

The Effect of Water and Ethanol Extracts of Ginger and Garlic on the Nutritional Quality and Physico-Chemical Properties of Stored Soymilk

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Abstract: Soymilk is an aqueous, white, creamy extract produced from soybeans (*Glycine max*). It is a highly nutritious food drink which contains protein, fat, carbohydrates vitamins and minerals. Recently, there has been an increased demand for foods having long shelf-life but with presence of minimum or no chemical food additives; therefore, the use of natural preservatives especially culinary herbs and spices in food products has increased. This study set out to investigate the effect of aqueous and ethanol extracts of ginger and garlic on the nutritional properties of soymilk before and after two weeks of storage. The soymilk was prepared using hot extraction method and each sample labeled A, B, C, D and E according to the extract they contain; ginger-ethanolic, ginger-aqueous, garlic-ethanolic and garlic-aqueous respectively while sample E served as the control containing no extract. The samples were stored at $\pm 4^{\circ}\text{C}$ for sixteen (16) days. The result obtained showed no significant difference ($P < 0.05$) in the % protein, % fat and % ash contents of all the fresh samples when compared with the control. However, after the storage period, there was significant ($P < 0.05$) decrease in the content, % fiber content, % protein content, % carbohydrate, and % fat contents of almost all the treated samples when compared with the control. A significant increase was also observed for the pH value for all treated samples after the storage period when compared with the control, although samples A and D showed a significant increase after the storage period. Samples A, C, and D, showed a significant reduction in % TTA when compared with the control after the storage period.

Keywords: Soymilk, Preservation, Ginger, Garlic, Nutrients

1. Introduction

Soymilk is a traditional oriental food beverage that is growing in popularity in the United States and the world [1]. It is the rich creamy liquid extract of soybean which resembles cow milk (conventional milk) in both appearance and consistency [2]. Soymilk is a popular nutritive and cheaper alternative to cow's milk [3] and has become a very interesting food due to its extraordinary nutritive value and health characteristics. It is a very rich source of highly valuable proteins, unsaturated fatty acids, soluble and insoluble dietary fibers, and isoflavones whose presence in everyday diet is very important [4]. In some countries, soymilk is intended for the population who cannot digest milk due to lactose intolerance, allergy to milk proteins, or vegetarian way of diet. Soymilk is made by soaking soybeans

in water before grinding and straining.

The importance of protein in the diet of growing children and its continuous supply is very vital in Africa where the occurrence of protein energy malnutrition (PEM) is very rampant. Soybean, a highly proteinous seed has been identified over the years as a cheap and readily available source of protein that could be exploited to supply much of the needed nutrient. In developing countries and indeed in tropical regions of sub-saharan Africa (except East Africa), the production of milk and milk products are limited, scarce and expensive [5].

Milk is an excellent source of nutrients such as vitamins, amino acid, fats, minerals, proteins and sugar, making it an excellent medium for microbial proliferation [6]. Soybean seeds contains by weight approximately 32% carbohydrate, 20% fat, 5% minerals, 40% protein and 5% vitamins [7]. Its

lipid content is rich in polyunsaturated fatty acids like linoleic acid (52.3%) and linolenic acid (7.2%) [8]. These essential fatty acids reduce cholesterol content of blood thereby decreasing the possibilities of heart and blood vessel diseases in human beings [9].

Following the increasing awareness of the high nutrient value of soybean, there has been an increase in consumption and marketing of soybean products like soymilk, soy-cheese, soy-ogi, soy-cake, soy-flour and a host of others. Soymilk is the most popular of these products in Nigeria and efforts towards extending the shelf-life of soymilk is of utmost importance [10].

Maintaining or creating nutritional value, texture and flavour is an important aspect of food preservation. Although use of spices may not be employed as a primary preservative method, addition of spices aid in preserving foods.

Herbs and spices are rich in phenolic compounds and besides exerting antimicrobial effect they may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity [11, 12, 13, 14, 15]. A wide variety of phenolic substances derived from herbs and spices possess potent biological activities, which contribute to their preservative potential [16]. It has been observed that the use of garlic extracts inhibits growth of phytopathogenic fungi, *Aspergillus flavus*, *Curvularia lunata* and *Fusarium moniliforme* [17].

