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Efficacy of cloves (*Syzygium aromaticum*) and ginger (*Zingiber officinale*) extracts against microbial spoilage of locally produced soymilk at ambient storage

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ABSTRACT: There is a global concern at present with the use of synthetic chemical preservatives in foods due to carcinogenic and mutagenic related problems, resulting to the use of natural preservatives especially culinary herbs and spices in food products. Herein, efficacy of clove (*Syzygium aromaticum*) fruit and ginger (*Zingiber officinale*) rhizome was evaluated against rapid deterioration of locally produced soymilk at ambient storage by microorganisms and their bioactive compounds determined with gas chromatography and mass spectrometry (GC-MS). Interestingly, soymilk treated with 5% ginger extract among other tested concentrations had the least bacterial ($2.00 \pm 0.50 \times 10^4$ cfu/ml) and fungal ($2.00 \pm 0.00 \times 10^4$ sfu/ml) counts that did not exceed the acceptable limit for pasteurized milk (3×10^4 cfu/ml) by day 3 of storage when compared with untreated soymilk that recorded very high bacteria ($56.50 \pm 6.50 \times 10^4$ cfu/ml) and fungi ($13.50 \pm 1.50 \times 10^4$ sfu/ml) counts. The efficacy could be traced to beta-amyrin (24.15%), hexadecanoic acid (15.71%) and phytol (16.55%) that are major bioactive compounds detected in the ginger extract. Thus, the ginger extract offers prospect as promising alternative that can be applied in extending the shelf life of soymilk and other liquid processed foods.

Keywords: Soymilk; Plant extracts; Biopesticide; Microorganisms; Bioactive; GC-MS.

1. INTRODUCTION

Following the increasing awareness of the high nutrient value of soybean, there has been an increase in consumption and marketing of soybean products like soy yoghurt, soymilk, soy-cheese, soy-ogi, soy-cake, soy-flour, dadawa, animal feeds and a host of others [1, 2]. Soymilk, which is the most popular of these products in Nigeria is an aqueous, white, creamy extract which is similar to milk in appearance and consistency. It is a highly nutritious product which contains protein, fat, carbohydrates, vitamins and minerals [3] and this makes soymilk play an important role in the dietary pattern of people in most developing countries [4]. In fact, the increasing popularity of soymilk as a beverage worldwide is credited to its health benefits and comparatively low cost. The milk offers culinary diversity and a healthful nutritional profile, including essential omega-3 fatty acids and flavonoids that exert antioxidant, anti-inflammatory, and cardio protective properties.

However, the greatest problem with soy milk is its very short shelf life due to microbial activity. This is due to the fact that milk is highly nutritious and has a near neutral pH and high water activity [5]. Meanwhile,

various synthetic chemical preservatives are used to control microbial food spoilage and extend product shelf life in food industry. Examples of such include sodium benzoate, benzoic acid, nitrites, sulphites, sodium sorbate, and potassium sorbate and antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and propyl gallate [6, 7]. Nevertheless, there is a global concern at present with the use of synthetic chemical preservatives in foods due to carcinogenic and mutagenic related problems. They are toxic and can cause life threatening side effects and health hazards such as hypersensitivity, allergy, asthma, hyperactivity, neurological damage and cancer [8]. Besides, there is a health concern among consumers regarding the risk of using synthetic additives as preservatives and this has resulted to a sharp decrease in the use of these chemicals in food preservation [9].

Consequently, naturally occurring antimicrobials have gained attention among researchers and food manufacturers due to their safe and non-toxic status. Plants fulfil this requirement as herbs, and spices have been used traditionally to preserve food for long periods of time in different cultures [10, 11]. Even the emerging trend in food processing now focus on the use of natural compounds, which are considered as safe alternatives and satisfy the consumers' preferences for new eco-friendly methodologies that are free of chemical preservatives. Thus we produced soymilk locally and investigated the potency and the effectiveness of varying concentrations of aqueous extracts of ginger and cloves against its rapid deterioration by microorganisms at ambient storage. Additionally, we also determined the bioactive compounds of the aqueous extracts (GC-MS).

2. MATERIALS AND METHODS

2.1. Source of soybeans and preparation of soymilk

Soybean (*Glycine max*) seeds were purchased at Oja-Oba market, Owo, Ondo state, Nigeria. The method developed by International soybean program (INTSOY) and modified [12] was adopted for the preparation of the soymilk. About 250 g of soybean seeds were cleaned, winnowed and sorted (to remove cracked, damaged, pest infested and discoloured seeds). The cleaned seeds were then soaked in water for about 30 minutes, dehusked and thoroughly washed and soaked again for about 2 h. The seeds were then ground. About 10 litres of clean water was added to the grounded soybeans and mixed thoroughly. The mixture was sieved using a clean muslin cloth. The extracted milk (filtrate) was transferred into a clean pot and pasteurized at 63 °C for about 30 minutes. The milk was then allowed to cool gradually to a temperature of about 40 °C before dispensing them aseptically into sterile bottles at 90 ml per bottle.

