IJABR Vol. 14(1): 74 - 86 (2023)

Original article

EXTENDING THE SHELF LIFE OF SOYMILK

***Olarongbe, G.O., Akindele, M.A. and Oyagbola, E.O.**

**Department of Science Laboratory Technology, Institute of Applied Sciences.
Kwara State Polytechnic, Ilorin, Kwara State**

Submitted: January, 2023; Accepted: June, 2023; Published: June, 2023

ABSTRACT

Soymilk, a plant-based drink, contains high nutritional content, including protein, fat, carbohydrates, vitamins, and minerals. However, its limited shelf life as a result of microbial activities poses a significant challenge. It is a fact that plant extracts inhibit the growth of microorganisms. The objective of this research work was to evaluate the assessment of spoilage in soymilk and explore techniques to extend its shelf life. Samples of sweetened and unsweetened soymilk were acquired from different locations, and transported to the laboratory for analysis. The pour plate method was employed to determine the microbial count and identified the microorganisms. The bacteria isolated include; *Staphylococcus aureus*, *Klebsiella* spp, and *Pseudomonas* spp, while the fungi isolated were *Aspergillus* spp, *Fusarium* spp, and *Candida albicans*. Furthermore, various plant extracts were tested against the organisms that had the highest population, and it was discovered that *Azadirachta indica* inhibited the growth of *Staphylococcus aureus*, whereas *Sesamum indicum* inhibited the growth of *Aspergillus* spp. This research finding indicated a substandard hygienic level in soymilk production, which contributed to spoilage caused by microorganisms. It also emphasizes the role of plant extracts from *Azadirachta indica* (Neem leaf) and *Sesamum indicum* (Sesame Leaf) in extending the shelf life of soymilk, albeit with potential effects on its organoleptic properties.

Keywords: Soymilk, Plant extract, Shelf life, organoleptic properties and Food safety

---

***Corresponding author's email**: linto4real@gmail.com, michaelakixe@gmail.com, myoyetayo@gmail.com, 08033755580, 07067405594, 08060204046

INTRODUCTION

Appropriate nutrient consumption supports healthy living. Food security via agricultural intensification and industrial food supply aims to prevent food scarcity and malnutrition. Cereal crops like maize, wheat, rice, etc., provide global food energy due to their essential nutrient composition [1]. Milk and dairy products are known as nutrient-dense foods, supplying energy and high-quality protein with a range of essential micronutrients [2]. Milk minerals are crucial for human health and development as well as in dairy processes as cheese-making and for all traits involving salt-protein interactions. Soybean (*Glycine max*), as an economically important food and oilseed crop, is known by different names in different parts of the world, soya beans (Nigeria), Chinese pea (China), Churia bean (Manchuria). It is the major source of plant-derived dietary protein, oil and bio-functional components [3]. The soybean seed contains all essential amino acids required for human consumption. It is enriched with macro- and micronutrients and provides an environmentally friendly protein source for both humans and monogastric livestock operations [4]. It has been determined that obesity can be effectively prevented and treated by the administration of soy protein. Soybean is also a good source of phenolic compounds with antioxidant properties and has an extraordinarily high amount of isoflavones [5]. Saponins and phytic acid present in soybeans are reported to be effective in preventing chronic diseases, including arteriosclerosis, cardiac diseases, diabetes, senile dementia, cancer, and osteoporosis [6]. However, food quality control affects safety, shelf life, texture, palatability, and acceptability food. Pathogenic bacteria in processed foods affect safety and shelf life, reducing consumer choice and demand [7].

A very popular soybean product is soymilk. Soymilk is a plant-based milk gotten from soybean seed which have an exceptional nutritional value. Soymilk has a smooth creamy texture with an off-white emulsion-like suspension containing water-soluble vitamins, proteins and carbohydrates. Soy milk possesses a balanced nutrient combination, which is similar to cow's milk in compositions and physical appearance, but free of cholesterol, gluten and lactose, plus favourable phytochemical compounds linked to health [8, 9]. When soymilk is properly processed, it offers a lot of nutraceutical and health benefits. Some of those health benefits may include, low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention of heart disease, and certain cancers [10, 11]. Nutrients such as omega-3, fatty acid, dietary fibre, iso-flavones, Vitamin C, proteins, carotenoids and oligosaccharides are abundant in the Soymilk [12]. Soymilk have been suggested as an alternative probiotic food carrier to dairy milk due to their low cost, availability, suitability for vegetarians and health benefits without the side effects often associated with commercial drugs [13]. The protein content of soymilk is close to that of cow's milk and some phytochemicals present in soybeans that confer health benefits are brought into the soymilk hence, it can be recommended as naturally substitute for cow milk in adults [14, 10]. Soya milk can be produced traditionally from soybean seeds. The soybean seeds undergo a thorough cleaning and sorting process to remove defective seeds, pebbles, and foreign materials. It is then washed with tap water and soaked overnight before the water is drained [15]. To facilitate the removal of the seed coat, the seeds are rubbed between the hands (just the same way beans is de-hauled). Using flowing tap water and hands, the soybean seed coat is then sieve out and rinse with clean water several times [16]. At this stage, the beans are ready for milk extraction. The seeds are transferred to blender and some amount of water is added and blended together until smooth [15]. The resulting mixture is filter through a fine sieve and double-layer cheesecloth to ensure the separation of undesired particles from the liquid [15]. The filtered soya milk is transferred into a pot and boiled for about 15-20 minutes, with occasional stirring to prevent scorching. Once removed from the heat, it is filtered to remove the insoluble soy pulp fibre (Okara) and leave to cool [16]. Finally, the cooled soymilk is carefully packed in transparent bottles and tightly sealed for storage at both ambient and refrigeration temperatures.