The aim of this study is to investigate the effect of aqueous and ethanol extracts of ginger and garlic on the nutritional properties and physico-chemical attributes of soymilk before and after two weeks of storage.

Specific objectives were to:

- 1) To assess the effect of water and ethanol extracts of ginger and garlic on the nutritional quality of soymilk before and after two weeks of storage.
- 2) To evaluate the effect of water and ethanol extracts of ginger and garlic on the physico-chemical attributes of soymilk stored for two weeks at $\pm 4^{\circ}\text{C}$.
- 3) To evaluate the effect of water and ethanol extracts of ginger and garlic on the antioxidant activities of soymilk for two weeks at $\pm 4^{\circ}\text{C}$.

2. Materials and Methods

2.1. Source of Raw Materials

The soybean seeds, ginger rhizomes and garlic bulbs used in this study were all obtained from Bodija market, Ibadan and identified in the Department of Botany, University of Ibadan.

2.2. Preparation of Ethanol Extract of Ginger and Garlic

Ginger rhizomes were washed, peeled and air-dried for one week. The dried ginger was grounded to a fine powder in an electric blender. 40g of the powdered ginger was extracted with 200ml of 95% ethanol for 72 hours at room temperature in a beaker. The beakers were covered with foil paper and shaken vigorously at regular intervals. The extract was filtered using filter papers to remove residue and then

evaporated in a water bath at 40°C . The extract obtained after evaporation of ethanol was used as natural antioxidant in the soymilk samples.

2.3. Preparation of Aqueous Extract of Ginger and Garlic

Ginger rhizomes were washed, peeled and air-dried for 72 hours. The dried ginger was grounded to a fine powder in an electric blender and sieved. 40g of the powdered ginger was soaked in 200ml of warm distilled water for 48 hours at room temperature in a beaker. The beakers were covered with foil paper and shaken vigorously at regular intervals. The extract obtained after evaporation of water was used as natural antioxidant in the soymilk samples.

2.4. Preparation of Soymilk

The method developed by INTSOY (International soybean program) and modified by [18] was used. The soybeans were cleaned in order to remove dirt and also some impurities like other seed stalks etc. The beans were blanched in hot water for 30 minutes for the following reason; to soften the seeds and aid in seed coat removal, to reduce the beany flavour and eliminate the anti-nutritional factors. The beans were then dehulled and milled with water using the Kenwood blender, about 3 parts of water was added to the slurry and filtered using a Muslim cloth, the filtrate was allowed to simmer on fire about 30 minutes it was then bottled and allowed to cool.

2.5. Sample Preparation and Storage

250mls each of soymilk were placed in five separate sterile transparent plastic bottles respectively and treated with the different concentration of the extracts.

Sample A: 0.6g of the ethanol ginger extract was weighed into a beaker containing 20ml of hot sterile sugar solution placed in a boiling water bath and stirred continuously until the extract is completely dissolved. The solution was then added to 250ml of the prepared soymilk in a transparent plastic bottle and shaken vigorously before storage.

Sample B: 1.5g of the water ginger extract was used.

Sample C: 0.32g of the ethanol garlic extract was used.

Sample D: 1.5g of the water garlic extract was used.

Sample E (Control - No treatment)

The samples were stored at $4^{\circ}\text{C} \pm 2$ in a refrigerator in the laboratory throughout the storage period.

2.6. Physico-Chemical Analysis

2.6.1. pH

pH of the sample were determined using a standard pH meter throughout the storage period. 10ml of the samples was measured into a clean sample bottle into which the electrode was immersed and allowed to balance after standardizing the pH with the buffer solution (3M KCl).

2.6.2. Total Titratable Acidity (TTA)

TTA was determined by titrating 10ml of the milk with 0.1N NaOH after 1ml of the sample was diluted into 100ml volumetric flask, 1% phenolphthalein added and shaken

properly. It was then titrated.

2.6.3. Proximate Analysis

Crude Protein was determined according to micro-Kjeldahl Procedure according to method described by [19]. Fat, carbohydrate, ash and moisture protein contents were determined using methods described by [9], carbohydrate by difference.

