

EFFECT OF SODIUM BENZOATE CONCENTRATION ON THE QUALITY AND RANCIDITY OF 'WARA' (A LOCAL CHEESE PRODUCED FROM SOYMILK) DURING STORAGE

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Abstract

*The effect of sodium benzoate on the quality and rancidity of "Wara" from soymilk was investigated. Soymilk samples with varying quantities of sodium benzoate (0 % 0.6 %, 0.8 %, 1.0 % and 1.2 %) were coagulated with Sodom apple (*Calotropis procera*) leave extract as enzyme source to produce "Wara" products. The "Wara" samples were further subjected to ambient temperature (28-35 °C) and refrigerated temperature (4±1 °C) storage during which some physico chemical properties (pH, total titratable acidity and thiobarbituric acid) were evaluated at 2 days intervals. Results showed that total titratable acidity and thiobarbituric acid value increased with storage period while pH decreased. The titratable acidity of the Wara samples ranged from 0.14-0.18 % and 0.14- 1.35% at the end of storage at ambient and refrigeration temperature respectively. The residual sodium benzoate in the "Wara" samples were below limit of 0.1% stipulated as preservative in such products. The concentrations levels of 0.05 % and 0.06 % sodium benzoate should be used for preserving Wara as it improved chemical properties and shelf life to 6 and 15 days for ambient and refrigeration storage respectively.*

Keywords: chemical properties, wara, soybean, soymilk

Introduction

Milk is rich in nutrients and is used in the production of different food products, one of which is cheese. The production of cheese in Africa is not properly standardized and is dictated largely by tradition. Cheese production has increased and about one third of the total milk volume is used for the purpose (FAO, 2002). In Nigeria, milk is mainly produced by the nomadic Fulani people. Due to lack of refrigeration facilities, the Fulani women process surplus fresh milk into soft, unripened cheese called 'Warankas' or 'Wara' (Adetunji & Babalobil, 2011). Due to scarcity of cow milk with attendant high cost, alternative milk-like products from plants have been used in production of Wara (Chikpah *et al.*, 2015). One of such milk-like products is soymilk. In recent times, the consumption of soymilk and soy curd has increased, perhaps due to the perceived health related problems such as heart diseases, obesity, high cholesterol and other cardiovascular diseases associated with high consumption of animal products (Chikpah *et al.*, 2015). West African soft cheese uses the juice extract of the plant Sodom apple (*Calotropis procera*) as the milk coagulant. Other coagulants used include lime, calcium oxide and steep water (fermented pap water).

Traditionally, Wara is produced by curdling fresh hot soy milk with either lime juice, alum or corn steep water (Fasoyiro *et al.*, 2012). It is preserved traditionally in its whey which last for barely a day or two. The traditional processing method for making Soy Wara does not take into consideration the quality control measures. Unhygienic conditions of processing of cheese make

the risk of microbial contamination very high. These contribute not only to the short shelf life of the products but also more importantly its potential health hazards (Ashaye *et al.*, 2006).

The use of acid and salt preservatives has been reported by Zamal *et al.* (2013) to extend the shelf life of soy curd products. However, these preservatives (Potassium-meta-bi-sulphite (KMS), Sodium-benzoate and potassium sorbate) are usually prepared as solution and added directly to the curd when it had been formed. This may result to uneven distribution of the preservative in the curd. Sodium benzoate is a sodium salt used to inhibit the growth of molds, yeasts and bacteria, found in a variety of products such as sauces, beverages and juices (Turkoglu, 2007). It is odorless and soluble in water and ethanol (Lennerz *et al.*, 2015). The Food and Drug Administration (FDA) considers the preservative, sodium benzoate to be safe (FDA, 2017). Thus, maximum limit of sodium benzoate as preservative is 0.05 g/100 mg or 0.05 g/100 mL (Zhang and Ma, 2013). Therefore, the objective of this study was to determine the effect of sodium benzoate concentration on the quality and rancidity of *wara* (a local cheese produced from soymilk) during storage.

