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Effect of Local Preservatives on Improving the Shelf Life of Soymilk

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Abstract

The ensuing increase in soymilk consumption has led to small-scale production of milk under household conditions, with little or no consideration of quality control measures. Local preservatives are nowadays applied to preserve many local drinks. This study determines the effect of local preservatives on improving the shelf-life of soymilk. Clean soybean seeds collected from local markets were soaked, blended, and filtered to obtain soymilk. Extracts of certain spices (garlic, ginger, and clove) were added at various concentrations, singly and in combination, coupled with pasteurization and refrigeration. Standard microbiological techniques such as the pour plate method and culturing were used to enumerate and isolate spoilage microorganisms in the sample over the test period. Results obtained showed that the combination of ginger-clove extract was more effective, it preserved the soymilk sample for 10 days. The spices could be used for a certain period to extend the shelf life of soymilk and they could also be used as an alternative source of natural antimicrobial substance in Soymilk. The microorganisms isolated from the spoiling soymilk after 10 days include Pseudomonas spp. *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Aspergillus fumigatus, Aspergillus lentulus, Aspergillus niger, Rhizopus stolonifer* and *Trichosporon asahil.* The isolation of these spoilage organisms after a given period implies that the preservative effect of the local spices used in this study is time dependent. The synergetic use of ginger, clove, and other local preservatives as preservatives for local drinks should be explored further and optimized.

Keywords Soymilk, ginger, clove, preservative efficiency

1.0 INTRODUCTION

People in many developing nations eat mostly starchy roots, cereals, and a few legumes as their primary sources of nutrition. Unfortunately, animal sources of protein, which are needed to complement starchy foods, are sometimes too expensive and unaffordable for low-income families, especially in underdeveloped and developing nations [1]. Recently, it has been discovered that different edible legume varieties have high plant protein, so they can help solve many nutrition-related problems around the world [1,2]. For large populations in poor tropical countries, grains and legumes are a cheap source of protein. In an effort to increase the availability of milk-like products, particularly in regions where milk is in short supply, numerous legume-based milk and milk derivatives have been produced. Legumes are crucial sources of reasonably priced protein, thus the development of imitation milk products derived from them will make protein deficiencies easier to develop [2].

The development of milk substitutes derived from beans is an option for the production of acceptable nutritious foods. Soybean (Glycine max) which is known as a legume plant aborigine to East Asia is widely cultivated as an edible legume that has multiple uses. Soy cheese, soymilk, soy vegetable oil, and soy *nono* are some examples of soy goods. A thick, creamy soybean extract called soymilk is comparable to industrial cow milk. It is a well-liked nutritious substitute for cow's milk and is even less expensive than cow's milk [3]. Consequently, it has very good health properties and a high nutritional value. In addition to having a substantial amount of protein, it also contains a wealth of nutritious fibre, both soluble and insoluble and isoflavones, which are crucial for every diet [4]. The bean can help avoid several heart diseases, cancer, bone loss, and menopausal symptoms because it has low cholesterol and lactose levels [5].

Men who consume a diet high in soy are less likely to get prostate cancer. During menopause, women's natural production of estrogen will be minimized. The rapid reduction of estrogen has brought many health problems to postmenopausal women. Women who have had menopause are more susceptible to heart disease, diabetes, and obesity. Similarly, they are more prone to psychological issues like sleeplessness, mood swings, and despair. Soybeans contain phytoestrogens which work well to replace estrogen. Soy consumption is a great approach to preventing and treating certain postmenopausal disorders [6].

Nigeria has not yet maximized the potential of soy milk as a substitute for cow milk as an inexpensive and high-quality protein source. Therefore, it is obvious that encouraging the production and consumption of soymilk will yield high economic returns for the country [5, 7]. More people will get cheaper high-quality protein for good health. The nutritional properties of soy milk make it susceptible to microbial attack, so its shelf life is limited. A large number of microorganisms such as aerobic mesophilic bacteria, coliform bacteria and fungi can lead to spoilage of soymilk and result in undeserved spoilage [8]. Extending the shelf life of soy products saves time and effort in their manufacture [9]. By controlling the development of microorganisms coupled with food rot and pathogenicity, this can be achieved.

