Assessment of the Effect of Different Preservatives on the Physico-chemical Quality of Soy milk Stored at Different Temperatures

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ABSTRACT: The aim of this study was to assess the effect of different preservatives on the physico-chemical quality of Soy milk stored at different temperatures. The utilization of soy bean for the production of soy milk was evaluated. Soy milk was extracted from whole and dehulled seed, pasteurized and fermented. Physicochemical qualities of the soy milk samples were evaluated to determine the physical and chemical quality of the products. All the samples were treated with potassium sorbate and sodium benzoate (in combination) with propyl gallate and Ascorbyl palmitate (singly and in combination), organoleptically evaluated and stored at ambient temperature and 4 ± 2^{O}C for 16 days and were subjected to physical examination and chemical analyses such as pH and titratable acidity (TTA). There was a gradual decrease in the pH and an increase in the total titratable acidity of the samples. For both pH and TTA, there was significant difference ($P<0.05$) between all the treatments and the length of storage. At ambient temperature, a rapid decline in pH was dominant especially for samples treated with $NaHCO_3$. Samples treated with Na_2CO_3 had a higher pH stability compared to its counterpart. At refrigeration temperature, there was a slow decrease in pH over time. Samples treated with Na2CO$_3$ also had a stable pH during the first few days of storage (4-10). For TTA, samples treated with $NaHCO_3$ had the highest increase in TTA value compared to samples treated with Na_2CO_3 at ambient temperature. The control showed least increase (from 0.026-0.036) for samples treated with Na_2CO_3 and 0.027-0.037 for sample treated with $NaHCO_3$. At refrigeration, due to longer storage time, there was a higher value for TTA. Ascorbyl Palmitate treated samples showed marked increase in TTA values in comparison with those treated in combination and singly. However, in all the samples, the control showed the least increase (for Na_2CO_3 0.026-0.039 and 0.027-0.037 for $NaHCO_3$). No direct relationship was observed between pH values and titratable acidity. The study showed that the overall changes in pH and TTA observed for all the stored soy milk samples in the study may be suggesting that the fermentative activities of the soy milk starters still continued in storage. Generally, improved combined preserving techniques can in particular improve keeping quality; lessen the risk of microbial food poisoning.

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1. INTRODUCTION

Grain legumes serve as a cheap source of protein to a large proportion of the population in poor countries of the tropics (Massawe *et al*, 2005). In recent years, different edible varieties of legumes have been identified that have high nutritional value and therefore could help to address a number of diet related problem globally (Kolapo and Oladimeji, 2008). Development of milk substitutes extracted from legumes serves as an alternative way of producing an acceptable nutritious food based on vegetable. Soybean (Glycine max) is recognized as one of the crops with huge potential the world over. This plant has been exploited for the manufacture of food products such as soybean fortified garri and tapioca and cereals-based traditional foods (Kolapo and Oladimeji, 2008; Iwe, 2003; Ikpeme *et al*, 2009).

Soymilk yoghurt serves as a very good alternative to the expensive cowmilk yoghurt (Nsofor and Maduako, 1992; Ashaye *et al*., 2001; Jimoh and Kolapo, 2007; Osundahunsi *et al*., 2007; Farinde *et al*., 2008, 2009, 2010). Soymilk has a characteristic beany flavour and this offflavour has often made it less acceptable than cow milk, but this has reportedly been reduced by lactic acid fermentation (Mital *et al*., 1974; Pithang *et al*., 1980; Farinde *et al*., 2010). Lee *et al*. (1990) reported the health benefit of lactic acid fermentation of soymilk to include reduced level of cholesterol. Chang *et al*. (2005) also reported that intake of fermented soymilk improves the ecosystem intestinal tract by increasing the amount of probiotics. Various processing methods have been developed to reduce syneresis in soy-yoghurt and improve its acceptability (Moor, 1985; Lee et al., 1990; Collins et al., 1991; Jimoh and Kolapo, 2007; Farinde *et al*., 2010).

