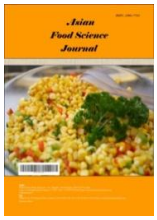

Asian Food Science Journal

15(4): 21-31, 2020; Article no.AFSJ.57063
ISSN: 2581-7752

Effect of Addition of Citric Acid and Sodium Benzoate on pH and Microbial Profile of Soymilk

Johnpaul I. Agbaka[1*], Charles N. Ishiwu[2] and Ajibola N. Ibrahim[3]

[1]College of Food Science and Nutritional Engineering, China Agricultural University, Haidian District, Beijing, China.
[2]Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[3]Department of Food Science and Technology, Federal University Wukari, Wukari, Taraba State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors JIA and CNI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CNI contributed in the supervision of the experiment. Authors JIA and CNI managed the analyses of the study. Authors JIA and ANI managed the literature searches, grammar correction and submission of the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/AFSJ/2020/v15i430158
Editor(s):
(1) Dr. Chouitah Ourida, University of Mascara, Algeria.
Reviewers:
(1) K. D. MINI, Mahatma Gandhi University, India.
(2) Ahmad Gambo, Bayero University Kano, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57063

Original Research Article

Received 19 March 2020
Accepted 24 May 2020
Published 01 June 2020

ABSTRACT

Aim: To study the synergistic effect of chemical preservatives on the keeping quality of soymilk.
Study Design: Ten soymilk samples were prepared and treated with different concentrations of citric acid and sodium benzoate and stored at ambient conditions.
Place and duration of Study: The present study was conducted at the Department of Food Science and Technology, Nnamdi Azikiwe University, Awka between March 2015 and June 2016
Methodology: Ten (10) soymilk samples were prepared. Soybean seeds (2 kg) that are free of dirt and stones were weighed and steeped in 4 L of tap water, a 12 h steeping regime was adopted. Each soymilk sample was formulated by adding different concentrations of sodium benzoate and citric acid, while the control sample had no treatment. All soymilk samples were then boiled at 75^{o}C for 15 minutes and stored in storage bottles. Standard microbiological techniques were employed

**Corresponding author: Email: jagbaka@cau.edu.cn;*

in the isolation and enumeration of potential spoilage organisms in soymilk samples. pH analysis was conducted throughout the storage period.
Results: There was a decrease in pH of all soymilk samples with increasing storage time. pH at day 0 ranged between 6.2 to 7.2. Isolated bacteria in 10 soymilk samples included *Streptococcus sp., Pseudomonas sp., Proteus sp., Bacillus spp., Staphylococcus aureus, Klebsiella sp., Escherichia coli,* and *Enterobacter species*. However, results obtained showed that soymilk could keep up to 7 days at ambient temperature, encouraging the use of citric acid and sodium benzoate as chemical preservatives.
Conclusion: In the present study, preservation of soymilk samples from a combination of citric acid and sodium benzoate which are chemical preservatives was found to be more effective than several organic preservatives. Hence, they represent an alternative source of chemical antimicrobial substances for use in food systems to prevent the growth of food borne microorganisms and extend the shelf-life of processed food.

Keywords: Soymilk; chemical preservatives; shelf stability; microbial profile.

1. INTRODUCTION

The expensive nature of the animal source of proteins has unfortunately made them quite unaffordable to low-income earners [1], who could have used them as diet complements for starchy foods.

Soybean (*Glycine max. L.*) is one of the most important bean seed in the world, a potential source of bioactive peptides, an alternative source of protein for lots of people for over 4000 years ago, and also an important source of ingredients for several industrial chemical products [2]. As a good alternative for vegetarians, soybean protein is significantly rich in all essential amino acids except methionine and cysteine [3], however, it is a good source of riboflavin.

These huge benefits most probably have stimulated many studies on the incorporation of soybean into indigenous meals such as soybean fortified garri and tapioca [4,5], soy *daddawa* [6,7] and soy-yogurt [8,9]. of these diet incorporation, soymilk has been identified as one of the promising products [10,11].

Soymilk, a traditional oriental food beverage [12], is an aqueous, off-white, creamy extract produced from soybeans (dehulled or undehulled), [13], resembling cow milk in both appearance and consistency [14,15,16,17], and rich in water-soluble protein, carbohydrate, and oil. Soymilk can be produced from different methods. The most common method is the Illinois method, which involves grinding of the soybean in hot water to obtain the milk [14]. Other methods include wet extraction and dry extraction methods [18].

