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Characterization of Fresh *Moringa oleifera* Beverage

Phyllis Naa Yarley Otu[1*] Firibu Kwesi Saalia[2] Emmanuel Adu Amankwah[3]
1. Department of Science Laboratory Technology, Accra Polytechnic, PO box GP 561, Accra, Ghana
2. Department of Food Science and Nutrition, University of Ghana, PO box LG 25, Accra, Ghana
3. Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Private Mail Bag, Kumasi, Ghana
* E-mail of the corresponding author: phyllisotu@gmail.com

Abstract
To determine the nutritional composition of fresh Moringa leaf beverage (50% moringa extract, 38% pineapple juice and 12% carrot extract) and assess the keeping quality. Proximate analysis, chemical analysis and shelf stability studies under three different storage conditions of temperature were conducted on the beverage. Fresh Moringa oleifera beverage recorded 2.9g/100ml of protein, 1.02mg of iron and 159.14mg/100ml of vitamins C. After 8weeks of storage 78% of vitamins C was still retained even under the most severe storage condition (sunlight). There was no microbial growth under all the conditions of storage, and the product was still acceptable. Fresh Moringa oleifera beverage is therefore an excellent means of distributing nutrients to the malnourished and other consumers.
Keywords: Analysis, content, nutritional composition, keeping quality

1. Introduction

Analyses of raw Moringa leaves composition has revealed them to have significant quantities of calcium, iron, protein, vitamin A, B, and C (Ramachandran *et. al.,* 1980). Meanwhile, as many as 800 million persons worldwide are affected by malnutrition. More than half the childhood deaths in developing countries are related to malnutrition (Benson, 2004).

Furthermore, across the continent increasing numbers of individuals live with or die - prematurely - from stroke, hypertension, diabetes and cancers. In countries such as Ghana a greater number of medical admissions and deaths result from chronic non-communicable diseases than from communicable diseases such as HIV and tuberculosis (Aikins, 2008).

In 2004, the World Health Organization (WHO) adopted the Global Strategy on diet to help prevent or minimize the occurrence of non-communicable diseases. It recommended that individuals achieve energy balance and a healthy weight; limit energy intake from total fats and shift fat consumption away from saturated fats to unsaturated fats and towards the elimination of trans-fatty acids; increase consumption of fruits, vegetables, legumes, whole grains, and nuts; limit the intake of free sugars; and limit salt consumption from all sources and ensure that salt is iodized (Lachat, 2013).

In response to the above mentioned recommendation by WHO, 2004, fresh Moringa leaves (extract) beverage (50% moringa extract, 38% pineapple juice and 12% carrot extract) was developed by optimizing its acceptability (Article Title: Optimizing Acceptability of Fresh *Moringa oleifera* Beverage – about to be published). Knowledge on the nutritional composition and keeping quality of this beverage is therefore paramount to trigger its usage as a vehicle for availing nutraceutical benefits to many consumers.

2. Material and Methods

2.1 Proximate Analysis

2.1.1 Determination of moisture content

Moisture contents of the samples were determined in triplicate, at 103 ± 2^{o}C for 2 hours using the air oven drying method in accordance with AOAC (1990) method 977.11 and results recorded in grams (g).

2.1.2 Determination of fat content

The fat content were determined in duplicate by the gravimetric Werner-Schmid process as described in Pearson's Chemical Analysis of foods (1987) and results recorded in grams (g).

2.1.3 Determination of protein content

The macro kjeldahl procedure based on the AOAC (1990) method 984.13 was used. The protein content of samples was determined in duplicates by analyzing for total nitrogen and then converting it to protein using the conversion factor 6.25. The results were recorded in grams (g).

2.1.4 Determination of carbohydrate content

Carbohydrates were determined by difference method and results recorded in grams (g).

2.1.5 Determination of ash

The dry ashing method in accordance with AOAC (1990) was used in this determination using Gallenkamp Muffle Furnace, England and results recorded in grams (g).

2.2 Analytical procedure for shelf-life study

2.2.1 pH measurement

The pH of ten milliliters (10 mls) of juice was determined using a pH meter (Model pHep3, µicropHep).