However, due to its nutritious content, it is vulnerable to microbial attack during processing or stocking which lead to limited shelf life of the product as well as the risk of public health hazard if not properly processed [17]. Some producers of soymilk do not undergo detailed processes of producing soymilk and still produce and sell under unhygienic environment thereby exposing the soymilk to high levels of contamination by pathogenic and other spoilage organisms. The presence of certain Bacteria such as *Bacillus* spp., *Enterobacter* spp. and *Escherichia coli* has been isolated in soymilk at room and refrigeration temperatures [17]. [18] also isolated *Staphylococcus* spp, *Klebsiella* spp, *Pseudomonas* spp. and fungi isolates of the genera *Aspergillus*. [19] examined the microbial quality of locally and industrially produced soybean products sold in Port Harcourt Metropolis, Nigeria. Their results revealed significantly higher bacteria and fungi counts in locally prepared samples compared to industrially prepared samples. They reported that *Pseudomonas* spp., and *Staphylococcus* spp., were the most common bacteria in locally processed samples.

Soymilk is stable up to two days at room temperature without addition of any preservatives and by using different types of preservatives in different levels the shelf life can be increased up to 14-17 days at refrigeration temperature. Potassium-meta-bi-sulphite (KMS), Sodium-benzoate and potassium sorbate can be used individually or in a mixture of the three for preservation of soymilk [20]. However, potassium-meta-bi-sulphite may cause fatal allergic reactions if inhaled or swallowed by some asthmatics and other 'sulfite-sensitive' individuals but the colour and the flavour remains constant. Similarly, Sodium-benzoate can increase the risks of inflammatory and also reduce the potential of soymilk to treat obesity. According to [21] who conducted Antibiotic sensitivity test against isolated organisms from soymilk, *Staphylococcus aureus, Streptococcus faecalisare*, *Salmonella* spp, and *Klebsiella* were all sensitive to antibiotics used but when employed as a preservatives agent could affect soymilk texture and the taste due to the contents of antibiotics. However, the use of plant extracts against food pathogens and spoilage microorganisms has been greatly studied [22]. The phytochemical compounds such as nimbolide, gedunin, terpenoids, etc. are known to have antibacterial activity without drug resistant problem [23], the leaves, flowers, seeds, fruits, roots and bark of neem tree are used for the treatment of infections on skin, teeth and gums [24]. In addition, [25] reported that thyme oil extract could decrease the growth of *Candida albicans* and *Pseudomonas aeruginosa.* Due to the phenolic and terpene molecules present in ginger, the ethanolic extract has been used against various bacteria including *E. coli* and *S. typhi* [26-27]. Furthermore, the

composition of Sesame has been extremely studied thereby make its effective against microorganisms include: *S. aureus, Fusobacterium nucleatum, K. pneumonia* [28-29]. It has also been demonstrated that, the compounds such as alkaloids (betacyanins and betaxanthin), polyphenols (flavonoids, steroids, catechuic acids and tannins), terpenoids (cerasinone and norecasantalic acid) and saponins present in *Amaranthus* have broad-spectrum anti-bacterial activity [30-31].
Therefore, the aim and objective of this research are to isolate and identify the possible microorganisms causing spoilage in soymilk and see how its shelf life can be extended using plant extracts

MATERIALS AND METHOD

Collection of Samples and Shelf-Life Studies

Two different soymilk sample (sweetened and unsweetened) were purchased from Ilorin, kwara State and stored at room temperature (27+20°C) under aerobic and anaerobic condition. The soymilk samples were observed every day until the organoleptic properties changed.