2.6.4. Determination of Phytic Acid

Phytic acid was determined by the procedure of [20]. 2ml of the sample was measured into a 250ml conical flask. 100ml 2% concentrated HCl was used to soak sample for 3hours and then filtered with Whatman No 1 filter paper. 50ml of filtrate and 10ml of distilled water were added in each case to give proper acidity. 10ml of 0.3% ammonium thiocyanate solution was added into the solution indicated and titrated with standard Iron III chloride solution containing 0.00195g Iron/ml, endpoint was observed to be yellow which persisted for 5minutes. The percentage phytic acid was calculated thus:

$$\% \text{ phytate} = y \times 1.19 \times 100$$

$$\text{Where } y = \text{titre value} \times 0.00195\text{g}$$

2.6.5. Determination of Oxalate

The determination of oxalates was carried out by the titration method of [19]. 2ml of sample was suspended in a mixture of 190ml of distilled water and 10ml of 6N HCl in a 250ml volumetric flask and digested for one hour at 100°C, cooled and made up to 200ml with distilled water. The digest was being filtered through Whatman No 1 Filter paper using a suction pump. A duplicate proportion of 125ml of the filtrate was measured into 250ml beakers and four (4) drops of methyl red indicator add into each beaker. Concentrated NH_4OH or NH_3 solution was added drop wise until the test solution changed from its salmon pink colour to faint yellow colour (pH 4-4.5). Each proportion was heated up to 90°C and 10ml of 5% CaCl_2 was added while being stirred constantly. After heating, it was cooled and left overnight at 5°C. The supernatant was decanted and the precipitate completely distilled in 10ml of 20% (v/v) H_2SO_4 solution. At this point, the filtrate resulting from digestion of the sample was combined and made up to 300ml. Aliquots (125ml) of the filtrate was heated until near boiling and then titrated against 0.05M standard KMnO_4 solution to a faint pink colour that persisted for 30 seconds. Oxalic acid content was calculated using the formula:

$$\text{Oxalate (mg/100g)} = \frac{T \times (\text{Vme}) (\text{Df}) \times 10_3}{\text{ME} \times \text{Ms}}$$

Where:

T= Titre of KMnO_4

Vme= volume-mass equivalent (i.e., 1ml of 0.05M KMnO_4 solution is equivalent to 0.0022g anhydrous oxalic acid)

Df= the dilution factor

ME= the molar equivalent of KMnO_4 in oxalic acid (KMnO_4 redox reaction is 5)

Ms= the mass of sample used Determination of antioxidant

2.7. Antioxidant Assay

2.7.1. Determination of Total Flavonoid

Total flavonoid was estimated spectrophotometrically using the method based on the formation of flavonoid-aluminium complex with some modifications [21]. 0.5ml of 2% ethanolic aluminium chloride (AlCl_3) was added to 0.5ml of sample. After 45minutes of incubation at room temperature, the absorbance of the reaction mixture was measured at 420nm. Total flavonoid content was calculated from the equation of the plot and expressed as equivalent to quercetin in mg/g of the juice samples.

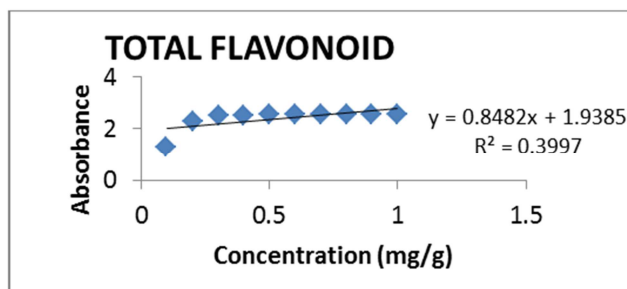


Figure 1. Plot of the standard (Quercetin) Absorbance versus concentration (mg/g).

2.7.2. Determination of Total Phenolic Content

Total phenolic content was determined by the spectrophotometry method. 1ml of the juice was mixed with 1ml of Folin-Ciocalteu reagent. After 5minutes, 10ml of 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13ml of distilled water and mixed thoroughly. The mixture was kept in the dark for 90minutes at 25°C after which the absorbance was recorded at 750nm. Total phenolic content was evaluated from a gallic acid standard curve and expressed as gallic acid equivalent/1ml (GAE/ml) of sample.