Unfortunately, fresh soymilk has a very short shelf-life [19,20], this has indirectly limited its consumption to locations within the range of its production source.

An immense number of microorganisms such as coliforms, mesophilic aerobic bacteria, moulds, and yeasts are responsible for the spoilage of soymilk and can produce undesirable changes in milk [21,22]. This is due to the fact that the nutritive nature of soymilk makes it prone to microbial attacks as it contains all that is required for these microorganisms to survive and multiply especially if not properly processed and/or stored.

Thermal processing is considered to be a common practice in soymilk preservation [23,24] based on its ability to inhibit the metabolism of vegetative pathogens and several other bacteria. However, this method has been found to have a detrimental effect on the nutritional and quality attributes of soymilk with a resultant off-flavor development [25], thereby negatively encouraging the use of this method of preservation. Refrigeration has been found to have a great positive impact on the keeping quality of soy milk [26], however, in a country like Nigeria where there is an unstable power supply coupled with the increasing population of low-income earners, home ready methods of preservation should be encouraged.

Microbial activities of microorganisms in foods and drinks can be inhibited by the addition of preservatives thereby extending their life span by preventing attacks of these microorganisms [27]. Fortunately, chemical preservatives; substances with an inherent property that tends to destroy microorganisms and preventing further fermentation and spoilage of foods with no

negative effect on the consumers can be used for the preservation of soymilk and other dairy products. The most available chemical preservatives in the dairy (yoghurt and soymilk) industry are potassium sorbate and sodium metabisulphite.

While much research [26,28,29,30] has been devoted to the preservation of soymilk quality using chemical preservatives and/or processing methods coupled with refrigeration, little attention has been paid to improving and extending shelf-life stability.

In a bid to create an unfavourable environment for the growth and multiplication of bacteria, chemical preservatives; citric acid, and sodium benzoate were used as preservatives in this study. Citric acid is used as a food ingredient in the production of fruit products, juices, oils and fats, and many other food products where it functions as an acidulant, pH control, flavoring, and sequestrant. In the United States as reported by AAFCO, [31], sodium benzoate is designated as generally recognized as safe (GRAS) by the Food and Drug Administration. Sodium benzoate is also allowed as an animal food additive at up to 0.1%, per the Association of American Feed Control Officials [32]. The International Programme on Chemical Safety found no adverse effects in humans at doses of 647–825 mg/kg of body weight per day, [33,34].

Therefore, in this study, we investigated the preservative efficiency of chemical preservatives on shelf life extension of soymilk.

2. MATERIALS AND METHODS

Soybean seeds (yellow variety), the major material used for this research work were purchased from Eke-Awka market, Anambra state, Nigeria.

2.1 Preparation of Soymilk Samples

Ten (10) soymilk samples were prepared using a modified method as described by Odu and Egbo, [26]. Soybean seeds (2 kg) that are free of dirt and stones were weighed and steeped in 4 L of tap water, a 12 h steeping regime was adopted. At an interval of 3 h, steep water was drained off and replaced with a fresh one until the end of the 12 h steeping regime. After the steeping step, the soybean grains were blanched in hot water at 75°C(to reduce the beany flavor of the soybean), dehulled, washed with water, then ground to paste in a blender (Kenwood). Sixlitres of clean water were added to the paste and thoroughly mixed to produce a slurry. The homogenized slurry was filtered through a muslin cloth to obtain the milk, then divided into 10 parts. Each soymilk sample was then formulated byadding different concentrations of sodium benzoate and citric acid, while the control sample had no treatment (Table 1). All samples (treated and untreated milk samples) were then boiled at 75^{o}C for 15 minutes and stored in storage bottles for further analysis.

2.1.2 Storage conditions

The soy milk samples were stored at ambient temperature (27°C ± 2°C) for 14 days.

2.2 pH Measurement

The pH value determination of the soymilk samples was carried out in triplicates to check for the effect of storage time on pH of the samples. Values were checked on a daily basis (day 0 to 13) using a hand pH meter (H198128 pHep ®).

2.2.1 Procedure

The pH meter was standardized against a known solution (water) of pH (buffer 7). After standardizing, the electrode of the pH meter was rinsed with distilled water in a washed bottle and was then immersed into the test sample. The pH of the sample was shown on the pH scale. After each measurement, the electrode was washed and returned to the buffer solution of 7.

2.3 Microbiological Analysis of Samples

Throughout storage, all soymilk samples were subjected to microbiological analysis to investigate the effect of treatment on their microbial profile.

