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CASSAVA ADDITION FOR AMYGDALIN ENRICHMENT IN DAIRY BEVERAGES

H. F. Forsan [1]* M. M. El Abd [2] W. B. Elsabie [1] H. M. Sobhy [3]

[1] **Dairy Chemistry Research Department, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza, Egypt**
[2] **Dairy Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt**
[3] **Department of Natural Resources, Faculty of African Post Graduate Studies, Cairo University, Giza, Egypt**

***Correspondence to:** Hagar F. Forsan, Dairy Chemistry Research Department, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza, Egypt.
Email: hagarfathy@pg.cu.edu.eg

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Abstract

The cassava plant is known for its numerous medicinal and nutritional benefits. This study used cassava to enhance the nutritional content of milk and enrich it with amygdalin (vitamin B17). Properties associated with amygdalin include anticoagulants, atherosclerotic, hepatic protective, anti-tumor, antidiabetic, antifungal, anti-inflammatory, anticancer, antibacterial, anti-aging, and antioxidants. Cassava paste was prepared by boiling its roots in water for 1 hour and subsequently mashing them. Raw cow's milk, 5 % sugar, and 1 sachet of vanilla (1 gm) were mixed and heated at 80°C for 10 minutes. Fresh and refrigerated samples were tested for amygdalin concentrations at 6 ± 1°C. The amygdalin content in 5% cassava milk was 3.51 ± 0.01 mg/100 g and 3.52 ± 0.01 after 1 week of refrigeration (6± 1°C). Whereas, for 10% cassava milk it increased from 7.01 ± 0.01 mg/100 g when freshly prepared to 7.02 ± 0.02 after 1week's refrigeration at 6 ± 1°C. This slight increase could be attributed to water evaporation during the one-week refrigeration.

Keywords: Dairy beverags, Cassava, Amygdalin, B17, Nutritive value.

اضافة الكسافا لتدعيم المشروبات اللبنية بالاميغدالين

هاجر فتحي فرسان [1]* منير محمود العبد [2] وفاء بديع السبع [1] حسن محمد صبحي [3]

[1] قسم بحوث كيمياء الالبان، معهد بحوث الانتاج الحيواني، مركز البحوث الزراعية، الجيزة، مصر

[2] قسم تكنولوجيا الالبان، كلية الزراعة، جامعة القاهرة، الجيزة، مصر

[3] قسم الموارد الطبيعية، كلية الدراسات الافريقية العليا، جامعة القاهرة، الجيزة، مصر

***المراسلة الى:** هاجر فتحي فرسان، قسم بحوث كيمياء الالبان، معهد بحوث الانتاج الحيواني، مركز البحوث الزراعية، الجيزة، مصر.

البريد الالكتروني: hagarfathy@pg.cu.edu.eg

الخلاصة

الكسافا نبات له فوائد طبية وغذائية متعددة، لذا تهدف هذه الدراسة إلى تدعيم اللبن بالأميغدالين (فيتامين ب17) وزيادة القيمة الغذائية للبن باستخدام الكسافا. يعتبر الأميغدالين مضاد للبكتريا ومضاد للأورام والفطريات وللالتهابات التخثر وتصلب الشرايين الشيخوخة ومرض السكر ومضادًا للأكسدة كما أن له دور في حماية الكبد. يتم تحضير معجون الكسافا عن طريق الغلي في الماء الساخن لمدة ساعة ثم الهرس. يتم تسخين حليب البقر الطازج مع اضافة 5% سكر وكيس واحد من الفانيليا (1 جم) عند درجة حرارة 80 درجة مئوية لمدة 10 دقائق. تم التقسيم الى ثلاث اجزاء من اللبن الجزء الاول هو الكنترول والثاني بإضافة 5٪ من مهروس الكسافا، والثالث بإضافة 10٪ من مهروس الكسافا. تم اختبار العينات الطازجة والمبردة مع تقدير الأميغدالين عند 6 درجات مئوية ± 1. أشارت النتائج إلى أن محتوى الأميغدالين في مشروب اللبن المدعم بالكسافا بنسبة 5٪ كان 3.51 ± 0.01 مجم/ 100 جم عندما يكون طازجًا و3.52 ± 0.01 عند التبريد (6 ± 1 درجة مئوية) لمدة اسبوع، في حين أظهر محتوى فيتامين ب 17 في مشروب اللبن المدعم بالكسافا بنسبة 10٪ زيادة طفيفة من 7.01 ± 0.01 ملغم/ 100 جم عندما يكون طازجًا و7.02 ± 0.02 عند التبريد (6 ± 1 درجة مئوية) لمدة اسبوع. ويمكن أن تعزى هذه الزيادة الطفيفة إلى تبخر الماء أثناء التبريد (6 ± 1 درجة مئوية) لمدة اسبوع.

