



Development and Nutritional Evaluation of Walnut (*Juglans regia* L.) Milk During Storage

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ABSTRACT

Walnut (*Juglans regia* L.) have proven health benefits as it contain essential fatty acids (omega 3, omega 6), proteins carbohydrates, minerals, vitamins, antioxidants, and phenolic compounds which provides many health benefits. The objectives of study were to develop walnut milk and application of preservation techniques to increase its shelf life and quality evaluation of developed product during storage. After soaking, skin removal, grinding, walnut milk was pasteurized at 65 °C for 15 minutes and then stored at refrigeration and ambient temperature according to treatment plan i.e To (Walnut milk without preservative), T₂ (Walnut milk with 0.08% sodium benzoate), T₄ (Walnut milk with 0.02% sodium azide), T₆ (Walnut milk with 0.04% sodium benzoate with 0.01% sodium azide) were stored at room temperature while T₁ (Walnut milk without any preservative), T₃ (Walnut milk with 0.08% sodium benzoate), T₅ (Walnut milk with 0.02% sodium azide) and T₇ (Walnut milk with 0.04% sodium benzoate with 0.01% sodium azide) were stored at refrigeration temperature. The physicochemical (pH, acidity, fat, protein, ash, total solids, total phenols and antioxidant), sensory (taste, color, flavor and overall acceptability) and microbiological (TVC, TFC) were assessed after every 5 days of storage. The results of the study demonstrated that T₅ walnut milk with 0.02% sodium azide at refrigeration temperature scored 7.2×10^3 at day 25th TVC, and scored 1.87 which was considered as the most acceptable treatment which compared to other treatments has improved shelf life up to 25 days of storage.

Keywords: Walnut milk, *Juglans regia* L., preservation techniques, sodium azide, sodium benzoate, physicochemical properties, sensory evaluation, microbiological analysis, shelf life, antioxidant activity

INTRODUCTION

Walnut (*Juglans regia* L.) is the most wide spread nut trees in the world and is native in a region expanding from the Balkans Eastward to the Western Himalayan chain (Taha and Mohammed, 2011). In Pakistan, the total area under walnut fruit production is 1214 hectares. Out of that, Khyber Pakhtunkhwa has 1114 hectares of the total area with the production of 9926 tons annually. (Aslam and Samiera, 2013). However, it is also produced in Gilgit Baltistan and AJ&K.

The edible part of walnut (kernel) is worldwide acknowledged for its nutritional, health, and sensory characteristics (USDA, 2018). They are high in Fat (61.97 to 70.92%), protein (15.17 to 19.24 %), rich source of carbohydrates (8.05 to 13.23%), and water (3.25 to 3.91%) and minerals, bio active compounds

such as flavonoids, sterols, peptic substances, phenolic compounds are also present in walnuts (Gulcan and Koyuncu, 2005). Walnuts are also rich source of omega-3 fatty acid, vitamin E and other antioxidants which are preferable for heart and reduce the growth of cancer cell (Iwamoto *et al.*, 2000; Martinez *et al.*, 2010). They also help to conserve the immune system, nerve and bone function. It also defeats cancer development in kidney, colon, breast and prostates (Albert *et al.*, 2002). In Iranian traditional medicine, walnut kernel has been used for the treatment of inflammatory bowel disease. The kernel of this fruit is used for the treatment of diabetes, asthma and to treat the prostate and vascular disturbance in Palestine (Taha and Mohammed, 2011).

The development and characterization of walnut beverage (walnut milk) depicts compositional and nutritional values. Potentially walnuts were also used to

obtain vegetable milk and not only able to exert health benefits but also as an alternative to dairy based products (Popovici *et al.*, 2016). Walnut contain diverse range of bio active compounds and unsaturated fatty acid therefore the development of walnut milk can be a very good and dairy substitute for people suffering from lactose intolerance and hypocholesterolemia. Walnut consumption is not only high in Pakistan but also greatly consumed in AJK and Gilgit Baltistan.

MATERIAL AND METHODS

Current study was conducted in the Department of Food Science and Technology Faculty of Agriculture, University of Poonch Rawalakot. The walnut samples were collected from different locations of District Shigar, Gilgit Baltistan, Besil Basha, Saisko, Dogro, Tissar, Alchori and Gulapur were transported to laboratory of the Department of Food Science and Technology. The research study was divided in two phases, in the 1st phase the proximate analysis of the samples was done and in 2nd phase walnut milk was prepared and preservatives (Sodium azide and Sodium Benzoate) were applied at ambient and refrigeration temperature. The effect of temperature and preservatives on its chemical and physical appearance like taste, color, was analyzed after every 5 days of storage and spoilage factors was monitored by microbiological analysis.

Physical Analysis of Freshly Harvested Walnut Fruit: The Dry walnut fruit was analyzed for weight of whole walnut with shell weight of kernel and kernel shell ratio by using digital balance in laboratory.

Biochemical Analysis of Walnut Fruit: Biochemical analysis including crude fiber, ash content, carbohydrate content, moisture, nitrogen content or protein, and crude

fat were determined through the methods given by AOAC (1990).

Development of Walnut Milk

Preparation of walnut milk: The technology for the preparation of walnut milk, the walnut kernel was soaked in drinking quality water at 20-80°C for 6 to 8 hours. Then the thin shell of the kernel was removed manually, then added water and was shredded in the mixer for 5 minutes. The resulting suspension was filtered through a double layered thin muslin cloth to obtain walnut milk the milk was pasteurized at 73°C for 15 minutes and applied preservatives sodium benzoate and sodium azide with different ratios then packed into different containers according to requirements.

Preservation of walnut milk: The samples were treated with different concentrations of preservatives and stored at different temperatures to check the shelf life.

