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Improving the Microbial Keeping Quality of Home Made Soymilk Using a Combination of Preservatives, Pasteurization and Refrigeration

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Abstract: The greatest problem consumers encounter with soy milk remains its very short shelf life due to microbial activity. The combination of different preservation methods may just be the solution to the problem. The effect of certain preservatives at various concentrations within their maximum permissible levels along with pasteurization and refrigeration storage on the microbial keeping quality of home- made soy milk was therefore, studied. This is with the intention of determining which combination is best for a prolonged shelf life. Standard microbiological techniques were employed in the enumeration of potential spoilage organisms in soy milk samples preserved with permissible levels of sodium benzoate and sodium metabisulphite over a period of time. Results obtained showed that soy milk can keep for up to 13 days at refrigeration temperature during which no reasonable multiplication of mesophilic aerobes above $3x10^3$ cfu/mL was observed and a total inhibition of yeasts and molds were achieved when preserved with between 700-800 parts per million (ppm) of sodium benzoate, pasteurization and refrigeration while a combination of 400 ppm of sodium benzoate and 175 ppm of sodium metabisulphite can achieve a preservation of the milk for about 11 days.

Key words: Pasteurization, preservatives, quality, refrigeration, Soy milk

INTRODUCTION

Soymilk is an aqueous, white, creamy extract produced from soybeans which resembles cow milk both in appearance and consistency. It is a highly nutritious food drink which contains protein, fat, carbohydrates vitamins and minerals. It is because of this nutritious value and comparative low cost, (Wilson, 1995), that soymilk plays an important role in the dietary pattern of people in most developing countries.

Recently, the consumption of soymilk has greatly increased for reasons which include poverty alleviation and because it is recommended for people that cannot tolerate lactose since it does not contain lactose. It is continuously being used as a substitute to cow milk in most remote areas of Nigeria and indeed Africa. This may also be because it has a few other known advantages over cow milk e.g., It has a beneficial effect in the prevention of protein energy malnutrition in infants and growing young children as well as in the prevention of osteoporosis and kidney diseases (Messina, 1995).

The nutritious nature of soymilk however, makes it prone to microbial attack if not properly processed and stored as the nutrients it contains are also required for the growth of most spoilage organisms. A large number of microorganisms such as mesophilic aerobic bacteria, coliforms, yeasts and moulds are known to be responsible for the spoilage of soymilk, producing undesirable changes in the milk (Osuntogun and Aboaba, 2004). In Nigeria and most West African countries, soymilk is produced mostly at home under not very hygienic conditions and is thus prone to contamination and spoilage by the microflora of the raw materials and utensils. The metabolic products of these organisms as well as their presence in soy milk, pose health hazards to the consumers. The addition of preservatives, pasteurization and refrigeration however, are processes used to either prevent the proliferation and or to eliminate these harmful and spoilage organisms which may bring about undesirable fermentation or cause diseases to consumers in milk and milk products.

This study presents the results of the efforts to determine the most appropriate concentrations of certain preservatives that can be used along with refrigeration and pasteurization to improve the microbial keeping quality of soy milk.

MATERIALS AND METHODS

Materials: The major materials used for this work include Soya bean seeds (purchased from central market, Kaduna-Nigeria), Sodium benzoate, Sodium Metabisuphite, Domestic pulveriser (blender), muslin cloth, weighing balance (Gallenkamp, England) and water bath (Gallenkamp, England). These materials were assembled at the Microbiology laboratory of the Department of Food Technology of Kaduna Polytechnic where the work was done.

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Methods:

Production of Soy milk: Cleaned, sorted (To remove cracked, damaged and discoloured seeds) and winnowed soybean seeds were rinsed and soaked in water about three times the volume of the beans for about 10 h. The water was however, changed at three hours intervals. The beans were then parboiled in water for a few minutes with constant agitation. The boiled beans were cooled, dehusked, thoroughly washed and further homogenized with clean water into a paste. The paste obtained, was extracted, (sieved) using a clean muslin cloth to separate the milk (filterate) from the paste. The milk (Soymilk) so produced was boiled to remove the beany flavor which is characteristic of soy milk. Sodium benzoate and sodium metabisulphite were then added at different concentrations (Taking note of their maximum permissible levels in foods). The product were then packaged and pasteurized.