The application of preservatives in food has been in use for many years. The effectiveness of chemical preservatives depends on the concentration used, the composition of the food, nutrients, and the type of spoilage microorganisms. In low concentrations, the chemical preservative sodium benzoate can slow down or inhibit the activity of microorganisms. The ability of benzoate to combat bacteria and mold depends on pH, so it shows antibacterial activity at low pH. Its antibacterial action is due to the fact that the entire molecule of these compounds is inactive at neutral pH. However, it has been established that using chemical preservatives may adversely affect consumers [10]. Adverse effects of sodium benzoate on human health, including cellular damage, have been confirmed [10, 11]. This has led to increased consumer concern about foods that contain little or no chemical preservatives as they can be toxic [10].

Nowadays, consumers express a desire for goods with a lengthy shelf life and no risk of contracting a foodborne illness. In an attempt to achieve their objectives for microbiological safety, the food industries are compelled by this viewpoint to pursue the elimination of chemical preservatives which are sometimes detrimental to consumers' health and the introduction of natural alternatives [11]. Therefore, there is great interest in natural preservatives that are less expensive and do not pose toxicological or environmental concerns [12]. This study aimed to determine the effectiveness of some local preservatives such as ginger, garlic and cloves to extend the shelf-life of soymilk.

2.0 Materials and Methods

2.1 Production of Soymilk

About 5 kg of soybean grain was purchased from a local market in Minna, Nigeria. The beans were sorted to remove the dirty particles, cracks, stones, and discolored seeds and washed with distilled water. They were soaked in about 4 liters of sterilized water for 12 hours to soften the seed. The outer layers of the beans were removed by holding the beans between the producer’s clean palms and rubbing them against one another under clean water. The bean seeds were washed and rinsed. The bean was mashed into a paste using a pristine electric blender, and the milk was then removed (sieved) from the paste using a clean muslin cloth. The milk obtained was heated steadily at about 100°C in a heavy-bottomed pan for 20 minutes with occasional steering to prevent sticking. This crucial step eliminated the beany taste of the soybean and made it consumable [13].

2.2 Extraction of Spices

Peeled, cleaned, thinly sliced, and air-dried rhizomes of ginger, clove and garlic were used. Using a pristine electric blender, the dried spices were ground. Then, the extracts were made by soaking 100 g of each of the powders for ginger, garlic, and cloves in 60 mL of distilled water and soaked for 72 hours at room temperature. Extracts were collected in conical flasks, filtered using filter papers and then, refrigerated following the techniques demonstrated by Panpatil et al. [14].

2.3 Addition of Preservatives

The soymilk was allowed to cool and dispensed into 25 separate sterilized bottles, a total of 24 bottles received preservation treatment, while the 25th bottle acts as the control. Each of the three preservatives - garlic, ginger, and clove extracts as well as combinations of the three - garlic, ginger, and clove extracts, clove extract, and garlic extract - as well as acetic acid were employed. The concentrations of these preservatives utilized were 1%, 3%, and 5% v/v. The preservatives were included in the bottled soymilk, which was also correctly labelled and kept chilled.

2.4 Total Microbial Counts of Soymilk Treated with Preservatives

To evaluate the number of microbial cells and the effect of various extracts of preservatives on the shelf life of soymilk, the total bacterial count was conducted following the basic microbiological procedures. Briefly, the soymilk samples were serially diluted, 0.1 mL of each dilution was poured plated on nutrient agar and incubated over 24 hours. The resulting colonies on the plate were counted and multiplied by the dilution factor and expressed as colony forming unit (CFU) per mL These were carried out right away after soymilk production and subsequently every five days.