Chemical Analysis and Sensory Evaluation of Product: The biochemical parameters mentioned in the walnut kernel analysis was determined after five days interval. Sensory test was carried out on preserved samples after four days interval using ten member panel. The samples taste, aroma, color, flavor and general acceptability was checked by panelist using hedonic scale ranging 1 extremely like to 9 for extremely dislike.

Physiochemical analysis of Walnut milk

pH: pH of the samples was checked by pH meter according to (AOAC, 1990). pH meter was calibrated by dipping electrode in buffer solutions of pH 4.0-9.0.

Titrateable acidity: Total acidity (TA) was expressed in terms of equivalent malic acid to walnut samples was estimated by (AOAC, 1990).

$$\% \text{ TA} = \frac{\text{ml of NaOH} \times \text{N of NaOH} \times \text{Eq. wight of malic acid}}{\text{Sample volume used}}$$

Fat content: The Gerber method analysis was used to assess the fat content in walnut milk (AOAC, 1990). For this purpose, milk sample (10mL) was taken and added it into butyrometer, then added 1mL of Iso amyl alcohol and shake it well. After this, butyrometer was put into Gerber machine for 2-3 minutes. Lower end of fat column on to a main graduation mark were brought by slightly withdrawing stopper of butyrometer. The difference between the two readings stretches the percentage by mass off at in walnut milk.

Protein content: The protein contented in walnut milk sample was calculated by using Kjeldhal method (99I.20) of AOAC (2016). 10mL of walnut milk was taken and kept in digestion tube, for digestion of

sample, two digestion tablet (comprising 5g K₂SO₄ and 0.05 g selenium) and 20mL N-free H₂SO₄. Acid and digestion tablets were added in one tube with-out sample (blank). Then tubes were moved to the digestion block and heated at 430 °C. Digestion process was continued till the digest cleared.

Then digestion were performed by distillation unit in occurrence of 40% sodium hydroxide, distillate was collected in conical flask which contained 4% boric acid solution. At the last content in the flask were titrated across 0.1N sulphuric acid and volume used was observed, till the pink color appear. The nitrogen was calculated by the following equation;

$$\begin{aligned} \text{N\%} &= 0.0014 \times \text{Volume (blank sample) Sulphuric acid} \times 100 \\ &\text{Weight of sample} \\ \% \text{ Protein content} &= \text{N\%} \times 6.381 \end{aligned}$$

Antioxidant activity: The DPPH technique was used to test sample's Antioxidant activity (Alvites-Misajel *et al.*, 2019). Take 0.5ml of the walnut milk sample and 1ml of newly made DPPH solution were made as well as extract of various concentration 0.5 ml of the walnut milk sample and 1 ml of freshly made DPPH solution were created as well as extracts of various

concentrations (100 microgram per ml) for this purpose (0.25mm) Each sample was given 1ml of ethanol and was left at room temperature for 30 minutes in a dark environment. Then using spectrophotometer measured the absorbance at 517nm, each mixture was examined for its capacity to scavenge DPPH radicals. After that, a formula was used to determine the antioxidant activity

$$\text{Activity (\%)} \text{ DPPH Scavenging} = \left(AO - \frac{AS}{AO} \right) \times 100$$

Where,

AO = Absorbance of the control, As = Absorbance of the sample

Total phenol: The total phenolic content was calculated as Gallic acid equivalent (GAE (Ramos-Escudero *et al.*, 2012; Castro-López *et al.*, 2016). It was created to use Folin-reagent Ciocalteau's (v/v). 0.5 ml of an aqueous fruit extract was added after 2.5 ml of 10% Folin-reagent Ciocalteau's and 2 ml of 7.5% sodium carbonate. This mixture was incubated at 45 °C for 40 minutes, and the absorbance at 765 nm was determined in a spectrophotometer. Gallic acid was applied similarly to a regular phenol. The total phenol content was reported as mg of gallic acid equivalent/gram extract using the mean of three readings.

Microbial analysis

Total Bacterial Count and Total Fungal Count: The plate count method, as described by (AOAC, 2016), was used to determine the level of microbials contamination. To create a stock solution, an amount of 1 ml of each sample was added one at a time into test tube which already had 9 ml of distilled water in it. It was serially diluted five times. The plate count was conducted using the following media. PDA for the total fungal count and nutrient agar medium for total viable count. Spread plate technique was employed. Using a sterile spreader,

one cc of the sample was applied to the surface of each medium. For the 24 hour bacterial count and the 48 hour fungal count, the plates were incubated at 35 °C. After counting the colonies, the dilution factor was multiplied by the number of colonies

RESULTS AND DISCUSSION

Physicochemical analysis of walnut: Before the development of milk walnut kernel was examined for several tests physically like fruit weight, kernel weight, shell weight and checked kernel shell ratio and biochemically examine fat, protein, carbohydrate, fiber, moisture and ash content of different walnut collected from district Shigar Gilgit Baltistan. The physical assessment of walnut fruits collected from different locationof district, Shigar Gilgit Baltistan, L₁ (Alchori), L₂ (Besil), L₃ (Dogro), L₄ (Gulapor), L₅ (Saisko) and L₆ (Tissar) was done and the result shown in Table 1. The physical assessment of walnut collected from six different locations is given in Table 2. The biochemical assessment of walnut fruits collected from different location of district, Shigar Gilgit Baltistan, L₁ (Alchori), L₂ (Besil), L₃ (Dogro), L₄ (Gulapor), L₅ (Saisko) and L₆ (Tissar) was done and the result shown in Table 3.

Table 1: Treatment plan used for preservation of walnut milk.