2.2.2 Titratable acidity measurement

Ten milliliters (10 mls) of juice was mixed with 100ml distilled water. The mixture in triplicate was then titrated against 0.1M NaOH using 1% phenolphthalein as indicator. Acidity was calculated as acetic acid (%).

2.2.3 Determination of alcohol content

The pycnometer procedure based on AOAC (1984) method 9.037 was used. The corresponding percentage of alcohol was determined by weight in distillate from table 52.005 (AOAC, 1984). The result was multiplied by weight of distillate and divided by weight of sample and recoded in percentages (%).

2.3 Chemical Analysis

2.3.1 Mineral Analysis

A wet digestion method was used to eliminate all organic matter from the sample before sample was analyzed for the various minerals. About 1 ml of the sample was measured into a 250 mls beaker. Twenty five milliliters (25 mls) concentrated HNO_3 was added and the beaker was covered with a watch glass. The sample was digested with care on a hot plate in a fume chamber until all the organic matter had been oxidized (20-30 mins). The pale yellow solution was cooled and 1ml 70% $HClO_4$ was added with care. Digestion was continued until the solution was almost colorless (until all the HNO_3 was removed).

The solution was then cooled slightly after the digestion process, and about 30 mls distilled water was added and allowed to boil for about 10 mins then filtered when hot through No. 4 Whatman filter paper into a 100 mls volumetric flask. The beaker was washed well with distilled water and filtered. The flask was then cooled and made up to the 100 mls mark. This solution was used for all the mineral analyses. The following minerals; Magnesium (Mg), Calcium (Ca), Potassium (K) and Iron (Fe) were all determined using the PerkinElmer Atomic Absorption Spectrophotometer (AAS; Model AAnalyst 400, Minneapolis, U.S.A.) and results recorded in milligram (mg).

2.3.2 Determination of Provitamin A

Provitamin A analysis were determined by HPLC method as described in Pearson's Composition and Analysis of Foods (1987) and results recorded in milligram (mg).

2.3.3 Determination of Vitamin B_2

HPLC techniques were used as described in Pearson's Composition and Analysis of Foods (1987) and results recorded in milligram (mg).

2.3.4 Determination of Vitamin C

Titration procedures as described in Pearson's Composition and Analysis of Foods (1987) and results were recorded in milligram (mg).

2.3.5 Determination of Color

The color of the juice were determine using the Minolta Chroma Meter (Minolta CR 300 series) using the L*a*b* color system. The Chroma meter was calibrated with a standard white tile (L* = 97.95, a* = -0.12, b* = +1.64).

2.4 Microbial Analyses

The juice was tested for their microbiological safety by determining the Total Plate Count (TPC), Yeasts /Molds, Total Coliforms and *Staphylococcus aureus* using procedures outlined in the Quality Assurance Procedure Manual of Ghana Standards Board, Okponglo, Legon.

2.4.1 Total Plate Count

The total population counts of the mesophilic bacteria were determined using the Total Plate Count Method, on a Plate Count Agar (pH 7.0 from Oxoid Ltd., Basingstoke, Hampshire – England). The plate was incubated at 35^{o}C for 48 ± 2 hrs. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

2.4.2 Yeasts and Molds

Malt Extract Agar (pH 6.6 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the yeasts and molds population in the sample. The plates were incubated at 25^{o}C for 5 days. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

2.4.3 Total Coliforms (Presumptive Test)

Lauryl Tryptose Broth (pH 6.8 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the

presence of Coliforms. Fermentation tubes with inverted Durham tubes in them were used. The tubes were incubated at 35°C for 48 ± 2 hrs. The presence of gas trapped in the Durham tubes would indicate a positive test for Coliforms.

2.4.4 Staphylococcus aureus

Baird Parker Agar (pH 7.2 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the presence of *Staphylococcus areaus.* The plates were incubated at 37°C for 48 ± 2 hrs. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

3. Results and Discussion

3.1 Proximate Analysis

The composition of fresh Moringa leaves (extract) and the final composite juice is as presented on Table 1. The data of Moringa leaves from literature (Campden and Chorleywood, 1998), pineapple and carrot (USDA SR-21) are presented alongside for easy comparison. Moringa leaves clearly have significant amounts of protein and minerals, and can be a good source of nutrients if incorporated as an ingredient in food product formulation.