Media preparation

The media used namely Nutrient agar (NA), MacConkey's agar (MCA), Eosin methylene blue agar (EMB), Simmon's Citrate agar (SCA) and Urea agar (UA) were prepared according to the manufacturer instructions and sterilized by autoclaving at 121°C for fifteen minutes.

Isolation of bacteria

The pour plate method described by [32] was adopted. The samples were serially diluted in ten test tube, five test tube for each sample and 1ml of each dilution were introduced into sterile petri dishes. Nutrient agar was added and the plates were rotated gently for easy mixing of the samples and the media. The plates also contained ketoconazole at a concentration of 0.05mg/l to inhibit fungal growth. The plates were incubated in an inverted manner at temperature of 37°C for 24 hours. The resulting colonies were sub-cultured and then transferred into agar slants for further analysis.

Characterization and Identification of the Bacterial Isolates

The isolates were subjected to morphological and biochemical tests and were identified by comparing their characteristics with those of known taxa as described by [33]. Gram staining, sugar fermentation, (glucose, lactose, sucrose), catalase test, citrate test, urease test, Methyl red, indole test and oxidase test were carried out as described by [34].

Isolation of fungi

Cheesbrough's pour plate method (2006) was adopted. The samples were serially diluted in ten test tubes, five test tubes were used for each sample, with 1ml of each dilution were introduced into sterile Petri dishes. Prepared Potato Dextrose Agar was added and the plates were rotated gently for easy mixing of the samples and the media. The PDA media was modified with streptomycin to inhibit bacterial contamination. The plates were then incubated at temperature of 25°C for 48-67hours. The resulting colonies were sub-cultured and then transferred into agar slants for further analysis.

Identification of fungi

Fungi counts were determined on Potato dextrose agar plates (PDA). Fungi were then identified by cultural and morphological characteristics (using a microscope) based on mycelium structure, branch conditions, conidiophore presence, shape etc. according to [35].

Preparation of Plant-Extracts

The plant-extracts used were from Neem leaves (*Azadirachta indica*), Green amaranth leaves (*Amaranthus hybridus*), Thyme leaves (*Thymus vulgaris*), sesame leaves (*Sesame indicum*) and Ginger leaves (*Zingiber officinale*). Ten grams (10 g) of leaves of neem pants were thoroughly washed in clear water to remove any undesirable particles and soaked in 1 liter of boiled (100^0C) water for 8 hours for extraction of the leaves bioactive constituents. The resulting extract was stored at 25^0C until usage. The same extraction method was employed for the other plant leaves.

Sensitivity test against isolated organisms using Plant extracts

The sensitivity test was done against the isolated organisms. Plant extracts were used instead of distilled water to prepare Nutrient agar according to the manufacturer`s instructions for the isolation of bacteria. The Nutrient agar was poured into a plate labeled *S. aureus, Klebsiella,* and *Pseudomonas*. Using the streak method, a discrete colony was picked from each isolated colony and aseptically streaked onto the NA plates prepared with extracts. The plates were then incubated at 37°C in an inverted manner for 24 hours. Similarly, Potato Dextrose Agar was prepared with plant extracts against the isolated fungi at room temperature (20-25°C) in an inverted manner for 72 hours.

Shelf Life Study Using Plant Extracts as a Preservative Agent at 25^0C

1ml concentration of extracts of each plant extract was obtained and incorporated into 750 ml of fresh soymilk samples; also, a combination of two extracts, each 0.5 ml was introduced into 750 ml of freshly collected samples, and its shelf life was studied every 12 hours until the organoleptic properties changed.

RESULTS AND DISCUSSION

From my table 1, it will be observed that presence or absence of air during storage of soymilk does not have any effect on its shelf life because both the aerated and unaerated samples keep well until the same period i.e. spoilage occur about the same time. The organisms isolated in this research work includes *Staphylococcus* spp*, Pseudomonas* spp*, Klebsiella* spp, *Aspergillus* spp and *Fusarium* spp. This result is in line with the work of [17, 36], they also reported the presence of *Staphylococcus aureus, Klebsiella* and *Pseudomonas* from their isolated samples. This research is also in accordance with [21] who studied the Antibiotic Susceptibility Screening of Pathogenic Bacteria Isolated from Locally Produced Soybean (*Glycine max*) Milk and the organisms isolated were *Staphylococcus aureus* and *Klebseilla* spp. The presence of *Staphylococcus aureus,* and *Klebsiella* spp*.,* has been found to align with [9] researched from the sample from Eke-Awka, Amenyi, Ifite, Aroma and Permanent Site markets all in Awka, Anambra State, Nigeria. The microbial load of the analyzed soymilk exceeds the recommended standard of bacterial count (2.0×10^4 CFU/ml) established by the Soy Foods Association of America (SFAA) and the acceptable limit for pasteurized milk (3.0×10^4 CFU/ml), thus rendering them unsuitable for consumption. *Staphylococcus aureus* had the highest percentage 50% of occurrences of bacteria in the soymilk samples show that there is a poor personal hygiene in the production processed making it unsafe for consumption and limit its shelf life. The isolation of *Aspergillus* spp which had the highest percentage 64% of occurrence after 72 hours at room temperature indicate that