T ₀	Walnut milk without preservative at room temperature
T ₁	Walnut milk at refrigeration temperature
T ₂	Walnut milk with 0.08% sodium benzoate at room temperature
T ₃	Walnut milk with 0.08% sodium benzoate at refrigeration temperature
T ₄	Walnut milk with 0.02% sodium azide at room temperature
T ₅	Walnut milk with 0.02% sodium azide refrigeration temperature
T ₆	Walnut milk with 0.04% sodium benzoate with 0.01% sodium azide at room temperature
T ₇	Walnut milk with 0.04% sodium benzoate with 0.01% sodium azide at refrigeration temperature

Table 2. Means of the physical assessment of walnut collected from six different locations of district, Shigar Gilgit Baltistan

Location	Fruit weight (g)	Kernel weight (g)	Shell weight (g)	Kernel –shell ratio
L1	11.5±1.5	5.4±0.5	6.0±0.5	5.4:6
L2	8.6±1.5	4.29±0.5	3.65±0.5	4:3.6
L3	12.4±1.5	5.5±0.5	6.9±0.5	5.5:7
L5	10.6±1.5	5.9±0.5	4.7±0.5	6:4.7
L6	14.9±1.5	5.52±0.5	9.4±0.5	5.5:9

Gilgit Baltistan, L1 (Alchori), L2 (Besil), L3 (Dogro), L4 (Gulapor), L5 (Saisko) and L6 (Tissar)

Effect Of Treatment and Storage on Physiochemical Properties Of Walnut Milk

pH: pH is the main reference to categorization of food acidity relations. Walnut milk is non-dairy plant based dairy substitute. The effect of treatment and storage intervals on pH level of walnut milk was significantly interacted and the results are mentioned in Table 4. The highest value of pH was recorded in T₇=Walnut milk with 0.04% sodium benzoate with 0.01% sodium azide at refrigeration temperature. During storage the pH

value decreased from 6.74 at 1st day to 6.25 at 25 day of storage. However, among treatments highest value (6.13) was recorded in T₀ (walnut milk without preservatives at room temperature) at day 1st while lowest value (5.13) at 25 day of storage. The reason behind this decrease was might be the metabolic activity of acid forming organisms and their continuous multiplication inside the walnut milk which ultimately reduced the pH of the milk in refrigerated sample with preservatives. These findings are confirmed by the study of Yang *et al.* (2022).

Table 3: Means of Biochemical analysis of walnut kernel collected from District Shigar Gilgit Baltistan

Location	Fat %	Protein %	Fiber %	Moister %	Ash %	Carbohydrate
L1	57s.5±1.52	12.15±1.52	7.03±0.52	10.15±0.52	3.6±0.12	10.5±0.12
L2	60.1±1.52	12.05±1.52	6.7±0.52	10.9±0.52	3.1±0.12	6.9±0.12
L3	61.2±1.52	9.9±1.34	7.0±0.52	8.59±0.5	3.8±0.12	8.6±0.12
L4	55.2±1.52	12.53±1.34	7.12±1.34	12.16±0.5	2.5±0.12	10.5±0.12
L5	59.8±1.52	11.0±1.34	5.8±0.52	8.69±0.5	5.5±0.12	9.21±0.12
L6	60.04±1.52	10.5±1.34	5.4±0.52	9.5±0.5	5.4±0.12	10.5±0.12

Titrateable acidity: Acidity in food product is due to the presence of organic acid or produced in foods during storage period. In food when acidity increase the pH will decreases and vice versa. The result of analysis of variance of titrateable acidity and the different treatments have significance effect on the results of acidity of

different treatment and storage are showed in Table 5. It is clear from the result that the acidity of walnut milk T₀ (walnut milk without preservatives) while the lowest value of acidity was in T₅=Walnut milk with 0.02% sodium azide refrigeration temperature 0.11 this decrease in acidity due the addition of preservatives and uses of low temperature during storage the result of (AOAC, 2016) represented that the reduction of acidity in tiger nut milk during storage

Table 4: Effect of treatments on pH value of walnut milk during storage.

Treatment pH	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	6.13±0.01jm	6.14±0.01jm	6.12±0.04km	5.9±0.05mn	5.66±0.05n	5.13±0.04o	5.84 D
T ₁	7.3 ±0.01ab	7.04±0.01be	6.86±0.04cg	6.13±0.05jm	5.99±0.05km	5.9±0.043mn	6.54 BC
T ₂	6.65±0.01di	6.57±0.02fj	6.49±0.04gk	6.32±0.05im	6.32±0.05im	6.53±0.04gk	5.13 C
T ₃	7.11±0.01bc	6.65±0.03di	6.83±0.04cg	6.78±0.05ch	6.7±0.052ci	5.98±0.04in	5.93 B
T ₄	5.98±0.01di	6.6±0.03gk	6.51±0.05gk	6.49±0.05ci	6.68±0.04gk	6.48±0.04hl	6.53 C
T ₅	7.12±0.02bc	7.04±0.01be	7.06±0.05bd	7b±0.05f	6.98±0.04bf	6.76±0.04ch	5.98A
T ₆	6.63±0.02di	6.51±0.04gk	6.49±0.05gk	6.68±0.05ci	6.48±0.04gk	6.61±0.04ei	6.4 BC
T ₇	7.03±0.01be	6.98±0.01bf	7.6±0.05 a	7.76±0.05ci	6.53±0.05gk	6.51±0.04ei	6.76 A
Mean	6.7435 A	6.695 B	6.745 AB	6.6325 C	6.34 D	6.25 E	

Fat: Fat content is the most important nutritional constituent of any food items which provide high energy to the body in nuts there are highly contained unsaturated fatty acid which are the good for health and have the ability to reduce LDL in the body, Table 6 that the storage factors and treatment was not significantly interacted in previous research (AOAC, 2016) reported that the amount of un saturated fatty acid and total fat are not changed during storage as well as not noticeable differences with treatment