2.4.1 Total Viable Count

After a thorough shaking, 1 mL of the soymilk sample was taken using a sterile pipette. Sterile test tubes were placed in a plastic rack that contained 9 mL of sterile distilled water. A tenfold serial dilution was performed by placing 1 mL of the sample into the first test tube with the label 10^{-1}. This was mixed thoroughly. Once more, 1 mL was taken from the 10^{-1} dilution tube and put into the 10^{-2} test tube. As a result, the dilution reached 10^{-5}. Before each transfer, the test tubes were individually firmly shaken.

2.4.2 Inoculation

The various samples were plated using the pour plate technique. Using a sterile pipette, 0.1 mL of the dilution 10^{-3} was placed into a sterile petri dish. Assorted Petri dishes containing 0.1mL of diluted sample were filled with molten nutrient agar and potato dextrose agar prepared in accordance with the instructions provided by the manufacturer. The plates were turned clockwise to make it simple to combine the sample and media. On the bench, all plates were allowed to solidify. Then, duplicate samples of each plate were taken. While plates for bacterial isolation were kept in a separate incubator at 37°C for 18–24 hours, the potato dextrose agar plates were placed at 25 C for 3-5 days in another incubator.

2.4.3 Counting of Colonies

After each plate had been incubated, the colonies in each plate were counted manually using a tally counter. The count's mean value was calculated, and it was then multiplied by the proper dilution factor. The count's average value was calculated and multiplied by the dilution factor.

2.4.4 Isolation of Microorganisms from the Soymilk Sample

As previously mentioned, the soymilk samples were serially diluted. All the samples were plated using the pour plate culturing technique. Using a sterile pipette, 0.1 mL of serially diluted sample was injected into each sterile Petri dish from the 10^{-3} dilution tube. The dishes were filled with molten potato dextrose and nutrient agar. All the plates were then left to solidify on the bench. The remaining plates were placed into a different incubator at 37°C for 18–24 hours [23], while the potato dextrose agar plates were all moved into an incubator at 25°C for 3–5 days. Every day, the bacterial growth, and the mycelia on all the incubated plates were checked. Discrete colonies from bacteria plates were taken out after the incubation period and subcultured onto freshly made nutrient agar plates using a flamed wire loop. Similarly, new PDA plates were subcultured with various colors of mycelia development from potato dextrose agar plates using a flamed loop wire. Accordingly, all plates were incubated. The PDA plates were cultured at 25°C for 3–5 days, whereas all the nutrient agar plates were placed in an incubator at 37°C for 18–48 hours. The pure cultures of mycelia and colonies that developed were transferred to slants and preserved properly for characterization and identification.

2.4.5 Characterization of Purified Fungal Cultures

The physical properties of the mycelia were examined for their structure and color. The morphological structure was also used to determine microscopic features following the Wet Mount technique that was used before viewing with ×40 objective lens of the microscope [22]. The morphological structures include sporangiophores, fruiting bodies, septate and non-septate structures, as well as unique organisms like rhizoids and others. Each isolate's morphological structure was compared with a known taxa using the Atlas of Food Microbiology for identification [15].

2.4.6 Characterization of Purified Bacterial Culture

Each pure bacterial specimen underwent macroscopical, microscopic, and gram staining examinations to assess its gram status. Through a few routine biochemical tests, the bacterial isolates were characterized. Additionally, tests for sugar fermentation were performed and reported. The findings were compared to an atlas of bacterial species [16].

3.0 RESULTS AND DISCUSSION

3.1 Total Bacteria Count of the Treated Soy Milk

The total bacterial count is displayed in Table 1. It was observed on day 1 that ginger extract 3% had the least bacterial count of 0.40×10^3 from the single extract, while ginger-clove 5% had the least count of 0.30×10^3 from the combined extract. Garlic