Materials and Methods

Sample collection and preparation

Soybeans (*Glycine max*) was purchased from Kure market, Minna Nigeria. The coagulant (*Calotropis procera*) leaves were freshly harvested from Federal University of Technology, Minna farm. The preservative (sodium benzoate) used is of analytical grade and safe for consumption. The coagulant, leaf extract of the sodom apple (*Calotropis procera*) was prepared by the method described by Olorunnisomo and Ikpinyang (2012). The extract was obtained by finely grinding 120 g leaves of Sodom apple (*Calotropis procera*) using a laboratory mortar and pestle. The ground leaves were then transferred into 500 ml of the distilled water and was allowed to soak for 10 minutes after which the mixture was sieved to obtain the leaves extract. Also, higher concentration of sodium benzoate above the maximum limit (0.05 g/100 mL) was used because the preservative is highly soluble in water, thus will be dissolve in the whey. sodium benzoate solution was prepared by separately weighing 0.6 g, 0.8 g, 1.0 g and 1.2 g of sodium benzoate with a weighing balance. This was then added into each 100 ml of distilled water in a beaker. The solution was mixed thoroughly to have a homogenous solution. The soymilk was prepared according to the method described by Ikuomola *et al.* (2013). Raw soybeans (500 g) were handpicked to remove stones and dirt and then soaked in 2 litres of water for 24 hours at room temperature (32 ± 2 °C). The soaked soybeans were drained and wet-milled with the aid of a blender (Kenwood BL440, 500 W, China) at high speed, then sieved, using muslin cloth to separate the chaff from the soymilk. The ratio of soybean to water used during wet-milling was one ratio eight (1:8). One litre each of the soy milk was then put in clean pot for processing to *wara*.

The *Wara* samples were prepared by modification of the method of Ikuomola *et al.* (2013). Five samples labeled as A, B, C, D and E; each containing one litre of soymilk were separately put into a pot and placed on a burner and heated to 70°C, and held at that temperature for 30 minutes. Then, different concentration of sodium benzoate 0%, 0.6%, 0.8%, 1.0% and 1.2% (higher concentration was used because sodium benzoate is soluble in water, thus will be dissolve in the whey) were added to Samples A, B, C, D and E respectively, and the samples were allowed to continue heating for 5 minutes after which 100ml of the coagulant was added to each sample and again the samples were allowed to continue heating until soy curd was formed. Immediately after forming, each of the samples was emptied from the pot into a muslin

cloth in order to separate the curd from the whey by pressing technique. The separated curds were separately sliced, fried and allowed to cool at room temperature. Thereafter, each of the samples was divided into two parts and separately packed into polyethylene bag and then sealed. One part of each sample was stored at room temperature (28-35°C) for 9 days while the other part was stored by refrigeration (4±1°C) for 27 days. Slice of each part of the samples were collected at interval of 2 days for determination of pH, Titratable Acidity (TTA), and Thiobarbituric Acid (TBA).

Determination of Residual Sodium Benzoate in *Wara* Samples

This was carried out using the titrimetric method described by AOAC (2000). Ten (10g) of the sample was weighed and mashed in 20 ml of distilled water using mortar and pestle then filtered. The filtrate was transferred into a conical flask and 1ml of 10% NaOH solution and 12 g NaCl was added. Distilled water was added to bring the volume up to 50 ml and allowed to stand for 30 minutes with frequent shaking. Two drops of phenolphthalein indicator were added to change the color and 3 drops of HCl was added until the colour change disappeared with the addition of excess 3 ml HCl. The mixture was transferred into a separating funnel and 25ml of chloroform was added and allowed to stand for 30 minutes with frequent shaking. Chloroform layer (12.5 ml) (low layer) was transferred into a conical flask and the chloroform was evaporated off on a water bath. Fifty (50 ml) of 50 % ethanol solution was added and titrated with 0.05 M NaOH using phenolphthalein as indicator. Sodium benzoate retained in the samples was calculated using the formula below:

$$\% \text{ of sodium benzoate} = \frac{x}{\text{weight of sample}} \times 100$$

$$\text{weight of sodium benzoate (x)} = \text{Titre ml of NaOH} \times 0.0072$$

Determination of pH, TTA and TBA of *Wara* Samples

The pH of the *wara* samples was determined according to the procedure described by Onwuka (2005). About 5g of each sample was mashed and weighed into 50 ml of distilled water in a beaker to form a homogenous solution. It was allowed to stand for 30 minutes in 40 °C water bath. The samples were then filtered using Whatman No. 1 filter paper and the supernatant dispensed into a 50 ml beaker, mixed thoroughly and the pH measured with pH meter. The pH meter was calibrated with standard buffer solution of pH 4.0 and 7.0. The TTA of the *wara* samples was determined according to AOAC (1990) method. Each *wara* sample (1.0 g) was mixed with 5 ml of warm water and volume was made up to 10 ml in 100 ml conical flask; each sample was shaken vigorously and filtered. The filtrate was titrated with 0.1 N NaOH using phenolphthalein as indicator. Percentage acidity was calculated by using the following expression:

$$\text{TTA}(\% \text{ lactic acid}) = \frac{0.0090 \times \text{volume of NaOH used} \times 100}{\text{weight of sample}}$$

The TBA of the *wara* samples was determined using Onwuka, (2005) method. The *wara* samples (10 g each) were macerated with 50 ml of distilled water for 2 min and washed into distillation flask with 47.5 ml of water. The pH was brought down to 1.5 by adding 2.5 ml of 4M HCl acid followed by an antifoam preparation and a few glass beads. The flask was heated by means of an electric mantle; 50 ml distillate was collected in 10 minutes from the time boiling commenced. 5 ml of the distillate was pipetted into a glass stopper tube, 5 ml of TBA reagent (0.2883 g, 100 ml of 90 % glacial acetic acid) was added to the glass stopper, shaken, and heated in boiling water for 3 5 min. A blank was prepared similarly using 5 ml water reagent,

then tubes were cooled in water for 10 min and the absorbance (D) was measured against the blank at 538 nm using 1 cm cells.

$$\text{TBA number (malonaldehyde per g sample)} = 7.8 \times D$$

where D = Absorbance

Result and Discussion

Residual Sodium benzoate in *Wara* Samples

The residual sodium benzoate in *Wara* samples is as presented in Table 1. The samples had residual concentration of sodium benzoate lower than 0.1% stipulated levels by Food and Drug Administration (FDA). There was significant ($p \leq 0.05$) difference in the concentrations retained by the samples and increased with increasing concentration of the sodium benzoate. The higher level (0.05 % and 0.06 %) retained in the samples D and E may be due to higher quantity of the sodium benzoate added, while the low level of 0.02 % retained in sample B may be as a result of low quantity added. This could also be as a result of solubility of the sodium benzoate in the whey as they are highly soluble in water. During salting process, moisture is expelled from the cheese and a percentage of salt is lost to the whey (Grummer *et al.*, 2012).

Table 1: Residual Sodium Benzoate Retained in *Wara* Samples

Samples	Sodium benzoate(g/l)	Residual concentration (%)
A	0	ND
B	0.6	0.02±0.00 ^c
C	0.8	0.04±0.00 ^b
D	1.0	0.05±0.01 ^{ab}
E	1.2	0.06±0.01 ^a
LSD		0.01

Values are means $\pm$ standard deviation of duplicate determinants. Means followed by different superscript in the same column are significantly different ($p \leq 0.05$); ND = Not Detected

Key: Sample A= control (no preservative); Sample B = 0.6% sodium benzoate; Sample C= 0.8% sodium benzoate; Sample D = 1.0% sodium benzoate; Sample E = 1.2% sodium benzoate

Effect of concentrations of sodium benzoate on the pH of *Wara* samples stored at ambient and refrigeration temperatures

The effect of concentrations of sodium benzoate preservative on the pH of the samples at ambient and refrigeration temperatures are as shown in Figures 1 and 2. The pH was observed to increase with increase in concentration of sodium benzoate and decreased with extended storage period in both storage methods. It was also observed that Sample E recorded the highest pH (6.28) at the end of storage while Sample A had the lowest (5.86) at ambient temperature storage. The increase in pH on the day of production may be due to buffering capacity of the sodium benzoate because pH of the treated samples increased as the concentration of sodium benzoate was increased. This result agreed with Everard *et al.* (2008) who reported that increasing the emulsifying salt in processed cheese leads to increase in pH and firmness. The decrease in pH recorded in all samples throughout the storage period could be as a result of the activities of microorganism associated with the products during storage

(Badriah *et al.*, 2013). However, storage at ambient temperature recorded higher decrease in pH compared to refrigerated temperature, which may be due to the fact that low temperature (refrigeration) slows down activities of microorganism thereby lowering the rate of acid production.