2.2. Preparation of aqueous extracts of cloves and ginger

Clove fruits and ginger rhizomes were dried and ground into powder. Varying concentrations (1%, 3% and 5%) of extracts were prepared. For 1%, 1 g of each powdered extract was dissolved in 99 ml of distilled water and 3 g of each powdered extract was dissolved in 97 ml of distilled water for 3% while 5g of each powdered extract was dissolved in 95 ml of distilled water for 5%. The mixture was allowed to stand for 30 minutes and later filtered with muslin cloth and the filtrate collected in a conical flask was kept as stock.

2.3. Addition of varying concentrations of clove and ginger extracts to soymilk

Before use, all the extracts were pasteurized at 63 °C for about 30 minutes and 10 ml of each varying concentrations of the extracts, after cooling was aseptically added to the sterilized bottles containing 90 ml of prepared soymilk samples. They were later stored at 28 °C and relative humidity of 90% and observed daily for microbial counts.

2.4. Microbial analysis

Serial dilution was carried out by aseptically transferring 1 ml of treated soymilk sample to obtain primary dilution factor ($\times 10^1$). The procedure continued until $\times 10^4$ dilution factor was obtained. One (1) ml of the $\times 10^4$ dilution factor was then pipetted aseptically into sterile Petri dishes. This was followed by pouring already prepared agar medium into the Petri dishes (nutrient agar was used for bacteria and potato dextrose agar for fungi). The Petri dishes were carefully swirled for homogeneity of the content. The medium was allowed to solidify and later incubated upside down at 37 °C for 24 h for bacteria and at 26 °C for 72 h for fungi. After incubation, all the plates were observed for colony growth and the colonies were counted using a colony counter (Gallenkamp Model, UK) and results expressed as colony forming unit per ml (cfu/ml) for bacteria and spore forming unit per ml (sfu/ml) for fungi. Meanwhile, untreated soymilk and samples treated with sodium benzoate served as negative and positive controls respectively.

2.5. Isolation and identification of microbial isolates

The observed colony of each microbial isolate was then sub-cultured several times until pure colonies were obtained. Individual bacteria isolate was later identified using biochemical methods and microscopy [13] as described below:

2.5.1. Gram staining test

A heat fixed smear of the isolated organism was made on a clean slide and flooded with crystal violet solution for one minute. This was rinsed under slow running tap water, and then flooded with Lugol's iodine solution for one minute. The slide was rinsed under slow running tap water and then decolourised drop wise with 70% ethanol for two minutes. The glass slide was rinsed under slow running tap water and then counter stained with safranin for 30 seconds. The slide was rinsed, air dried and then viewed under the Olympus microscope with a magnification of $\times 100$ using oil immersion.

2.5.2. Catalase test

The catalase test was done by putting two drops of 3% hydrogen peroxide on a glass slide. Using a sterile wire loop, bacterial cells were carefully picked from the plates and smeared on the slide. A positive result is indicated by instant bubbles on the slide. No bubble production is an indication of negative result.

2.5.3. Starch hydrolysis test

A few colonies of the test organism were picked using a sterile loop. These were streaked across the width of a sterile starch plate. The plate was then incubated at 37°C for 24-48 hours. Two drops of 10% iodine solution was directly added onto the edge of the colonies. A characteristics purple-black colouration in the medium indicates positive result.

2.5.4. Coagulase test

A smear of bacterial cell was made on a clean slide containing plasma using a flamed wire loop. Clotting indicates positive coagulase test while no agglutination indicates negative results.

2.5.5. Sugar fermentation test

Purple broth was inoculated with isolated bacterial colonies and incubated at between 35-37°C for 5 days. The medium was observed daily for development of a yellow colouration in the medium. The

development of a yellow colour in the medium indicates a positive result while lack of yellow colour in the medium indicates negative result.

2.5.6. Urea test

The surface of a urea agar slant was streaked with a bacterial colony. With the cap loosely tightened, it was incubated at 37°C for 5 days. Development of a pink colour indicates a positive result while no colour change indicates a negative result.

2.5.7. Citrate test

Simmon's citrate agar medium slant was carefully streaked with bacterial inoculum. This was incubated at 37°C for 72 hours. The development of blue colour and growth on the medium indicates positive result. If there is no growth and the colour remain the same, then the result is negative.

2.5.8. Indole test

A sterilized test tube containing 4mls of tryptophan broth was aseptically inoculated with bacterial colony and incubated at 37°C for 24 hours. About 0.5 ml of Kovac's reagent was added to the broth culture. Formation of pinkish red layer colour ring on top of the medium within few seconds of adding the reagent indicate positive reaction, no colour change indicates negative reaction.

2.5.9. Hydrogen sulphide test

An inoculum from a pure isolate was transferred aseptically to a sterile triple sugar iron agar (TSIA) slant. The tube was incubated at 37°C for 24 hours. Formation of black precipitate on the medium indicates a positive result. No blackening on the medium indicates a negative result.

2.5.10. Cotton blue lactophenol technique

Cotton blue lactophenol technique was adopted for fungal identification using the criteria outlined by Frazier and Westhoff [14] by placing a drop of the stain on clean slide. Thereafter, a small portion of the mycelium from the fungi isolate was removed and placed in a drop of the lactophenol on the slide. The mycelium was carefully spread on the slide and a cover slip was gently placed over it with little pressure to eliminate air bubbles. The slide was then mounted and observed under Olympus microscope using × 40 objective lens.