5% has the highest count of 0.90 x 10^3, while ginger-garlic-clove 5% has the highest count from the combined extract. On day 5, clove 3% had the least count of 1.00 x 10^4 from the single extract, while ginger-clove 3% had the least count of 1.04 x 10^4 from the combined extract. Ginger 1% had the highest count of 1.80 x 10^4 from the single extract, while garlic-clove 1% had the highest count of 1.82 x 10^4 from the combined extract. On day 10, clove 5% had the least count of 0.50 x 10^4, from the single extract, while ginger-clove 3% had the least count of 1.40 x 10^4 from the combined extract. Ginger 1% had the highest count of 2.90 x 10^4 from the single extract, while ginger-clove 1% had the highest count of 2.30 from the combined extract. On day 15, clove 5% had the least count of 0.76 x 10^4, while ginger-clove 3% had the least count of 0.65 x 10^4 from the single extract. Garlic 1% had the highest count of 2.88 x 10^4, while ginger, garlic-clove had the highest count of 3.00 x 10^4 from the combined extract. It is evident that at all sampling time, soymilk samples were found to undergo several changes in viable bacteria count. There was a general increase in the count as storage times increased, and the spices' ability to preserve food was likewise reduced with longer storage times. It was observed from the bacteria load in some samples that the higher the concentration of preservatives/spices, the lower the bacteria count. Sagdic *et al.* [17] have earlier discovered these; they found that as the concentration of Zingiber officinale (ginger), *Monodora myrystica* (Ehuru), and *Syzygium aromaticum* in zobo beverages increased, the microbial load decreased.

Table 1 Bacterial total plate count for treated soymilk sample.

Soymilk Sample	Concentration of Extract (ml)	Days of Storage and Diluting Factor			
		Day 1 (10^3)	Day 5 (10^4)	Day 10 (10^4)	Day 15 (10^3)
Ginger	1	0.64	1.80	2.90	TNTC
	3	0.40	1.10	1.80	2.60
	5	0.88	1.52	2.00	2.80
Garlic	1	0.86	1.40	TFTC	2.88
	3	0.74	1.34	1.75	TNTC
	5	0.90	1.08	TFTC	1.68
Clove	1	0.89	1.78	2.00	1.12
	3	0.68	1.00	0.67	0.92
	5	0.44	1.48	0.50	0.76
Ginger-Garlic	1	0.68	1.64	2.16	2.88
	3	1.00	1.52	TFTC	2.60
	5	0.84	1.68	2.25	2.12
Ginger-Clove	1	0.56	1.52	0.68	2.04
	3	0.84	1.04	0.40	0.65
	5	0.30	1.32	0.72	2.60
Garlic-Clove	1	1.08	1.82	2.30	2.80
	3	0.94	1.41	2.00	2.12
	5	0.62	1.12	TFTC	1.20
Ginger, Garlic, Clove	1	0.84	1.60	1.72	2.28
	3	0.50	1.30	TNTC	2.84
	5	1.20	1.18	TNTC	3.00
Acetic Acid	1	0.90	1.64	2.42	2.85
	3	0.55	1.36	2.15	1.80
	5	0.32	1.18	1.90	2.40
Control		1.01	2.10	2.57	1.32

3.2 Identification of Bacteria Isolates from Soymilk Sample

The results of cultural, morphological, and biochemical characteristics of bacteria isolates from the soymilk sample are presented in Table 2. The bacteria isolates identified include Pseudomonas spp, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Isolate 1 was negative rod shape, catalase, methyl red positive and citrate, coagulase, indole negative and was identified to be *E. coli*, isolate 2 was negative cocci shape, catalase, citrate, coagulase, indole positive and methyl red negative, it was identified to be Pseudomonas species. Isolate 3 was positive cocci shape, catalase, citrate, coagulase, indole positive and methyl red negative and was determined to be *S. aureus*. Isolate 4 was positive rod shape, catalase, citrate, coagulase, indole positive and methyl red negative and was identified as *B. cereus*. Isolate 5 was determined to be *B. subtilis* based on the biochemical characteristics displayed.

Table 2 Biochemical test.