The physicochemical characteristics of soymilk are the ones which affect the behavior of proteins in foods and such properties includes the protein content, viscosity, and pH content in the soymilk. Soymilk and cows milk contain approximately the same protein content (Smith and Circle, 1972, Piper and Morse, 1943). The degree of acidity or alkalinity of foods is also a very important factor in the properties and value of food and soymilk is not an exception to this. Studies show that the pH of soymilk produced by traditional oriental method is slightly acidic (5.38%) and lower in concentration when compared to the pH of normal cow's milk (Onuorah et al; 2007).

The aim of this study was to assess the effect of different preservatives on the physico-chemical quality of Soy milk stored at different temperatures.

2. MATERIALS AND METHODS

Soymilk was prepared using two methods modified from Illinois method.

2.1. Method 1: soybean was sorted to remove stones and damaged, deformed seeds. The soybean was washed and soaked in water (500g in 1 Liter) for 12 hours. It was rinsed and blanched in 1.25% $NaHCO_3$ for 30 minutes. The soybean was washed, manually dehulled and rinsed. The soybean seeds were ground in blender (kenwood) and expressed in the ratio of 3:1 to remove the okra. The resultant slurry was formulated by adding 0.1% of sodium benzoate and 0.1% potassium sorbate, 2% sucrose and propy gallate and Ascorbic pamitate at this ratios: 100ppm Ascorbic palmitate and 100ppm propyl gallate, 200ppm Ascorbic palmitate, 200ppm propyl gallate and Control (without preservative and antioxidant). The milk was heated at 71°C for 15 seconds and subsequently bottled and stored at ambient and refrigeration temperature.

2.2. Method 2: soybean was sorted to remove stones and damaged, deformed seeds. The soybean was washed and soaked in water (500g in 1 Liter) for 12 hours. It was rinsed and blanched in 1.25% Na_2CO_3 for 30 minutes. The soybean was washed, manually dehulled and rinsed. The soybean seeds were ground in blender (kenwood) and expressed in the ratio of 3:1 to remove the okra. The resultant slurry was formulated by adding 0.1% of sodium benzoate and 0.1% potassium sorbate, 2% sucrose and propy gallate and Ascorbic pamitate at this ratios: 100ppm Ascorbic palmitate and 100ppm propyl gallate, 200ppm Ascorbic palmitate, 200ppm propyl gallate and Control (without preservative and antioxidant). The milk was heated at 71°C for 15 seconds and subsequently bottled and stored at ambient and refrigeration temperature.

2.3. PHYSIOCHEMICAL ANALYSIS

The chemical composition of the sample was determined by standard methods in triplicate. pH and tritrable acidity were determined as described by Aderibigbe and Osegboun (2006), Olubamiwa et al. (2006) and Njoku *et al.* (1991) respectively.

2.3.1. pH

The pH was determined by the use of a pH meter every 2 days interval. The pH value was done by taking 10ml of soymilk from each of the sample into a beaker. The pH meter was then standardized using a buffer solution. The electrode of the pH meter was then washed with distilled water and dipped into each beaker with d samples (rinsing out the electrode with distilled water before introducing it into a new beaker). The pH value of each sample was read off.

2.3.2. TOTAL ACIDITY

It was determined by titrating suitable aliquots of the diluted sample against a 0.01N NAOH solution and was expressed as a percentage of lactic acid.

2.3.3. TITRATABLE ACIDITY

It was determined by titrating 1.0ml of sample against 0.1N NAOH using phenolphthalein as the indicator. The appearance of a pink colour marked the end point of titration. The titratable acidity (expressed as percent lctic acid) was determined using: 1ml 0.1N NaOH = 0.009008ml lactic acid (ACID FACTOR); TA=NORMALITY OF BASE X VOLUME OF BASE X ACID FACTORX100/VOLUME OF SAMPLE.

2.3.4. TOTAL SOLID

The samples were filtered and the filtrate evaporated to dryness in a weighing dish and was dried to constant weight at 180°C. The increase in dish weight represent total dissolve solid. It was calculated as:

Weight of the dried residue + dish, mg – weight of the dish x 1000.