Addition of preservatives and storage: Different concentrations of Sodium benzoate and sodium metabisulphite were added to the pasteurized products thus:

- To each tube of the milk sample, different concentrations of sodium benzoate (between 100-800 ppm) were added and each tube was duplicated.
- To another set of milk samples different concentrations of sodium metabisulphate (between 50-350 ppm) were added and each tube was also duplicated.
- To another set, a combination of sodium benzoate and sodium metabisulpite in the following combinations were added into each tube (in duplicates)

1) 400 ppm sodium benzoate +175 ppm sodium metabisulphite
2) 200 ppm sodium benzoate +87.5 ppm sodium metabisulphite

One set of the tubes were stored in the refrigerator while the second set were left at room temperature. The number of mesophilic aerobic bacteria, yeasts and molds in each tube was determined daily for 14 days.

Microbiological analysis:

Enumeration of mesophilic aerobic bacteria: The mesophilic aerobic bacteria in the product were enumerated using the standard plate count method as described by the food and Agricultural organization (FAO, 1986)

1ml of each milk sample was asceptically transferred into a sterile tube containing 9 mls of sterile ringers solution to obtain a sample of 1:10 dilution. Using separate sterile pipettes, decimal dilutions of $10^{-2,}$ 10^{-3}, $10^{-4,}$ 10^{-5} up to 10^{-9} were prepared by asceptically transferring 1ml of previous dilutions to 9mls of diluents in different test tubes. All the tubes were vigorously shaken. 1ml of each dilution was then transferred to separate marked petri dishes (in triplicates).

15 mls of plate count agar cooled to 45ºC was then added to each plate and gently rocked clockwise and anticlockwise. The plates were allowed to solidify and then incubated at 37ºC for 18 to 24 h. After incubation, the colonies growing on the plates we counted, and the colony forming units (cfu) in the original samples calculated.

Enumeration of Yeast and mold: The method employed is as described for the enumeration of mesophilic aerobic bacteria. The only differences are:

- The use of Potato dextrose agar in place of plate count agar
- The inclusion of10% tartaric acid into the culture medium to suppress the growth of bacteria.
- The incubation of the inoculated plates at 22-25ºC for 3-5 days suitable for the growth of molds and yeast.

RESULTS

Results of the enumeration of mesophilic aerobes, Yeasts and molds are as shown in Table 1-4.

Table 1: Result of Bacterial count after storage at Refrigeration temperature with the addition of preservatives

		Period of storage (in days)														
S. No.	Sample code	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aerobic plate count(cfu/mL)																
1	SB800	-	-	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	2.8×10^3	3.0×10^3	3.4×10^4
2	SB700	-	-	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	3.0×10^3	3.0×10^3	3.6×10^4
3	SB600	-	-	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	3.4×10^3	2.8×10^4	3.8×10^4
4	SB500	-	-	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	2.7×10^2	3.0×10^3	3.8×10^3	4.1×10^4	4.6×10^5
5	SB400	-	-	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	2.0×10^3	2.2×10^3	2.0×10^4	2.6×10^4	3.0×10^5	3.5×10^6
6	SM350	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times0^2$	$<3.0\times10^2$	3.0×10^4	2.7×10^4	1.0×10^5					
7	SB300	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times0^2$	$<3.0\times10^2$	2.1×10^3	2.8×10^3	3.2×10^4	3.4×10^4	30×10^5	3.6×10^5	4.0×10^6	4.1×10^6
8	SM250	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times0^2$	$<3.0\times10^2$	$<3.4\times10^2$	2.9×10^4	3.0×10^5					
9	SB200	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times0^2$	2.0×10^2	2.5×10^3	3.0×10^4	3.6×10^4	3.9×10^4	$<4.2\times10^6$	4.4×10^6	4.8×10^6	5.1×10^7
10	SM150	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times0^2$	2.8×10^4	3.0×10^4	3.0×10^5	4.7×10^5					
11	SB100	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times0^2$	2.6×10^2	2.9×10^3	3.3×10^4	3.8×10^4	3.9×10^4	4.7×10^7	5.0×10^6	1.2×10^7	5.5×10^7
12	SM50	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times103$	$1.0\times10_3$	2.6×10^3	3.0×10^3	3.1×10^4	3.0×10^5	4.4×10^6					
13	SB400 +SM 175							$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	2.6×10^2	3.0×10^3		4.0×10^4	4.0×10^5
14	SB200 +SM87.5					$<3.0\times10^2$	$<3.0\times10^2$	$<3.1\times10^2$	2.1×10^3	2.8×10^4	3.0×10^4	3.2×10^4	3.4×10^4	4.0×10^6	4.0×10^7	
CONTROL				$<3.0\times10^2$	$<3.0\times10^2$	3.0×10^2	3.6×10^2	3.8×10^3	4.4×10^4	2.8×10^5	3.0×10^6	2.9×10^7	3.0×10^2			