2.6. pH determination

This was done by inserting the electrode of a digital pH-meter (Jenway 550 model) directly into the soymilk samples and reading was taken at each day of storage.

2.7. Gas chromatography-mass Spectrometry (GC-MS)

The gas chromatography-mass spectrometry (GC-MS) analysis of the fruit of cloves (*Syzygium aromaticum*) and the rhizome of ginger (*Zingiber officinale*) was performed using a GC-MS (Modal; Agilent technologies 7890A) equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 mm film thickness. For GC-MS detection, an electron ionization system with energy of 70 eV was used. Helium gas (99.99%) was used as a carrier at a constant flow rate of 1 ml/min. injection and mass transfer line temperature were set at 200 and 240 °C respectively. The oven temperature was programmed at 80 °C to hold for 12 minutes at 10 °C/min to 240 °C to hold for 6 minutes. 2 ml of water solution of the samples was manually inserted in the split less mode, with a split ratio of 1:40 and with a mass scan of 50-600 amu.

The total running time of the GC-MS was 35 minutes. The relative percentage of each extract constituent was expressed as a percentage with peak area normalization. Interpretation of the mass spectrum of the plant extracts was conducted using the data base of the National Institute of Standard and Technology (NIST) library, having more than 62,000 spectral patterns [15].

2.8. Statistical analysis

All experiments were conducted in duplicates. Microbial counts were subjected to analysis of variance (ANOVA) using SPSS, version 26.0 and where significant, means were separated by Duncan Multiple Range Test at $p = 0.05$.

3. RESULTS

3.1. The total viable bacteria counts

The total viable bacteria count (TVBC) ($\times 10^4$ cfu/ml) of the treated and untreated soymilk sample is presented in Table 1. Results showed that there was a reduction in the TVBC of the treated soymilk on each day of storage as concentrations of cloves and ginger increase. For instance, soymilk treated with 1% clove after 24 hours of storage (day 1), the TVBC was 7.50 ± 0.50 which was not significantly different ($p > 0.05$) for soymilk treated with 3% cloves (7.00 ± 1.00) but significantly different ($p < 0.05$) from soymilk treated with 5% cloves (3.00 ± 1.00). Meanwhile, soymilk treated with sodium benzoate (positive control) had no TVBC but untreated soymilk sample showed high TVBC of 38.50 ± 2.50 . By day 3 of storage, the TVBC of soymilk treated with varying concentrations of cloves were 24.50 ± 1.50 (1%), 14.50 ± 1.50 (3%) and 11.50 ± 0.50 (5%) which were significantly different from one another. Besides, the TVBC of soymilk treated with benzoate had low TVBC of 2.00 ± 1.00 while the untreated sample had very high TVBC of 56.50 ± 6.50 . By day 5 of storage, the TVBC of soymilk treated with varying concentrations of cloves were 29.50 ± 1.50 (1%), 21.50 ± 2.50 (3%) and 16.00 ± 1.00 (5%) which were significantly different from one another. The TVBC of soymilk treated with benzoate had low TVBC of 2.00 ± 0.00 while the untreated sample had very high TVBC of 92.00 ± 4.00 (Table 1).

Furthermore, soymilk treated with 1% ginger after 24 hours of storage (day 1), the TVBC ($\times 10^4$ cfu/ml) was 4.00 ± 1.00 which was not significantly different ($p > 0.05$) for soymilk treated with 3% ginger (3.50 ± 0.00) and 5% ginger (0.50 ± 0.50). After 72 hours of storage (day 3), the TVBC of soymilk treated with varying concentrations of ginger were 11.50 ± 1.5 (1%), 8.00 ± 1.00 (3%) and 2.00 ± 0.50 (5%) which were not significantly different from one another but significantly different from benzoated treated sample (2.00 ± 0.00) and untreated soymilk (56.50 ± 6.50). By day 5 of storage, the TVBC of soymilk treated with varying concentrations of ginger were 15.00 ± 1.00 (1%), 15.00 ± 0.00 (3%) and 12.00 ± 1.00 (5%) which were not significantly different from one another but were significantly different from benzoated treated sample (2.00 ± 0.00) and untreated soymilk sample (92.00 ± 4.00).

However, results obtained equally showed that there was an increase in the TVBC of soymilk treated with each concentration of the extracts used as storage days increases (Table 1). For soymilk treated with 1% cloves, the TVBC increased significantly ($p < 0.05$) from 7.50 ± 0.50 (day 1) to 29.50 ± 1.50 (day 5). Also, the TVBC of soymilk treated with 3% cloves increased from 7.00 ± 1.00 (day 1) to 21.50 ± 2.50 (day 5) with no significant difference, while soymilk treated with 5% cloves recorded a significant increase in TVBC from 3.00 ± 1.00 (day 1) to 16.00 ± 1.00 (day 5). Soymilk treated with sodium benzoate, however, showed no significant increase in TVBC from 0.00 ± 0.00 (day 1) to 2.00 ± 0.00 (day 5) but the untreated sample recorded a significantly high increase in TVBC from 38.50 ± 2.50 (day 1) to 92.00 ± 4.00 .

Table 1. Total viable bacteria counts of the treated and untreated soymilk.

