ORIGINAL ARTICLE

Impact of Fermentation on the Microbiological and Phytochemical Properties of Functional Foods Juiced Together for A Healthy Beverage

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ABSTRACT

In this experimental study, the microbiological and phytochemical properties of functional foods (garlic, turmeric, bitter kola, neem leaf, jute leaf and pineapple) juiced together and fermented for 5 days was compared with the one not fermented. Garlic (*Allium sativum*), ginger (*Zingiberofficinate*), bitter kola (*Garcina kola*), turmeric (*Curcuma longa*), lime (*Citrus aurantifolia*), neem leaf (*Azadirachtaindica*) and onion (*Allium cepa*) were washed, dried, sterilized and grounded aseptically. At first, ginger 50g, turmeric 50g, garlic 20g, bitter kola 10g, onion 30g and neem leaf 30g were weighed into a stainless bowl. Two liters of sterile water at temperature 35^{0}C was used to soak the mixed powder for 2 hours, mixed together with mechanical stirrer and screw press was used to squeeze out the juice. Two thousand gram of peeled pineapple was washed, juiced and added to the prepared juice as sweetener. The mixture was divided into two and poured into a separate airtight vessel labeled A and B, both vessels were pasteurized at 62^{0}C for 30 minutes. *Saccharomyces cerevisae* (2g) was introduced to vessel B and allowed to ferment for 5 days at 27±2^{0}C. The microbial qualities and phytochemical properties of both fermented and non fermented juice were investigated. The pasteurized juice gave a better microbial quality result when compared with unpasteurized juice. The presence of photochemical in both fermented and unfermented juice indicates that the juice has antioxidant properties which offer protection that reduces the risk of many diseases. Therefore, the beverage pasteurized and not fermented is recommended for a healthy drink especially for malnourished or immunocompromised people.

Keywords: Antimicrobial quality, phytochemicals, antioxidant, immunocompromised, fermentation

International Journal of Human and Health Sciences Vol. 10 No. 03 July'26

DOI:https://doi.org/10.31344/ijhhs.v10i3.953

INTRODUCTION

For centuries, it has been known that certain plant produce can benefit human health when consumed in the right amounts. Plants like garlic, ginger, bitter kola, neem leaves, onions, moringa, and citrus, among others, have shown antimicrobial and antioxidant properties, making them valuable for promoting well-being[1] The aim of this research work is to investigate the effects of fermentation on the microbiological and phytochemical properties of functional foods juiced together for a healthy beverage. Combining garlic, ginger, bitter kola, neem leaf, onion, moringa, and citrus together to make a beverage can be used to naturally boost the immune system of man to combat immunodeficiency and other related diseases, since these plants produce have been known to have beneficial effect on man with little or no known adverse effect when consumed in right proportion.

Garlic (*Allium sativum*) has a long history of

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medicinal use and is now recognized for its cardiovascular, antineoplastic, and antimicrobial properties[2]. Fresh ginger (*Zingiber officinale*) has gastrointestinal benefits as well as anticancer and antidiabetic effects.[3,4] Turmeric (*Curcuma longa*) in the ginger family, a common spice, is useful in treating arthritis, digestive disorders, respiratory infections, allergies, liver disease, depression and many others[5]. Lime (*Citrus aurantifolia*) is high in vitamin C, with its pulp and peel containing a variety of phytochemicals, including polyphenols and terpenes[6]. Neem leaf (*Azadirachta indica*) has antifungal, antidiabetic, antibacterial, contraceptive and sedative properties[7]. It is used in preparing vegetable soup in India, it has a strong bitter taste. Evidence showed bitter kola (*Garcinia kola*) to contain anti-inflammatory, anti-allergic properties, anti-infective and caffeine[8]. Given the proven health benefits of these plant produce, it is worthwhile to investigate whether combining them into a single beverage could enhance their ability to boost the immune system. This could potentially help prevent immune deficiencies and related diseases.

METHODS

Sample collection: The samples were purchased from a farm located at the Benin-Owena multipurpose dam and farmland in Ondo State, Nigeria. These plant produce: garlic (*Allium sativum*), ginger (*Zingiber officinale*), bitter kola (*Garcinia kola*), turmeric (*Curcuma longa*), lime (*Citrus aurantifolia*), neem leaves (*Azadirachta indica*), onion (*Allium cepa*), and pineapple (*Ananas comosus*), were taken to the laboratory, washed, and stored in a cool, dry place for further treatment and use.

Preparation of the plants extract to make beverage: The plant produce: garlic (*Allium sativum*), ginger (*Zingiber officinale*), bitter kola (*Garcinia kola*), turmeric (*Curcuma longa*), lime (*Citrus aurantifolia*), neem leaves (*Azadirachta indica*), and onion (*Allium cepa*), were rinsed under running water and then soaked in Huwassan sterilizing fluid (containing 5% hydrogen peroxide) for 30 minutes. They were then sliced, dried in an oven at 40°C for 48 hours following the method described by Raja et al.[9], and ground into powder, following Zhu et al.[10]. The following quantities of the powdered plant products were weighed separately into a stainless steel bowl and then combined: 50 grams of ginger, 50 grams of turmeric, 20 grams of garlic, 10 grams of bitter kola, 30 grams of onion, and 30 grams of neem leaf. Two liters of sterile water, heated to 35°C, was added to the mixture and allowed to soak for 2 hours. The mixture was then stirred with a mechanical stirrer, and a screw press was used to extract the juice, yielding what is referred to as Extract A. To prepare Extract B, a mature pineapple (weighing 2000 g) was washed, peeled, and blended with 500ml of water using an electric blender. The mixture was then filtered through a sterile muslin cloth, yielding a total of 1.5 liters. Extracts A and B were then combined in a stainless steel bowl to form a beverage, which was subsequently divided into two portions. One portion of the beverage was pasteurized at 62°C for 30 minutes and then rapidly cooled, following the method described by Hunt[11], while the other portion remained unpasteurized. The pasteurized juice was then divided into two equal parts (2 liters each). *Saccharomyces cerevisiae* was added to one part, allowing it to ferment at room temperature for 5 days (resulting in Fermented beverage (B.05), while the remaining part was left unfermented (A.0).