2.3.1 Serial dilution

As carried out by Tunde-Akintunde and Souley, [35], each sample was serially diluted using sterile distilled water as diluents. Distilled water(9 ml) water was measured out into different test tubes, using separate sterile pipettes, 1 ml of the soymilk sample was measured out into the first test tube and was properly mixed. Using a different sterile pipette, 1 ml from the first test tube was pipetted into the second test tube already containing 9 ml of distilled water, this

continued following the same procedure till the third test tube. Using the spread plate method, 1 ml of each sample unit from the test tubes was pipetted into the sterile Petri dishes (already labeled) using separate sterile pipettes per sample with their duplicates, then into each Petri dish the prepared MacConkey agar was poured aseptically and the poured medium was rotated gently in the Petri-dishes to ensure proper mixing of the sample and the medium. This procedure was also carried out on Nutrient agar media. Flaming of the neck of the conical flask containing the agar was done after each of the dishes must have been plated to ensure sterility. Colonies were counted and multiplied by the dilution factor. They were subjected to further biochemical tests. The medium used for the enumeration of bacteria cells and also to maintain pure cultures was the nutrient agar based on the assumption that as many organisms as are on the samples will grow.

2.3.2 Characterization of bacterial isolates

Identification / Characterization of Isolates was based on cultural, morphological, and biochemical characteristics following standard methods (Table 5).

After incubation, the representative colonies on the plates were sub-cultured on fresh nutrients agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for biochemical identification. MacConkey agar was used primarily to differentiate lactose fermenters from non-lactose fermenters and also to suppress the swarming activity of proteus and other spreading organisms.

2.3.2.1 Gram staining

The method used was that described by Carpenter, [36] and Thomas, [37].

2.3.2.2 Motility test

This test was used to determine which of the isolates were motile. For this test, the hanging-drop technique was employedas described by Kirk et al. [38].

2.3.2.3 Urease test

The test is usually used to differentiate organisms like proteus from other non-urease positive organisms [39]. The method used was that described by Cowan, [40].

2.3.2.4 Catalase test

This test is usually used as an aid to differentiate *Staphylococc*i from *Streptococc*i and to differentiate other catalase-positive organisms from catalase-negative organisms [41]. The method employed here was that described by Speck [42].

2.3.2.5 Methyl red test

The test is usually used as an aid in the identification and differentiation of the *Enterobacteriaceae* [41]. This test was carried out as described by Kirk et al. [38].

2.3.2.6 Voges -Proskeur test (V.P. test)

The test is usually used to differentiate between Gram-negative organisms especially members of the *Enterobacteriaceae*, [41]. The test was carried out as described by Kirk et al. [38]. The positive reaction was indicated by a pink colour that appears immediately or within 5 minutes at the topmost part of the tube.

2.3.2.7 Indole test

The test is usually used as an aid in the differentiation of Gram-negative, *Bacilli* especially those of the *Enterobacteriaceae* [41]. The test was carried out as described by Kirk et al. [38].

2.3.2.8 Citrate utilization test

This test was used to identify isolates that can utilize citrate as the sole source of carbon for metabolism. The test is usually used as an aid in the differentiation of organisms in the *Enterobacteriaceae* and most other genres, [41].

2.3.2.9 Sugar fermentation

Since most bacteria especially Gram-negative bacteria utilize different sugars as a source of carbon and energy with the production of both acid and gas, or acid only the test is used as an aid in their differentiation. The growth medium used was peptone water and the method used was that described by Kirk et al. [38].

2.3.2.10 Spore stain

The malachite green staining method was used. The staining was carried out as described by Carpenter, [36].

Table 1. Concentrations (%) of preservatives in soymilk samples

Samples	Citric Acid %	Sodium benzoate %	Citric acid (g)	Sodium benzoate (g)
A	2.00	0.08	10.00	0.40
B	1.00	0.08	5.00	0.40
C	1.50	0.07	7.50	0.35
D	2.00	0.08	10.00	0.40
E	3.00	0.08	15.00	0.40
F	2.50	0.07	7.50	0.35
H	2.50	0.09	12.50	0.45
J	2.00	0.08	10.00	0.40
M	2.00	0.06	10.00	0.40
N	0.00	0.00	0.00	0.00

2.4 Statistical Analysis

All the assays were conducted at least in triplicate, and the results were expressed as the mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Effect of Storage Time pH of Soymilk

Table 2 illustrates the decreased rate of pH of all soymilk samples with increasing storage time. pH at day 0 ranged between 6.2 to 7.2 (Table 2). This agrees with the findings of Onuorah et al. [28]. However, slight decreases in pH values were observed during the first week (day 0 to 6) by approximately 0.1-0.3 units. The decrease in pH value of the soymilk samples could be due to an increase in titrable acidity [20,25,43], may have resulted from chemical interactions occurring in soymilk (e.g., lipolysis, proteolysis, etc.).