Sample	Concentration (%v/v)	Total viable bacteria count ($\times 10^4$ cfu/ml)		
		1	3	5
Soymilk + Cloves	1	7.50 ± 0.50^{Ca}	24.50 ± 1.50^{Db}	29.50 ± 1.50^{Db}
	3	7.00 ± 1.00^{Cb}	14.50 ± 1.50^{Cab}	21.50 ± 2.50^{Cb}
	5	3.00 ± 1.00^{Aba}	11.50 ± 0.50^{BCb}	16.00 ± 1.00^{BCb}
Soymilk + Ginger	1	4.00 ± 1.00^{BCa}	11.50 ± 1.50^{BCb}	15.00 ± 1.00^{Bb}
	3	3.50 ± 0.00^{BCa}	8.00 ± 1.00^{ABCb}	15.00 ± 0.00^{Bb}
	5	0.50 ± 0.50^{Aba}	2.00 ± 0.50^{ABb}	12.00 ± 1.00^{Bc}
Soymilk + Sodium benzoate (Positive control)		0.00 ± 0.00^{Aa}	2.00 ± 1.00^{Aa}	2.00 ± 0.00^{Aa}
Soymilk without preservative (Negative control)		38.50 ± 2.50^{Da}	56.50 ± 6.50^{Ea}	92.00 ± 4.00^{Eb}

Means followed by the same letter in superscripts along the column (upper case) and row (lower case) are not significantly different ($p > 0.05$) from one another using Duncan's New Multiple Range Test (DNMRT).

Also, for soymilk treated with 1% ginger, the TVBC increased significantly ($p < 0.05$) from 4.00 ± 1.00 (day 1) to 15.00 ± 1.00 (day 5). Also, the TVBC of soymilk treated with 3% ginger increased significantly from 4.00 ± 0.00 (day 1) to 15.00 ± 1.00 (day 5) while soymilk treated with 5% ginger recorded significant increase in TVBC from 0.50 ± 0.50 (day 1) to 12.00 ± 1.00 (day 5) (Table 1).

3.2. The total fungi counts

The total fungi counts ($\times 10^4$ sfu/ml) of the treated and untreated soymilk samples was presented in Table 2. Results showed there was a reduction in the total fungi counts of the treated soymilk on each day of storage as concentrations of extracts of both plants increase. For instance, by day 3 of storage, soymilk treated with 1% clove had total fungi count of 9.50 ± 1.50 which was not significantly different ($p > 0.05$) with soymilk treated with 3% cloves (6.00 ± 1.00) but significantly different from soymilk treated with 5% cloves (5.50 ± 1.50). However, these values were significantly different ($p < 0.05$) from sodium benzoated sample (positive control) which had very low fungi count (1.00 ± 0.00) and the untreated sample that recorded high fungal count of 13.50 ± 1.50 . By day 5 of storage, the fungi counts of soymilk treated with varying concentrations of cloves were 9.00 ± 2.00 (1%), 8.50 ± 0.50 (3%) and 7.50 ± 0.50 (5%) and they were not significantly different ($p > 0.05$) from one another but significantly different ($p < 0.05$) from sodium benzoated sample (5.00 ± 1.00) and the untreated sample (14.50 ± 0.50) (Table 2).

Similarly for ginger extract, soymilk treated with 1% ginger by day 3 of storage, had total fungi count of 7.00 ± 1.00 which was not significantly different ($p > 0.05$) from soymilk treated with 3% ginger (6.50 ± 0.50) but significantly different from 5% ginger (2.00 ± 0.00). Interestingly, the total fungi count of 5% ginger was not significantly different ($p > 0.05$) from sodium benzoated sample with low fungi count of 1.00 ± 0.00 but significantly different from the untreated with high fungi count (13.50 ± 1.50). By day 5 of storage, the soymilk treated with 1% ginger had total fungi counts of 9.50 ± 1.50 which was not significantly different ($p > 0.05$) from soymilk treated with 3% ginger (7.00 ± 1.00) but significantly different ($p < 0.05$) from 5% ginger (3.50 ± 0.00) (Table 2).

Furthermore, results obtained equally showed that there was an increase (though non-significant) in the total number of fungi of soymilk treated with each concentration of the extracts as storage days increase. For soymilk treated with 1% clove, the fungi count increased from 9.50 ± 1.50 (day 3) to 9.80 ± 2.00 (day 5).

Table 2. Fungi counts of the treated and untreated soymilk.

Sample	Concentration (%v/v)	Total fungi count ($\times 10^4$ sfu/ml)	
		Days of storage	
		3	5
Soymilk + Cloves	1	$9.50 \pm 1.50^{\text{Db}}$	$9.80 \pm 2.00^{\text{Cb}}$
	3	$6.00 \pm 1.00^{\text{BCDb}}$	$8.50 \pm 0.50^{\text{BCb}}$
	5	$5.50 \pm 1.50^{\text{BCb}}$	$7.50 \pm 0.50^{\text{BCb}}$
Soymilk + Ginger	1	$7.00 \pm 1.00^{\text{CDb}}$	$9.50 \pm 1.50^{\text{Cb}}$
	3	$6.50 \pm 0.50^{\text{BCDb}}$	$7.00 \pm 1.00^{\text{BCb}}$
	5	$2.00 \pm 0.00^{\text{ABb}}$	$3.50 \pm 0.00^{\text{Ab}}$
Soymilk + Sodium benzoate (Positive control)		$1.00 \pm 0.00^{\text{Aa}}$	$5.00 \pm 1.00^{\text{ABb}}$
Soymilk without preservative (Negative control)		$13.50 \pm 1.50^{\text{Eb}}$	$14.50 \pm 0.50^{\text{Db}}$

Means followed by the same letter in superscripts along the column (upper case) and row (lower case) are not significantly different ($p > 0.05$) from one another using Duncan's New Multiple Range Test (DNMRT).

