presence of tannins (CAJT 0.14 % and CAJT 0.12 %). An insoluble complex formed between protein and tannin inhibits protein digestibility (Osman and Gassem, 2013) and prevented the enzyme from breaking down, resulting in increased nitrogen removal through feces. This statement is compatible with the result of research by Kara *et al*. (2018).

3.2. Starch digestibility

Table 2 demonstrates how carbohydrate digestibility in experimental rats has decreased significantly after drinking cashew apple juice for 28 d. Yet, it is not as high as protein digestibility.

Table 2. Carbohydrate (starch) digestibility of experimental rat with cashew apple juice

Treatment	Carbohydrate digestibility Feed (%)	Carbohydrate content (starch) Feces (%)
CAJT 0.14 %	97.02 b	0.69 a
CAJT 0.12 %	97.05 b	0.68 a
Placebo	97.31 a	0.62 b

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %
CAJT 0.14 %: Pure Cashew Apple Juice
CAJT 0.12 %: Cashew Apple Juice with tannin reduction
Placebo: aqua dest

The significant increases in stool starch in Group 1 and Group 2 are evident in low carbohydrate digestibility rates. That tannic acid essentially inhibits glucose absorption in experimental rats' intestines, thus increasing their fecal starch levels, which was compatible with the results of research conducted by Amoako and Awika (2016) and reported by Saha *et al*. (2018) that tannic acid and catechins could be associated with starch, resulting in decreased digestibility *in vitro*.

3.3. Calcium excretion

Table 3 records the significant increase of fecal calcium levels in experimental rats after drinking cashew apple juice for 28 d.

Table 3. Calcium excretion of experimental rat with cashew apple juice

Treatment	Ca cashew apple juice (%)	Ca content feces (%)
CAJT 0.14 %	0.23	0.44 a
CAJT 0.12 %	0.21	0.43 a
Placebo	-	0.39 b

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %
CAJT 0.14 %: Pure Cashew Apple Juice
CAJT 0.12 %: Cashew Apple Juice with tannin reduction
Placebo: aqua dest

The high levels of calcium in the feces of Group 1 and Group 2 are evident that tannins can bind to calcium to form insoluble calcium-tanate; since the intestinal absorption of experimental rats was unable to absorb it, it was then excreted along with feces. This statement complies with Addisu (2016) that tannic acid could reduce the speed across the intestine due to the anti-nutritional inhibition of tannins. Further, the high loss of calcium through feces was due to decreased calcium absorption in the intestine triggered by the formation of complexes with tannins. This goes along with the assertion of Amalraj and Pius (2015) that calcium is very sensitive to even a tiny amount of tannins in feed or drink.

3.4. Rat weight gain

Table 4 logs the changes and percentages of experimental rats' weight gain after drinking cashew apple juice for 28 d.

Table 4. Weight gain of experimental rats with cashew apple juice

Treatment	Weekly weight gain (g)				
	0	I	II	III	IV
CAJT 0.14 %	126.81	142.37	166.47	186.10	205.66
CAJT 0.12 %	141.03	162.01	189.24	210.09	229.77
Placebo	112.14	133.17	164.69	187.74	213.63

CAJT 0.14 %: Pure Cashew Apple Juice
CAJT 0.12 %: Cashew Apple Juice with tannin reduction
Placebo: aqua dest

The result explains that the rats with cashew apple juice – both control and treatment – have lower weight gain rates than the placebo group. Focusing on the groups administered with cashew apple juice of different tannin contents, it is perceptible that the control group receiving more tannin has gained less weight than the treatment group. The ability of tannins to form insoluble complexes with protein (Table 1) and carbohydrates (Table 2) resulted in lower weight gain in rats. Details on weight gain percentage are revealed further in Table 5 below

Table 5. Weight gain percentage of experimental rats with cashew apple juice

Treatment	Weight Gain		Average Intake Feed (g d–1)
	%	g d–1	
CAJT 0.14 %	62.18 b	2.82 b	11.00 b
CAJT 0.12 %	62.92 b	3.17 ab	11.41 ab
Placebo	90.50 a	3.62 a	11.74 a

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %
CAJT 0.14 %: Pure Cashew Apple Juice
CAJT 0.12 %: Cashew Apple Juice with tannin reduction
Placebo: aqua dest

The lowest weight gain occurred in the group treated with CAJT 0.14 % cashew apple juice at 62.18 % (78.85 g), followed by the group with CAJT 0.12 % at 62.92 % (88.74 g). In comparison, the placebo rat group was 90.50 % (101.49 g) and significantly different ($P < 0.05$). This corresponds to the low digestibility of protein (Table 2) and starch (Table 2) as a result of the occurrence of complexes with anti-nutritional compounds that can bind protein and carbohydrates and reduce the activity of digestive enzymes, causing the rat's body weight gain to be below optimal during the trial. This phenomenon is in line with the study of Rivera-Méndez *et al*. (2017), stating that tannins could affect experimental rats' growth and weight gain (Nwaneri *et al*., 2016).

The rats' feed intake was affected by the rats' weight gain, which was lower on average at 11.00 g (CAJT 0.14 %) and 11.41 g (CAJT 0.12 %), while the placebo rat group was higher at 11.74 g. The low feed intake of the experimental rats is thought to be due to the astringent taste in the cashew juice that affects the experimental rats' appetite.

4. Conclusion

The tannin content in cashew apple juice can cut off protein and starch digestibility, increase calcium excretion, and suppress the rate of weight gain. Therefore, the results confirm cashew apple's potential to be a slimming agent. How cashew apple juice diminishes appetite and how to develop cashew apples from waste to functional food can be subjects for further research.

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