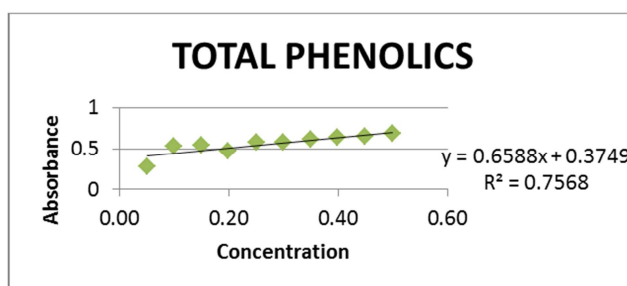


Figure 2. Plot of the standard (Gallic acid) Absorbance versus concentration (g/ml).

2.7.3. Determination of DPPH Radical-Scavenging Activity

The juice (100μl, /mg/ml) was added to 3.9ml of DPPH solution (0.025 g/l) and the reactants were incubated at 25°C for 30minutes. Instead of the juice, a positive control of ascorbic acid was used. The mixture was shaken and allowed to stand in the dark at room temperature for 35minutes. Free radical scavenging activity was calculated from absorbance values at 520nm [22] and expressed as inhibition percentage.

$$\text{DPPH radical – scavenging activity} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

2.8. Sensory Analysis

The Sensory evaluation of the soymilk samples were conducted in the Nutritional and Industrial Biochemistry Unit of the Department of Biochemistry, University of Ibadan. Ten (10) postgraduate students were randomly selected to rate the colour, taste, and odour of the samples using a nine-point hedonic scale, varying from “dislike extremely” (score 1) to “like extremely” (score 9) according to the method of [23]. The samples were served in a coded and transparent white plastic cups for proper assessment.

3. Results

Table 1. % Nutrients composition of soymilk before and after two weeks of storage.

		% MOISTURE	% PROTEIN	% FAT	% CHO
Sample A	BEFORE	87.00±0.10	5.07±0.06	1.53±0.06	5.57±0.21
	AFTER	90.90±0.02**	3.56±0.28*	2.82±0.04*	3.35±0.31*
Sample B	BEFORE	85.90±0.10	5.07±0.06	1.43±0.06	6.80±0.00
	AFTER	89.55±0.05*	3.48±0.03*	3.33±0.07**	3.44±0.05**
Sample C	BEFORE	86.57±0.15	5.03±0.12	1.40±0.10	6.17±0.21
	AFTER	90.34±0.13*	3.41±0.06*	2.72±0.03**	3.13±0.04**
Sample D	BEFORE	86.57±0.15	4.90±0.17	1.30±0.10	6.33±0.32
	AFTER	90.55±0.80**	2.27±0.01**	2.56±0.03*	4.41±0.4*
Sample E	BEFORE	86.07±0.15	4.87±0.12	1.23±0.06	7.10±0.26
	AFTER	90.70±0.03	3.20±0.31	3.46±0.03	2.24±0.06

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=3

*values differ significantly from the control.

Table 1 shows significant differences in the % nutrient content of the various soymilk samples before and after the storage period.

Table 2. pH of soymilk samples during storage.

SAMPLE	DAY 1	DAY 4	DAY 8	DAY 12	DAY 16
A	6.73±0.028*	6.62±0.021*	6.62±0.021*	6.62±0.021*	5.52±0.021*
B	6.56±0.014*	6.31±0.014*	5.92±0.028*	5.82±0.021*	4.56±0.219
C	6.43±0.042*	6.53±0.028*	6.51±0.028*	5.98±0.035*	4.84±0.156
D	6.42±0.014*	6.31±0.028*	6.25±0.035*	5.96±0.092*	5.69±0.332*
E	5.52±0.021	4.56±0.219	4.84±0.156	4.62±0.021	4.39±0.156

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=2

*Values differ significantly from control (P < 0.05)

Table 2 shows the pH of the soymilk samples taken every four days of storage. There were significant differences in the pH of the treated samples when compared with the control.