كلمات مفتاحية: المشروبات اللبنية، الكسافا، الاميغدالين، فيتامين ب 17، القيمة الغذائية.

Introduction

Cassava belongs to the Plantae kingdom, of the Euphorbiaceae family called genus Manihot, and of the M. esculenta species (25). It is found in Mexico and Central America and recognized as manioc, mandioca, or yucca in South America and tapioca in Asia (19). Cassava is a significant tropical crop that grows on more than 30 million hectares and produces over 315 million tons of crops annually. Africa produces 65% of the world's cassava, with Nigeria being the leading producer at up to 20% of the total (29). Angola, China, Brazil, Ghana, Mozambique, Indonesia, Thailand, and Vietnam are some of the other main cassava producers (29).

Over 800 million people in rural regions rely on cassava as a key source of food and income due to its high carbohydrate content and its ability to grow in poor soil (27). After wheat, rice, potatoes, and maize, it is one of the main sources of staple food for human consumption. It is also used to make commercial starch and biodegradable polymers and for animal feed. A flowering, hardy perennial shrub native to South America, cassava was domesticated some 8000 years ago and introduced to West Africa by Portuguese traders in the sixteenth century (26). Research revealed that humans consume 70% of the cassava crop, with the other 30% being used by industry to make starch, glucose, and alcohol for producing paper, adhesives, and textiles (10). Cassava output has increased to almost match that of maize, one of the primary food grain crops. Studies reveal that compared to major food crops like rice, maize, and sorghum, cassava production has the least negative effects on the environment as a whole (28).

For humans and livestock, cassava offers an alternative source of macro- and micro-elements. The high nutritional content in its roots makes it one of the best sources of energy with 1-3% crude proteins, 0.1-0.5% crude fat on fresh weight, and 80 - 90% carbohydrates on dry weight. Cassava has less carbohydrate content than potatoes, wheat, rice, yellow maize, and sorghum. About half its crude protein is made up of complete proteins, and the other half of free amino acids, primarily glutamic and aspartic acid. Cassava roots provide about 140 Kcal/100 g (25). There are two cyanogenic substances in cassava, namely lotaustralin and linamarin. The difference in amygdalin content in cassava plants has several reasons, including the type grown and practices during cultivation. The maximum amygdalin value in cassava roots is 48.33 mg/gm (5). Cyanide is produced as a byproduct of its metabolism (14). It contains poisonous hydrogen cyanide (HCN) (13) which has a lethal dosage at 0.54 mg/kg for humans (12).

Amygdalin's pharmacological activities, have been extensively studied over the years such as those against fibrosis (15 and 20), inflammation (18), analgesia (4), auxiliary anticancer (11), immune regulation (23), anti-atherosclerosis (1), anti-cardiac hypertrophy (1), anti-ulcer (18), and hypoglycemia (1). Significantly, a growing body of research has shown its anticancer properties, and in recent years, has garnered more attention to its curative properties for cancers of the prostate, lungs, bladder, colon, and rectal regions (22). This study focuses on enhancing the amygdalin and nutritional values of cassava beverages. Amygdalin is considered a popular but controversial anti-tumor therapy because it produces prunasin and mandelonitrile, which eventually break into deadly benzaldehyde and hydrocyanic acid (16). As such, this study aimed to use cassava in enhancing the nutritional value of milk and supplementing it with amygdalin (vitamin B17).

Materials and Methods

Materials: Fresh cow's milk was obtained from the Animal Production Research Institute of the Ministry of Agriculture. sucrose sugar, vanilla and cassava from the local market. Sigma-Aldrich provided the methanol, diethyl ether 98%, amygdalin (98% purity), and HPLC-grade ethanol 98%.

Preparation of the cassava milk beverage: Cassava paste was made by mashing small slices of the plant's roots and boiling them at a ratio of 1:5 water for 1-hour. A sachet of vanilla (1 gram), fresh cow's milk, and 5% sucrose sugar were heated at 80°C for ten minutes, and the milk divided into three equal portions. Then, 5% and 10% cassava paste were added to the second and third portions, respectively, with the first portion used as the control. The chemical properties of the prepared cassava-paste milk beverage and its control were evaluated while it was fresh and after one week's refrigeration at 6± 1°C.