3.2 Effect of Storage Time on the Microbial Profile

The results of the microbial analysis show that soymilk's microbial population increased with storage time (0 to 13 days). Total Viable Counts (TVC) and Total Coliform Counts (TCC) were used to determine the population of microorganisms that must have gained entrance into the soymilk from the storage environment and/or by contamination through processing and handling of the soymilk respectively. The microbial loads of the samples were within fair ranges between days 0 and 7 (Table 3 and Table 4) with sample H having the least TVC while sample F had a lower microbial load in terms of TCC. The results of the microbial analysis showed that the microbial load of the control sample (Sample N) increased rapidly when compared to its microbial load at Day 0 this agrees with the findings of Okafor [44] where it was suggested that a high microbial load

Table 2. pH of soymilk samples

pH of Soymilk Samples										
Days	**Soymilk Samples**									
	A	**B**	**C**	**D**	**E**	**F**	**H**	**J**	**M**	**N**
0	6.20	6.80	6.80	6.30	6.50	6.00	6.60	6.50	6.70	7.20
1	6.10	6.70	6.60	6.10	6.20	5.90	6.50	6.30	6.50	6.60
2	5.80	6.50	6.40	5.80	6.00	5.60	6.00	5.90	5.90	6.00
3	5.50	6.30	6.00	5.40	5.80	5.30	5.60	5.50	5.70	5.50
4	5.20	5.70	5.70	5.30	5.20	5.40	5.50	5.20	5.50	5.10
5	5.00	5.30	5.30	5.10	5.20	5.10	5.20	4.90	5.10	4.70
6	5.00	5.00	5.00	4.90	5.00	4.80	5.10	4.70	4.80	4.50
7	4.50	4.30	4.60	4.30	4.50	4.30	4.60	4.40	4.20	4.20
8	4.30	4.00	4.30	4.00	4.20	4.00	4.20	4.00	4.00	3.90
9	3.90	3.80	3.80	3.70	3.40	3.50	3.40	3.30	3.20	3.60
10	3.40	3.50	3.50	3.20	3.00	3.40	3.10	3.00	3.00	3.30
11	3.20	3.20	3.10	3.00	3.00	3.10	3.00	3.00	3.00	3.00
12	3.00	3.00	3.00	2.90	2.80	3.00	3.00	2.90	2.80	2.80
13	2.90	2.80	2.70	2.60	2.80	2.90	2.60	2.70	2.50	2.40

**Results are mean values of triplicate reading*

Table 3. Total viable counts of samples (×10^4)CFU mL^{-1}

Soymilk samples	Storage duration (Days)			Mean ±SD
	0	7	14	
A	0.80	1.40	4.00	2.07±1.7
B	0.80	1.80	3.80	2.13±1.53
C	0.90	1.70	2.70	1.77±0.9
D	1.00	1.60	3.90	2.17±1.53
E	0.80	1.80	3.80	2.13±1.53
F	1.00	1.00	3.00	1.67±1.15
H	0.50	1.50	3.50	1.83±1.53
J	0.60	1.70	4.50	2.27±2.01
M	1.00	1.60	3.30	1.97±1.19
N	0.80	2.00	5.00	2.6±2.16

**Results are mean values of triplicate readings*

Table 4. Total coliform counts of samples (×10^4) CFU mL^{-1}

Soymilk Samples	Storage duration (Days)			Mean ±SD
	0	7	14	
A	0.10	1.60	4.60	2.1±2.29
B	0.20	1.20	4.20	1.87±2.08
C	0.10	1.00	3.00	1.37±1.48
D	0.10	1.00	3.10	1.4±1.54
E	0.50	1.50	3.50	1.83±1.53
F	0.06	1.00	4.00	1.69±2.06
H	0.40	1.40	3.40	1.73±1.53
J	0.20	1.20	3.70	1.7±1.8
M	0.20	1.10	4.00	1.77±1.99
N	0.20	1.70	4.90	2.27±2.4

**Results are mean values of triplicate readings*

is associated with unpreserved soymilk. Some samples were out of range with TVC ranging from 4.0 x 10^4 CFU mL^{-1} to 5.0 x 10^4 CFU mL^{-1}thereby making them unsafe for human consumption. Soymilk samples maintained a fair range of microbial populations until day 7. Given that the maximum acceptable microbial load of soymilk is 3 x 10^4 CFU mL^{-1} [45] and considering this standard critical limit, the spoilage of soymilk and thereby shelf-life of soymilk samples was decided.