Isolates	Gram staining	Catalase	Citrate	Coagulase	Methyl red	Indole	Probable organism
1	ROD -	+	-	-	+	-	*E. coli*
2	Cocci -	+	+	+	-	+	*Pseudomonas spp*
3	Cocci +	+	+	+	-	+	*S. aureus*
4	Rod +	+	+	+	-	+	*B. cereus*
5	Rod +	+	+	+	-	+	*B. substilis*

3.3 Identification of fungi isolates

The result of the fungal identification is shown in Table 3. The fungi found to be associated with soymilk in this research work are *Trichosporona asahil*, *Aspergillus fumigatus*, *Aspergillus lentulus*, *Aspergillus niger* and *Rhizopus stoloniffer*. The morphological features of the isolated fungi are also displayed in Table 3.

Table 3 Cultural and morphological characteristics of fungi isolate from soymilk sample.

Isolates	Cultural characteristics	Morphological characteristics	Probable organism
1.	Dark brown colony with whitish reverse side	Conidiophores are septate and unbranched	*A. niger*
2.	Whitish long hyphae, growing rapidly	Aseptate hyphae with branched sporangiospores	*R. stoloniffer*
3.	Smaller globose vesicles	Small conidial heads with diminutive vesicles	*A. lentulus*
4.	White filamentous showing dark grey reverse	Smooth wall conidiophores	*A. fumigatus*
5.	White to creamy, semi-shiny, smooth fissures with fimbriate margin	Septate hyphae with arthroconidia	*T. asahil*

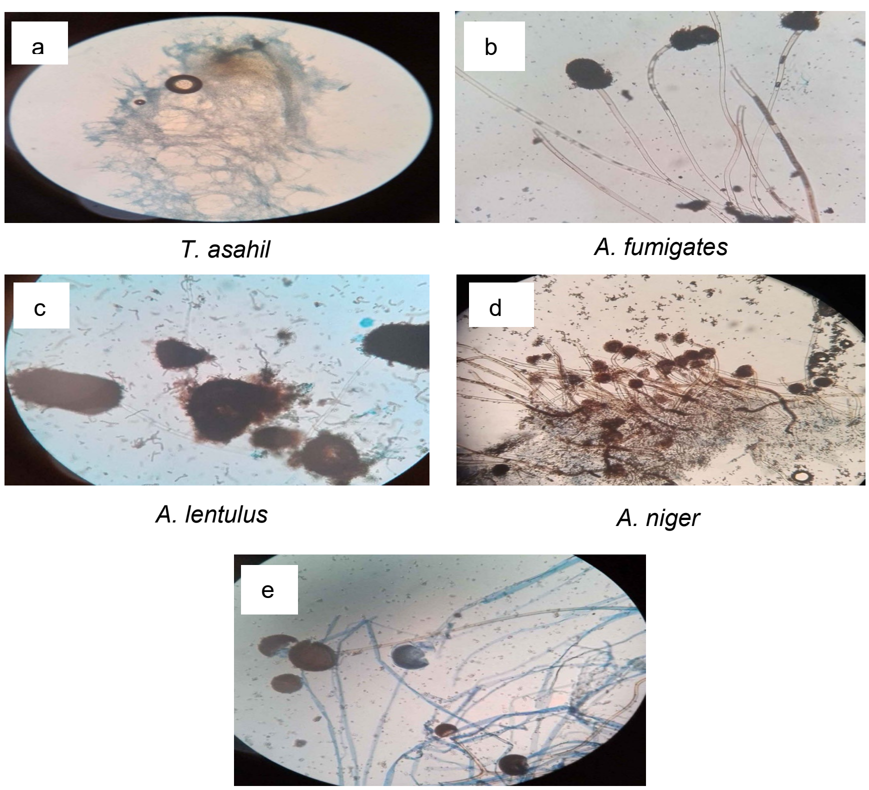

Figure 1 The microscopical features of the isolated fungi (a) *T. asahil (b) A. fumigates (c) A. lentulus (d) A. niger (e) R. stoloniffer*

The microbial contaminants isolated in this study are of great importance because of their pathogenic effect. The isolated bacteria include *E. coli*, *Pseudomonas spp, B. cereus, B. subtilis, S. aureus*, fungi isolates include *T. asahil., A. fumigatus, A. lentulus, A. niger and R. stoloniffer* as illustrated in Figure 1. Some of these sets of fungi have earlier been reported by Odu *et al.* [18], who reported that the dominant fungal flora associated with spoilage of soymilk include *Saccharomyces cerevisiae* and Aspergillus sp. The likely sources of microorganism contamination may be through soil, air, method of production, storage condition, food handler and processing equipment.