3.1.1 Carbohydrate content

Results of the proximate composition of carbohydrate of the fresh Moringa leaf extract showed 8.7 g/100g was extracted, which is more than half the total amount in the fresh leaves reported in literature. However, both carrots and pineapple have lower amounts of carbohydrate (Table 1) and therefore caused a reduction of carbohydrates in the final composite juice to 4.91 g/100g. The centrifugation of the Moringa leaf extract during the processing also took out the insoluble fiber which is a form of carbohydrate.

3.1.2 Protein content

According to Aurand and Woods (1973) the colloidal dimensional structure of proteins makes it uneasy to pass through semi permeable membranes. Also some proteins are not water soluble and therefore could not have been extracted in the aqueous medium. As much as 4.8 g/100g of proteins was extracted from the fresh leaves. The relatively very low protein composition of the pineapple and carrot of 0.54 g/100g and 1.0 g/100g respectively (USDA SR-21) further decreased the total composition in the final mixture to 2.9 g/100g.

3.1.3 Fat content

Fat is soluble in organic solvents like petroleum ether. Since water was used in extraction, only 0.83 g/100g was extracted from the fresh leaves. The addition of fat from the pineapple and carrot accounts for the increase in fat content of the final composite juice of 1.81 g/100g.

3.1.4 Water content

The total water content of the final juice was 86.82 g/100g. Though the water content of fresh Moringa leaves reported in literature was 75 g/100g, the use of water in slurring of the leave increased the water content to 81.30 g/100g. The water composition of raw pineapple and carrot are 86 g/100 g and 87.5 g/100 g respectively (USDA SR-21). The pineapple was pressed and pulp and was allocated almost 40 percent, whiles the carrot which extracted with 600mls of water per 100 g was allocated 10 percent of the final juice. The increment of water recorded in the composition of the final juice was thus expected.

3.2 Mineral analysis

The mineral analysis (magnesium, potassium, calcium and iron) revealed that there was general reduction after extraction from leaves. However, from literature (Campden and Chorleywood, 1998) a good amount of potassium, 115 mg/100 g and 240 mg/100 g, has been found in raw pineapple and carrot respectively which helped boost the content of the minerals in the juice. This resulted in potassium recording 77 mg/100 g, the highest amount among the minerals of the final composite juice.

3.2.1 Vitamins content

Adequate absorption of fat – soluble vitamins (vitamin A) depends on efficient fat absorption (Wardlaw and Insel, 1996). A total of 48.8% fat extracted from the leaves was able to extract along 5.98 mg/100 g of provitamin A. The level of provitamin A in carrot made a positive impact on the Moringa juice by increasing the total amount of the provitamin A to 6.64 g/100 g.

Water – soluble vitamins, like riboflavin and particularly ascorbic acid is easily destroyed by heat, light, exposure to air, cooking in large amounts of water and alkalinity (Wardlaw and Insel, 1996). The extraction medium (i.e. water) for riboflavin (vitamins B_2) and ascorbic acid (Vitamins C) strongly reflected in the values recorded. A total amount of 215 mg/100 g of riboflavin and 220 mg/100 g of ascorbic acid were extracted from the fresh leaves. However, exposure to air during preparation and the use of heat for pasteurization destroyed some of the water- soluble vitamins and hence reducing the amount of ascorbic acid to 159.14 mg/100 g in the final juice (Table 1). The appreciable amount of vitamin C present in the pineapple juice was an additional source in the final product.