Microbial results showed that the total viable counts were generally higher than the total coliform counts which suggested that environmental conditions as a result of processing conditions and/or handling, had a higher impact on the microbial population of the soymilk samples.

3.3 Bacteria Isolated

Several bacteria were isolated from the various soymilk samples. Some of the organisms isolated were gram-positive cocci in pairs or chains, non-spore, non- motile, aerobic, catalase-negative and oxidase-negative.

Isolated colonies resulting from plate cultures on nutrient agar were subjected to conventional morphological characterization and biochemical tests, specifically to identify the probable bacteria present in the samples. Morphological characteristics (Table 5) were used in the identification of the probable organisms isolated from the soymilk samples, these characteristics gave a hint on the probable micro-organism that was present in the culture during bacterial analysis using nutrient and MacKonkey agar and further spread plate as the enumeration method. Probable organisms isolated (Table 6) included *Klebsiella spp., Streptococcus spp., Escherichia coli, Bacillus spp., Staphylococcus aureus, Pseudomonas spp., Enterobacter spp., Lactobacillus bulgaricus, and Proteus spp.* Processing and storage conditions influence the presence or absence of microorganisms that can cause undesirable changes or effects on soymilk

Table 5. Characterization of bacterial isolates from soymilk samples after 14 Days of Storage

													Sugar Fermentation						
Isolate	**Grams reaction**	**Citrate**	**Methyl red**	**Vogues proskeur**	**Hydrogen sulphid**	**Mortility**	**Coagulase**	**Indole**	**Catalase**	**Urease**	**Spore strain**	**Starch hydrolysis**	**Lactose**	**Glucose**	**Fructose**	**Sucrose**	**Mannitol**	**Maltose**	**Probable organisms**
1	-RODS	+	-	-	-	-	-	+	-	-	-	+	AG	AG	AG	AG	AG	AG	*Klebsiellaspp*
2	+COCCI In chain	-	+	-	-	-	-	-	-	-	ND	-	-	A	A	-	-	-	*Streptococcus sp*
3	-RODS	-	+	-	-	-	-	+	-	-	-	+	AG	A	AG	AG	AG	A	*Escherichia coli*
4	+RODS	+	+	-	+	+	-	-	+	+	+	-	-	A	AG	AG	AG	AG	*Bacillus spp*
5	+COCCI	+	-	-	+	-	+	+	+	-	-	+	A	A	A	A	AG	A	*Staphylococcus aureus*
6	-RODS	-	+	+	-	+	-	-	-	-	ND	+	A	A	A	A	A	A	*Pseudomonas sp*
7	-RODS	+	+	+	+	-	-	-	+	-	ND	+	-	A	-	-	AG	AG	*Enterobactersp*
8	+RODS	-	-	-	-	+	-	-	-	+	-	+	AG	AG	AG	AG	AG	AG	*Lactobacillus bulgaricus*
9	-RODS	-	-	+	-	+	-	-	+	+	+	+	-	AG	-	-	-	-	*Proteus sp*

Key: +=positive, -=negative, A=acid production, AG= acid and gas production, ND=not determined

Table 6. Bacteria isolated from soymilk samples

Bacteria Isolated	Sample									
	A	B	C	D	E	F	H	J	M	N
Klebsiella sp	-	+	-	+	-	+	+	+	+	+
Streptococcus sp	-	+	+	+	-	+	-	+	+	-
Escherichia coli	-	+	+	-	-	+	+	-	-	+
Bacillus spp	+	+	-	+	+	-	+	+	-	+
Staphylococcus aureus	+	-	+	+	-	+	+	+	+	-
Pseudomonas sp	+	-	+	-	+	+	+	+	+	-
Enterobacter sp	-	-	+	-	+	+	+	+	+	+
Proteus sp	-	+	+	+	-	-	-	-	+	+

Key: + = Present, - = Negative

been suggested that the soymilk condition [46,47,48]. It has favoured the growth of bacteria at pH of 7.2, this was observed in the present work. Likewise, the detection of *Escherichia coli* and *Staphylococcus aureus* in the soymilk sample represented a poor hygienic standard in its production process [48] which also agrees with the findings of Adeleke et al. [49]. As observed from the decline to the acidity of pH values (Table 2) of the soymilk samples, it has been suggested [48] that the occurrence of *Bacillus spp. and Lactobacillus spp.* could initiate spoilage of soymilk and a noticeable increase in acid production.