Aspergillus spp. contribute to soymilk spoilage. This, in turn, limits the shelf life. Due to the significant importance of nutritional content of soymilk, the challenge with its handling is in the processing and storage as the presence of spoilage organisms multiply and cause undesired effects which thereby limits its shelf life.

The sensitivity test against the isolated bacteria after 24 hours of incubation indicated that *Staphylococcus aureus* is more sensitive to plant-extracts (Neem leaves extracts) which had just one colony count against the NA prepared with Neem leaves compared to other extracts. However, it shown resistance against sesame leaves extracts (Table 6). Similarly, among all the isolated fungi, *Aspergillus* spp. was seen to be more sensitive to sesame leaves extracts compared to other organisms and plant extracts, this is due to the antimicrobial content of the leaves, which inhibits the growth of the microorganisms (Table 7). Aligned with this research, Antibiotic Susceptibility Screening of Pathogenic Bacteria Isolated from Locally Produced Soybean (*Glycine max*) Milk Sold in Port Harcourt, Nigeria recorded *Staphylococcus aureus* to be the most sensitive to all antibiotics particularly Ampiclox (80%) [21].

The synergistic effects of the powder leaves extracts when incorporated into a fresh soymilk samples showed that the combination of Neem and Sesame leaf powder greatly increased soymilk shelf life for more than 48 hours (Table 8). Based on this research, when soy milk is stored at 25^{0}C for more than 48 hours without any preservative agent, it leads to microbial proliferation, which significantly shortens its shelf life. Using plant extracts against the most proliferation organisms that render them susceptible to spoilage can be an effective way of extending its shelf life.

Table 1. Physical characteristics of soymilk stored in ambient under aeration and anaerobic condition

Time	Storage condition	Colour	Taste	Odour
24hrs	Ambient (anaerobic)	White, there is no separation of whey.	Good	Good
	Ambient (aeration)	White, there is no separation of whey.	Good	Good
48hrs	Ambient (anaerobic)	White, there is separation of whey.	Good	Good
	Ambient (aeration)	White, there is no separation of whey.	Good	Good
72hrs	Ambient (aeration)	Whitish whey separated	Bad	Bad
	Ambient (anaerobic)	Whitish whey separated	Bad	Bad

Table 2: Plate Count on Nutrient Agar/Potato Dextrose Agar

Selected Plates	Number of days (CFU/ml) (24hrs) (NA)	Selected Plates	Number of days (CFU/ml) (72hrs) (PDA)
10^{-5} (ANO)	1.5×10^8	10^{-4}	1.3×10^6
10^{-4} (AA)	3.8×10^3	10^{-4}	5.0×10^4
10^{-4} (AA)	7.0×10^5	10^{-4}	3.0×10^3
10^{-5} (ANO)	4.7×10^6	10^{-5}	7.0×10^5

Keys= Ambient (Aeration) (AA)
Ambient (Anaerobic) (ANO)
Potato Dextrose Agar (PDA)
Nutrient Agar (NA)

Table 3: Cultural characteristics of isolated bacteria and Fungi

Isolated Bacteria		Isolated Fungi	
Isolated plates	Cultural Features on NA	Isolated plates	Cultural Features on PDA
10^{-5}	Creamy, small, round, shiny and smooth edges	10^{-4}	Creamy, small, round, shiny and smooth edges Flat, filiform, filamentous, black
10^{-4}	Milky, flat, shiny, small, round with irregular shape and surface with rigid edges	10^{-4}	Milky, flat, shiny, small, round with irregular shape and surface with rigid edges
10^{-4}	Creamy, yellowish, small, shiny and smooth surface, irregular shape with rigid edges	10^{-4}	Creamy, yellowish, small, shiny and smooth surface, irregular shape with rigid edges
10^{-5}	Creamy, round, Small, Yellowish, shiny with smooth edges	10^{-5}	Creamy, round, Small, Yellowish, shiny with smooth edges