Table 1. Proximate composition of fresh Moringa leaves extract, final composite juice and also literature on fresh Moringa leaves[1], pineapple[2] and carrot[2] (per 100g)

Composition	**Fresh Moringa leaves extract**	**Final composite juice**	**Literature on Fresh Moringa leaves[1]**	**Literature on pineapple[2]**	**Literature on carrot[2]**
Carbohydrate (g)	8.7 ± 0.06	4.91 ± 0.02	13.4	12.63	9.0
Protein (g)	4.8 ± 0.08	2.9 ± 0.06	6.7	0.54	1.0
Fat (g)	0.83 ± 0.03	1.81 ± 0.01	1.7	0.12	0.20
Water(g)	81.30 ± 0.04	86.82 ± 0.05	75.0	86.0	87.5
Magnesium(mg)	24.44 ± 0.02	12.30 ± 0.03	24.0	12.0	18.0
Potassium(mg)	98.16 ± 0.15	77.01 ± 0.28	259.0	115.0	240.0
Calcium(mg)	124.4 ± 0.03	60.65 ± 0.05	440.0	13.0	33.0
Iron(mg)	3.05 ± 0.01	1.02 ± 0.05	7.0	0.28	0.66
Pro-vitamin A(mg)	5.98 ± 0.01	6.64 ± 0.02	6.8	0.03	8.29
Vitamin B_2(mg)	0.05 ± 0.01	0.03 ± 0.03	0.05	0.03	0.05
Vitamins C(mg)	215 ± 0.27	159.14 ± 0.12	220	36.20	7.0

[1]Campden and Charleywood (1998)

[2] USDA SR-21

3.3 Shelf-Life Analysis

The optimized final composite juice was pasteurized (62^{o}C for 30 mins), bottled and was closely monitored to determine the shelf – life under three different storage conditions; refrigerator (5^{o}C), room temperature (26 ^{o}C) and sunlight (above 37 ^{o}C) for eight (8) weeks. The parameters monitored during this period included; ascorbic acid, titrable acidity, pH, alcohol content, color and microbial analysis. The data generated from the attributes were subjected to Analysis of Variance, and summarized in Table 3.

3.3.1 Vitamin C

The data for ascorbic acid in the beverage stored under different conditions is displayed in (Fig 1). The figure shows that the rates of ascorbic acid degradation were lower when the beverage was stored in the refrigerator than at ambient conditions or in the sun. The data was fitted into linear trendline models and the trends show that when stored under refrigerator condition, the rate of ascorbic acid degradation was 2.5mg/week. The rate of ascorbic acid degradation in the juices increased to 4mg/week when it was stored at room temperature and 6mg/week when stored in the sun, as shown in Table 2.

Analysis of variance and multiple range tests by LSD (Table 3 and 4 respectively) showed that the storage conditions had significant ($p \leq 0.05$) effects on the Ascorbic acid. Storage of the juice in the refrigerator preserved ascorbic acid content better than at room temperature conditions. The room temperature conditions also significantly ($p \leq 0.05$) preserved ascorbic acid in the juices better than when stored in the sun. The effect of storage time on the ascorbic acid content of the juices was also significant ($p \leq 0.05$). Ascorbic acid under all storage condition degraded with time, but even under sunlight, 78% of the original amount of ascorbic acid was retained in the juice after eight weeks of storage.

Table 2. Degradation rates for ascorbic acid in Moringa juice during storage

Storage condition	**Linear model for ascorbic acid**	**R – Squared**
Refrigeration (15°C)	Y = -2.51X + 142	0.79
Room Temperature (25°C)	Y = -4.29X + 142	0.97
Sun (above 35°C)	Y = -6.22X + 142	0.95