2.4. DATA ANALYSES

The data obtained were subjected to analysis of variance (ANOVA) using Graph Pad Prism Software, version 5.01. Significant difference between means were determined at $p<0.05$. The result of the study collated at the end of the storage was analyzed using statistical means to determine if there were any significant differences among their means. T-test was used to determine the relationship (difference) between the different temperatures of storage for both the market and sample soymilk. This was because t-test measure's the differences between the means of two variables. Also t-test was used to analyze if significant differences exist between the soymilk treated with acid salt ($NaHCO_3$) and the soymilk treated with alkaline salt (Na_2CO_3). Two way ANOVA was also used to determine if differences among the individual samples, in terms of pH and TTA.

3. RESULTS ANALYSIS

In this study, sample A was $NaHCO_3$ Soymilk treated with Propyl Gallate, sample B was $NaHCO_3$ Soymilk treated with Ascorbyl Palmitate, sample C was $NaHCO_3$ Soymilk treated with both Propyl Gallate and Ascorbyl Palmitate, sample D was $NaHCO_3$ Control, sample E was Na_2CO_3 Soymilk treated with Propyl Gallate, sample F was Na_2CO_3 Soymilk treated with Ascorbyl Palmitate, sample G was Na_2CO_3 Soymilk treated with both Propyl Gallate and Ascorbyl Palmitate while sample H was Na_2CO_3 Control.

3.1. CHANGES IN pH

The pH of the entire sample followed the same decreasing pattern as storage progressed (Table 1). At ambient temperature, a rapid decline in pH was dominant especially for samples treated with $NaHCO_3$. In all samples the control has the most significant decrease. Samples treated with Na_2CO_3 had a higher pH stability compared to its counterpart. In all, there were no significant variations ($P<0.05$) in the individual samples treated with propyl gallate and asorbyl palmitate.

Table 1: pH of sample soymilk at ambient temperature

	pH values							
	Samples							
Days	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0	7.02	7.01	7.02	7.00	7.22	7.22	7.21	7.21
2	6.52	6.55	6.53	6.50	6.43	6.46	6.41	6.44
4	5.43	5.42	5.30	4.82	5.65	5.64	5.65	5.60
6	4.82	4.85	4.82	4.75	4.96	4.94	4.97	4.00
8	3.75	3.73	3.72	3.10	3.97	3.98	3.98	3.10
10	3.63	3.63	3.62	3.00	3.85	3.84	3.84	3.02

At refrigeration temperature (Table 2), there was a slow decrease in pH over time. Samples treated with Na2CO_3 also had a stable pH during the first few days of storage (4-10). Samples treated with $NaHCO_3$ had a slow decrease but with lower values.

Figure 1 illustrates changes in sample pH at ambient temperature. Figure 2 shows variations of pH values in the individual samples and changes in pH over time (storage). However, the control samples showed marked decrease with that treated with $NaHCO_3$ only being most apparent. These changes in pH seem to be stabilized at day 8 and 10 (where pH values ranged from 3.9-3.02) for both Na_2CO_3 and $NaHCO_3$. The individual sample that showed the least decrease (7.01-3.84) was sample F (soymilk treated with Ascorbyl Palmitate).

Table 2: pH of sample soymilk at refrigeration temperature

	pH-refrigeration							
	Samples							
days	A	B	C	D	E	F	G	H
0	7.02	7.01	7.02	7.00	7.22	7.22	7.21	7.21
4	6.12	6.18	6.18	6.10	6.33	6.32	6.35	6.30
6	6.02	6.01	6.05	6.00	6.24	6.28	6.24	6.10
8	6.04	6.02	6.03	5.90	6.19	6.18	6.19	6.00
10	6.00	6.00	6.00	5.60	6.02	6.01	6.02	5.98
12	5.75	5.76	5.75	4.00	5.94	5.94	5.92	4.50
14	5.64	5.64	5.65	3.61	5.87	5.86	5.83	3.80
16	4.56	4.56	4.54	3.39	4.77	4.76	4.77	3.50

