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Formulation and Quality Evaluation of Beverage Based on Germinated Amaranth Flour

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Abstract
Amaranth (Amaranthus spp.), a nutrient-dense pseudo cereal, is gaining prominence in the development of functional foods and beverages due to its high-quality protein, essential amino acids, and antioxidant components. This study aimed to formulate and evaluate a ready-to-serve therapeutic beverage using germinated amaranth flour (GAF). Amaranth grains were germinated, processed into flour, and used to develop beverages at three different concentrations of GAF to water (T_1: 1:4, T_2: 1:5, T_3: 1:6). The beverages were analyzed for their physicochemical composition, storage stability, and sensory acceptability. Results showed that germination significantly enhanced the protein content of amaranth flour while reducing crude fiber and fat. Among the formulations, T_1 exhibited the highest nutritional density, particularly in protein, fat, fiber, and carbohydrate content. During refrigerated storage (6 days), a significant increase in titratable acidity and total soluble solids (TSS), and a decrease in pH was observed across all samples. Sensory evaluation revealed that T_1 had the highest overall acceptability, especially in taste and flavor, although all treatments experienced a slight decline in sensory attributes over time. The findings suggest that germinated amaranth flour can be effectively used to develop a nutritious, acceptable, and functional plant-based beverage with good storage stability.

Keywords: *Amaranth; Germination; Beverage; Physicochemical properties; Sensorial properties*

I. Introduction

Amaranth is a pseudo cereal crop that grows annually, and its name comes from the Greek word Amaranthus. It is hardy, weedy, fast-growing, and herbaceous. It is a member of the *Amaranthacee* family. Because of its nutritional qualities, amaranth can be used as a potential food crop. It can also be used to extend or enhance the dietary value of other cereals. The seeds of amaranth are round in structure. The starch-rich perisperm is surrounded by the germ or embryo, which together with the seed coat make up the bran portion, which is high in protein and fat. Seeds are incredibly high in dietary fiber, iron, and calcium. Amaranth seeds are a great source of balanced protein due to their high amino acid content. Amaranth, in particular, boasts elevated amounts of tryptophan, arginine, lysine, and sulfur-containing amino acids. Specifically, the lysine concentration in amaranth is double that found in wheat and threefold higher than maize. Furthermore, its nutritional value surpasses that of barley, maize, and wheat (Gorinstein et al., 2002). Amaranth is composed of 62% carbohydrate, 5-9% fat, and 15–17% protein. The fractions of glutelins, globulins, albumins, and prolamins in amaranth range from 22.4–42.3% and 13.7–18.1%, 48.9-65% and 1.0-3.2%, respectively, of the total protein (Januszewska-Jóźwiak and Synowiecki, 2008; Paśko et al., 2008). Because it includes phenolic components including flavonoid, glycosides and anthocyanins, as well as a variety of antioxidants, amaranth is a stronger radical scavenger than other cereals. The demand for the health beverages is expanding day by day. The therapeutic beverage industry sector is said to be the fastest growing segment in the soft drink industry. The useful effects and the health benefits of amaranth are known to the worldwide. A ready to serve drink being refreshing in nature is liked by most of people. In recent times, the interest in using the herbal products is increasing day by day in the food industries. This type of beverages is hitting the interest of the consumers in health as well as wellness. Peoples are becoming more health conscious nowadays and they are focusing on all the health benefits in one drink. Demand for the soft drink increasing as a trend and it seems that there is a great demand for the development of value-added beverages using nutritional food with medicinal benefits in them. The therapeutic beverage sector has been determined to be the fastest growing sector in the soft drink industry sector (Chandra et al. 2018). Everyone has a desire to get nutritional benefits from whatever we take in our diet whether it be a soft drink or any food.