Also, the total fungi count of soymilk treated with 3% clove increased from 6.00 ± 1.00 (day 3) to 8.50 ± 0.50 (day 5) while soymilk treated with 5% clove recorded an increase in total fungi count from 5.50 ± 1.50 (day 3) to 7.50 ± 0.50 (day 5). In the same vein, the fungi count of soymilk treated with 1% ginger increased from 7.00 ± 1.00 (day 3) to 9.50 ± 1.50 (day 5) while, 3% ginger showed an increase in fungi count from 6.50 ± 0.50 (day 3) to 7.00 ± 1.00 (day 5) and fungi count for 5% ginger recorded an increase from 2.00 ± 0.00 (day 3) to 3.50 ± 0.00 (day 5). Soymilk treated with sodium benzoate however showed a significant increase in total fungi count from 1.00 ± 0.00 (day 3) to 5.00 ± 1.00 (day 5) while the untreated sample showed a non-significant increase (though high fungi count) from 13.50 ± 1.50 (day 3) to 14.50 ± 0.50 (day 5) (Table 2).

3.3. The pH values

The pH values of the treated and untreated soymilk samples are presented in Table 3. Results showed that there is a decrease (though not significant) in the pH of the treated soymilk on each storage day as concentrations of extracts increase. For example, the pH by day 1 of storage of soymilk treated with 1, 3 and 5% cloves were 6.17 ± 0.24 , 6.10 ± 0.20 and 6.07 ± 0.23 respectively while soymilk treated with 1, 3 and 5% ginger had respective pH values of 6.59 ± 0.20 , 6.41 ± 0.29 and 6.39 ± 0.31 . Meanwhile, benzoated treated sample had pH 6.40 ± 0.10 while the untreated sample had highest pH of 6.71 ± 0.27 .

In the same vein, the pH values by day 3 of storage for soymilk treated with 1, 3 and 5% cloves were 6.10 ± 0.50 , 6.02 ± 0.15 and 6.02 ± 0.12 respectively while soymilk treated with 1, 3 and 5% ginger had respective pH values of 6.51 ± 0.27 , 6.33 ± 0.29 and 5.21 ± 0.33 . Meanwhile, benzoated treated sample had pH of 6.10 ± 0.10 while the untreated sample had the highest pH of 6.66 ± 0.06 among all the various samples. Also, by day 5 of storage, the pH values of soymilk treated with 1, 3 and 5% of cloves were 5.90 ± 0.41 , 5.98 ± 0.19 and 5.85 ± 0.27 respectively while soymilk treated with 1, 3 and 5% ginger had respective pH values of 6.22 ± 0.35 , 5.95 ± 0.36 and 5.19 ± 0.30 (Table 3). Meanwhile benzoated treated sample had pH of 5.35 ± 0.15 while the untreated sample had highest pH of 6.47 ± 0.10 among all the various samples.

Similarly, results also showed that there was a decrease in the pH values of treated soymilk as days of storage increase. For instance, the pH values from day 1 to day 5 of storage reduced significantly for soymilk treated with 1% cloves ($6.17 \pm 0.24 - 5.90 \pm 0.41$), 3% cloves ($6.10 \pm 0.20 - 5.89 \pm 0.19$), 5% cloves ($6.07 \pm 0.23 - 5.85 \pm 0.27$), 1% ginger ($6.59 \pm 0.20 - 6.22 \pm 0.35$), 3% ginger ($6.41 \pm 0.29 - 5.95 \pm 0.36$), 5% ginger

($6.39 \pm 0.31 - 5.19 \pm 0.30$), benzoated treated soymilk ($6.40 \pm 0.10 - 5.35 \pm 0.15$) and untreated soymilk sample ($6.71 \pm 0.27 - 6.47 \pm 0.10$) (Table 3).

Table 3. pH of treated and untreated soymilk.

Sample	Concentration (%v/v)	pH values		
		1	Storage days 3	5
Soymilk + Cloves	1	6.17 ± 0.24^{aB}	6.10 ± 0.50^{aA}	5.90 ± 0.41^{aA}
	3	6.10 ± 0.20^{aB}	6.04 ± 0.15^{aA}	5.89 ± 0.19^{aA}
	5	6.07 ± 0.23^{aB}	6.02 ± 0.12^{aA}	5.85 ± 0.27^{aA}
Soymilk + Ginger	1	6.59 ± 0.20^{aB}	6.51 ± 0.27^{aA}	6.22 ± 0.35^{aA}
	3	6.41 ± 0.29^{aB}	6.33 ± 0.29^{aA}	5.95 ± 0.36^{aA}
	5	6.39 ± 0.31^{aB}	5.21 ± 0.33^{aA}	5.19 ± 0.30^{aA}
Soymilk + Sodium benzoate (Positive control)		6.40 ± 0.10^{aB}	6.10 ± 0.01^{aB}	5.35 ± 0.15^{aA}
Soymilk without preservative (Negative control)		6.71 ± 0.27^{aB}	6.66 ± 0.06^{aB}	6.47 ± 0.10^{aA}

Means followed by the same letter in superscripts along the column (lower case) and row (upper case) are not significantly different ($p>0.05$) from one another using Duncan’s New Multiple Range Test (DNMRT).