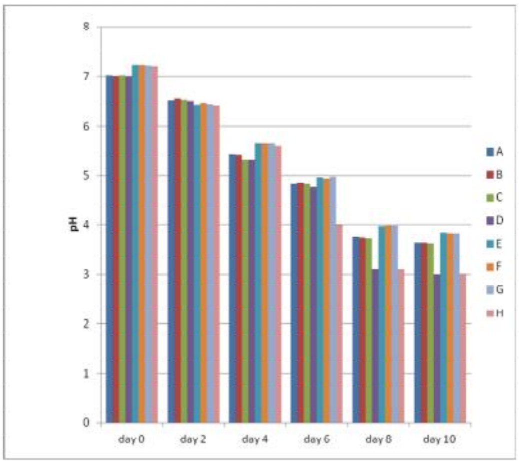

Figure 1: pH of sample soymilk at ambient temperature

Figure 2 illustrates changes in sample pH at refrigeration temperature. It showed that there was a slow decrease in pH over time.

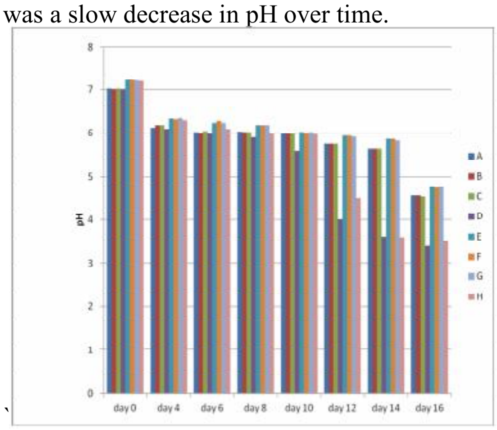

Figure 2: pH of sample soymilk at refrigeration temperature

3.2. CHANGES IN TITRATABLE ACIDITY

The changes in titratable acidity showed similar increase in acidity with storage time (Table 3). At ambient temperature, samples treated with $NaHCO_3$ had the highest increase in titratable acidity value compared to samples treated with Na_2CO_3. Individually, samples treated with Ascorbyl Palmitate had a higher increase in titratable acidity values in comparison with those treated propyl gallate, in combination. The control showed least increase (from 0.026-0.036) for sample control treated with Na_2CO_3 and for sample treated with $NaHCO_3$ (0.027-0.037).

Table 3: Titratable Acidity (TTA) of sample soymilk at ambient temperature

Titratable Acidity (TTA) - ambient								
Sample code								
days	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0	0.033	0.033	0.033	0.027	0.030	0.030	0.030	0.026
2	0.032	0.033	0.033	0.030	0.031	0.032	0.031	0.028
4	0.035	0.036	0.035	0.032	0.034	0.034	0.034	0.030
6	0.037	0.037	0.037	0.033	0.036	0.036	0.035	0.031
8	0.038	0.039	0.035	0.039	0.037	0.038	0.037	0.035
10	0.039	0.039	0.039	0.037	0.039	0.039	0.038	0.036

Figure 3 illustrates the variations in the titratable acidity of produced soymilk at ambient temperature. The study also showed variations in the individual samples titratable acidity values were negligible (they have close values).

Table 4 shows the values of the titratable acidity (TTA) of sampled soymilk at refrigeration temperature. At refrigeration temperature (Table 4), due to longer storage time, there was a higher value for titratable acidity. Ascorbyl Palmitate treated samples showed marked increase in titratable acidity values in comparison with those treated in combination and singly. However, in all the samples, the control showed the least increase (for Na_2CO_3 0.026-0.039 and for $NaHCO_3$ 0.027-0.037).

Figure 4 illustrates the variations in the titratable acidity of produced soymilk at refrigeration temperature. Here also the variations in titratable acidity values of the individual samples were negligible. It showed changes in titratable acidity as storage progressed.