3.4 Evaluation of Shelf-Life Quality of Soymilk

Shelf-life studies (Table 4) indicated that refrigerated soymilk samples lacking preservatives (control) were found suitable for consumption 5 days after production compared to the unrefrigerated and unpreserved soymilk samples that got spoiled after 24 hours. This is due to the combined effects of pasteurization and refrigeration [19,21]. Most growing organisms in the soymilk product may have been severely harmed by pasteurization, which would have led to spoiling sooner than what was actually observed and controlled microbial proliferation at low temperatures. This is consistent with the findings of Mbajiuka *et al.* [19], who observed that soymilk that had been pasteurized and refrigerated was safe to consume four days after the day it was produced. Ginger extract 5% preserved the soymilk sample for 8 days, 3% preserved it for 6 days, while 1% preserved it for 4 days. This agrees with the work of Akani *et al.* [20], who reported that the prolonged shelf life of ginger treated soymilk at room temperature was due to its antimicrobial property. Clove extract at 5% and 3% preserved the soymilk sample for 8 days, and 1% preserved it for 6 days. The result is similar to the work of Kabiru et al. [21], who opined that soymilk samples might be conserved with clove extract for up to 8-days in the refrigerator.

Garlic 5% and 3% preserved soymilk sample for 6 days while 1 % preserved it for 3 days. Ginger-Garlic extract 5% and 3% preserved soymilk sample for 5 days while 1% preserved it less than 5 days. Ginger-clove extract 5% and 3% preserved soymilk sample for 10 and 8 days, respectively, while 1% preserved it for 4 days. Garlic-clove extract 5 and 3% preserved the soymilk sample for 5 and 4 days, while 1% preserved it for 3 days. Ginger, garlic-clove extract 5% preserved the soymilk sample for 5 days, while 3 and 1% preserved it for 4 days. Acetic acid 5 and 3% extract preserved soymilk sample for 10 days while 1% preserved it for 6 days. Preservation of soymilk sample using a combination of ginger-clove extract was found to be more effective than other combinations and also, the single extract extended the shelf life of soymilk for 10 days. Thereby indicating synergistic effect of the two extracts. Mbajiuka *et al*. [17] reported similar synergistic effects of ginger and garlic in extending the shelf life of “kunun-zaki” and “burukuru”. respectively.

Table 4 Changes in soymilk sample and evaluation of shelf-life quality of soymilk.

Sample	Concentration of extract (%)	Storage time (days)	Appearance/odour
Soymilk 1		1	Creamy, curdling off odour
Control		5	Creamy, non-curdling, beany
Ginger	1	4	Creamy, curdling, off odour
	3	6	Creamy, non-curdling beany
	5	8	Creamy, non-curdling, beany
Clove	1	6	Tea-brown, non-curdling, beany
	3	8	Tea-brown, non-curdling, beany
	5	8	Tea-brown, non-curdling, beany
Garlic	1	3	Creamy, curdling, off odour
	3	6	Creamy, curdling, off odour
	5	6	Creamy, non-curdling, beany
Ginger-Garlic	1	4	Creamy, curdling, off odour
	3	5	Creamy, curdling, off odour
	5	5	Creamy, curdling, off odour
Ginger-Clove	1	4	Creamy, curdling, off odour
	3	8	Creamy, non-curdling, beany
	5	10	Creamy, non-curdling, beany
Garlic-clove	1	3	Tea-brown, curdling, off odour
	3	4	Tea-brown, curdling, off odour
	5	5	Tea-brown, curdling, off odour
Ginger, Garlic-Clove	1	4	
	3	4	
	5	5	
Acetic acid	1	6	Creamy, non-curdling, beany
	3	10	Creamy, non-curdling, beany
	5	10	Creamy, non-curdling, beany