SM = Sodium Metabisulphite; SB = Sodium Benzoate, Each sample code also denotes the type of preservative and its concentration added

Table 2: Result of Bacterial count after storage at room temperature with the addition of preservatives

Period of storage (in days)																
S. No.	Sample code	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aerobic plate count(cfu/mL)																
1	SB800	-	$<3.0\times10^2$	$<3.0\times10^2$	3.2×10^2	3.3×10^3	3.0×10^5	3.0×10^5								
2	SB700		$<3.0\times10^2$	$<3.0\times10^2$	3.3×10^2	3.6×10^3	3.2×10^4	3.6×10^5								
3	SB600	-	$<3.0\times10^2$	$<3.0\times10^2$	3.5×10^2	3.8×10^3	4.0×10^4	3.9×10^6								
4	SB500	-	$<3.0\times10^2$	$<3.0\times10^2$	3.8×10^2	4.0×10^3	4.8×10^4	5.0×10^5								
5	SB400	-	$<3.0\times10^2$	$<3.0\times10^2$	4.0×10^2	4.6×10^3	2.2×10^5	2.0×10^6								
6	SM350	-	$<3.0\times10^2$	3.2×10^2	3.0×10^3	3.4×10^4	3.1×10^5	4.02×10^6								
7	SB300	-	$<3.0\times10^2$	3.0×10^2	3.0×10^3	3.4×10^4	3.0×10^5	4.0×10^2								
8	SM250	-	$<3.0\times10^2$	3.3×10^2	3.3×10^3	3.5×10^4	3.9×10^5	5.0×10^6								
9	SB200	-	$<3.0\times10^2$	3.3×10^2	3.2×10^2	3.6×10^4	4.0×10^5	5.2×10^6								
10	SM150	-	$<3.0\times10^2$	3.4×10^2	3.6×10^3	4.5×10^4	4.0×10^5	7.0×10^6								
11	SB100	-	$<3.0\times10^2$	3.4×10^2	3.7×10^3	4.4×10^4	2.6×10^6	3.0×10^7								
12	SM50	-	-	$<3.0\times10^2$	1.0×10^3	1.0×10^3	2.6×10^3	3.0×10^3	3.1×10^4	3.0×10^5	4.4×10^6					
13	SB400 +SM 175		$<3.0\times10^2$	$<3.0\times10^2$	3.9×10^2	4.1×10^3	4.1×10^4	2.0×10^6								
14	SB200 +SM87.5		$<3.0\times10^2$	3.2×10^2	3.0×10^3	3.4×10^4	3.8×10^5	5.0×10^6								
CONTROL			3.0×10^2	3.4×10^2	3.6×10^6	3.6×10^6	4.0×10^7	8.0×10^8								

SM= Sodium Metabisulphite; SB= Sodium Benzoate

Table 3: Results of Yeast and Mold count after storage at refrigeration temperature with the addition of preservatives