Protein content: Protein is the one of the most important and functional part of the diet. Walnut is the s

one of rich source of protein. The result concern about protein are mentioned in Table 7 that treatment wise there was not significantly different. Whereas the means of storage intervals showed that the protein content in walnut milk was reduced gradually at day 1st the maximum value recorded was 2.47 which gradually reduced and 1.36 was at day 25 of storage this changes was occurred same in the research of (AOAC, 2016). The reduction of level of protein was might be the due to photolytic bacteria found in walnut milk and might be they showed the proteolysis action in walnut milk and which caused spoilage of milk

Table 5: Effect of treatments on Total acidity of walnut milk during storage

Treatment Total acidity	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	0.07±0.01pu	0.12±0.01kq	0.56±0.01ef	0.37±0.02hi	0.60±0.02ef	0.60±0.01ef	0.446 A
T ₁	0.07±0.01pu	0.10±0.01ns	0.54±0.01f	0.33±0.02i	0.58±0.02ef	0.62±0.01e	0.3761 C
T ₂	0.06±0.01ru	0.03±0.01tu	0.2±0.012j	0.43±0.02gh	0.88±0.01c	1.00±0.01a	0.396 C
T ₃	0.08±0.01pu	0.02±0.01u	0.21±0.02j	0.44±0.02g	0.81±0.01d	0.91±0.01bc	0.4150 B
T ₄	0.11±0.01lr	0.09±0.01ot	0.14±0.02ko	0.04±0.02ru	0.13±0.01kq	0.16±0.01jn	0.2333 D
T ₅	0.10±0.01mn	0.0±0.017qu	0.07±0.02pu	0.95±0.027ab	0.05±0.01ru	0.13±0.01kp	0.1144 F
T ₆	0.07±0.01pu	0.18±0.01jk	0.13±0.02kp	0.16±0.02jn	0.17±0.01jm	0.18±0.01jk	0.1513 E
T ₇	0.04±0.01su	0.18±0.01jk	0.16±0.02jn	0.17±0.02jm	0.17±0.01jl	0.18±0.01jk	0.1517 E
Mean	0.0780 F	0.1008 E	0.2579 D	0.3650 C	0.4271 B	0.4758 A	

Table 6. Effect of treatments on Fat content of walnut milk during storage.

Treatment Fat	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	7.56±0.03a	7.36±0.04a	7.4±0.03a	7.40±0.04a	7.0±0.03a	7.06±0.05a	7.33A
T ₁	7.66±0.05a	7.86±0.04a	7.63±0.03a	7.46±0.04a	7.53±0.03a	7.17±0.05a	7.64A
T ₂	7.65±0.04a	7.17±0.04a	7.37±0.03a	7.30±0.04a	7.23±0.03a	7.17±0.05b	7.26B
T ₃	7.30±0.03a	7.23±0.04a	7.23±0.03a	7.26±0.04a	7.35±0.03a	7.17±0.05a	7.17B
T ₄	7.33±0.03a	7.30±0.04a	7.30±0.03a	7.30±0.04a	7.23±0.03a	7.0±0.05ab	7.19B
T ₅	7.340±0.03a	7.64±0.04a	7.30±0.03a	7.26±0.04a	7.37±0.03a	7.0±0.05ab	7.29AB
T ₆	7.30±0.03a	7.30±0.04a	7.30±0.03aa	7.23±0.03a	7.30±0.03ab	7.0±0.05ab	7.30AB
T ₇	7.30±0.03a	7.30±0.04a	7.33±0.05a	7.30±0.05a	7.30±0.05ab	7.06±0.05ab	3.31AB
Mean	7.46A	7.64A	7.36A	7.32A	7.19AB	7.20AB	

s

Total solids: The substance which are found any food either dissolved or in suspended form. There is no significance effect during storage interval but slightly changes occurred was noticed. The result for total solid content of different treatment and storage intervals are showed in Table 8. Total solid content has no significantly affected treatment vise but slightly reduced

from storage with the respect to time the means of storage was reduced from 12.8 at 1st day and at the 25th day the total solid content reading reached to 10.0. The reduction of solid content of walnut milk is might be due the utilization of milk solid by microorganism which converts substrate to nonsolid form. These finding was mostly similar to AOAC (2016) according to their research on tiger nut milk development and nutritional assessment.

Table 7: Effect of treatments on Protein content of walnut milk during storage.

Treatment protein	Days of storage						Means
	1	5	10	15	20	25	
T ₀	1.23±0.02a	1.30±0.04a	1.31±0.4a	1.53±0.4ab	1.40±0.4ab	1.37±0.2a	1.38A
T ₁	1.61±0.04a	1.43±0.02a	1.59±0.4a	1.31±0.4ab	1.44±0.2ab	1.37±0.2a	1.46A
T ₂	1.97±0.04a	1.53±0.2a	1.49±0.02a	1.45±0.2a	1.54±0.4a	1.50±0.4a	1.45A
T ₃	1.95±0.04a	1.23±0.2ab	1.31±0.2a	1.40±0.02a	1.54±0.02a	1.51±0.02ab	1.39A
T ₄	1.84±0.02a	1.48±0.2a	1.97±0.04ab	1.49±0.04ab	1.35±0.4a	1.38±0.04ab	1.53A
T ₆	1.75±0.2a	1.40±0.02a	1.36±0.02a	1.48±0.4a	1.38±0.4a	1.66±0.04a	1.44A
T ₆	1.10±0.2a	1.46±0.2ab	1.30±0.4ab	1.35±0.02ab	1.37±0.02a	1.48±0.4ab	1.34A
T ₇	1.30±0.2a	1.27±0.2ab	1.38±0.4ab	1.54±0.4ab	1.31±0.4ab	1.35±0.02ab	1.36A
Means	2.47A	1.94AB	1.84AB	1.41AB	1.38AB	1.36AB	

Total ash content: The ash content of any food products are analyzed to check the un soluble unburnable material content in food in ash contain all

minerals which was to check in walnut milk there are least amount of minerals.. The analysis of variance of this study the results were shown in Table 9. The mean values of storages and treatment are not significantly different. This finding was related to the research of AOAC (2016).