Determination of total lipids, protein, cyanide, fiber content, and acidity percentage was done according to (21), total flavonoids according to (31), and phenolic according to (3). The mineral contents of zinc, magnesium, calcium, and potassium were measured using a novel microwave digestion technique, followed by Thermo Scientific's ICP-AES (inductively coupled plasma) system. For the amygdalin calibration curve a stock solution of 100 μg mL^{-1} was prepared by dissolving the amygdalin standard in distilled water and stored at 20°C until analysis. A calibration curve was created using six standard solutions containing amygdalin concentrations of 1, 5, 10, 20, 40, and 50 μg mL^{-1} according to (6). For the extraction of amygdalin, two grams of cassava were placed in a 200 mL conical flask and immersed in a water bath with continuous shaking at 37°C after 50 mL of water was added. Amygdalin extraction took 40, 80, 100, 120, and 180 minutes. The extracts were filtered through Whatman No.1 filter paper and then placed into 50 ml disposable polypropylene tubes. Twenty milliliters of n-hexane were added and then vortexed thrice for 1 minute to remove fat. A tabletop Eppendorf 5810R centrifuge was used at 3250 rpm for ten minutes. The supernatants were mixed and discarded. With the help of a rotary evaporator running at low pressure at 35°C and 7 mill bars, the remaining hexane was extracted from the sample according to (6).

Sensory evaluation: All samples were evaluated for organoleptic properties, namely flavor (20 points), body and texture (10 points), and color and appearance (10 points), according to (2).

Statistical analysis: The COSTAT program was used for a one-way analysis of variance to test for differences between samples, and the Excel program was utilized for calculating the standard deviation (SD).

Results and Discussion

Chemical composition of cassava: Table 1 illustrates the chemical composition of cassava before and after boiling for 1 hour. The total protein in fresh cassava and boiled cassava paste did not change according to boiling heat treatment. After subjecting both fresh cassava and boiled cassava paste to heat treatment, the total lipid content remained unchanged.

According to boiling heat treatment, the total fiber content of both fresh cassava and boiled cassava paste remained unchanged. According to boiling heat treatment, the calcium content of both fresh cassava and boiled cassava paste remained unchanged.

According to boiling heat treatment, the potassium content of both fresh cassava and boiled cassava paste remained unchanged. Both fresh cassava and cooked cassava paste retained their magnesium content after being subjected to heat treatment.

Boiling heat treatment did not alter the zinc content of either fresh cassava or boiled cassava paste. Amygdalin levels in boiling heat-treated fresh cassava and cassava paste remained unchanged. These results for total protein, total lipids, and zinc agree with those (17). Ca, k, mg, and HCN were similar to the results of (17).

Total polyphenol and flavonoid content decreased when the cassava was freshly boiled for 1 hour according to the heating temperature and time (30).

Fresh cassava has 0.0038gm/100 gm cyanide and soaking in water reduces its content, possibly due to its solubility. Small slices of cassava were boiled in water at a 1:5 ratio for 1 hour and filtered. HCN decreased to 0.0011 gm/100 gm (5). This value is considered safe because the lethal dose is 0.54 mg/kg of human body weight (7 and 8).

Boiling the fresh cassava for 1 hour decreased total polyphenols and flavonoids from 1.348 gm/100gm and 0.068 gm/100gm to 0.547 gm/100gm and 0.005 gm/100gm, respectively, based on their sensitivity to heat treatment (9).

Table 1: Chemical composition of cassava.

Chemical Composition	Fresh Cassava	Cassava Paste
Total protein (gm/100gm)	2.9 ± 0.10	2.9 ± 0.10
Total lipid (gm/100gm)	0.8 ± 0.05	0.8 ± 0.05
Total fiber (gm/100gm)	5.7 ± 0.10	5.7 ± 0.10
Calcium (gm/100gm)	0.370 ± 0.15	0.370 ± 0.15
Potassium (gm/100gm)	0.063 ± 0.1	0.063 ± 0.1
Magnesium (gm/100gm)	0.590 ±0.1	0.590 ±0.1
Zinc (gm/100gm)	0.001 ± 0.1	0.001 ± 0.1
Amygdalin (mg/gm)	0.08 ± 0.01	0.08 ± 0.01
Total phenols (gm/100gm)	1.348 ± 0.01	0.547 ± 0.02
Total flavonoids (gm/100gm)	0.068 ± 0.01	0.005 ± 0.00
HCN (gm/100gm)	0.0038 ± 0.01	0.0011± 0.01

Each value represents the mean ± S.E (Standard Error) and mean of three replicates.

