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Estimation of some active compounds in functional cardamom drink produced using probiotics

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Abstract

The study was conducted in the laboratories of the Department of Food Sciences / College of Agriculture / Tikrit University, for the purpose of producing a natural cinnamon drink enhanced with a bio-bio-active substance using a mixture of probiotic bacteria including *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium*. The study included fermenting the product for four weeks T₂, T₃, T₄ and T₅, respectively. then cooling at a temperature of 5°C and comparing it with the control drink T₁, and conducting several periodic tests weekly of storage in addition to sensory tests to determine the quality and keeping `quality of the product, the results showed a gradual decrease in the concentration of total soluble solids (TSS) of the cardamom drink during the fermentation weeks due to the consumption of microorganisms of the components of the produced drink, especially simple carbohydrates. The increase in the percentage of total acidity in the functional product is due to the bacteria producing organic acids, by lactic acid bacteria. The results showed a gradual increase in the values of all active compounds, including total phenols and flavonoids, in addition to vitamins. Sensory tests were conducted on the product, including taste, color, smell, texture, and general acceptance, the results were acceptable after four weeks of fermentation.

Keywords: Cardamom drink, active compounds, total phenolics, vitamins, probiotics

Introduction

Cardamom (*Elettaria cardamomum*) is called the emperor or the king of spices ^[1], as it is one of the most expensive and valuable spices in the world ^[2]. It is a perennial plant with thick, fleshy root stems with underground buds and fibrous roots. It is a monocotyledonous plant belonging to the ginger family ^[3]. Known to Arabs as cardamom. Cardamom contains large leaves that vary in color between white, light green, brown, and yellow. The part used is the seeds inside the fruit. It usually grows in the shade under forest trees and in high, humid places at a temperature ranging between 20-25°C, as it grows in warm tropical forests. The countries that produce it in large quantities are India, Sri Lanka, and Guatemala ^[4]. Cardamom contains volatile compounds that are responsible for its distinctive aroma, and it has many medicinal benefits, as it is an appetite stimulant and is used to improve the flavor of medicines and perfumes. Natural cardamom drink contains many effective aromatic compounds such as cineole, limonene, alpha-pinene, terpenes, and flavonoids such as quercetin and cambiferol, as well as vitamins, the most important of which are vitamin C and the vitamin B group ^[5]. In addition to containing antioxidants, antibacterials and antimicrobial compounds such as staphylococci present in the mouth, which can cause tooth decay, gum and throat infections, it has therapeutic properties for heart disease, high blood pressure and digestive problems ^[6]. It is used as a pain reliever, gas expeller, aids digestion, prevents nausea and vomiting and strengthens the stomach ^[7]. With the increase in health awareness and the avoidance of medical drugs as much as possible due to their negative side effects, reliance has begun on functional foods produced from natural sources and their use as alternative treatments for many diseases and their prevention. Many benefits of these foods, especially fermented functional beverages, have emerged. Recent years witnessed significant progress in this field, especially after it was confirmed that these food products have the ability to treat and prevent many diseases ^[8].

Many functional products have been developed and produced that fall within the field of fermented beverages, and to increase consumer demand for these healthy foods [9]. Due to importance of this product to health and nutritional perspective, and the lack of studies on this topic, the study aimed to produce a bio-enhanced therapeutic cardamom drink, study some of its biochemical properties [10], following it during the fermentation stages, and determine its shelf life.

Materials and Methods

Production of natural bio-enhanced cardamom drink: Cardamom seeds were used, from the local markets of Tikrit city, cleaned from dust and suspended matter and ground using a GOSONIC grinder. 10g of cardamom powder was taken and added to 1 liter of filtered and sterilized water with specific specifications. The mixture was then left for 24 hours at a temperature of 25°C, then filtered and the total soluble solids concentration of the product was adjusted by adding sucrose according to the Iraqi standard specifications to 12%. Then it was filled into previously sterilized, hermetically sealed glass bottles and pasteurized at 80°C for 20 seconds, then quickly cooled, and kept refrigerated at 5°C, then inoculated with a mixture of (*L. rhamnosus*, *L. acidophilus*, *L. casei*) and *Bifidobacterium* at a concentration of 1.5×10^8 cells/ml according to McFarland test under controlled sterile conditions, then the products were kept in the refrigerator until the study was conducted.