Table 4. Biochemical Test for bacterial identification

Isolated plates	Appearance	Gram staining	Catalase test	Indole test	Citrate test	Urease test	Oxidase test	Methyl red	Glucose	Sucrose	Lactose	Probable microorganisms
10^{-5}	Nil	+Rod	+	+	+	+	_	_	+	+	+	*Klebsiella oxytoca*
10^{-5}	Nil	+cocci	+	_	+	+	_	+	+	+	+	*Staphylococcus aureus*
10^{-4}	Milky	+cocci	+	_	+	+	_	+	+	+	+	*Staphylococcus aureus*
10^{-4}	Yellowish	+Rod	+	_	+	_	+	_	_	_	_	*Pseudomonas* spp
10^{-3}	Yellowish	+Rod	+	_	+	_	+	_	_	_	_	*Pseudomonas* spp
10^{-3}	Milky	+cocci	+	_	+	+	_	+	+	+	+	*Staphylococcus aureus*

Table 5: Percentage of occurrence of bacterial and fungal isolates

Isolates for bacterial			Isolates for Fungi		
Probable organisms	Number	%	Probable organisms	Number	%
Staphylococcus aureus	3	50	*Aspergillus* spp	7	64
Klebsiella oxycota	2	33.3	*Fusarium* spp	2	18
Pseudomonas spp	1	16.6	*Candida* spp	2	18
Total	6	100	Total	11	100

Table 6: Sensitivity Test of the plant extracts against the isolated bacteria after 24 hours' incubation

Plant Extracts	*Staphylococcus aureus*	*Klebsiella oxycota*	*Pseudomonas* spp
Neem leaves	1	7	6
Green amaranth leaves	7	5	9
Sesame leaves	9	10	8
Ginger leaves	5	8	5
Thyme leaves	5	12	8

Table 7: Sensitivity Test against the isolated fungi after 72 hours incubation

Plant Extracts	*Aspergillus* spp	*Fusarium* spp	*Candida* spp
Neem leaves	9	8	9
Green amaranth leaves	11	5	7
Sesame leaves	0	9	8
Thyme leaves	6	7	5
Ginger leaves	5	9	12

Table 8. (Study of Soymilk Sample Using Plant Extracts as a Preservative At 25^0C)

Plant extracts	Fresh Soymilk Samples										
	12h	24h	36h	48h	60h	72h	84h	96h	120h	135h	144h
Neem leaves	Excellent	Excellent	Very good	Very good	Good	Good	Fair	Fair	Doubtful	Doubtful	Poor
Thyme leaves	Very good	Good	Fair	Fair	Doubtful	Poor	Very poor	Bad	Bad	Bad	Bad
Ginger leaves	Excellent	Very good	Good	Fair	Doubtful	Doubtful	Poor	Poor	Very Poor	Bad	Bad
Sesame leaves	Excellent	Very good	Very good	Good	Good	Good	Fair	Fair	Doubtful	Poor	Bad
Green amaranth leaves	Very good	Good	Fair	Fair	Doubtful	Poor	Very Poor	Very Poor	Bad	Bad	Bad
NL + AL	Excellent	Very good	Good	Good	Fair	Doubtful	Poor	Poor	Very Poor	Bad	Bad
SL + AL	Excellent	Good	Good	Good	Fair	Doubtful	Poor	Very poor	Bad	Bad	Bad
GL + TL	Very good	Good	Good	Fair	Doubtful	Poor	Very poor	Bad	Bad	Bad	Bad
NL + TL	Excellent	Very good	Good	Good	Fair	Fair	Doubtful	Poor	Very Poor	Bad	Bad
TL + SL	Very good	Very good	Good	Good	Good	Fair	Doubtful	Poor	Poor	Very poor	Bad
SL + GL	Excellent	Very good	Very good	Good	Fair	Fair	Doubtful	Poor	Very poor	Bad	Bad
NL + SL	Excellent	Excellent	Excellent	Very good	Very good	Good	Good	Good	Good	Fair	Doubtful
GL + AL	Very good	Good	Good	Fair	Doubtful	Doubtful	Poor	Very poor	Bad	Bad	Bad
TL + AL	Very good	Good	Fair	Fair	Doubtful	Poor	Very poor	Bad	Bad	Bad	Bad
GL + NL	Excellent	Very good	Good	Good	Fair	Fair	Doubtful	Poor	Very poor	Bad	Bad

Keys = Sesame leaves (SL)
Green Amaranth leaves (AL)
Neem leaves (NL)
Thyme leaves (TL)
Ginger leaves (GL)
Hour(h)