Chemical composition of cassava milk: Table 2 illustrates that the average total protein content in cassava milk increased with the addition of 5% and 10% cassava portions from 3.44 gm/100 gm to 3.55 gm/100 gm, respectively. After 7 days of refrigeration at 6±1°C, total protein increased slightly, possibly due to water evaporation. The evaporation of water is due to plastic cup packages that are not tightly closed, which allows moisture to evaporate during storage.

The mean average total fat content in fresh cassava milk increased from 3.9 gm/100 gm to 4 gm/100 gm following the addition of 5% and 10% cassava, respectively. Total fat content increased slightly, perhaps as a result of evaporating water. Water evaporates when plastic cup packaging is not sealed tightly, allowing moisture to evaporate while being stored. Amygdalin was not detected in the control but registered 3.51 gm and 7.01 gm per 100 gm, respectively following the 5% and 10% cassava addition.

Table 2: Chemical composition of cassava milk.

Treatments	Control	5% Cassava	10% Cassava
Fresh cassava beverage			
Total protein gm/100 gm	3.44 ± 0.03c	3.55 ± 0.01b**	3.70 ± 0.003a*
Total lipid gm/100 gm	3.9 ± 0.03	4.0 ± 0.03	4.0 ± 0.03
Amygdalin gm/gm	Not detected	3.51 ± 0.01b	7.01 ± 0.01a
Stored cassava beverage (after 7 days)			
Total protein gm/100 gm	3.45 ± 0.03c	3.60 ± 0.01b*	3.72 ± 0.003a*
Total lipid gm/100 gm	3.9 ± 0.03	4.0 ± 0.03	4.0 ± 0.03
Amygdalin mg/gm	Not detected	3.52 ± 0.01b	7.02 ± 0.01a

Each value represents the mean ± S.E (Standard Error) and mean of three replicates. Values in the same column with the same letter are not significant at $p \leq 0.05$.

The acidity of cassava milk: As seen in Table 3, the significant acidity of 5% and 10% cassava milk increased after storage at 6 ± 1°C for 7 days as the higher bacterial load produced acids in the medium. As the microbial populations increased, the treatable acidity value increased.

This increase illustrates the implication of using treatable acidity as an indication of undesirable bacterial growth in milk.

Table 3: Acidity of cassava milk.

Treatments	Control	5% Cassava	10% Cassava
Fresh cassava milk beverage			
Acidity %	0.16 ± 0.1a**	0.16 ± 0.1a**	0.17 ± 0.1a**
Stored cassava milk beverage (after 7 days)			
Acidity %	0.17 ± 0.1a*	0.17 ± 0.1a*	0.18 ± 0.1a*

Each value represents the mean ± S.E (Standard Error) and mean of three replicates. Values in the same column with the same letter are not significant at $p \leq 0.05$.

Sensory evaluation of cassava milk: Table 4 lists the scores of the milk beverage's sensory properties. These properties were assessed for texture, aroma, flavor, acceptability, and appearance both when the beverage was fresh and after a week’s storage.

Sucrose is added to improve sensory acceptance of many foods, increasing the sweetness of the final products. Mixed drinks made with different proportions of cassava had the best sensory acceptance. The highest value was found to be the beverage containing 10% fresh cassava milk. Adding cassava that contains starch presented the highest apparent texture values.

Table 4: Sensory evaluation of cassava milk.

Treatments	Acceptability (20)	Appearance (20)	Aroma (20)	Flavor (20)	Texture (20)	Total scores (100)
Fresh cassava milk						
Control	18 ± 1	18 ± 1	18 ± 1	18 ± 1	17 ± 1	89 ± 1
Milk with 5% cassava	19 ± 1	18 ± 1	18 ± 1	18 ± 1	18 ± 1	91 ± 1
Milk with 10% cassava	19 ± 1	19 ± 1	19 ± 1	19 ± 1	19 ± 1	95 ± 1
Stored cassava milk (after 7 days)						
Control	15 ± 1	16 ± 1	15 ± 1	15 ± 1	15 ± 1	76 ± 1
Milk with 5% cassava	17 ± 1	17 ± 1	17 ± 1	18 ± 1	18 ± 1	87 ± 1
Milk with 10% cassava	18 ± 1	18 ± 1	18 ± 1	17 ± 1	19 ± 1	90 ± 1

Conclusions

The findings of this study reveal the possibility of producing cassava-supplemented dairy beverages that are rich in various vital dietary components such as vitamins, amygdalin, minerals, proteins, fats, and fibers.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

All authors have contributed in writing the manuscript. Monier M. El Abd was responsible for revising and editing the manuscript.

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The authors declare no conflict of interest.

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