4. CONCLUSION

Without the addition of preservatives, soymilk on the average can keep for 48 hours [22], this is the average shelf life reported generally for most milk and milk-based products. Fortunately, the shelf life of soymilk can be extended by the use of different preservation techniques [50]. Several researchers are exploring the use of chemical preservatives for prolonging the shelf life of soymilk [51].

This study confirms that the use of citric acid and sodium benzoate in combination can appreciably prolong the shelf lifeof soymilk. In line with other studies [26,50,51], it was demonstrated that processing method, storage temperature, and storage duration have significant combined effects on the keeping quality of soymilk. The pH 7.2 for the control sample (sample N) [52] falls within the range obtained in this study, which incidentally favoured bacterial growth. Most strains of *Staphylococcus aureus* are known to be pathogenic mostly due to the heat-stable enterotoxin [53] they produce in direct relationship to their inoculum level [54]. Considering the notoriety of the resistance of *Staphylococcus aureus* to methicillin, other penicillins (an antibiotic) and cephalosporins [55,56,57], its detection regularly in the soymilk samples analyzed, poses a serious health hazard to consumers, hence, a call for aseptic processing and handling of soymilk. In contrast however, Tortora et al., [58] in their findings suggested that some of these microbes may not pose harmful effects to consumers since *Lactobacillus* has been found to assist in the enzymatic breakdown of food while some others synthesize useful vitamins. They also went further to suggest that *Staphylococcus aureus* is a normal microbiota of humans and animals.

In this study, preservation of soymilk samples from a combination of citric acid and sodium benzoate which are chemical preservatives was found to be more effective than several organic preservatives [59] where extracts of cloves were found to increase the shelf life of soymilk by 2 days while the extracts of guinea pepper extended it by a day and a combination of the extracts of cloves and guinea pepper extended the shelf life by 2 days.