Table 3. % total titratable acidity of soymilk samples during storage.

SAMPLE	DAY 1	DAY 4	DAY 8	DAY 12	DAY 16
A	0.065±0.007	0.085±0.021	0.275±0.007*	0.360±0.000*	0.385±0.035*
B	0.060±0.000	0.085±0.007	0.430±0.000*	0.645±0.021*	0.650±0.042*
C	0.060±0.000	0.105±0.021	0.305±0.007*	0.400±0.014*	0.320±0.028*
D	0.075±0.007*	0.090±0.000	0.275±0.007*	0.365±0.021*	0.420±0.014*
E	0.055±0.007	0.085±0.007	0.355±0.007	0.535±0.007	0.545±0.007

A= Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=2

*Values differ significantly from control (P < 0.05)

Table 3 shows the % total titratable acidity of the soymilk samples taken every four days of storage. There were significant differences in the % total titratable acidity of the treated samples when compared with the control.

Table 4. Total phenolics composition of soymilk sample.

GROUPS	CONCENTRATION (GAE/ml)
SAMPLE A	3.8340±0.327*
SAMPLE B	3.7940±0.537*
SAMPLE C	3.7805±0.421*
SAMPLE D	4.1205±0.163*
SAMPLE E	3.5555±0.424

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=2

*Values differ significantly from control (P < 0.05)

Table 4 shows the total phenolic composition of the fresh soymilk samples. There was no significant difference in the total phenolic content of the treated samples when compared with the control.

Table 5. Total flavonoids composition of soymilk samples.

SAMPLES	CONCENTRATION (mg/ml)
A	0.835±0.005
B	0.838±0.002
C	0.829±0.004*
D	0.839±0.003
E	0.842±0.002

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=2

*Values differ significantly from control (P < 0.05)

Table 5 shows the total flavonoid content of the fresh soymilk samples. There was significant difference in the total flavonoid content of treated samples C when compared with the control.

Table 6. Oxalate composition of the soymilk samples.

GROUPS	CONCENTRATION (mg/100g)
SAMPLE A	2.9175 ± 0.117*
SAMPLE B	3.8300±0.071*
SAMPLE C	10.373 ± 0.032
SAMPLE D	5.5610 ± 0.849*
SAMPLE E	6.0670 ± 2.830

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=2

*Values differ significantly from control (P < 0.05)

Table 6 shows the oxalic acid concentration of the soymilk samples. There were significant differences in the oxalate composition of some of the treated soymilk samples when compared with the control.

Table 7. % phytic acid composition of soymilk samples.

GROUPS	% PHYTIC ACID
SAMPLE A	0.0750 ± 0.007
SAMPLE B	0.0650±0.007
SAMPLE C	0.0550 ± 0.007
SAMPLE D	0.0650 ± 0.007
SAMPLE E	0.0670 ± 0.009

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=2

*Values differ significantly from control (P < 0.05)

Table 7 shows the %phytic acid composition of the soymilk samples. There was no significant difference in the %phytic acid composition of the treated soymilk samples when compared with the control.

Table 8. Sensory evaluation of soymilk samples.

Sample	Colour	Taste	Odour
A	8.50±0.71	6.70±1.89	6.70±1.70
B	7.90±1.20	7.90±0.57	8.10±0.88
C	7.30±1.50	5.50±1.65*	4.80±1.69*
D	6.70±2.75	4.60±2.12*	5.20±2.15*
E	7.80±0.79	7.40±0.97	7.20±1.81

A=Ethanollic Ginger extract, B= Aqueous ginger extract, C= Ethanollic Garlic extract, D = Aqueous Garlic extract, E = Control

The data are expressed as mean ± SD, n=10

*Values differ significantly from control (P < 0.05)

Table 8 shows the result of sensory evaluation of fresh soymilk containing different natural preservatives. There were significant differences in the taste and odour of some of the treated various samples when compared with control.

4. Discussion

For the fresh samples, the significant increase observed in the % moisture composition of samples A, C, and D and the increase % fat content of samples A and B when compared with that of the control, could be attributed to the additional nutrients from the extracts as supported by the study carried out by [24].