3.4. The chromatogram of aqueous extract of cloves and ginger

The chromatogram of aqueous extract of cloves and ginger obtained using the GC-MS is shown in Figures 1 and 2 respectively.

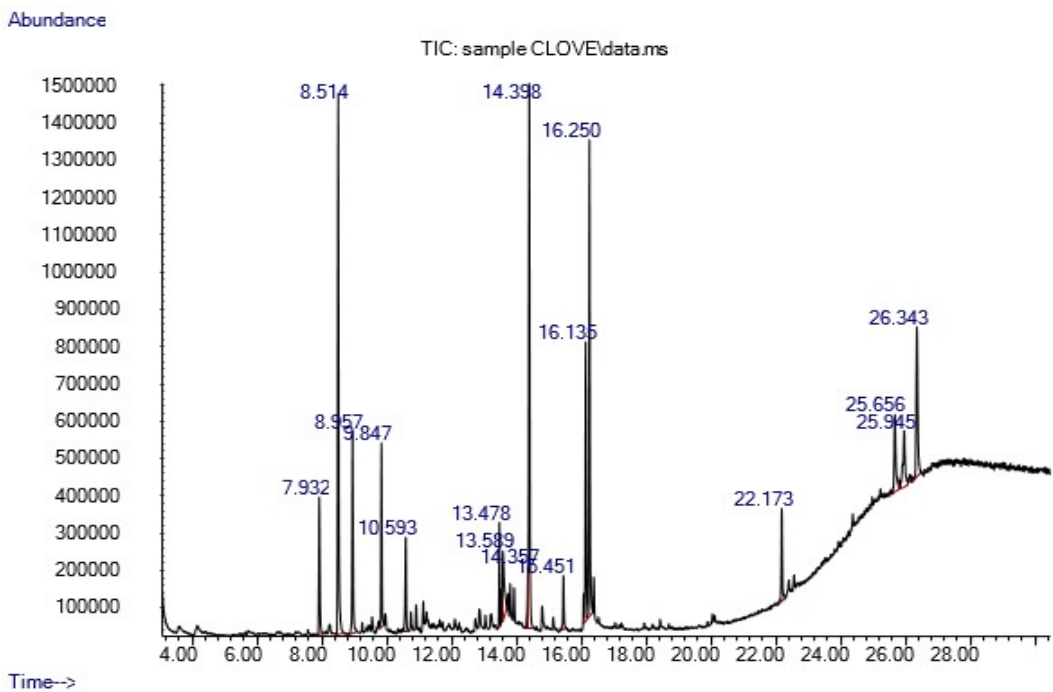


Figure 1. Chromatogram of aqueous extract of clove (*Syzygium aromaticum*).

Besides, bioactive compounds with their peak area (%) detected in clove extract included alpha-cubebene (4.35), caryophyllene (16.59), humulene (6.28), 6-hexahydronaphthalene (4.95), caryophyllene oxide (2.97), 3-hexadecyne (2.27), methyl hexadec-9-enoate (1.49), hexadecanoic acid (15.71), geranyl-alpha-

terpinene (1.77), octadecatrienoic acid (8.26), 1,4-eicosadiene (12.99), supraene (3.07), cyclotrisiloxane hexamethyl (4.27), tris (tert-butyldimethylsilyloxy)arsane (3.92) and decamethyltetrasiloxane (7.16) (Table 4) while neophythadiene (3.12), 2-pentadecanone,6,10,14-trimethyl (1.70), hexadecanoic acid (18.54), 8,11-octadecadienoic acid (2.26), 9,12,15-octadecatrienoic acid (14.11), phytol (16.55), methyl stearate (3.75), squalene (2.83), decamethyltetrasiloxane (1.32), cyclotrisiloxane hexamethyl (3.52), tris(tert-butyldimethylsilyloxy)arsane (8.16) and beta-amyrin (24.15) were obtained from the ginger extract (Table 5).