Hence, they represent an alternative source of chemical antimicrobial substances for use in food systems to prevent the growth of food borne microorganisms and extend the shelf-life of processed food. Therefore, encouraging home preparation of soymilk and shelf storage. This could be a great solution to people who cannot afford to purchase power generating sets to compliment the epileptic nature of power supply in Nigeria. Following aseptic steps and the addition of the chemical preservatives used in the present studies, individuals could economically keep soymilk for a longer period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kolapo AL, Oladimeji GR. Production and quality evaluation of soy-corn milk. Journal of Applied Biosciences. 2008;1(2):40-45.
2. Vij S, Hati S, Yadav D. Biofunctionality of probiotic soy yoghurt. Food and Nutrition Sciences. 2011;2(5):502-509. DOI:10.4236/fns.2011.25073
3. Anderson JW, Bush HM. Soy protein effects on serum Lipoproteins: A Quality Assessment and Meta-Analysis of Randomized, Controlled Studies. Journal of the American College of Nutrition. 2011; 30(2):79-91
4. Sanni OM, Sobamiwa AO. Processing and characteristics of soybean fortified garri. World Journal of Microbiology and Biotechnology. 1994;10: 268-270.
5. Kolapo AL, Sanni MO. Soyabean fortified garri and tapioca. African Journal of Biotechnology. 2005;6(8):94-97.
6. Popoola OS, Akuesh CO. Micro-organisms associated with the fermentation of soybean for the production of Soybean 'Daddawa' (a condiment). Nigerian Food Journal. 1985;2:194-196.
7. Popoola OS, Kolapo AL, Afolabi OR. Biochemical deterioration of soybean daddawa- A condiment. Journal of Food Agriculture and Environment. 2007;5(1): 67- 70.
8. Nsofor LM, Nsofor ON, Nwachukwu KE. Soya- yoghurt starter culture Development from Fermented Tropical Vegetables. Journal of the Science of Food and Agriculture. 1992;60:515-518.
9. Olubamiwa AO, Kolapo AL, Odetoyinbo BB. Effect of different Starter Cultures on the chemical composition and acceptability of Soy-Yoghurt. Proceedings of the Being a paper delivered at the 37th Annual Conference of Nutrition Society of Nigeria, Abeokuta, Nigeria. 2006; 8-11.
10. Gianluca R, Luciana B. Soy, soy foods and their role in vegetarian diets. Nutrients, 2018;10(1):43.
11. Osundahunsi OF, Awor OC. Nutritional evaluation, with emphasis on protein quality, of maize-based complementary foods enriched with soybean and cowpea tempe. International Journal of Food Science and Technology. 2003;38:809 - 813.
12. Jimoh KO, Kolapo AL. Effect of different stabilizers on acceptability and shelf-stability of soy-yogurt. African Journal of Biotechnology. 2007;6:1000-1003.
13. King RA, Bignell CM. Concentrations of Isoflavones phytoestrogens and their glucosides in Australian soya beans and soya foods. Australian Journal of Nutrition and Dietetics. 2000;57:70–78.
14. Iwe MO. The science and technology of soybean. Rejoint communication Services Ltd., 2003;145.
15. Peæalvo JL, Castilho MC, Irene MN, Silveira MC, Esperanza MT. Fatty acid profile of traditional soymilk. European Food Resource and Technology. 2004; 2(19):251–253.
16. Adebayo-Tayo BC, Adegoke AA, Akinjogunla OJ. Microbial and Physico-chemical quality of powdered soymilk samples in AkwaIbom, South Southern Nigeria. African Journal of Biotechnology. 2009;8(13):3066- 3071.
17. Oladimeji GR, Kolapo AL. Evaluation of proximate changes and Microbiology of defatted residues of some selected Nigerian oil seeds. African Journal of Agricultural Research. 2008;3(In Press).
18. Hackler LR, Steinkraus KH, Van Buren JP. A comparison of the nutritional value of protein from several soybean fractions. Journal of Nutrition. 1962;80:63.
19. Egbo NN. M.Sc thesis in the Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria. 2012;97
20. Smita UK, Kiran SS, Govind BY. Studies on shelf-life of fresh soymilk. International Journal of Processing and Post-Harvest Technology. 2014;5(2):120-126.
21. Osuntogun B, Aboaba OO. Micro-biological and physico-chemical evaluation of some non-alcoholic beverages. Pakistan Journal of Nutrition, 2004;3(3):188-192.
22. Kayode RMO, Akinyoseye FA, Arotupia DJ. Comparative evaluation of the bacteria isolated from decomposing Cow milk and Soymilk. Journal of Agriculture Research and Development. 2005;4(2):40–45.
23. Lozano PR, Drake M, Benitez D, Cadwallader KR. Instrumental and sensory characterization of heat-induced odorants in aseptically packaged soymilk. Journal of Agricultural and Food Chemistry. 2007; 55(8):3018-3026.
24. Kohli D, Shahi NC. Food processing by pulse electric field: A Review. Advances in Research. 2017;9(2):1-6.
25. Achouri A, Boye JB, Zamani Y. Changes in soymilk quality as a function of