- **Total acidity test:** Total acidity was estimated by titration against the base NaOH and phenolphthalein indicator, as shown by [11].
- **Total soluble solids test:** Total soluble solids (TSS) were estimated using a Hand Refractometer as stated in [12].
- **Total phenols test:** Total phenols were determined using Follen's reagent using a UV-Vis Spectrophotometer as stated in [13].
- **Flavonoids test:** Flavonoids were determined by dissolving the sample in ethanol, using a UV-Vis Spectrophotometer as reported in [14].
- **Vitamin Test:** Vitamins (C, B6, B12) were determined using HPLC technique according to the report of [11].
- **Sensory tests:** The sensory evaluation was conducted immediately after the production process, and then after storage in the refrigerator for 4 weeks by 20 evaluators from graduate students at the College of Agriculture/Tikrit University, where the degrees of attributes were evaluated according to what was mentioned in [15], and the evaluation form was used to monitor the degrees of color, smell, taste, body, and overall acceptance, where the evaluation degrees ranged between 9-1.
- **Statistical analysis:** The experiment was conducted according to the CRD design to determine the significant differences between the treatments at the probability level (0.05) using the statistical program Minitab version (2010).

Results and Discussion

Chemical tests

T₁: Represents the control sample (free of probiotic bacteria), T₂: The first week of probiotic fermentation, T₃: the second week of probiotic fermentation, T₄: the third

week of probiotic fermentation, T₅: the fourth week of probiotic fermentation.

Table 1: Estimates of TSS% and total acidity% for samples of fermented beverages for four weeks compared to standard beverages

Treatments	Acidity (%)	TSS (%)	Characteristic
T ₁	0.35±0.05	12.00±0.00	a
T ₂	0.45±0.05	11.55±0.05	b
T ₃	0.65±0.05	10.95±0.05	c
T ₄	0.75±0.05	10.45±0.00	d
T ₅	0.95±0.05	9.75±0.00	e

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

Table 1 shows the values of total soluble solids (TSS). The results of the statistical analysis of the data show that there are significant differences between the value of treatment T₁ and treatments T₃, T₄ and T₅, between the value of treatment T₂ and treatments T₃, T₄ and T₅, between treatment T₃ and treatment T₅, and between the values of treatment T₄ and treatment T₅. While no significant differences were observed between the values of T₁ and T₂ and the values of T₃ and T₄, the same table shows significant differences in the acidity characteristic between the value of treatment T₁ and the values of T₃, T₄ and T₅, between the value of treatment T₂ and the values of T₃, T₄ and T₅, between the value of treatment T₃ and the value of T₅, and between the values of T₄ and T₅.

The results of the study show a significant increase in the percentages of total acidity values during the fermentation weeks, as a result of the consumption of the probiotics used in the fermentation process of the simple sugars present in the functional drink and thus the production of lactic acid [16]. The results of the study also showed a gradual decrease in the percentages of total soluble solids (TSS) values during the fermentation weeks. This is due to the consumption of simple components represented by carbohydrates by the probiotics for the purpose of growth and reproduction, as simple sugars are the basic material for the growth and reproduction of these microbes and for the purpose of carrying out the fermentation process [17]. Fermentation is a desirable change due to the microbial activity of the microorganisms used in the production process, thus improving food digestion in the digestive system and increasing nutrient absorption, as its enzymes work to digest food components and improve the metabolism and absorption of nutrients within the human digestive system [18].

Table 2: Estimation of active compounds of fermented beverage samples for four weeks compared to standard beverages

Treatments	Total Phenols (g/100ml)	Flavonoids (g/100ml)	Characteristic
T ₁	118.50±0.50	93.50±0.50	e
T ₂	129.50±0.50	101.50±0.50	d
T ₃	142.50±0.50	112.50±0.50	c
T ₄	157.50±0.50	119.50±0.50	b
T ₅	169.50±0.50	125.50±0.50	a

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

T₁: Represents the control sample (free of probiotic bacteria), T₂: The first week of probiotic fermentation, T₃: The second week of probiotic fermentation, T₄: The third

week of probiotic fermentation, T₅: the fourth week of probiotic fermentation.

Table 2 shows the values of flavonoids and total phenolic compounds. The results of the statistical analysis show that there are significant differences between the sum of the values of these characteristics during the fermentation weeks. It is noted that there are significant differences between the value of treatment T₁ and treatments T₂, T₃, T₄ and T₅, and between the value of treatment T₂ and treatments T₃, T₄ and T₅, and between the value of treatment T₃ and treatments T₄ and T₅, and between the values of treatments T₄ and T₅.

The results of the study show a gradual increase in the concentrations of total phenolic compounds and flavonoids in the functional beverage models compared to the standard beverages during the fermentation weeks. The reason for the increase in the concentrations of these components is due to the activity of the probiotics used in the production process and their metabolic activity during the fermentation and bio production process [19]. It has the ability to oxidize the components of the fermentation medium and produce complex components such as pigments, antioxidants and other vital compounds, and this technology is effective in increasing the concentrations of these compounds [16].