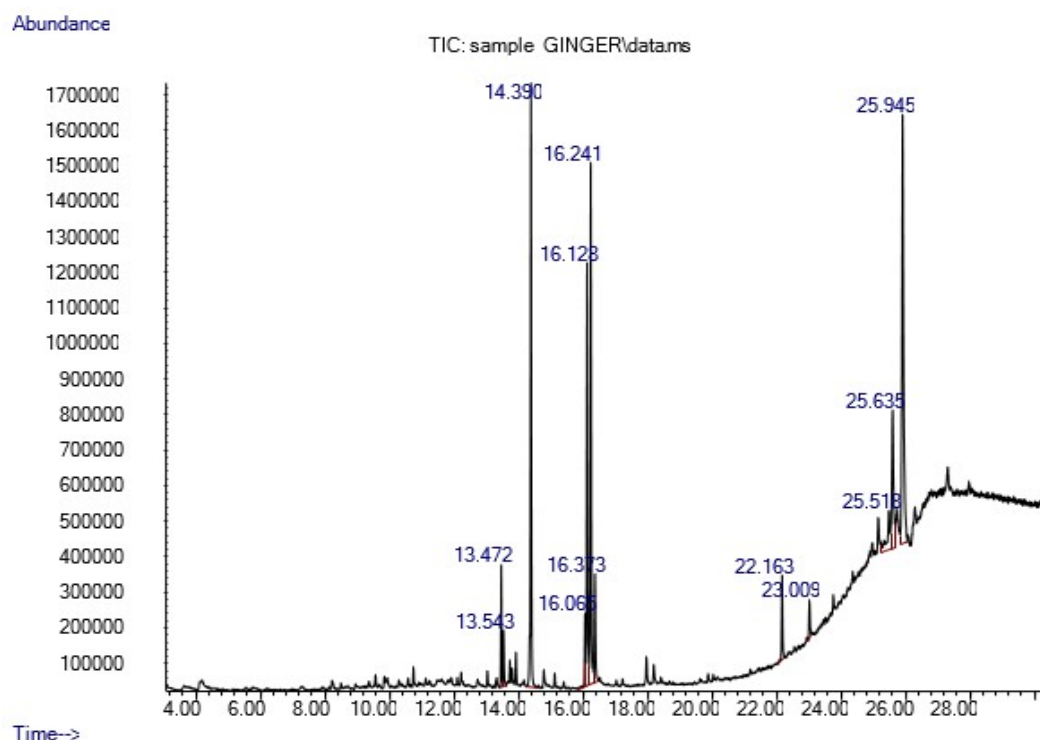


Figure 2. Chromatogram of aqueous extract of ginger (*Zingiber officinale*).

Table 4. Bioactive compounds of cloves using GC-MS.

S/N	Rt (Min)	Peak Area (%)	Compounds	Molecular Formula	Molecular weight (g/mol)
1	7.932	4.35	Alpha-cubebene	C ₁₅ H ₂₄	204
2	8.514	16.59	Caryophyllene	C ₁₅ H ₂₄	204
3	8.957	6.28	Humulene	C ₁₅ H ₂₄	204
4	9.847	4.95	6-Hexahydronaphthalene	C ₁₀ H ₁₄	134
5	10.593	2.97	Caryophyllene oxide	C ₁₅ H ₂₄ O	220
6	13.478	2.27	3-Hexadecyne	C ₁₆ H ₃₀	222
7	14.357	1.49	Methyl hexadec-9-enoate	C ₁₇ H ₃₂ O ₂	268
8	14.398	15.71	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270
9	15.451	1.77	Geranyl- alpha-terpinene	C ₂₀ H ₃₂	272
10	16.135	8.26	Octadecatrienoic acid methyl ester	C ₁₉ H ₂₂ O ₂	292
11	16.250	12.99	1,4-Eicosadiene	C ₂₀ H ₃₈	278
12	22.173	3.07	Supraene	C ₃₀ H ₅₀	410
13	25.656	4.27	Cyclotrisiloxane, hexamethyl	C ₆ H ₁₈ O ₃ Si ₃	222
14	25.945	3.92	Tris(tert-butyldimethylsilyloxy)arsane	C ₁₈ H ₄₅ AsO ₃ Si ₃	468
15	26.343	7.16	Decamethyltetrasiloxane	C ₁₀ H ₃₀ O ₃ Si ₄	310

Table 5. Bioactive compounds of ginger using GC-MS.

S/N	Rt (Min)	Peak Area (%)	Compounds	Molecular formula	Molecular weight (g/mol)
1	13.472	3.12	Neophythadiene	C ₂₀ H ₃₈	278
2	13.543	1.70	2-Pentadecanone,6,10,14-trimethyl	C ₁₈ H ₃₆ O	268
3	14.390	18.54	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270
4	16.065	2.26	8,11-Octadecadienoic acid	C ₁₉ H ₃₄ O ₂	294
5	16.128	14.11	9,12,15-Octadecatrienoic acid	C ₁₉ H ₂₂ O ₂	292
6	16.241	16.55	Phytol	C ₂₀ H ₄₀ O	296
7	16.373	3.75	Methyl stearate	C ₁₉ H ₃₈ O ₂	298
8	22.163	2.83	Squalene	C ₃₀ H ₅₀	410
9	23.009	1.32	Decamethyltetrasiloxane	C ₁₀ H ₃₀ O ₃ Si ₄	310
10	25.518	3.52	Cyclotrisiloxane, hexamethyl	C ₆ H ₁₈ O ₃ Si ₃	222
11	25.635	8.16	Tris(tert-butyldimethylsilyloxy)arsane	C ₁₈ H ₄₅ AsO ₃ Si ₃	468
12	25.945	24.15	Beta-amyrin	C ₃₀ H ₅₀ O	426

4. DISCUSSION

The increase in the TVBC and total heterotrophic fungi counts of all the treated soymilk samples as days of storage increase, with more bacteria counts than fungi spores was in agreement with the previous work [16] who recorded more bacterial counts than fungal counts in their own soymilk samples treated by heat. Also, Adebayo-Tayo et al. [3] affirmed that more bacteria were isolated from their work on powdered soymilk samples. The observed more bacterial counts in this study must have been influenced by pH as buttressed by [17] that pH favours bacteria growth. Findings from this work showed that the pH range of all the soymilk samples were between 5.19-6.75 and this consequently resulted in higher bacteria counts than fungi which are more acid tolerant.