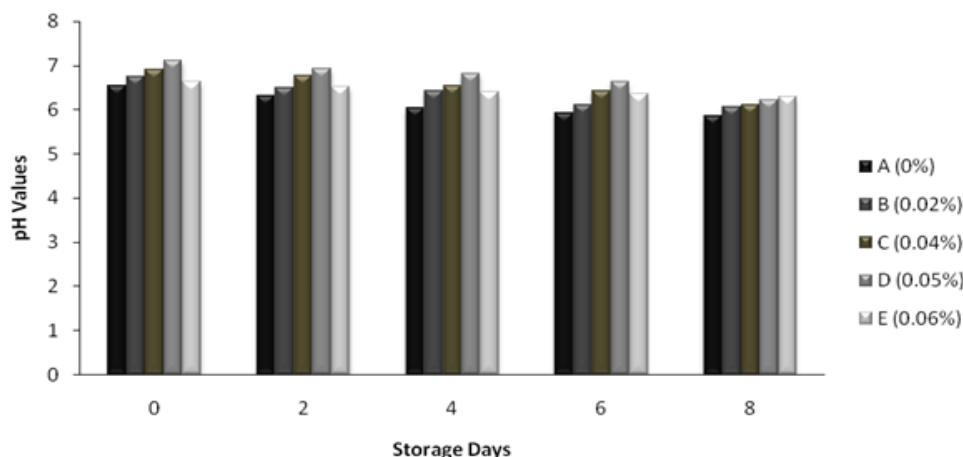


Fig.1: Effect of Concentrations of Sodium Benzoate on pH of *Wara* Samples during Storage at Ambient Temperature

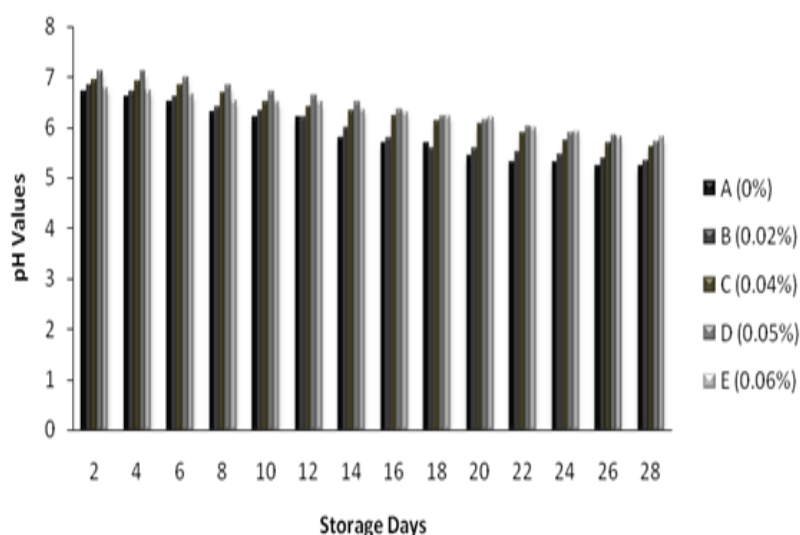


Fig. 2: Effects of Concentrations of Sodium Benzoate the pH of *Wara* Samples during Storage at Refrigeration Temperature

Effect of concentrations of sodium benzoate on the TTA of *Wara* samples during storage at ambient and refrigeration temperatures

Figures 3 and 4 show the effect of concentrations of sodium benzoate on the TTA of *Wara* samples during storage at ambient and refrigerated temperature. It was observed that the total titratable acidity (TTA) of all the samples were affected by the increasing concentrations of the sodium benzoate. The TTA was observed to decrease with increasing concentration of residual

sodium benzoate and increased with storage days at different storage temperatures. The titratable acidity of the *Wara* samples ranged from 0.14-0.18 % on day 0, and was observed to increase gradually with storage time. The high acidity recorded by the control samples on the day of production was probably because there was no inhibition of microbial growth. The development of acidity is caused by the production of organic acids as a result of breakdown of carbohydrate fat and protein by microorganisms (Alfred, 2010). It was also noted that storage at ambient temperature resulted to an increase in the titratable acidity compared to samples stored at refrigerated temperature. This may be due to the fact that low temperature of storage slowed down activity of microorganism thereby lowering the rate of acid development (Hamid, 1998).