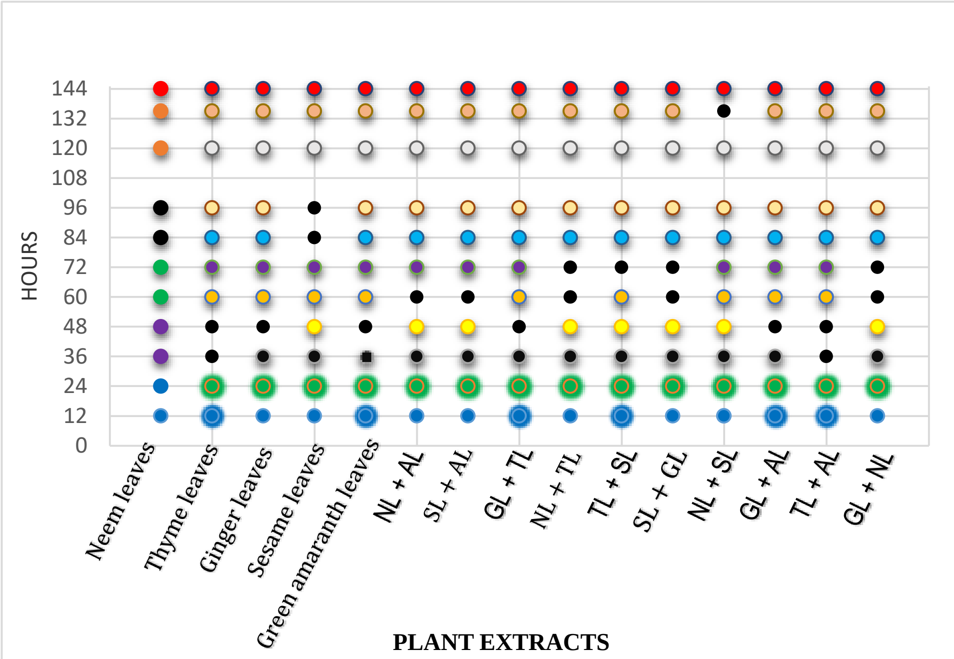

Figure 1: (Chart Showing the Shelf life of Soymilk sample using plant extracts as a preservation at 25°C)

Keys: Excellent (Blue)
Very good (Purple)
Good (Green)
Fair (Black)
Doubtful (Orange)
Poor (Red)
Very poor (Dark Red)
Bad (Dark Red)

CONCLUSION AND RECOMMENDATION

A variety of bacteria and fungi, including *Staphylococcus aureus, Klebsiella* spp., *Pseudomonas* spp., *Fusarium* spp., *Aspergillus* spp., and *Candida* spp., were found in the soymilk Samples, which limit its shelf life. *Staphylococcus aureus*, which tends to be the most predominant bacteria, can be attributed to the producers' poor personal hygiene, as well as the unhygienic conditions of the processing machinery and raw materials, which favor microbial proliferation. However, plant extracts are an effective way to extend soymilk shelf life without any side effects for the consumer compared to commercial preservatives, but the organoleptic properties remain affected due to the limonoid content present in Neem leaf. If a standard hygienic practice can be employed in the production and the combination of Sesame and Neem plant extracts can be added during the boiling of the soymilk, a product with an extended shelf life can be made.

Authors Contribution

GO and EO conceptualized the study. GO, EO, and MA, designed the study. EO and MA participated in fieldwork and sample collection. GO and EO participated in plant extracts collection. GO and MA studied the Sensitivity Test against the isolated bacteria. GO, EO and MA studied the Soymilk Sample Using plant extracts as a preservative. GO and MA performed the data analysis; GO, EO and MA interpreted the data. MA prepared the first draft of the manuscript, reviewed by GO and EO. All authors contributed to the development of the final manuscript and approved its submission.

REFERENCES

1. Bathla, S., Jaidka, M., & Kaur, R. (2019). Nutritive Value. *Intechopen,* doi: 105772/intech.88963

2. Gorska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019). Milk and Dairy Products and their Nutritional Contribution to the Average Polish Diet. *Multidisciplinary Digital Publishing Institute, 11*(8), 1771. https://doi.org/10.3390/nu11081771

3. Babalola, F. U., & Fajoye, O. E. (2021). Temperature and Time Model for Optimum Nutritional Contents in Soymilk Processing. *European Journal of Biology and Biotechnology*, 2684-5199. DOI: 10.24018/ejbio.2021.2.2.163

4. Singer, W. M., Zhang, B., M.A. Rouf Mian, M. A., & Huang, H. (2019). Soybean Amino Acids in Health, Genetics, and Evaluation. *Intechopen*, DOI: 10.5772/intechopen.89497.