Table 8: Effect of treatments on Total Solid content of walnut milk during storage.

Treatment Total solid	Days of storage						Mean
	1	2	3	4	5	6	
T ₀	13.6±1.15a	12.33±1.15a	12±1.15a	11±1.15a	10.6±1.15a	10±2.15a	11.6A
T ₁	11.3±2.15a	11.3±2.1a	12±2.15a	9.6±3.15a	12±1.15a	10.6±2.15a	11.16A
T ₂	11.6±2.15a	12.3±2.5a	11.6±2.15a	9.6±3.15a	10.6±3.15a	9.6±4.15a	11.1A
T ₃	13.6±1.15a	12.6±1.15a	9.6±3.15a	9.6±3.15a	10.6±3.15a	10.6±3.15a	11.3A
T ₄	13±1.15a	12.3±3.15a	11.3±3.15a	10.3±3.15a	9.33±3.15a	11.3±3.15a	11.2A
T ₅	13±1.15a	13±1.15a	11.3±3.15a	11±3.15a	11±3.15a	9.33±3.15a	11.4A
T ₆	12±1.15a	16.0±1.15a	13±1.15a	10.3±3.15aa	9.33±3.15a	10±1.15a	11.7A
T ₇	12.6±3.15a	12.6±3.15a	11.6±1.15a	10.3±1.15a	9.33±1.15a	9.33±1.15a	10.38A
Mean	12.8A	11.6A	11.5A	10.5A	10A	10A	

Antioxidant activity: Antioxidant is the fragment that delays the corrosion of further particles. Oxidation is the biochemical reaction in which the produce free electron which lead the uncontrolled division of cells in Table 10. The result of antioxidant activity was not significantly affected by treatment vise but the storage

vise changes occurred showed in the means of storage at day 1st the walnut milk 20.37 and this value of antioxidant DPPH scavenging activity reduced gradually to the day 25 and noted 15.5 which was significantly interacted. This finding is nearly related to (Alvites-Misajel *et al.*, 2019). In that research they have also found no significant difference on effect of preservatives in juice of guava.

Table 9. Effect of treatments on Ash content of walnut milk during storage.

Treatment Ash	Days of storage						Means
	1	5	10	15	20	25	
T ₀	0.27±0.04a	0.29±0.04ab	0.26±0.04a	0.58±0.04a	0.25±0.04a	0.30±0.04a	0.32A
T ₁	0.25±0.04a	0.29±0.04a	0.40±0.04a	0.17±0.04a	0.37±0.04a	0.29±0.04a	0.29A
T ₂	0.38±0.04a	0.17±0.04a	0.25±0.04a	0.21±0.04a	0.39±0.04a	0.30±0.04a	0.28A
T ₃	0.17±0.04a	0.34±0.04a	0.26±0.04a	0.33±0.04ab	0.25±0.04a	0.32±0.04a	0.28A
T ₄	0.25±0.04a	0.29±0.04a	0.28±0.04a	0.34±0.04ab	0.26±0.04a	0.17±0.04a	0.28A
T ₅	0.33±0.04a	0.25±0.04a	0.27±0.04a	0.13±0.04a	0.25±0.04a	0.25±0.04a	0.26A
T ₆	0.25±0.04a	0.40±0.04ab	0.25±0.04a	0.29±0.04a	0.44±0.04a	0.17±0.04a	0.26A
T ₇	0.25±0.04ab	0.25±0.04ab	0.25±0.04a	0.30±0.04ab	0.16±0.04a	0.34±0.04a	0.30A
Means	0.274AB	0.28AB	0.28A	0.29AB	0.30A	0.27AB	

Total phenols: Phenols are the good source of numerous bio active compound including flavonoids, phenolic acid and phenolic. The bioactive compound is related to the freshness of sources fruits, vegetables nuts and other herbal sources. The data shows that there is significantly different in means of antioxidant but between treatment there is not significantly changes occurred observed in Table 11 the maximum phenol

content was found 12,49 at day 1st and gradually reduced with the passage of time and the lowest value was 9.54mg GAE of walnut milk sample .walnut milk store at refrigeration temperature showed the minimum decrease with respect to others.it also shows that the reduction in total phenol is also responsible to the loss of antioxidant activity of the walnut milk . The results is correlate to the research findings of (Ramos-Escudero *et al.*, 2012; Castro-López *et al.*, 2016).

Table 10. Effect of treatments on Antioxidant activity (%) of walnut milk during storage

Treatment AO	Days of storage						Means
	1	5	10	15	20	25	
T ₀	21.0±1.6	20.0±1.5	23.66±2.6	19.1±1.5	16.0±1.6	15.6±2.8	19.25A
T ₁	22.6±2.6	24.6±1.6	25.3±2.6	20.1±1.5	17.3±2.6	13.9±1.5	20.66A
T ₂	22.2±1.5	23.3±1.6	19.0±2.8	21.321	19.5±1.5	16.0±2.8	19.81 A
T ₃	22.3±1.5	19.2±1.6	19.2±1.5	20.5±1.6	17.9±1.5	15.8±1.5	18.21 A
T ₄	12.2±2.6	20.8±2.8	21.6±2.6	18.6±1.6	17.3±1.6	14.16±1.5	18.48 A
T ₅	20.1±2.8	19.3±2.8	22.5±2.8	18.1±2.8	16.2±2.8	16.33±1.5	18.94A
T ₆	18.2±1.5	21.6±1.5	20.6±1.5	17.6±1.5	16.33±1.5	15.0±1.5	18.25 A
T ₇	20.0±1.5	19.6±1.5	20.0±1.5	22±1.5	17.0±1.5	17.3±1.5	19.87 A
Means	20.37A	21.0A	21.53A	19.94	17.05B	15.52B	