After the storage period however, a significant increase was observed in the % moisture content of all the samples when compared to the values obtained for the fresh sample (Table 1) which could be indicative of increased microbial activities as supported by an earlier study carried out by [25] on *C. capio*, whose finding showed an increase in moisture content from an initial value of 79.35% to 82.60% after three weeks of storage at refrigeration temperature. Also, there was significant increase in the % fat content of all the samples when compared with the results obtained for the fresh samples (Table 1). This could also be indicative of increased microbial activities in all the samples. However, when compared with the control, the % fat content was significantly reduced in all the treated samples (A, B, C, and D) as supported by the study carried out by [24]. This could be attributed to lesser microbial activities in the four treated samples than in the control.

A significant reduction was observed for the % protein composition of all the samples after the storage period (Table 1). This could be attributed to increased microbial activities in all the samples. Nonetheless, when compared with the control, the % protein composition of sample A was significantly higher, while that of sample D was significantly lower (Table 1). The higher protein composition observed in sample A could be attributed to the potent antioxidant and antimicrobial activities the ginger ethanol extract present in the sample which has efficiently reduced protein oxidation and the hydrolytic action of proteases produced by the

spoilage bacteria. Increase in moisture and decrease in protein were observed in spotter seer during ice storage by [25]. A decrease in protein fraction in fish during ice storage was also reported by [26].

The significant decrease observed for % carbohydrate composition in all the samples after two weeks of storage could be attributed to increased microbial activity leading to breakdown of carbohydrate and sugar. The most prominent microbe involved in this is the *Lactic Acid Bacteria* (LAB) which ferments carbohydrate into lactic acid and CO₂. Yet, the % carbohydrate content of all individual treated samples was significantly higher than that of the control group which is in congruence with a study carried out by [24].

All the treated groups showed a significantly higher pH value when compared with the control on the first day of production. This could be ascribed to the nature of the extracts which is most likely basic. The gradual decline observed in the pH values of all the samples on the fourth day, the eighth day and the twelfth day, could be attributed to increase in fermentation of sugars by LAB. However, the pH of Samples A and D were significantly higher on the sixteenth day when compared with the control. This could be credited to the potency of the extracts as supported by the observation of [27] in their work on the preservation of West African soft cheese with chemical treatment.

The % total titratable acidity value increased for all the samples all through the storage period. However, on the fourth day, there was no significant difference between the values obtained for the treated groups when compared with the control. Samples A, C and D showed significant reduction in the % total titratable acidity values obtained on the eighth, twelfth and sixteenth day when compared with the control while Sample B showed significant increase in the % total titratable acidity on the eighth day, twelfth day and sixteenth day of storage when compared with the control.

The total phenolic content of the treated samples were significantly higher than that of the control. This can be credited to the phenolic content of the extracts which functions as antioxidants.

There was no significant difference in the total flavonoids content of the treated samples when compared with the control except for sample C which showed a significant decrease. For the antinutrient assay, samples A, B, and D showed a significant decrease in the oxalate composition when compared with the control while all the treated groups showed no significant difference in the phytic acid composition when compared with the control.

The sensory analysis indicated that there is no significant difference in the colour of the treated soymilk samples when compared with the control, however samples C and D which contained aqueous and ethanol extract of garlic showed a lower preference in taste and odour when compared with the control as shown in Table 8. This lower acceptability of sample C and D's taste and odour could be due to the strong pungent taste of garlic.

5. Conclusion

Although several studies have been carried out on the antimicrobial and antioxidant activities of *Allium sativum* and *Zingiber officinale* [28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40], not much has been done on its ability to preserve fluid food products. This study was able to reveal the efficacy of the aqueous and ethanolic extract of *Z. officinale* and *Allium sativum* in retaining the nutritional composition and physicochemical attributes of soymilk after the two weeks of storage. All the extracts used in this study showed a potential to retain the nutritional composition and physicochemical attribute of soymilk, most importantly, the ethanolic extract of *Zingiber officinale* which was found to be most efficient. However, it is recommended that further studies should be done on the best concentration of the extract that will elicit the highest preservative activity while still maintaining good organoleptic property.

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