Table 3: Estimation of vitamins for fermented beverage samples for four weeks compared to standard beverages

Treatments	V C (g/100ml)	V B3 (g/100ml)	V B6 (g/100ml)	Characteristic
T ₁	9.35±0.05	1.15±0.05	0.65±0.05	e
T ₂	10.65±0.05	1.35±0.05	0.95±0.05	d
T ₃	12.95±0.05	1.55±0.05	0.15±0.05	c
T ₄	14.65±0.05	1.75±0.05	1.35±0.05	b
T ₅	16.95±0.05	2.05±0.05	1.55±0.05	a

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

T₁: Represents the control sample (free of probiotic bacteria), T₂: The first week of probiotic fermentation, T₃: The second week of probiotic fermentation, T₄: the third week of probiotic fermentation, T₅: the fourth week of probiotic fermentation.

Table (3) shows the values of vitamins C, B3 and B6. The results of the statistical analysis show that there are significant differences between the sum of the values of these vitamins during the fermentation weeks. It is noted that there are significant differences between the value of treatment T₁ and treatments T₂, T₃, T₄ and T₅, and between

the value of treatment T₂ and treatments T₃, T₄ and T₅, and between the value of treatment T₃ and treatments T₄ and T₅, and between the values of treatments T₄ and T₅ for all vitamins. The results of the study show a gradual increase in vitamin concentrations during the fermentation weeks compared to standard beverages, as probiotic bacteria have a high potential to consume the components of the fermentation medium and produce many vital products such as vitamins and flavor compounds [20]. Probiotic bacteria have important effects during the fermentation process [16], and this was confirmed by [21] regarding the possibility of these probiotics to improve the vitamin content of fermented products, especially B-complex.

T₁: Represents the control sample (free of probiotic bacteria), T₂: The first week of probiotic fermentation, T₃: The second week of probiotic fermentation, T₄: the third week of probiotic fermentation, T₅: the fourth week of probiotic fermentation.

Table 4 shows the results of the sensory tests for the functional cardamom drink model. The results of the statistical analysis of the data show that there are significant differences in the taste and smell characteristics between the value of treatment T₁ and treatments T₃, T₄ and T₅, and between the value of treatment T₂ and treatments T₃, T₄ and T₅, and between treatment T₃ and treatment T₅, and between the values of treatment T₄ and treatment T₅, while no significant differences were observed between the values of T₁ and T₂ and the values of T₃ and T₄. Regarding the color characteristic, the results of the statistical analysis showed that there were significant differences between the value of treatment T₁ and treatment T₅, between the value of treatment T₂ and treatment T₅, between the value of treatment T₃ and treatment T₅, and between the value of treatment T₄ and treatment T₅, while no significant differences were observed between the values of treatments T₁, T₂, T₃, and T₄. With regard to the texture characteristic, the results showed significant differences between the value of treatment T₁ and treatments T₄ and T₅, between the value of treatment T₂ and treatments T₄ and T₅, and between the value of treatment T₃ and treatments T₄ and T₅, while no significant differences were observed between the values of treatments T₁, T₂, and T₃ and treatments T₄ and T₅. As for the general acceptance characteristic, the results show significant differences between the value of treatment T₁ and treatments T₃, T₄ and T₅, and between the value of treatment T₂ and treatments T₃, T₄ and T₅, while no significant differences were observed between the values of treatments T₁ and T₂ and treatments T₃, T₄ and T₅.

Table 4: Sensory tests of fermented beverage samples for four weeks compared to standard beverages

Treatments	Taste	Color	Smell	Consistency	General Admission	Characteristic
T ₁	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	a
T ₂	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	a
T ₃	8.00±0.00	9.00±0.00	8.00±0.00	9.00±0.00	8.00±0.00	b
T ₄	8.00±0.00	9.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	b
T ₅	7.00±0.00	8.00±0.00	7.00±0.00	8.00±0.00	8.00±0.00	b

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

The results of the study showed a slight decline in the sensory evaluation results in the functional beverage models produced, as a result of the increase in acidity first and the production of other compounds that participate in giving the flavor to the product, as confirmed by [19]. On the possibility of probiotics to ferment food and maintain the health and

nutritional qualities of the product for four weeks with a slight decline in the sensory evaluation results, as a group of volatile compounds is produced that contribute to the production of a mixture of flavors that make the fermented food less acceptable when the storage period increases [22].

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