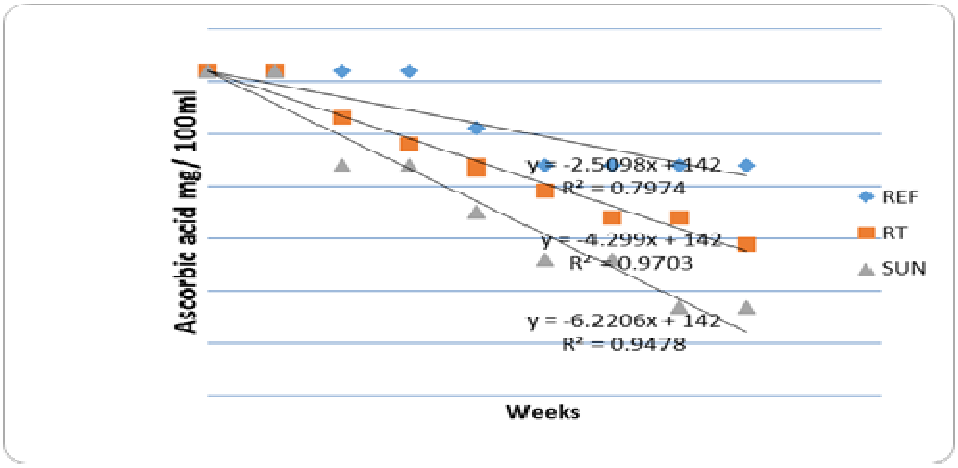
Where, X= Storage time

Y= Total amount of ascorbic acid

Table 3. Anova summary for shelf-life study

Source of variance	**Parameters**					
	Vitamin C	pH	Titrable acidity	Color L*	Color a*	Color b*
Storage condition	56.96**	7.74**	48.00**	56.16**	83.82**	60.55**
Storage time (week)	48.43**	51.97**	53.66**	122.26**	68.91**	343.300**

* Significant at 95 confidence interval (CI); **significant at 99% CI



REF= Refrigerator RT= Room temperature SUN= Sunlight

Fig 1. Ascorbic acid content under different storage conditions and time

3.3.2 pH and Titratable acidity

Titratable acidity and pH of the juice is a measure of the sourness of the product and it also reflects on the stability of the product with regards to deterioration during storage. The analysis of the data for pH showed significant effects ($p \leq 0.05$) of storage conditions and time. The multiple range analysis showed that while there were no significant difference ($p \geq 0.05$) in pH between refrigerator and room temperature storage (4.02 and 4.04), the pH of the beverage stored under sunlight was significantly higher (4.09). This could be due to heat and/or sunlight induced degradation of some components like protein that will affect the pH. Such a reaction or degradation could not have been due to microbial activity because there was no microbial growth.

Storage time did not show significant effects on the titratable acidity of the juices. On the other hand, there were significant differences between the titratable acidity of the juices stored at different conditions. Titratable acidity at the end of storage of the final composite juice in the refrigerator (0.27%) was significantly lower than that

stored at room temperature (0.28%) and sunlight (0.29%). Though juice stored in the sun recorded the highest titratable acidity its corresponding pH was also the highest. This trend is difficult to explain, but could suggest some buffering effects of the juice proteins.

3.3.3 Color

Color is one of the most important quality attributes of food. The first impression of the quality and acceptability of a particular food is judged upon its appearance (Neilsen, 1998). Preliminary study revealed that some consumers make choices of juices based on color. The juices were all yellowish-green in color. L* is a color parameter that measures the extent of lightness, thus L* when zero (0) would indicate black, and when one hundred (100) would indicate white; a* when positive signifies reddish color coordinate and when negative signifies greenish color coordinate; b* value when positive signifies yellowish color coordinate and bluish color coordinate when negative.

*3.3.4 Color L**

The L* values for the juice were significantly ($p \leq 0.05$) affected by the storage conditions as well as storage time. The Multiple Range Test by LSD at the end of storage showed that refrigerator significantly preserved the L* value (44.19) of the juice better than the other storage conditions. L* values for the juices stored at room temperature (43.79) and sunlight (42.95) were all significantly different. Thus samples stored in the sun turned darker compared to those stored at ambient and refrigerator conditions. The darkening observed under sunlight could be due to degradation of the chlorophyll by UV light and temperature or non-enzymatic browning in an aqueous environment that has proteins and reducing sugars.

*3.3.5 Color a**

The analysis of variance for a* values showed significant effects of storage conditions and storage time. A negative a* value suggests green coloration, and this was significantly higher in absolute terms for juices stored in the refrigerator (-33.63) than at room temperature (-33.53) and sunlight (-33.30) at the end of storage.