Germination is a beneficial process that enhances the nutritional quality of cereals by improving the bioavailability of nutrients. This process not only improves the digestibility and absorption of nutrients from milled grains but also activates hydrolytic enzymes that decompose complex polymers into simpler, more accessible compounds. Steeping and germination treatments are used to boost the nutritional value and bioactive compounds in pseudocereals, while also reducing the presence of anti-nutritional elements. According to Argüelles-Lopez et al. (2018), a beverage was formulated using germinated and extruded amaranth along with chia (*Salvia hispanica*). Their findings revealed that germinated amaranth and chia seeds contained more protein than those subjected to extrusion. Amaranth is commonly used as a carbohydrate-rich ingredient in the production of alcoholic drinks and fermented products, such as *ogi*, a traditionally fermented cereal porridge popular in parts of Africa or even as an alternative to soy in *shoyu* preparation (Mlakar et a., 2009; Rovina et al., 2017). Additionally, amaranth-based protein beverages have been developed to cater to the dietary needs of vegans, individuals with gluten intolerance (coeliac disease), and people who are lactose intolerant (Figueroa-González et al., 2022, Manassero et al., 2020). Because of its nutritional, physiochemical and functional properties, amaranth is a good alternative for the development of plant-based beverages. Keeping in view the health benefits of germinated amaranth flour, the research work was planned to formulate the beverages from germinated amaranth flour and analyze the physicochemical properties of developed beverage. The effect of storage on the functional parameters of beverage was also studied in this research work.

II. Materials and Methods

2.1 Collection of material

Amaranth grains and brown sugar were purchased from a local market, Rohtak, Haryana. Grains were cleaned and stored in an airtight container in refrigerator till further used. All the chemicals used in the study were of analytical grade.

2.2 Preparation of germinated amaranth flour

The amaranth seeds were washed with water (2-3 times) and soaked in water at a 1:2 ratio (rice to water) for 4 hours at 30°C. After soaking, the seeds were allowed to germinate for 48 hours at a temperature of 25°C. Once germination was complete, the seeds were air-dried. The dried germinated samples were then ground into a powder using a grinder, sieved, and kept in an airtight glass container for further use.

2.3 Preparation of beverage

The beverage was developed using germinated amaranth flour (GAF) in different concentrations. The three concentrations of beverage were prepared and labeled as T_1 with 1:4 ratio of GAF and distilled water respectively, T_2 with 1:5 ratio of GAF and distilled water respectively, and T_3 with 1:6 ratio of GAF and distilled water respectively. The beverage was prepared by adding water into GAF, followed by adding 4.5% of brown sugar, the content of brown sugar was constant in all beverages. The mixture was filtered through double layered muslin cloth. The beverage obtained after filtration was filled in pre-sterilized glass bottles and subjected to pasteurization at 80°C /30 sec. Further, it was cooled and stored at refrigerated temperature (7±1°C).

2.4 Physiochemical analysis

Beverage was examined for its proximate analysis such as ash content, moisture content, curde fiber, crude fat, and protein content following standardized protocols (AOAC, 2000). Carbohydrate content was calculated using the subtraction method. Beverage was examined for its physicochemical properties including total soluble solids, pH, and titrable acidity. TSS of fresh beverage was determined with the help of Abbe's hand refractometer by directly placing the sample on the refractometer and the degree brix was recorded. Using a digital glass electrode pH meter, the pH of the beverage was determined at room temperature. Before measuring the sample pH, the pH meter was calibrated using buffer solutions with pH values of 4.0 and 7.0. Titratable acidity of beverage was estimated using the method given by AOAC (2000).

2.5 Sensory analysis of beverage

The 9-point hedonic rating scale was used to evaluate the sensory evaluation of beverages. The degree of likeness on the 9-point hedonic scale runs from 1 to 9, with 1 denoting dislike extremely, 5 denoting a neutral range, and 9 denoting like extremely. The beverage samples were evaluated based on five criteria: flavor, taste, color, appearance, and overall acceptability.