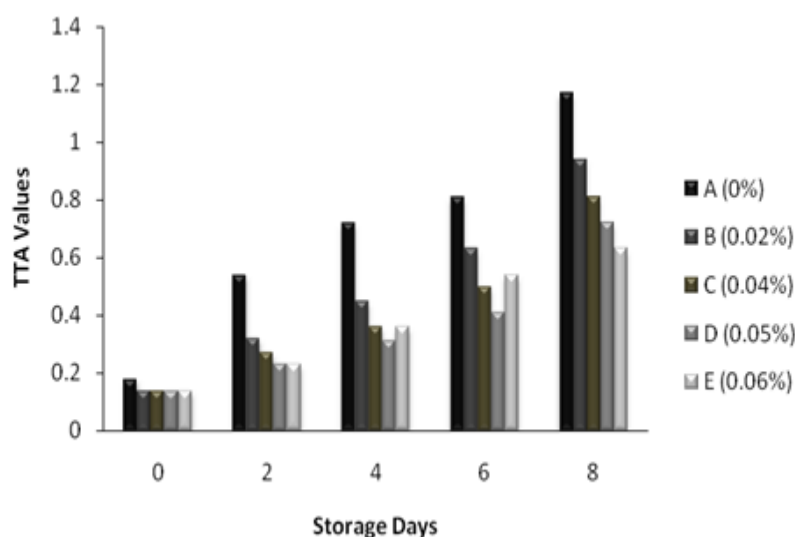


Fig 3: Effect of Concentrations of Sodium Benzoate on the TTA of *Wara* Samples during Storage at Ambient Temperature

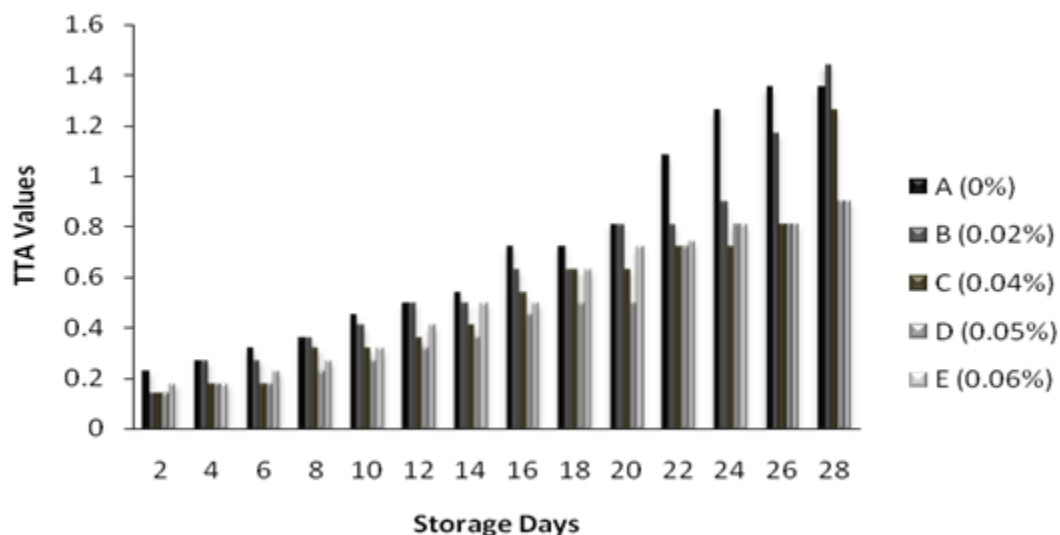


Fig. 4: Effect of Concentrations of Sodium Benzoate on the TTA of *Wara* Samples during Storage at Refrigeration Temperature