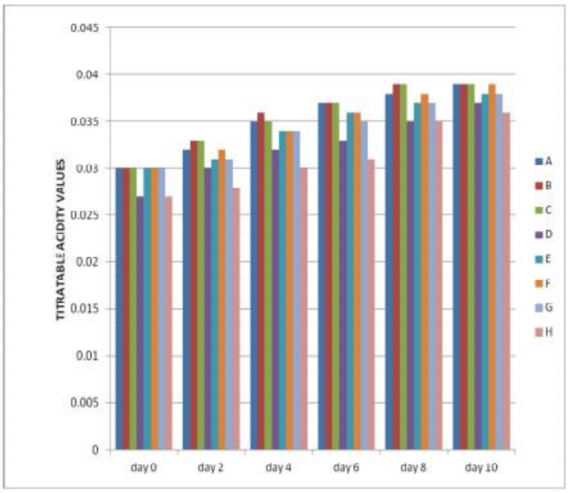

Figure 3: TTA of sample soymilk at ambient temperature

Table 4: TTA of sample soymilk at refrigeration temperature

TTA-refrigeration								
Sample code								
days	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0	0.030	0.030	0.030	0.027	0.030	0.030	0.030	0.026
4	0.033	0.033	0.032	0.029	0.031	0.032	0.031	0.028
6	0.035	0.034	0.035	0.030	0.033	0.034	0.034	0.030
8	0.037	0.037	0.037	0.032	0.035	0.035	0.035	0.031
10	0.038	0.039	0.038	0.033	0.037	0.038	0.037	0.036
12	0.039	0.040	0.039	0.034	0.038	0.038	0.038	0.036
14	0.040	0.040	0.040	0.035	0.039	0.039	0.039	0.037
16	0.041	0.042	0.041	0.037	0.040	0.041	0.040	0.039

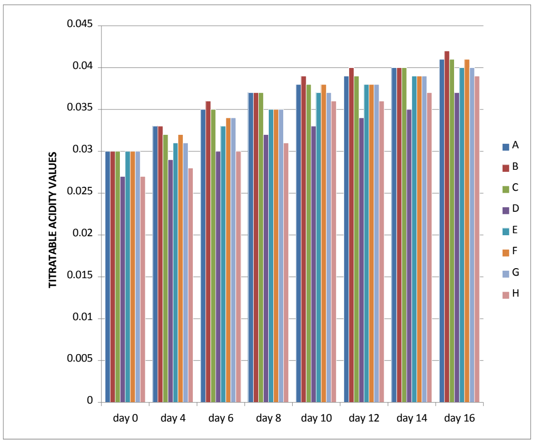

Figure 4: TTA of sample soymilk at refrigeration temperature

4. DISCUSSION

The physicochemical quality of produced soymilk during storage at ambient temperature and at refrigeration temperature was evaluated. There was a significant difference ($p<0.05$) in pH of soymilk stored at ambient temperature and those stored at refrigeration temperature. The average value of pH of "soy milk" at day 0 was 7.11. The pH values being neutrality, which agrees with the reports of several other workers (Fraizer and Westhoff, 1985; Gesinde et al., 2008), may account for the growth of bacterial cells observed since bacteria thrive best on such media (Gesinde et al., 2008).

The difference in pH and titratable acidity levels during storage at refrigeration temperature were not significant for those treated with different preservatives and the controls. As can be deduced from the physical assessment, colour change did not occur until after day 2 and gas production did not occur until day 6. Watery texture and curdling occurred on day 8. This show a much better report than that of Iwe (2003) and Lo *et al*. (1968), where they indicated that whey separation, curdling and production of gas occurred after 48 hours (2 days) at ambient temperature. Therefore, it can be deduced from this study that the combined use of potassium sorbate and sodium benzoate (in combination) with propyl gallate and Ascorbyl palmitate (singly and in combination) has demonstrated the benefits of preservatives treatment. This was evident in the slight gradual decrease in pH and gradual increase in TTA, unlike other reports of Lo *et al*. (1968), where it was reported that *Lactobacillus and Streptococcus spp* brought about rapid/drastic increase in acidity and decrease in pH. The pH values of the samples were reasonably justified and suitable for soy milk marketed in tropical areas because of the expected effect of bad storage conditions such as high temperatures encountering in some zones in Nigeria which can affect the acidity of yoghurt (Olugbuyiro and Oseh, 2011).