2.6 Storage study of beverage

Beverage prepared from germinated amaranth flour was stored as refrigerated temperature (7±1°C) and analyzed for physicochemical and organoleptic characteristics during 6 days storage period at the interval of 3 days.

a. **Statistical analysis**

b. The data were analyzed using OPSTAT statical software. ANOVA was applied in a completely randomized model. The values were represented as means ± SD. The mean was compared at 5% level of significance.

III.Results and Discussion

3.1 Chemical composition of native and germinated amaranth flour

The chemical composition of native (NAF) and germinated amaranth flour (GAF) is given in Table 1. The percentage of ash, moisture, protein, fat, and crude fiber in NAF was 2.78, 8.09, 15.10, 7.20, and 5.16, respectively. The percentage of ash, moisture, protein, fat, and crude fiber in GAF was 2.25, 9.07, 16.99, 6.50, 2.59, respectively. The crude fiber content of amaranth decreased significantly after germination. GAF has the high value of protein than NAF, which is consistent with findings noted by Siwatch et al. (2019). The carbohydrate content ranged from 61.67 to 62.6%.

Table 1. Chemical composition of native and germinated amaranth flour

Parameters (%)	NAF	GAF
Moisture	8.09 ± 0.02^{a}	9.07 ± 0.05^{b}
Ash	2.78 ± 0.03^{b}	2.25 ± 0.25^{a}
Fat	7.20 ± 0.05^{b}	6.50 ± 0.09^{a}
Protein	15.10 ± 0.75^{a}	16.99 ± 0.46^{b}
Crude fiber	5.16 ± 0.52^{b}	2.59 ± 0.07^{a}
Carbohydrates	61.67 ± 0.02^{a}	62.60 ± 0.03^{b}

The values are expressed as mean of ± three independent determination.
NAF: Native amaranth flour; GAF: Germinated amaranth flour

3.2 Chemical composition of beverage prepared from germinated amaranth flour

The nutritional content such as moisture, ash, protein, fat, crude fiber, and carbohydrate content of freshly prepared beverages formulated using different concentration of GAF was found significantly different. Moisture content of freshly prepared beverage varied from 71.76 to 75.22% (Table 2). Among the treatments, the T_3 showed high moisture content which might to be due to high amount of water used (1:6 ratio of GAF: Water) for the preparation of T_3 beverage. T_1 reflected the lowest value of moisture content. Ash content of freshly prepared beverage varied from 0.11 to 0.80%. A non-significant difference in ash content of various samples of beverage was found.

Table 2. Chemical composition of beverage prepared from germinated amaranth flour

Parameter	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fiber (%)	Carbohydrate (%)
T_1	71.76 ± 0.49^{a}	0.50 ± 0.00^{a}	0.15 ± 0.01^{c}	4.88 ± 0.00^{a}	1.90 ± 0.17^{c}	20.81 ± 0.02^{c}
T_2	73.35 ± 0.00^{b}	0.51 ± 0.00^{a}	0.14 ± 0.00^{b}	4.82 ± 0.01^{b}	1.86 ± 0.23^{b}	19.32 ± 0.03^{b}
T_3	75.22 ± 0.02^{c}	0.51 ± 0.00^{a}	0.12 ± 0.01^{a}	4.32 ± 0.00^{c}	0.96 ± 0.12^{a}	18.87 ± 0.05^{a}

The values are expressed as mean of ± three independent determination.
Different superscripts within the same column denote significant differences ($p < 0.05$).
T_1 = 1 flour: 4 waters; T_2 =1 flour: 5 water; T_3 = 1 flour: 6 water

Fat content of freshly prepared beverage was varied from 0.12 to 0.15% (Table 4.3). Among the blends, highest fat content was observed in T_1 (0.15%) while, lowest fat content recorded in the treatment T_3 (0.12%). The protein content of beverage varied from 4.32 to 4.88%. The high protein content in beverage is due to good amount of protein present in GAF. Among all the blends, highest protein content was observed in sample T_1 (4.88%), while lowest protein was observed in T_3 (4.32%). The crude fiber content of freshly prepared beverage was varied from range 0.96 to 1.9%. Among the blends, highest crude fiber was reported in sample T_1 (1.9%) and lowest was observed in sample T_3 (0.96). Similar result observed by Manassero et al. (2020) in high protein beverage based on amaranth (0.4 to 1.9%). The carbohydrates content of freshly prepared beverage was varied from 18.87 to 20.21% Among all blends, highest value of carbohydrate content is observed in sample T_1 (20.21%) and lowest in sample T_3 (18.87%). The reduced value of carbohydrate content in T_3 beverage is due to increased water proportion used in preparation of beverage comparison to T_1 and T_2.