Effect of concentrations of sodium benzoate on the TBA of *Wara* samples during storage at ambient and refrigeration temperatures

The effect of different concentrations of preservative on the thiobarbituric acids of samples stored at ambient temperature and refrigeration temperature are as presented in Figures 5 and 6. It was observed that the TBA slightly decreased with increasing concentrations of sodium benzoate. Thiobarbituric acid (TBA) of products determines the rate of rancidity which is due to oxidation of unsaturated fatty acid (Alfred, 2010). However, values with no preservative showed a higher increase in TBA value (2.96) than treated samples (Sample D) which recorded the lowest value (0.82) at the end of storage period at ambient and refrigeration temperatures. Thiobarbituric acid value increased in all the samples during storage irrespective of the concentration of residual benzoate and storage temperature. This may be as a result of fat deterioration as a result of reaction of fat with moisture or oxygen. The high level of rancidity recorded by the control sample may be due to non-inhibition of microorganism. Increased level of rancidity is attributed to non-inhibition of microbial growth resulting in enhanced lipolysis to produce more fatty acids (Alfred, 2010). The type of rancidity associated with these products is hydrolytic rancidity in which lipid react with water resulting in odor that develops when triglycerides are hydrolyzed and free fatty acids are released. The low values of thiobarbituric acid recorded in the *wara* samples containing 0.05 % and 0.06 % sodium benzoate compared to the *wara* samples containing 0.02 % and 0.04 % sodium benzoate, may be attributed to the fact that higher concentration of the sodium benzoate may have slowed down chemical reaction resulting in microbial inhibition and loss of lipolytic activity.

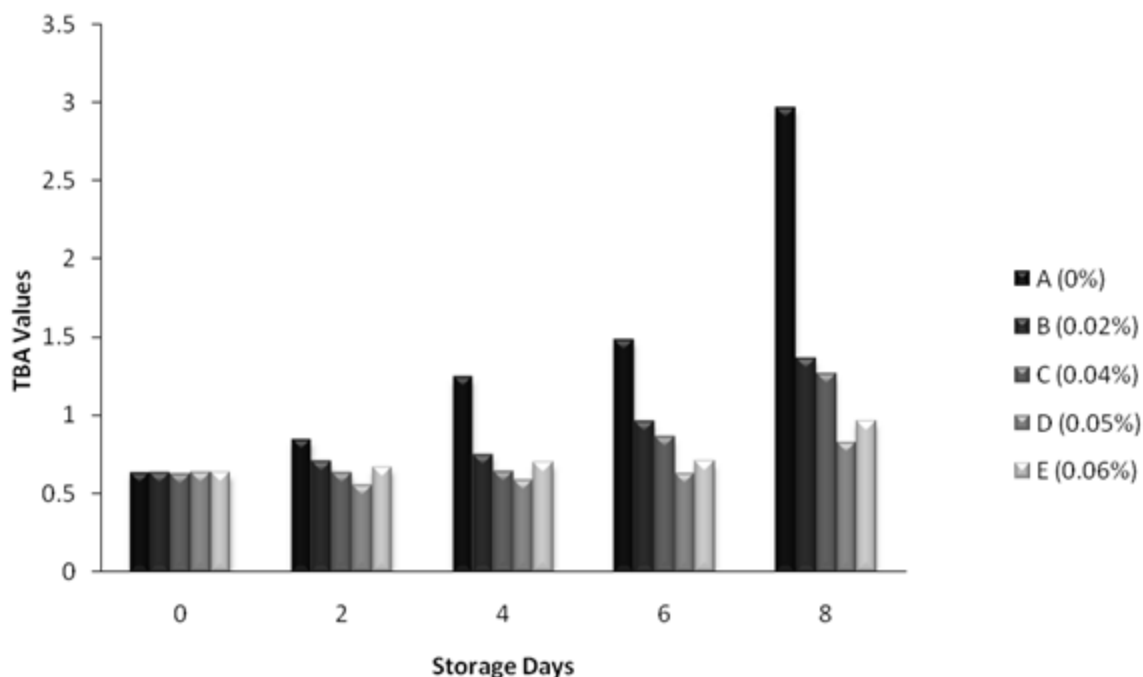


Fig. 5: Effect of Concentrations of Sodium Benzoate on the TBA of *Wara* Samples during Storage at Ambient Temperature