*3.3.6 Color b**

Positive b* values are a measure of the yellowness or blueness of the product. Analysis of variance showed that storage time and storage temperature significantly affected the yellowness of the juices. Samples at the end of storage in the refrigerator had significantly higher b* values (12.99), than those stored at room temperature (12.66) and sunlight (12.23). Thus storage temperature significantly influences color of the juice with time.

3.3.7 Alcohol content

The three different storage conditions were not significantly different in alcohol production with storage time. There were no detectable amounts of alcohol in the juice under any of the storage conditions, for the entire eight weeks shelf life study period. Indeed the microbial analysis confirmed that there were no growths at any of the storage conditions.

3.4 Microbial analysis

The results for growth of Total Coliform, *Staphylococcus areaus*, Yeast /Mold and Total Plate Count (Table 5) revealed that there was no microbial growth under all conditions for the eight weeks period of study.

Table 4. Summary table for multiple range test by storage condition for shelf-life

Parameter	Storage condition	LS Mean	Nutritional content (week 8)
Vitamin C (mg/100ml)	Refrigerator	$132.78^{a} \pm 0.05$	124
	Room temp.	$124.94^{b} \pm 0.04$	109
	Sunlight	$117.00^{c} \pm 0.05$	97
pH	Refrigerator	$4.02^{a} \pm 0.01$	4.24
	Room temp.	$4.04^{a} \pm 0.01$	4.44
	Sunlight	$4.09^{b} \pm 0.02$	4.47
Titrable acidity (%)	Refrigerator	$0.27^{a} \pm 0.01$	0.26
	Room temp.	$0.28^{b} \pm 0.02$	0.28
	Sunlight	$0.29^{c} \pm 0.01$	0.29
Color L*	Refrigerator	$44.19^{a} \pm 0.08$	42.38
	Room temp.	$43.79^{b} \pm 0.08$	42.03
	Sunlight	$42.95^{c} \pm 0.07$	41.70
Color a*	Refrigerator	$-33.63^{a} \pm 0.02$	-33.22
	Room temp.	$-33.53^{b} \pm 0.02$	-33.11
	Sunlight	$-33.30^{c} \pm 0.01$	-33.03
Color b*	Refrigerator	$12.99^{a} \pm 0.05$	11.88
	Room temp.	$12.66^{b} \pm 0.05$	11.33
	Sunlight	$12.22^{c} \pm 0.04$	11.02

NB: Different alphabets (a, b, c) assigned to LS Mean values for the 3 different conditions of a parameter represents significant differences ($p \leq 0.05$); whiles same alphabets represents insignificant differences ($p \leq 0.05$).

4. Conclusion

Comparing the nutrient composition of some blended juices from literature with the final composite juice, the latter is a more nutritious beverage considering the protein, Iron, vitamin A and C content.

The keeping quality of the Moringa juice in the refrigerator (5^{o}C), at room temperature (25^{o}C), or even when exposed to the sun (above 37^{o}C) for eight weeks, was good. All attributes were better preserved when the juice was stored in the refrigerator. The rate of degradation of vitamin C was slow even under harsh conditions, when almost 80% of the vitamin was retained after 2 months of storage.

Table 5. Microbial analysis for shelf-life study

STORAGE CONDITION	STORAGE TIME (WEEK)	YEAST AND MOULDS (10^{-1})	TOTAL COLIFORMS (10^{-1})	TOTAL PLATE COUNT (F/S)	*STAPHYLOCOCCUS AREAUS* (10^{-1})
Refrigerator	0	< 10	0	1	<10
Room Temp.	0	< 10	0	1	<10
Sunlight	0	< 10	0	1	<10
Refrigerator	2	< 10	0	2	<10
Room Temp.	2	< 10	0	1	1
Sunlight	2	< 10	0	<10	<10
Refrigerator	4	< 10	0	<10	<10
Room Temp.	4	< 10	0	<10	1
Sunlight	4	< 10	0	<10	<10
Refrigerator	6	< 10	0	<10	<10
Room Temp.	6	< 10	0	3	<10
Sunlight	6	< 10	0	3	<10
Refrigerator	8	< 10	0	2	<10
Room Temp.	8	< 10	0	1	<10
Sunlight	8	< 10	0	1	<10

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