3.3 Effect of storage period on the physicochemical properties of beverage

Effect of storage period on pH of beverage is presented in Figure 1. The pH value of freshly prepared beverage samples was ranged from 6.2 to 6.3. pH of different beverage samples T_1, T_2, and T_3 was 6.2, 6.3, and 6.3, respectively. The freshly prepared beverages showed non-significant difference for pH value. A significant reduction in the pH of all beverage samples was found with the increase in storage period. The lowest pH value was recorded to be 4.2 for T_3 sample after the storage of 6 days. These results were in agreement with Megelapriya and Vahini (2022). TSS of freshly prepared beverage treatment T_1, T_2, and T_3 was noted as 6.16, 4.73 and 3.80 °Brix, respectively as shown in Figure 2. The observed value represented that storage period and different treatment significantly influenced the TSS value of beverage. TSS value of various beverages increased during the storage of 6 days. The higher value of TSS was observed as 8.2° Brix for T_1 at the end of 6 days of storage. Several researchers have observed an increase in TSS of pseudocereal based beverage during storage. The rise in TSS of beverage during the storage may be possibly through the conversion of polysaccharide into soluble sugars. Similar result was obtained by Megalapriya and Vahini (2022) who reported an increase in TSS of amaranth and soybean-based beverage during storage.

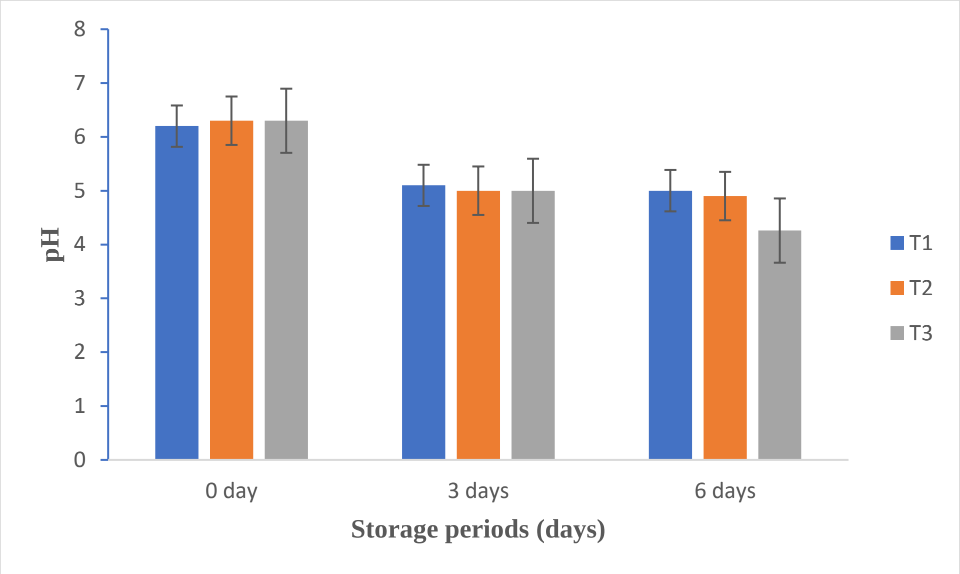

Figure 1. Effect of storage period on pH of germinated amaranth flour-based beverage

Effect of treatment and storage period on acidity of germinated amaranth flour-based beverage is shown in Figure 3.