Total viable count: The theoretical discussion about the total bacterial count is assessment of viable bacteria found in any food item which effect the spoilage factors of that food items specially the liquid food has the lots of chances of spoilage due to those bacterial or fungal behavior inside them. In this study Total viable count was checked and the result is mention in the Table 12. It is cleared that the bacterial colony was reduced significantly with the uses of preservative as well as the

effect of temperature during of storage in table 11 shows that total viable count of T₁=walnut milk without preservatives at room temperature is 0.9×10³ at day 1st day was noticed increase to 9.1×10³ at day 25 while the minimum viable count was found in T₅= Walnut milk with 0.02% sodium azide refrigeration temperature has 0.07×10³ at day 1st and 7.2×10³ at day 25th the same result were found in Loss *et al.* (2012) in their study they have used the same preservatives in assessment of quality attributes Tigernut milk.

Table 11. Effect of treatment on Total phenol mg GAE /ml of walnut milk during storage.

Treatment total phenol	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	12.6±1.59a	14.13±1.45a	11±1.95a	9.91±1.95a	9.73±1.95a	9.56±1.59b	11.17A
T ₁	12.9±1.45a	12.8±1.45a	11.2±1.59ab	11.3±1.95a	10.86±1.45a	9.12±1.59c	11.28A
T ₂	13.5±1.95a	12.8±1.59a	9.35±1.95ab	12.9±1.95a	10.77±1.45a	9.21±1.59c	11.48A
T ₃	12.6±1.95a	12.8±1.95a	10.5±1.45a	11.3±1.59a	9.97±1.95bc	9.46±1.45c	11.45A
T ₄	12.3±1.95a	12.7±1.59a	12.0±1.59a	11±1.95a	11.8±1.95a	10.4±1.45ac	11.40A
T ₅	13.0±1.45a	13.7±1.59a	11.8±1.95a	10.7±1.95a	9.4±1.95ac	11.9±1.45ac	11.79A
T ₆	10.6±1.59a	10.4±1.45a	13.9±1.45a	12±1.95a	9.45±1.45bc	7.48±1.45c	10.69A
T ₇	12.11.59ab	11.7±1.45a	12.2±1.45ab	10.7±1.95ab	9.03±1.45bc	9.17±1.45c	10.83A
Mean	12.49AB	12.56A	11.54AB	11.29AB	10.15BC	9.54C	

Total Fungal Count: The fungus growth inside the food content is directly related to the water activity inside the food will cause the spoilage of the food in dairy product, they have lots of chances of growth of microbial including bacterial and fungal. In fungal strain, yeast and mold are very commonly found in food and cause spoilage factors inside the food. Walnut milk is one dairy substitute that one commercially consumed

as a good source of non-dairy milk. The effect of treatment on the growth of microbial are not significance but the storage factor significantly interacts with the fungal growth which is given in the Table 13. T₂ = Walnut milk with 0.02% Sodium azide at refrigeration temperature has minimum fungal growth 0.7×10³ on 1st day of storage has increased with respect to time and the maximum fungal count was found to = 9.1×10³ Walnut milk control at room temperature without preservatives. These results are related to the Oluwayemisi *et al.* (2022)

Table 12. Effect of treatment on Total viable Count (×10³ cfu/ml) of walnut milk during storage.

Treatment TBC	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	0.81±0.01oq	2.02±0.01nq	2.23±0.02mq	5.20±0.04di	6.65±0.04bg	9.15±0.05a	4.34 AB
T ₁	0.79±0.01oq	1.40±0.01nq	2.07±0.02nq	4.27±0.04hm	6.23±0.04ch	7.48±0.05ac	3.37 BC
T ₂	0.76±0.01oq	1.41±0.01nq	2.83±0.02jo	4.73±0.04fk	6.38±0.04bh	6.18±0.05ch	3.37 BC
T ₃	0.80±0.01oq	1.71±0.01nq	2.52±0.02o	5.34±0.04ci	6.88±0.04bf	8.50±0.05ab	2.29 AC
T ₄	0.85±0.01oq	2.52±0.01lo	2.64±0.02ko	5.16±0.04di	6.80±0.04bf	8.43±0.05ae	4.40 A
T ₅	0.07±0.01q	0.23±0.01pq	2.08±0.02mq	3.49±0.04in	4.96±0.04ej	7.23±0.05ad	3.01 D
T ₆	0.83±0.01oq	1.43±0.01nq	2.37±0.02p	4.49±0.04gl	5.93±0.04ch	7.47±0.05ac	3.75 AC
T ₇	0.79±0.01oq	0.78±0.01oq	3.36±0.02in	4.52±0.04gl	5.45±0.04ci	7.00±0.05ae	3.65 D
Mean	0.71 F	1.44 E	2.51 D	4.65 C	6.16 B	7.68 A	

Sensory Evaluation: Sensory characteristic is the most important attributes of product quality. Walnut milk was assessed organoleptically by 7 judges and all samples were checked out using 9 points Hadonic scale 1 is used for extremely like and 9 is used for extremely dislike.

Walnut samples were sensorily assessed at 1st 5th 10th 15th 20th and 25th days of storage respectively. Then the collected data was managed for statistical examination and get results (Zhi *et al.*, 2016).

Table 13. Effect of treatment on Total Fungal Count (×10³ cfu/ml) of walnut milk during storage.