Period of storage (in days)																
S. No.	Sample code	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aerobic plate count(cfu/mL)																
1	SB800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	SB700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	SB600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	SB500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	SB400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	SM350	-	-	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	$2.8X10^3$	3.0×10^3	3.1×10^4	3.3×10^4	3.3×10^4	3.6×10^5	4.8×10^7
7	SB300	-	-	-	-	-	-									
8	SM250	-	-	-	-	-		$<3.0\times10^2$	2.2×10^3	$2.9X10^3$	3.2×10^4	3.8×10^4	1.5×10^5	2.4×10^6	6.6×10^6	7.0×10^7
9	SB200	-	-	-												
10	SM150	-	-	-	-	-	-	$<3.0\times10^2$	3.0×10^3	$3.2X10^3$	3.4×10^4	4.0×10^4	2.0×10^5	3.0×10^6	2.0×10^7	2.5×10^8
11	SB100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	SM50	-	-	-	-	-	-	3.1×10^4	3.2×10^3	$3.6X10^3$	4.1×10^4	2.0×10^5	2.6×10^5	3.4×10^6	4.0×10^7	4.0×10^8
13	SB400 +SM 175	-	-	-	-	-	-	-	-	-	-		-	-	-	-
14	SB200 +SM87.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CONTROL		-	-	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	$<3.0\times10^2$	3.0×10^2	3.0×10^3	3.0×10^5	4.2×10^5	4.8×10^6	-	-	-	-

SM= Sodium Metabisulphite; SB= Sodium Benzoate

Table 4: Result of Yeast and Mold count after storage at room temperature with the addition of preservatives

Period of storage (In days)																
S.No.	Sample code	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aerobic plate count(cfu/ml)																
1	SB800	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	3.3×10^2	3.6×10^2	3.4×10^3	3.8×10^4	4.4×10^5				
2	SB700	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	3.5×10^2	4.0×10^2	3.6×10^3	4.0×10^4	4.8×10^5				
3	SB600	-				$<3.0\times10^2$	$<3.0\times10^2$	4.0×10^2	4.6×10^2	3.8×10^3	4.4×10^2	5.0×10^5				
4	SB500	-	-			$<3.0\times10^2$	$<3.0\times10^2$	3.0×10^3	3.2×10^3	4.0×10^4	4.9×10^4	2.0×10^6				
5	SB400	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	3.2×10^3	3.6×10^3	4.2×10^4	5.0×10^4	2.4×10^6				
6	SM350	-	$<3.0\times10^2$	3.0×10^2	3.2×10^3	3.0×10^4	3.0×10^4	3.0×10^5	4.1×10^5							
7	SB300	-				$<3.0\times10^2$	3.0×10^2	3.3×10^3	3.9×10^3	5.0×10^4	5.1×10^4	3.6×10^6				
8	SM250	-	$<3.0\times10^2$	3.4×10^2	3.8×10^3	3.4×10^4	3.7×10^7	4.0×10^5	4.6×10^5							
9	SB200	-	-	-	$<3.0\times10^2$	3.1×10^2	3.3×10^3	3.6×10^3	2.0×10^4	1.5×10^5	2.2×10^6	4.6×10^6				
10	SM150	-	$<3.0\times10^2$	3.6×10^2	4.0×10^3	3.6×10^4	4.0×10^5	4.4×10^5	2.0×10^6	-	-	-				
11	SB100	-	$<3.0\times10^2$	3.0×10^2	3.2×10^2	3.6×10^4	3.8×10^2	2.6×10^4	3.5×10^5	3.0×10^6	2.0×10^7					
12	SM50	-	3.0×10^2	3.8×10^2	6.0×10^3	4.4×10^4	6.0×10^5	2.0×10^6	1.2×10^7							
13	SB400 +SM 175	-	-	-	-	$<3.0\times10^2$	$<3.0\times10^2$	3.0×10^2	3.2×10^3	3.8×10^4	4.8×10^4	6.0×10^5				
14	SB200 +SM87.5	-	-	-	-	$<3.0\times10^2$	3.2×10^2	3.4×10^3	1.9×10^4	6.0×10^4	4.0×10^5	7.0×10^5				
CONTROL		-	-	3.0×10^2	3.0×10^3	4.2×10^4	3.3×10^5	4.6×10^6	5.5×10^7	6.1×10^8	-	-	-	-	-	-