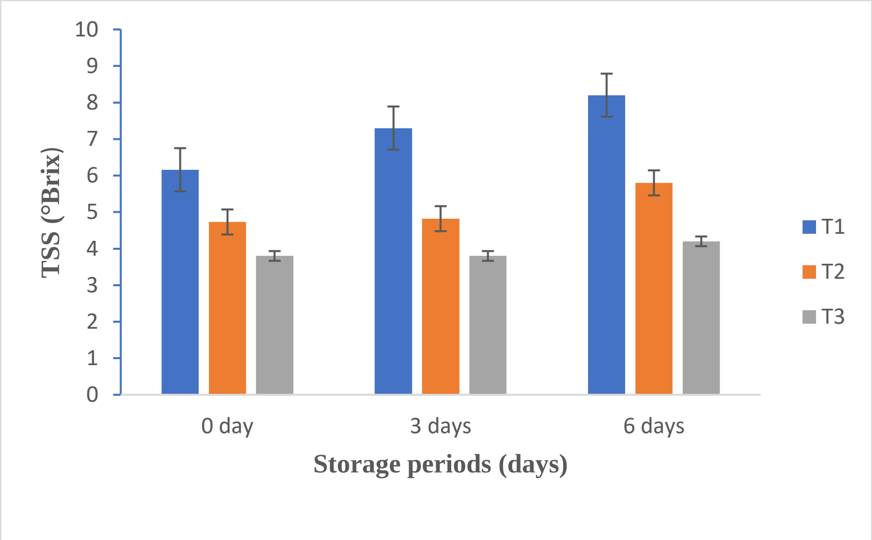

Figure 2. Effect of storage period on TSS of germinated amaranth flour-based beverage

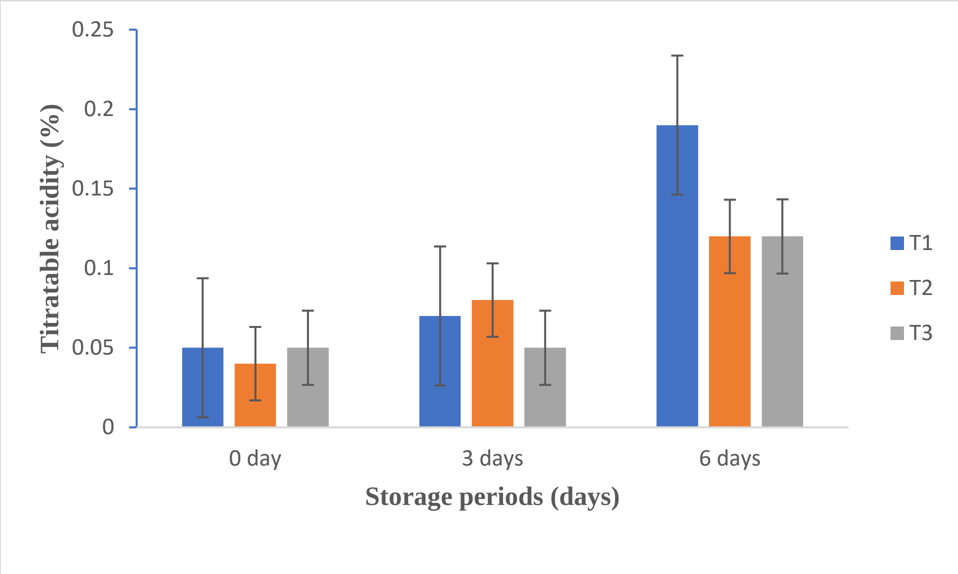

Figure 3. Effect of storage period on % acidity of germinated amaranth flour-based beverage

The freshly prepared beverages showed non-significant difference for titrable acidity value. Acidity of freshly prepared beverage samples T_1, T_2, and T_3 was recorded as 0.05%, 0.04%, and 0.05%, respectively. During storage an increase in titrable acidity was observed for all the samples. The sample T_1 (0.19%) containing highest percentage of acidity during the storage of 6 days. There was a proportional decrease in pH value with an increase in acidity during storage as presented in Figure 1. These findings were also supported by the observation of Megalapriya and Vahini (2022), who found an increase in % acidity (0.3% to 0.16%) of amaranth and soybean-based beverage during storage period.