5. Akhagbi, M., Zare, M., & Nouripour, F. (2017). Effect of Soy and Soy Isoflavones on Obesity-Related Anthropometric Measures: A Systematic Review and Meta-analysis of Randomized Controlled Clinical Trials. *American Society for Nutrition*, 8, 705-17. https://doi.org/10.3945/an.117.015370

6. Kim, II-S., Yang, W., & Kim, C. (2021). Beneficial Effects of Soybean-Derived Bioaction Peptides. *International journal of molecular sciences, 22*(16), 8570. Doi: 10.3390/ijms22168570

7. Moloi, M., Lenetha, G. G., & Malebo, N. J. (2021). Microbial levels on street foods and food preparation surfaces in Mangaung Metropolitan Municipality. *National Institutes of Health*, 26, 1407. doi: 10.4102/hsag.v26i0.1407

8. Davy, P., & Vuong, Q. V. (2020). Soymilk by-product: Its Composition and Utilization. *Food Reviews International*, 28, 147-169. https://doi.org/10.1080/87559129.2020.1855191

9. Samuel, O., & Frederick, O. (2021). Bacteriological quality of unbranded soymilk sold in major markets in Awka, Anambra State, Nigeria. *Pharmaceutical and Chemical Journal*, *8*(1):1-8.

10. Niyibituronsa, M., Onyango, A. N., Gaidashova, S., Imathiu, S., Boevre, M. de, Leenknecht, D., Neirnck, E., Saeger, S. de, Vermeir, P., & Raes,

K. (2019). The Growth of Different Probiotic Microorganisms in Soymilk from Different Soybean Varieties and their Effects on Antioxidant Activity and Oligosaccharide Content. *Journal of Food Research*, *8*(1). https://doi.org/10.5539/jfr.v8n1p41

11. Zhu, Y. Y., Thakur, K., Feng, J. Y., Cai, J. S., Zhang, J. G., Hu, F., Russo, P., Spano, G., & Wei, Z. J. (2020). Riboflavin-overproducing lactobacilli for the enrichment of fermented soymilk: insights into improved nutritional and functional attributes. https://doi.org/10.1007/s00253-020-10649-1.

12. Begum, A. A., & Mazumder, M. A. (2016). Soymilk as source of nutrient for malnourished population of developing country: A review. *International Journal of Advanced Scientific and Technical Research*, 6(5). http://www.rspublication.com/ijst/index.html

13. Sairazi, N. S. M., & Sirajudeen, K. N. S. (2020). Natural Products and Their Bioactive Compounds: Neuroprotective Potentials against Neurodegenerative Diseases. *Evidence-based complementary and alternative medicine*, 6565396. https://doi.org/10.1155/2020/6565396

14. Li, Y. R., Yun, T. T., Liu, S., Qi, W. T., Zhao, L. Q., Liu, J. R., & Li, A. K. (2016). Analysis of water-soluble bioactive compounds in commonly consumed soymilk in China. *Journal of Food Composition and Analysis*, 46. https://doi.org/10.1016/j.jfca.2015.10.011

15. Jiang, S., Cai, W., & Xu, B. (2013). Food Quality Improvement of Soymilk Made from Short-Time Germinated Soybeans. *Foods,* 2 198-212. Doi: 10.3390/foods2020198

16. Murugkar, D. A. (2015). Effect of Different process parameters on the quality of soymilk and tofu from sprouted soybean. *Journal of food science and technology, 52*(5), 2886-2893. Doi: 10.1007/s13197-014-1320-z

17. Ezemba. C.C., Ajah. N.N., Ezejiofor. C.C., Ezemba. A.S., C. M. Ezeokoli. C.M., Osuala. O.J., Okoye. P.C., Udoye. I.W., Mmaduekwe. J.C., V. O. Etikudike. V. O., & V. N. Anakwenze. V.N. (2022). Assessment Of Microbiological Quality Of Soymilk Beverage. *Journal of Global Agriculture and Ecology*, *14*(4), 4-12.

18. Kakade. R. D., & Choudhary. A. (2022). A Study on the Determination of Shelf Life of Native Soybean Milk. *Journal of Innovation and Social Science Research*, 2591-6890. DOI: 10.53469/jissr.2022.09(09).01

19. Agwa. O. K., & Ossai-Chidi. L. N. (2016). Surveillance of the Microbial Quality of Soybean Products Sold within Markets in Port Harcourt Metropolis, Rivers State, Nigeria. *Food and Public Health*, *6*(5):399-400.

20. Kohli, D., Kumar, S., Upadhyay, S., & Mishra, R. (2017). Preservation and

processing of soymilk: A review. *International Journal of Food Science and Nutrition,* 2(6), 66-70.