3.5 Prevalence of Bacterial Isolates

The prevalence of bacterial isolates is depicted in Figure 2. The prevalence of bacteria isolates from this research work showed that *S. aureus* species has the highest occurrence of 56%, *Bacillus species* has the occurrence of 24%, *Pseudomonas species* has 12%, the least occurrence was *E. coli*, having 8% as shown in the chart below**.**

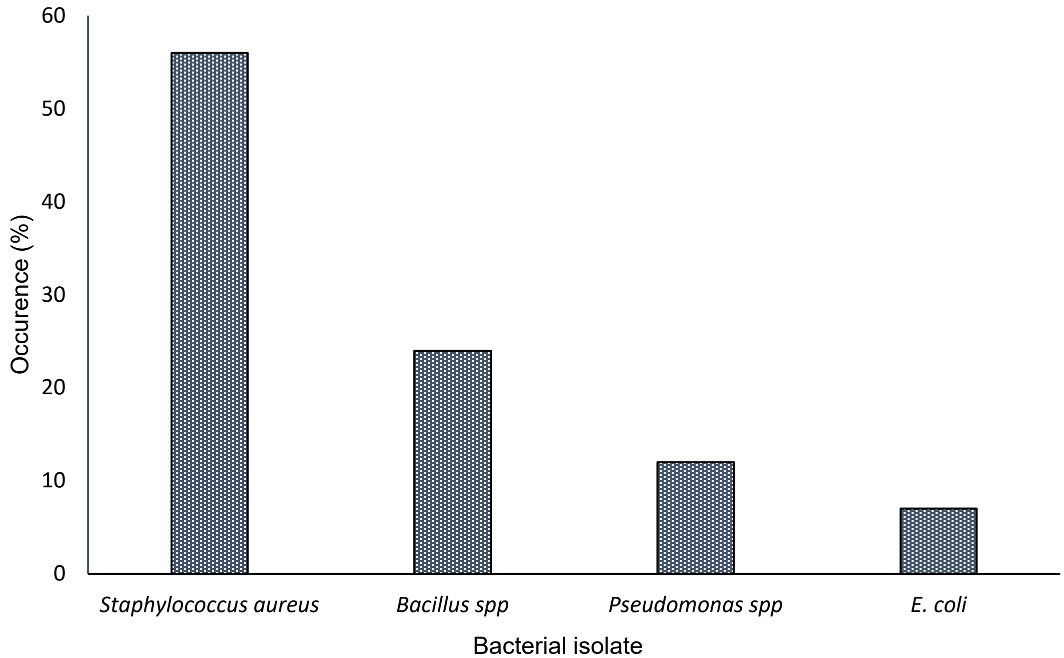

Figure 2 Prevalence of bacterial isolates in the soymilk

4.0 CONCLUSION

Shelf-life studies indicated that unrefrigerated and unpreserved soymilk samples got spoiled within 24 hours while refrigerated unpreserved soymilk were found suitable for consumption for only 5 days after production. Ginger-clove extract at 5% and 3% preserved soymilk sample for 10 and 8 days, respectively followed by clove extract at 5% and 3% which preserved the soymilk sample for 8 days and ginger extract at 5%, which preserved the soymilk sample for 8 days. These findings are comparable to acetic acid 5 and 3% extract that preserved soymilk sample for 10 days. Preservation of soymilk sample using combination of ginger-clove extract was found to be the most effective, thereby indicating a synergistic effect of the two extracts. Conclusively, from this research work, these spices could be used to extend the shelf life of soymilk and other related local drinks. The isolated bacteria from spoiled soymilk after 10 days include *E. coli*, *Pseudomonas spp, B. cereus, B. subtilis, S. aureus*, fungi isolates include *T. asahil., A. fumigatus, A. lentulus, A. niger and R. stoloniffer*. Adequate adherence to quality control measures during production, coupled with the use of natural preservatives, proper storage and acceptable standards should be adopted in the production and utilization of soymilk.

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