3.4 Effect of storage period on the organoleptic characteristics of beverage

Fresh beverage samples as well as stored samples were analyzed for organoleptic properties (Figure 4.). The sensory scores for taste, appearance, color, flavor, and overall acceptability of GAF-based beverages were found to be decrease during the storage of 6 days. The similar results have been reported by Boyapati (2019) for quinoa-based beverage. The mean scores for color of different treatment of GAF-based beverages was ranged from 6.0 to 7.33 during the storage of 6 days. The higher rating (7.33) for color was recorded for T_2 and T_3 samples after the storage period of 3 days. The appearance of different freshly prepared beverages showed no difference. The mean scores for appearance of different treatment of GAF-based beverages was ranged from 6.0 to 7.66 during the storage of 6 days. The higher rating (7.33) for appearance was recorded for T_3 sample after the storage period of 3 days. In case of taste and flavor of different beverage treatment it was clear from results that the higher mean score of taste and flavor was found in treatment T_1 because it contains 25% of GAF which gives a unique taste and flavor to beverage. But at the end of storage a specific decrease in taste and flavor of beverage sample was observed.

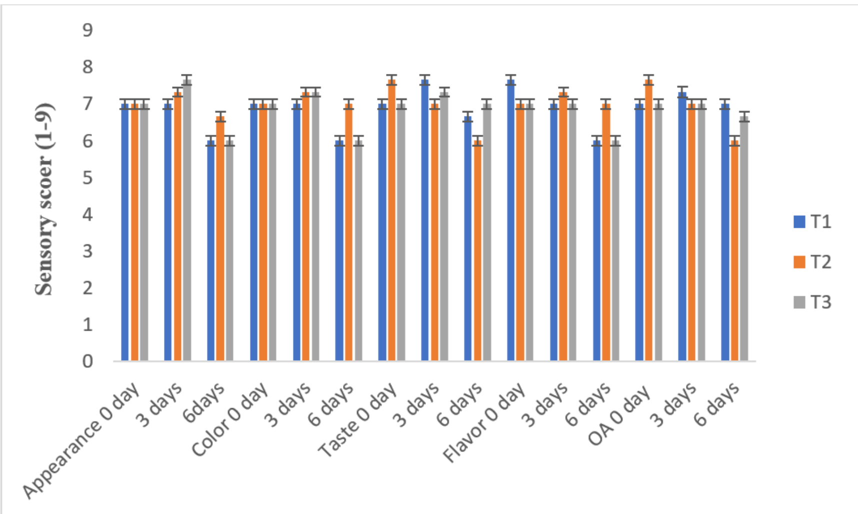

Figure 4. Effect of storage period on sensory parameters of germinated amaranth flour-based beverage

The mean scores for overall acceptability of different treatment of GAF-based beverages was ranged from 7.0 to 7.66 during the storage of 6 days. The higher rating (7.66) for overall acceptability was recorded for T_1 during storage period of 6 days.

IV.Conclusion

The study demonstrated that germinated amaranth flour is a promising ingredient for the development of a value-added, health-promoting beverage. Germination significantly improved the nutritional profile of amaranth by increasing protein content and reducing anti-nutritional factors. Among the different formulations, the beverage with a 1:4 ratio of GAF to water (T_1) emerged as the most nutritionally rich and organoleptically acceptable. Storage studies revealed that although there were changes in physicochemical and sensory properties over a 6-day refrigerated period, the beverage remained stable and acceptable. The research supports the potential of amaranth-based functional beverages in catering to the needs of health-conscious consumers, including those with dietary restrictions such as gluten or lactose intolerance. Future studies may explore fortification with natural flavoring agents or the inclusion of other functional ingredients to further enhance the sensory appeal and health benefits.

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