composition and storage. Journal of Food Quality. 2007;30(5):731-744.
26. Odu NN, Egbo NN, Okonkwo IO. Assessment of the effect of different preservatives on the shelf-life of soymilk stored at different temperatures. Researcher. 2012;4(6):62-69.
27. Messina TM. Soy products as common alternatives to cow milk products. Annals of Microbiology. 2013;50:43 -53
28. Onuorah CE, Adejare AO, Uhiara NS. Comparative physicochemical evaluation of soymilk and soycake produced by three different methods. Nigerian Food Journal. 2007;25(2):28-38.
29. Zamal SS, Uddin MB, Huda MS, Hannan A, Choudhury RU. Effect of preservatives on the shelf-life of soymilk. Eco-friendly Agriculture Journal. 2011;4(1):520-523.
30. Sebastian A, Barus T, Mulyono N, Yanti. Effects of fermentation and sterilization on the quality of soybean milk. International Food Research Journal. 2018;25(6):2428-2434.
31. AAFCO. Official Publication": 262.CFR - Code of Federal Regulations; 2004. Available:www.accessdata.fda.gov. (Accessed December 13, 2019)
32. CICA. Concise International Chemical Assessment Document 26, 2018: Benzoic acid and sodium benzoate; 2018. Available:Inchem.org. (Accessed December 13, 2019)
33. Cosmetic Ingredient Review Expert Panel Bindu Nair. Final Report on the Safety Assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. International Journal of Toxicology. 2001;20(3):23–50.
34. Code of Federal Regulations Title 21. Available:https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1733， (Accessed December 13,2019)
35. Tunde-Akintunde TY, Souley A. Effect of processing methods on quality of soymilk. Pakistan Journal of Nutrition. 2009;8(8): 1156-1158.
36. Carpenter PC. Microbiology, 4th edn. Saunders Company, Philadelphia. 1977; 401-402.
37. Thomas CGA. Medical microbiology, 3rd edn. Bauncere Tindall, London.1973;8-9.
38. Kirk CJ, Peel NR, James KR, Kershaw RN, Basic medical laboratory technology. Pitman Medical Publishing Co. Ltd., London. 1975;121-122.
39. MacFaddin JF. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA; 2000.
40. Cowan ST. Cowan and steel's manual for the identification of medical bacteria. 2nd edn. Cambridge University Press, Cambridge. 1974;46-67.
41. APHA. Standard methods for the examination of dairy products, 16th. American Public Health Association, Washington D.C; 1993.
42. Speck ML. Compendium of methods for the microbiological examination of foods. American Public Health Examination Association Inc. Washington, D.C. 1976; 566-567.
43. Achouri A, Boye JB, Zamani Y. Soybean variety and storage effects on soymilk flavour and Quality. International Journal of Food Science & Technology. 2008;43(1): 82-90.
44. Okafor N. Preliminary Microbiological Studies on the preservation of palm wine. Journal of Applied Bacteriology. 1975;38: 81-88.
45. IDF. Yoghur. Enumeration of characteristics micro-organisms colony count techniques at 37C. IDF standard 117, International Dairy Federation, Brussels; 1983.
46. Wilkens WE, Hackler LR. Effect of processing conditions on the composition of soymilk. Department of Food Science and Technology, Cornell University, New York. 1969;391-397.
47. Momoh JE, Udobi CE, Orunotan AA. Improving the microbial keeping quality of home made soymilk using a combination of preservation, pasteurization, and refrigeration. British Journal of Dairy Science. 2011;2(1):1-4.
48. Mbajiuka CS, Obeagu EI, Ifediora AC, Ugwu GU. Isolation and Identification of Microorganisms Involved in the Spoilage of Soymilk. Journal of Pharmacy and Biological Sciences. 2014;9(5):29-36.
49. Adeleke OE, Adeniyi BA, Akinrinmisi AA. Microbiological quality of local soymilk: A public health appraisal. African Journal of Biomedical Research. 2000;3:89-92.
50. Deepika K., Sanjay K, Shuchi U, Ritesh M. Preservation and processing of soymilk: A review. International Journal of Food Science and Nutrition. 2017;2(6):66-70.

51. Sumati RM, Shalini MR. Food Science. Wiley Eastern Limited, Bombay, India. 1986;196-207.
52. Davies JG. Dairy Microbiology. 1981;2: 42.
53. Stewart FS. Bigger's bacteriology and immunology for students of medicine 9thedn. ELBS and Bailliere, Tindal and Cassell; 1974.
54. Meyrand A, Boutrand-Loei S, Ray-Gueniot S, Mazuy C, Gaspard CE, Jaubert G, Perrin G, Lapeyre C, Vernozy-Rozand C. Growth and enterotoxin production of *Staphylococcus aureus* during the manufacture and ripening of Camembert-type cheeses from Raw Goats' Milk. Journal of Applied Microbiology. 1998;85: 537-544.
55. Adeleke OE, Odelola HA. Plasmid profiles of multiple drug-resistant local strains of *Staphylococcus aureus*. African Journal of Medical Sciences. 1997;26(7):191-121.
56. Allen JL, Cowan ME. Monitoring outbreaks of methicillin-resistant *Staphylococcus aureus*: Use of a commercial database and personal computer. British Journal of Biomedical Science. 1997;54:10-12.
57. Davies, S. Detection of methicillin-resistant *Staphylococcus aureus*: The evaluation of rapid agglutination methods. Journal of Biomedical Science.1997;54:13-15.
58. Tortora GJ, Funke BR, Case CL. Microbiology. An introduction. (5thedn.). Benjamin and Cummings Publishing Company Inc. California. 1995;285-298.
59. Kabiru YA, Makun HA, Saidu AN, Muhammad LH, Nuntah LC, and Amoo SA. Soymilk preservation using extracts of cloves *Syzygium aromaticum* Myrtaceae and Guinea-Pepper *Xylopia aethiopica Annonaceae*. Journal of Pharmacy and Biological Sciences. 2012;3(5):44-50

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57063