Interestingly, by day 3 of storage, soymilk treated with 5% ginger extract aside all other treated samples had the least bacterial and fungal counts that are not significantly different from the benzoated treated sample while untreated soymilk had comparatively higher bacteria and fungi counts. Remarkably, the microbial population obtained in both soymilk + 5% ginger extract and benzoated samples was below the acceptable limit of 2.0×10^4 cfu/g recommended for general bacterial count by the Soy Foods Association of America (SFAA) and did not also exceed the acceptable limit for pasteurized milk (3×10^4 cfu/ml). Thus, this showed that 5% ginger extract concentration was able to compete favourably with the chemical preservative by extending the shelf life of the treated soymilk for 72 hours at ambient storage as against the untreated that had already deteriorated after 24 hours of storage. In fact, the untreated samples had the highest bacterial / fungi load throughout the storage period when compared with all the treated samples.

The reduction in the microbial counts of treated samples cannot but be connected with the inhibitory effect of the extracts on the growth of microorganisms. This was supported by Tajkarimi et al. [18] who reported the use of antimicrobial herbs and spices in food. Similarly, Gottardi et al. [19] stressed the beneficial effects of spices in food preservation and safety. Spices contain chemical compounds which slows down or inhibit the growth of spoilage microorganisms. This assertion was also supported by Adeleke et al. [17] who confirmed the effectiveness of aqueous and ethanolic extracts of ginger and garlic on the nutritional quality of stored soymilk. Even, Wakoli et al. [20] reported low spoilage rate in foods treated with selected spices such as garlic, cumin, ginger, sage when compared with nontreated samples. This lower microbial load observed in this work (below the acceptable limit) in treated samples especially the 5% ginger could be traced to presence of bioactive

compounds in the extract which has antimicrobial activity and are able to inhibit growth of microorganisms. In fact, garlic, ginger, cinnamon and cloves have been shown to have bactericidal and antifungal properties. Their antimicrobial action is as a result of the phenolic compound content that bind to the microbial enzymes and destroy them [21].

The GC-MS of aqueous extracts of these herbs revealed the presence of caryophyllene (16.59%), hexadecanoic acid (15.71%) as the major bioactive compounds in clove extract indicated by their % peak area and beta-amyrin (24.15%), hexadecanoic acid (18.54%), 8,11-9,12,15-octadecatrienoic acid (14.11%), phytol (16.55%) were obtained from the ginger extract as the major bioactive agents. Interestingly, these active ingredients are phenol derivatives which have been identified to exhibit strong activities against several pathogens and insect pests [22]. Even Ngoci et al. [23] further corroborated the toxicity of these active compounds by reporting that they can acts as potential antifungal natural substitutes.

In fact, inhibitory activity of these extracts can be attributed to the presence of several constituents namely caryophyllene, beta-amyrin, hexadecanoic acid and phytol which could be described as potent antifungal and antibacterial compounds. Perhaps these bioactive agents were present in higher amounts in 5% ginger and therefore caused a significant reduction in the TVBC and fungi count of the treated soymilk even below the acceptable limit. Conversely, their lower amounts in lesser concentrations (1, 3%) of these extracts probably explained why the TVBC and fungi counts of the treated soymilk though significantly lower than the untreated samples, exceeded the acceptable limit for pasteurized milk (3×10^4 sfu/ml).

Although the following bacteria isolates were obtained from the soymilk samples: *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus* sp. and *Lactobacillus* sp., the TVBC on day 3 of storage for soymilk + 5% ginger ($2.00 \pm 0.00 \times 10^4$ cfu/ml) did not exceed the acceptable limit and the milk could thus be considered fit for consumption. Likewise, *Aspergillus flavus*, *A. niger*, *Saccharomyces cerevisiae*, *Cladosporium* spp. *Penicillium* spp. were the fungal isolates and fortunately, the total heterotrophic counts on day 3 of storage in soymilk + 5% ginger ($2.00 \pm 0.00 \times 10^4$ sfu/ml) did not also exceed the acceptable limit that could have rendered the drink unfit for consumption. This is because total microbial counts is an indicator of the hygienic quality of food products [24]. Besides, a large number of microorganisms such as mesophilic aerobic bacteria, coliforms and fungi, are to be present to initiate and cause spoilage of soymilk, producing undesirable changes [25]. Hence, this showed the efficacy of ginger over clove particularly the 5% concentration in extending the shelf life of soymilk by three days after preparation,

5. CONCLUSIONS

This study confirms that the use of aqueous extract of ginger (*Zingiber officinale*) and cloves (*Syzygium aromaticum*) appreciably prolong the shelf life of soymilk at ambient temperature with ginger having a better result by keeping soymilk for up to 72 h at ambient temperature. Hence, the extract of ginger represents an alternative source of natural antimicrobial substance that can be explored in food industries to prevent the growth of food borne microorganisms.

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