Treatment	Days of storage	Mean
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TFC	1	2	3	4	5	6	
T ₀	1.69±0.15ko	2.0±0.15jn	2.19±0.15jm	4.55±0.15fh	6.60±0.15ad	6.63±0.15ad	3.95AB
T ₁	0.79±0.05mp	1.39±0.05lp	1.69±0.05ko	4.11±0.05gi	6.16±0.05be	7.44±0.05ab	3.60B
T ₂	0.55±0.05np	0.86±0.05mp	2.2±0.05hk	4.64±0.05eh	6.27±0.05bd	6.01±0.05bf	3.49B
T ₃	0.70±0.05mp	1.66±0.05kp	2.14±0.05jn	5.11±0.05dg	6.08±0.05bf	7.50±0.05ab	3.86AB
T ₄	0.84±0.05mp	2.48±0.15jl	3.4±0.15hj	5.20±0.15dg	6.63±0.15ad	7.93±0.15a	4.42A
T ₅	0.07±0.15p	0.25±0.15op	2.03±0.15jn	3.4±0.15hj	5.5±0.15fh	6.70±0.15ad	3.55B
T ₆	0.80±0.04mp	1.51±0.04lp	2.1±0.04jn	4.2±0.04gh	5.70±0.04cg	6.69±0.04ac	6.82C
T ₇	0.78±0.04mp	0.70±0.04mp	3.2±0.04hk	4.1±0.04gi	5.20±0.04dg	6.83±0.04ac	3.47BC
Means	0.78F	1.36E	2.43D	4.42C	5.89B	7.0A	

Color: The observation of color is defined through color categories including (blue, red yellow-orange, and green) with acceptance of the respective product. The effect of treatment and storage on the color of walnut milk was highly significant in interaction. The results are given in Table 15, T5=walnut milk with 0.02 sodium azide with refrigeration, the temperature has been found 1.0has good color acceptable on the first day

and 3.0 at 25 days. In contrast, T0=control without premature walnut milk at room temperature color was changed got 2.0 on day 1st and 8.0 at 25 days of storage. These results might be due to the preservative effect and effect at temperature during storage. These results were similar to Milovanovic *et al.* (2020). In his study applied he applied some preservatives to soya milk for storage. The changes in color is might be enzymatic browning are also the effect of autolysis in food.

Table 14. Effect of treatment and storage interval on taste of walnut milk.

Treatment Taste	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	3.00±1.52f	4.0±1.5e	5.00±1.5d	6.00±1.5c	7.00±1.5b	8.00±1.5a	5.50 A
T ₁	2.00±1.5gh	2.0±1.5gh	4.0±1.5e	4.00±1.5e	5.00±1.75d	5.00±1.75d	3.66 C
T ₂	1.00±1.5j	4.00±1.e	4.00±1.d	5.00±1.5e	5.00±1.75d	7.00±1.75b	4.33 B
T ₃	1.00±1.5j	2.00±1.gh	1.00±1.5j	3.00±1.7f	4.00±1.75e	4.00±1.75e	2.50 E
T ₄	1.00±1.7j	1.00±1.7j	3.00±1.7f	2.33±1.7g	3.00±1.75f	4.00±1.75e	2.38 E
T ₅	1.00±1.51j	1.33±1.51ij	1.00±1.51j	1.00±1.51j	2.00±1.75±1.51gh	3.00±1.75±1.51f	1.55 G
T ₆	1.00±1.51j	2.00±1.51gh	2.00±1.51gh	4.00±1.51e	4.00±1.51e	5.00±1.51d	3.00 D
T ₇	1.00±1.51j	2.00±1.51gh	1.66±1.51hi	2.00±1.51gh	3.00±1.51f	3.00±1.51f	2.11 F
Mean	1.3750 F	2.2917 E	2.7083 D	3.4167 C	4.1250 B	4.8750 A	

Taste: The feeling observed by the human tongue and linked by aroma and texture is called taste. The human tongue has the receptors buds. The function of these buds is to check the sensory evaluation of food items. It is mentioned in Table 14, the results of different treatments and storage intervals showed significant interaction. Taste of the walnut milk sample significantly interacted with both treatment vice and storage duration also.T7= walnut milk with 0.04%

sodium benzoate with 0.01 percent Sodium azide at refrigeration temperature got the 1 mark show good in taste at 1st day and slowly changes occurred during storage throughout the 25 days and 3.0marks at the 25th day of storage which also was acceptable. This result might be due to the effect of the preservatives and refrigeration temperature of storage. These results found similar to Zhi *et al.* (2016).

Table 15: Effect of treatment and storage interval on color of walnut milk.

Treatment Color	Days of storage						Mean
	1	5	10	15	20	25	
T ₀	2.0±1.45gi	4.0±1.45e	3.0±1.55f	6.0±1.55c	6.0±1.75c	8.0±1.75a	4.83A
T ₁	1.0±1.45j	2.0±1.45gi	2.0±1.55gi	4.0±1.55e	5.0±1.75d	4.0±1.75e	3.00D
T ₂	1.0±1.45j	2.0±1.45gi	2.0±1.55gi	3.0±1.55f	5.0±1.75d	7.0±1.75b	3.33BC
T ₃	1.0±1.45j	3.0±1.45f	3.0±1.55f	4.0±1.55e	4.0±1.75e	4.0±1.75e	3.16BD
T ₄	1.00±1.45s	1.66±1.45hj	5.0±1.55d	2.66±1.55g	3.0±1.75f	5.0±1.75d	2.27E
T ₅	1.0±1.45j	1.33±1.45ij	1.0±1.55j	1.3±1.55fh	2.0±1.75f	3.0±1.75j	2.02G
T ₆	3.0±1.45f	1.00±1.45j	3.0±1.55f	1.0±1.55j	5.0±1.75d	4.6±1.75de	3.44B