There is an agreement between the result of the physical examination, pH and TTA changes of the stored soy yoghurt samples. The non significant changes in pH and TTA of the stored products within the first four day of storage might be responsible for the shelf stability of the stored soy milk within four days of storage; thus lending credence to the widely held opinion that chemical changes in foods grossly affect their shelf stability and consumer acceptability (Tomassi, 1988; Jimoh and Kolapo, 2007). The overall changes in pH and TTA observed for all the stored soy milk samples in the study may be suggesting that the fermentative activities of the soy milk starters still continued in storage (Jimoh and Kolapo, 2007). The simultaneous increase in pH and TTA in the present study is similar to that reported for another soyfood- soybean daddawa (Popoola et al., 2007), wherein the production of acid and ammonia were found to be superimposed in the course of storage; the balance between the two being responsible for the over all pH (Jimoh and Kolapo, 2007). The production of ammonia (which could increase the pH) in the present study becomes more probable as some yoghurt starter could produce ammonia from arginine (Collado et al., 1994; Jimoh and Kolapo, 2007).

The variation in the pH of soy mik stored at ambient temperature when compared with other samples stored at refrigeration could be due to its composition during production. The pH values observed in this study are comparable to other workers (Dublin-Green and Ibe, 2005; Hassan and Amjad, 2010; Jimoh and Kolapo, 2007; Olugbuyiro and Oseh, 2011). All the same, the pH results are in accordance with FDA specifications for the pH of yoghurt (4.6 or lower) (Olugbuyiro and Oseh, 2011). The pH obtained in this study is similar to values reported for soy yoghurt (Sugimoto and Van Buren, 1970; Buono, 1988) which range between 5 and 6. The difference in the rate of decrease in pH during production of yoghurt and soy milk is due to production of lactic acid by *Lactobacillus* (Adams and Moss, 1995) and other lactic acid bacteria. Related research carried out by Njoku et al. (1991) reported similar result. This could be attributed to metabolic activity of the fungi. In a study by Maduka et al. (2012), it was reported that the reduction in p^H during fermentation might be as a result of acidification by acetic acid fermentation of breadfruit which reduces the chance of microbial spoilage.

The values obtained for titratable acidity are generally below the standard which is 0.7% (FDA, 2009; Olugbuyiro and Oseh, 2011). No direct relationship was observed between pH values and titratable acidity as has been previously reported (Dublin-Green and Ibe, 2005; Olugbuyiro and Oseh, 2011). The gradual increase in titratable acidity during storage was also observed in Sudanese yoghurt (Manhal and Kamal, 2010). The titratable acidity values obtained during storage at refrigeration temperature is comparable to the values obtained by Davis and Mclachian (1974) and Younua *et al*. (2002), who reported mean values of titratable acidity in the range of 0.87 to 1.13. This was different from the values of 2.5 to 2.07 reported by Muhammed *et al*. (2009) for soy milk stored at refrigeration temperature.

The production of lactic acid after fermentation has the effect of lowering pH and thereby arresting any further development of pathogens and other toxic microorganisms, apart from having lethal and destructive effect on bacteria and arresting bacterial multiplication (Jayeola *et al.,* 2010). There was however no direct relationship observed between pH values and titratable acidity as has been reported by other workers (Robinson and Tamime, 1975) and this has been attributed to the presence of milk powder which increases the buffering capacity of the product. In a study by Saidu (2005), there was a substantial increase in the concentrations of lactic acid. This was attributed to production of these acids by microorganisms in the soak water and their subsequent diffusion into the beans (Saidu, 2005). There was also a decrease in pH of both the soak water and the beans due to the production of the organic acid. The changes that occurred in the organic acid and pH levels resulted in flavor alterations, since many of the organisms showed different patterns of growth ad survival (Saidu, 2005).

5. CONCLUSION

The study showed that the overall changes in pH and TTA observed for all the stored soy milk samples in the study may be suggesting that the fermentative activities of the soy milk starters still continued in storage. Generally, improved combined preserving techniques can in particular improve keeping quality; lessen the risk of microbial food poisoning.

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