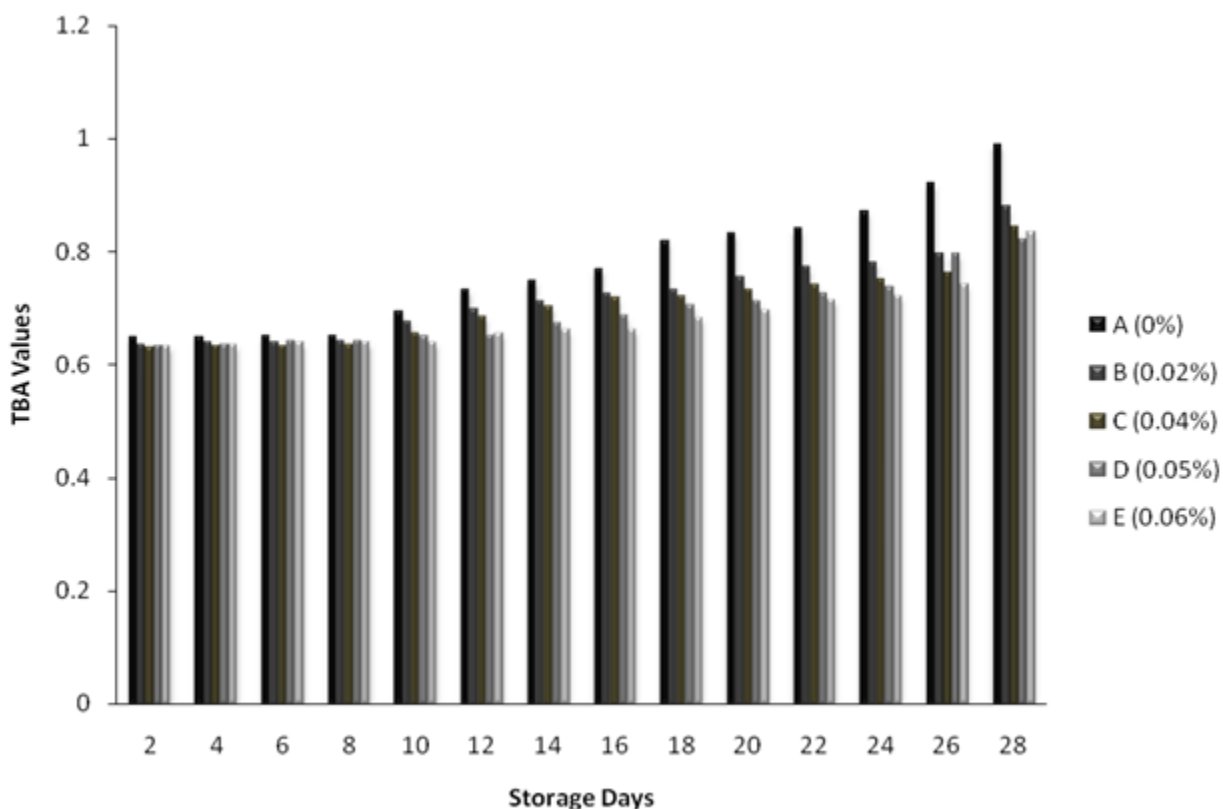


Fig. 6: Effect of Concentrations of Sodium Benzoate on the TBA of *Wara* Samples during Storage at Refrigeration Temperature

Conclusion

The residual sodium benzoate in the "*wara*" products were below limit of 0.1% stipulated as preservative in such products. Increase in concentration of sodium benzoate was observed to increase pH, TTA and TBA of the *wara* samples. However, TTA and TBA increased with storage time at different storage temperatures while pH decreased. The study has demonstrated that *Wara* should be preserved with concentrations of 0.05 % and 0.06 % sodium benzoate as it improved the chemical properties. Moreover, shelf life was also noted to be extended to 6 days for ambient storage and 15 days for refrigeration temperature.

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