21. Austin. A. O., & Felicia. O. O. (2023) Antibiotic Susceptibility Screening of Pathogenic Bacteria Isolated from Locally Produced Soybean (Glycine max) Mlk Sold in Port Harcourt, *Nigeria. Journal of Advances in Microbiology*, 23(*5*), 26-34. https://doi.org/10.9734/jamb/2023/v23i5723

22. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. *Frontiers Microbiology,* 9. https://doi.org/10.3389/fmicb.2018.01639

23. Ali, E., Islam, S., Hossen, I., Khatun, M., & Islam, A. (2021). Extract of neem (Azadirachta indica) lleaf exhibits bactericidal effect against multidrug resistant pathogenic bacteria of poultry. *Veterinary Medicine and Science*, *7*(5), 1921-1927. Doi: 10.1002/vms3.511.

24. Nagini, S., Nivetha, R., Palrasu, M., & Mishra, R. (2021). Nimbolide, a Neem Limonoid, is a promising Candidate for the Anticancer Drug Arsenal. *Journal of medicinal chemistry, 64*(7), 3560-3577. Doi: 10.1021/acs.jmedchem.0c02239

25. Vassiliou, E., Awoleye, O., Davis, A., & Mishra, S. (2023). Anti-Inflammatory and Antimicrobial Properties of Thyme Oil and Its Main Constituents. *International Journal of Molecular Sciences, 24*(8), 6936. Doi: 10.3390/ijms24086936

26. Ahmed, N., Karobari, M. L., Yousaf, A., Mohamed, R. N., Arshad, S., Basheer, S. N., Peeran, S. W., Noorani, T. Y., Assiry, A. A., Alharbi, A. S., & Yean, C. Y. (2022). The antimicrobial efficacy Against Selective Oral Microbes, Antioxidant Activity and Preliminary Phytochemical Screening of *Zingiber officinala*. *Infection and Drug Resistance,* 15, 2773-2785*.* https://doi.org/10.2147/IDR.S364175

27. Samiappan, S. C., Pandiyan, S., Ramalingam, S., Saravanan, R., Hameed, S.A. (2020). Targeting the extracellular polysaccharide production (EPS) by biofilm forming bacteria form orthodontic brackets and wires through antiquorum sensing action of bioactive compounds from curcuma longa and *Zingiber officinale*. *Biomedical and Pharmacology Journal, 13*(02), 1037-1045. Doi:10.13005/bpj/1973

28. Ozkan, A., Imali, A., Kocer, F., & Curat, D. (2010). Antimicrobial Activity of Some Sesame (Sesame indicum L) population Seed Oils. *Aromatic and Medicineal Plants of Southeast European Countries.*

29. Heidari-Soureshjani, R., Obeidavi, Z., Reisi-Vanani, V., Dehkordi, S. E., Fattahian, N., & Gholipour, A. (2017). Evaluation of antibacterial effect of sesame oil, olive oil and their synergism on *Staphylococcus aureus* in vitro. *Advanced Herbal Medicine*, *3*(3), 13-19.

30. Peter, K., & Gandhi, P. (2017). Rediscovering the therapeutic potential of *Amaranthus* species: A review. *Egyptian Journal of Basic and Applied Sciences,* 4, 196-205. Doi: 10.1016/j.ejbas.2017.05.001

31. Amornrit, W., & Santiyanont, R. (2016). Neuroprotective effects of Amaranthus lividus and Amaranthus tricolor and their effects on gene expression of RAGA during oxidative stress in SH-SY5Y cells. *Genetics and Molecular Research,* 15, 15027562. Doi:10.4238/gmr.15027562

32. Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries-Part 2. 2nd Edition, Cambridge University Press, New York.

33. Holt, J. G., Krieg, N.R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994) Bergey's Manual of Determinate Bacteriology *Baltimore, MD (USA) Williams & Wilkins.*

34. Samuel, O., & Frederick, O. (2018). Characterization and Identification of Fungi in the Sorrel Beverage (Zobo) Hawked in Ifite Awka, Anambra State, Nigeria. *International Journal of Homeopathy & Natural Medicines, 4*(1), 24-30. Doi: 10.11648/j.ijhnm.20180401.15

35. Alsohaili. S.A., & Bani-Hasan, B. (2018). Morphological and Molecular Identification of Fungi Isolated from Different Environmental Sources in the Northern Eastern. *Jordan Journal of Biological Sciences*, 11(3), 329-337.

36. Umeoduagu. N.D., Dimejesi. S. A., O. Nworie. O., Jerry. O. O., & R.O. Oti-Wilberforce. R. O. (2016). Microbiology Assessment of Soymilk Sold in Onitsha Metropolis. *African Journal of Basic & Applied Sciences*, *8*(2), 87-89. DOI: 10.5829/idosi.ajbas.2016.8.2.1165