SM = Sodium Metabisulphite; SB = Sodium Benzoate

DISCUSSION

Soymilk remains a very important local beverage in most parts of Africa and other parts of the world. Its high nutrient value has made it so irresistible that it is recommended very highly by nutritionists as a substitute to cow milk. The greatest problem encountered with soymilk however, has remained its relatively short shelf life. This is explained by the fact that the nutrient content of the milk are also known major requirements for the growth and proliferation of most of its spoilage microorganisms which are mainly bacteria, yeasts and molds. The processing and storage conditions of soy milk however, influence the presence or absence of these

spoilage organisms in the milk, where they multiply and cause unwanted effects. For example, yeasts and molds are problematic in foods in that they discolour food surfaces, cause off odours and off flavours as well as produce toxins in certain instances.

Chemical preservatives are included in food and pharmaceutical preparations to prevent microbial spoilage of the products and to minimize the risk of the consumer acquiring an infection when the preparations are taken. (Sean *et al*., 2004). These chemical agents affect microorganisms by disrupting critical cell factors e.g they may damage the plasma membrane or denature various cell proteins while others interfere with the functioning of nucleic acids thus inhibiting cell reproduction (Lansing *et al*., 2002). Benzoic acids(C_6H_5COOH), for instance has been employed in different forms as a preservative in foods among other organic acids because of its established antimicrobial properties especially against yeasts and molds and since its sodium salt is more soluble in water than other forms, benzoic acid is generally used in such form in foods (Ihekoronye *et al*., 1985) at levels not exceeding 0.1% (James *et al*., 2000; Lansing *et al*., 2002). The sodium salt of metabisulphites is also used as preservatives in foods at levels not exceeding 300ppm (Lansing *et al*., 2002). They are metabolized easily to sulphate and excreted in the urine without any obvious pathological result (Ihekoronye *et al*., 1985).

Results obtained showed that sodium benzoate at a concentration of 700-800 ppm along with refrigeration was able to control the proliferation of mesophilic aerobic bacteria maintaining them within the levels of 3×10^3 for up to 13 days of storage at refrigeration temperature (Table 1). At room temperature, it was possible to control the proliferation of mesophilic aerobes to 3×10^3 for only four days with up to 800 ppm of sodium benzoate (Table 2). Yeasts and molds were controlled to the permissible levels of 3×10^3 for 8 days when 600- 800 ppm of sodium benzoate was used while lower concentrations of 100-500 ppm were able to achieve preservation for only 7 days at room temperature (Table 4).

Sodium Metabisulphite at 250-350 ppm along with pasteurization and refrigeration was able to keep the mesophiles within the permissible level for around 8 days while lower concentration of 150 ppm was able to keep the product for around 6 days. At room temperature, sodium metabisulphite was able to control the mesophiles maintaining them at acceptable levels for only three days at a concentration of 150-350 ppm and for 2 days at a concentration of 50 ppm. The yeasts and molds were maintained at acceptable levels for 4 days at a concentration of 350 ppm and for 3 days at 50-250 ppm.

Preservatives have been used in foods in combinations. This is because the combines can have either a synergistic or a combined effect which will be better than the effect produced by one of them. Sodium benzoate and sodium metabisulphite combination (400 ppm+175 ppm) was able to maintain the mesophiles at acceptable levels for a reasonable 12 days and for 9 days at a concentration of 200 ppm sodium benzoate and 87.5 ppm sodium metabisulphite (Table 1). Generally, pasteurization must have destroyed most growing organisms in the product while the combined effects of the preservatives and the low temperature storage controlled the proliferation of those left. Microoganisms which are the known spoilage agents of soymilk can be controlled using a combination of pasteurization, preservatives and refrigeration.

CONCLUSION

When combined appropriately as per the recommendations of this work, it is possible for the products even though locally produced to keep for as much as 13 days in contrast to the known standard of only a few days.

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