T ₇	1.0±1.45j	1.66±1.45hj	2.33±1.55fh	4.0±1.55e	3.0±1.75f	2.33±1.75fh	2.07F
Mean	1.37E	2.0D	2.16D	3.33C	4.20B	4.75A	

Flavor: The flavor is the sensory aspect that was checked by taste and smell by nose and mouth partially both. The storage and treatment both significantly interact with the flavor of walnut milk. Storage and treatment both significantly interact with the flavor of walnut milk. The flavor acceptability was reduced with the time of storage product at T₅=walnut milk with 0.02% sodium azide at refrigeration temperature shows

highly acceptable with 1.01 at 1st day and gradually decrease to day 25 1.7±0.07 which was acceptable while T₀=walnut milk without preservatives at room temperature was badly effected during storage and was not acceptable which scored 8.0 at the 25 day changes and stability might be due ambient and favorable environment for microbial growth. These finding of Table 16 were related to Zhi *et al.* (2016).

Table 16: Effect of treatment and storage interval on flavor of walnut milk

Treatment Flavor	Days of storage						Means
	1	5	10	15	20	25	
T ₀	4.00±1.71e	3.00±1.45f	4.0±1.51e	7.0±1.71b	7.00±1.71b	8.00±1.71a	5.25 A
T ₁	1.0±1.45j	3.0±1.45f	4.0±1.51e	4.0±1.71e	4.00±1.71e	5.00±1.71d	3.30 C
T ₂	2.0±1.45gi	3.0±1.45f	3.0±1.51f	3.0±1.71f	6.00±1.71c	8.00±1.71a	3.91 B
T ₃	2.0±1.45gi	2.0±1.45gi	2.0±1.51gi	3.0±1.71f	4.00±1.71e	5.00±1.71d	2.86 E
T ₄	1.0±1.45j	2.0±1.45gi	2. ±1.51gi	2.33±1.71fh	2.66f±1.71g	3.00±1.71f	2.25 F
T ₅	1.0±1.45j	2.0±1.45gi	2.0±1.51gi	2.0±1.71gi	3.00±1.71f	4.00±1.71e	1.78 H
T ₆	2.0±1.45gi	1.0±1.45j	3.0±1.51f	3.0±1.71f	5.00±1.71d	5.00±1.71d	3.16 D
T ₇	1.3±1.45ij	1.6±1.45hj	1.6±1.51hj	2.0±1.71gi	3.00±1.71f	3.00±1.71f	2.00 G
Mean	1.50 F	2.0750 E	2.4625 D	3.3167 C	4.1792 B	4.8708 A	

Overall Acceptability: The overall acceptability of the walnut milk sample was significantly different as the results mentioned in Table 17. By using sensory evaluation, the overall acceptability 1.78 for T₅ containing 0.02% sodium azide with refrigeration temperature. While containing the walnut milk without preservatives at room temperature has score (5.2) lowest. The overall acceptability decreases with storage and increases with the addition of preservatives and

refrigeration temperature. The mean value of storage interval o overall acceptability is 8.0 minimum score at 25 days of storage. Compare with 1st day of storage (1.50) color, taste, and flavor were reduced during storage due to the formation of acid, lactic acid, and citric acid increase with the passage of time and affecting the overall acceptability of walnut milk (Zhi *et al.*, 2016).

Table 17: Effect of treatment and storage interval on overall acceptability of walnut milk

Treatment overall acceptability	Days of storage						Mean
	1	3	10	15	20	25	
T ₀	3.30±1.51l	3.60±1.51j	4.00±1.51i	1.30±1.51d	1.60±1.51c	8.00a±1.51	5.25A
T ₁	1.30±1.51qr	2.30±1.51n	3.00±1.51l	4.00±1.51i	4.60±1.51g	4.60±1.51g	3.30 C
T ₂	1.30±1.51qr	3.00±1.51l	3.00±1.51l	3.60±1.51j	5.30±1.51e	7.30±1.71b	3.91 B
T ₃	1.30±1.51qr	2.30±1.51n	2.00±1.71o	3.30±1.71k	4.00±1.71i	4.3±1.710h	2.86E
T ₄	1.00±1.51s	1.30±1.51qr	2.30±1.71no	2.30±1.71n	2.73±1.71m	4.0±1.710i	2.25 F
T ₅	1.00±1.51s	1.20±1.51qs	1.30±1.71qr	1.33±1.71q	2.60±1.71m	3.30±1.71k	1.78 H
T ₆	2.00±1.51o	1.30±1.51qr	2.60±1.71m	3.60±1.71j	4.6±1.710g	4.86±1.71f	3.16 D
T ₇	1.10±1.51rs	1.60±1.51p	1.60±1.71p	2.10±1.71no	3.00±1.71l	2.60±1.71m	2.00 G
Mean	1.5000 F	2.0750 E	2.4625 D	3.3167 C	4.1792 B	4.8708 A	

CONCLUSION AND RECOMMENDATIONS

During study it was concluded that walnut milk with 0.02% sodium azid eat 40C had more overall acceptability. It was observed and concluded that the walnut milk with 0.04% sodium benzoate and 0.01% sodium azide at refrigeration temperature 40C was also acceptable with respect to increasing shelf life with acceptable organoleptic properties. From the present

research, following conclusion have been drawn. Dairy substitute walnut milk can be prepared by addition of food grade preservative sodium azid up to 0.02% level. At room temperature the sensory and organoleptic characteristics of walnut milk was declined. The walnut milk with preservatives can be stored at refrigeration temperature 40 °C was acceptable for more than 25 days. It is recommended that the food grade stabilizer

utilization will increase the stability of walnut milk. Promote the development of plant based, nondairy milk (walnut milk) will be beneficial with respect to health

AUTHORS' CONTRIBUTION

All author have equal Contribution

CONFLICT OF INTEREST

All Authors have no any conflict of interest

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