Determinations of the microbial qualities of the fermented plant juice (B.05) and the unfermented plant extract (A.0): Both B.05 and A0.0 were subjected to microbial analysis, the method of Ruangpan et al.[12] was used to isolate the bacteria present and methods described by Samson et al.[13] were used to isolate the fungi. The plants extract were diluted in ratio 1:10, 1:100 and cultured using nutrient agar and eosin methylene blue agar for bacteria and malt extract for fungi. The bacterial plates were culture at 28±2^{0}C and 36±2^{0}C for 24 hours while the fungal plates were cultured at 25±2^{0}C for 72 hours.

Phytochemical analyses of the plants juice B0.5 and A0.0: The phytochemical properties of the plant extracts were also determined using the similar method described by Akkiraju et al.[14].

RESULTS

1. Microbiological quality of fermented juice (B.05) and unfermented plant juice (A.0): Unfermented

juice has no bacteria and fungi growth throughout the 5 days of experiment. The fermented juice has a bacterial load of 0.00×10^0 and fungi load of 1.5×10^1 on the zero (0) day of inoculation and increase to 0.00×10^0 for bacteria and 1.04×10^2 on the 5th day of inoculation (Table 1 & 2).

2. Phytochemical constituents of plant juice: The phytochemical constituents of fermented and unfermented juice were examined and the tannin content of the fermented was 0.245±0.007 and the unfermented gave 2.460±0.014, the flavonoids of fermented reduced significantly to 1.24±0.014 while unfermented remains at 1.14±0.0148 (Table 3).

Table 1: Viable counts for bacteria isolated from the plant extract

Day	A.0	B.05	BP
0	0.00×10^0	0.00×10^0	1.45×10^1
1	0.00×10^0	0.00×10^0	6.10×10^3
2	0.00×10^0	0.00×10^0	9.30×10^3
3	0.00×10^0	0.00×10^0	1.63×10^4
4	0.00×10^0	0.00×10^0	1.96×10^4
5	0.00×10^0	0.00×10^0	1.43×10^5

A.0=Pasteurized but not fermented; B.05=Pasteurized and fermented; BP=Bottled but not pasteurized

Table 2: Viable counts for fungi isolated from the plant juice

Day	A.0	B.05	BP
0	0.00×10^0	1.50×10^1	1.95×10^3
1	0.00×10^0	1.50×10^1	2.00×10^3
2	0.00×10^0	2.10×10^1	2.80×10^3
3	0.00×10^0	4.00×10^1	8.40×10^4
4	0.00×10^0	5.00×10^1	1.11×10^5
5	0.00×10^0	1.00×10^2	7.10×10^5

A.0=Pasteurized but not fermented; B.05=Pasteurized and fermented; BP=Bottled but not pasteurized

Table 3: Phytochemical constituents of the plant juice

Variables	A.0	B.05
Tannin	2.46±0.014	0.245±0.007
Phenol	nd	nd
Phytate	23.895±0.007	22.99±0.12
Oxalate	1.215±0.021	1.01±0.014
Saponin	2.125±0.021	1.36±0.014
Alkaloids	0.56±0.028	0.43±0.007
Flavonoids	1.24±0.014	1.14±0.014

nd=not detected; A.0=sample not fermented; B.05=fermented sample using 0.5g of yeast.

DISCUSSION

The importance of pasteurization is inevitable; this prevents a lot of food-borne diseases.[15] The microbial load found in the beverage bottled but not pasteurized might be as a result of processing and micro floral from the farm. In this study, the process of pasteurization was able to totally eliminate both the bacteria and fungi found in the beverage, which is supported by the findings of Rezaei et al.[16]. The fungal count in fermented plants extract was due to using yeast i.e., *Saccharomyces cerevisiae* (also known as brewer's yeast or baker's yeast), which was added to it before fermentation. Phytochemicals have antioxidants properties and offer protection that decreases the risk of many diseases[17]. The plants extract juiced together to make a beverage (both fermented and unfermented) were found to have appreciable value of phytochemicals, there is no recommended daily amount though[17]. The unfermented bevarege was found richer in phytochemicals than the fermented beverage as a result of leaching of some of the phytochemical present in the beverage during fermentation, supporting the findings of Ho & Redan[18].

CONCLUSION

Our study has shown that plants extract from Garlic (*Allium sativum*), ginger (*Zingiberofficinate*), bitter kola (*Garcina kola*), turmeric (*Curcuma longa*), lime (*Citrus aurantifolia*), neem leaf (*Azadirachta indica*) and onion (*Allium cepa*), mixed together to make a beverage and not fermented contains higher phytochemicals.

Therefore, the beverage pasteurized and not fermented is recommended for a healthy drink.

Conflict of Interest: The authors declared no conflict of interest.

Funding statement: No funding was received from anywhere for this work.

Ethical Approval: Not applicable.

Authors' Contributions: Conceptualization of this study: JF John-Isa, OO Adeoye; study design: JF John-Isa, OO Adeoye, RE Akindeko; data gathering and analysis: JF John-Isa, OO Adeoye, RE Akindeko; writing, editing and submission of the manuscript: JF John-Isa, OO Adeoye, RE Akindeko.

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