



## **PRESERVATIVE ACTIVITY OF ETHANOLIC EXTRACT OF GINGER IN WARA - A WEST AFRICAN TRADITIONAL SOFT (UNRIPENED) CHEESE**

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### **ABSTRACT**

The effect of ethanolic extract of ginger (*Zingiber officinale*) on sensory and storage qualities of wara was evaluated. Pasteurized fresh milk samples were used to prepare wara and; 1.6%, 2.4%, 3.2% and 0% ginger extracts (samples A, B, C and D respectively) were incorporated into the samples separately. The wara samples were then fried in hot palm oil as it is done traditionally and organoleptic properties of the samples were determined by a taste panelist. Also physico-chemical and microbiological changes during six (6) days storage at ambient tropical temperature ( $30 \pm 2^\circ\text{C}$ ) were determined. The samples (A, B and C) containing ginger were rated better than the control sample (D). Sample C had the highest overall acceptability of 4.0 while sample D had the least (2.8). During storage a significant decrease ( $P \leq 0.05$ ) in pH with a corresponding increase in titratable acidity (TA) was recorded for the wara samples. The pH ranged between 4.17 and 6.55 while the TA ranged between 0.018 and 0.099 (mg/100g). The peroxide values (POV) of sample D increased at a faster rate than samples A, B and C. The POV for sample D after 3 days of storage was 34.15 meq/kg while that of sample C was 25.33 meq/kg at the end of 6 days storage. Although there was general increase in the microbial loads (MLs) of all the samples, the MLs of samples A, B and C were significantly lower than that of sample D. Moreover samples A and B got spoiled by day 4 of storage; sample D by day 3 while sample C was still in good condition at the end of 6 days storage. This study therefore showed that incorporation of 3.2% ethanolic ginger extract significantly improved acceptability of wara and increased the shelf life of the product by 3 days.

**Keywords:** Cheese, Wara, Traditional, Preservation, Ginger, West Africa.

**Running Title:** Preservation of wara using ethanolic extract of ginger.

**Abbreviation:** POV, Peroxide value, TA, Titratable acidity, MLs, Microbial loads.

### **1. INTRODUCTION**

Cheese production is a household operation in many developing countries. Livestock farming in general and milk production still play an important socio-economic role in many developing countries. In Nigeria, the Fulani pastoralists process surplus fresh milk into various table products like wara, nono (fermented skimmed milk) and mai-shanu (Belewu *et al.*, 2005). The West

African soft cheese which is the special type of cheese found in Nigeria has a shelflife of 2 days when immersed in its whey. Various preservation methods are well documented in literature (Aworh and Egounlety, 1985). Joseph and Akinyosoye (1997) reported the use of 0.8% propionic acid and 0.8% sodium benzoate in the preservation of cheese for 8 days. However chemical preservatives have been shown to have adverse effects on consumers and the use of natural preservatives has become more popular as compared to synthetic antimicrobials and antioxidants (Ahn *et al.*, 2007). Since ancient times, spices and herbs have been added to foods as flavouring agents, folk medicines and food preservatives (Cutler, 1995; Marija and Nevena, 2009). Many natural occurring compounds known as phytochemicals found in edible and medicinal plants, herbs and spices such as ginger have been shown to possess antimicrobial activities against food spoilage and food-borne pathogens (Azzouz and Bullerman, 1982; Jay and River, 1984; Cowan, 1999; Seaberg *et al.*, 2003). Wara has a relatively short shelf-life due to the presence of some food borne microbial flora comprising of bacteria and fungi. Therefore, this study was carried out to evaluate the effect of ethanolic extract of ginger on the sensory properties and shelf-life of wara.

## **2. MATERIALS AND METHODS**

### **2.1. Experiment to Determine Quality Attributes of Wara Samples During Storage**

#### **2.1.1. Sample Collection**

Fresh milk samples were collected into a sterile container at Gaa-Apaara, Oyo State, Nigeria and then transported immediately to the laboratory for use in the preparation of wara. Ginger rhizomes were purchased from Bodija market in Ibadan, Nigeria.

#### **2.1.2. Preparation of Ginger Extract**

Ginger rhizomes were washed, peeled and oven-dried at 55°C for 24 hours. The dried ginger was grounded to a fine powder in a mill and sieved. 10 g of the powdered ginger was extracted with 100ml of ethanol overnight at room temperature. The extract was filtered to remove residue and then evaporated in a water bath at 40°C. The extract obtained after evaporation of ethanol was used as a natural antioxidant (Zia-Ur-Rehman *et al.*, 2003).

#### **2.1.3. Preparation of Wara**

Fresh cow milk samples were used to prepare wara using traditional method as described by Adesokan *et al.* (2009). The wara samples were cut into 50 g pieces and 1.6%, 2.4%, 3.2% (sample A, B and C) concentration of ginger extract were introduced into the wara samples by injection. Wara sample containing 0% ginger served as control (sample D). The wara samples were then fried in hot palm oil at 180°C for 5 to 10 minutes.

#### **2.1.4. Sensory Evaluation of Wara Samples**

Sensory evaluation was conducted using a panel of 10 judges who were familiar with the products. The judges evaluated the samples for appearance, taste, texture, colour and aroma using a 5 point hedonic scale (Adesokan *et al.*, 2009).

### 2.1.5. Storage Study on Wara Samples

Fried wara samples incorporated with different concentration of ginger extract were separately packed into thin transparent polythene bags, sealed and then stored at ambient temperature ( $30 \pm 2^\circ\text{C}$ ) for 6 days. Physico-chemical and microbiological analyses were carried out on the sample at 24 hours interval.

### 2.1.6. Microbiological Analysis

Samples of wara were serially diluted in sterile distilled water for the enumeration of microbial load of the sample by the pour plate method using appropriate culture media. Nutrient agar, MacConkey agar, potato dextrose agar (PDA) and de Mann, Rogosa and Sharpe (MRS) agar were prepared according to manufacturer's instruction and used for total viable, coliform, fungal and lactic acid bacterial (LAB) counts respectively.

### 2.1.7. Physico-Chemical Analyses

The pH of the wara sample was measured by homogenizing 1g in 10 ml sterile distilled water using a standardized pH meter (Crison MicropH). The titratable acidity of the wara samples was evaluated by dissolving 2 g sample in distilled water and making up to 25 ml with water followed by titrating 10 ml aliquots with 0.1M NaOH in the presence of phenolphthalein indicator (Achi and Akubor, 2000).

The peroxide value (meq/kg) was determined by the titrimetric method of Pearson (1981). 1 g of the wara samples was weighed into clean boiling tubes to which 1 g of powdered potassium iodide, 20 ml mixture of glacial acetic acid and chloroform were added at ratio 2:1 and held in boiling water for 30 seconds. The contents were then transferred into 250 ml conical flasks containing 20 ml of 5% potassium iodide solution. This was then titrated against 0.1M sodium thiosulphate solution using 1 ml of starch as indicator (AOAC, 1990).

## 3. RESULTS AND DISCUSSION

The sensory evaluation of the laboratory prepared wara samples as determined by a sensory panel are displayed in table 1. Sample C (fortified with 3.2% ginger extract) had the highest overall acceptability (4.0) followed by sample B (fortified with 2.4% ginger) which had 3.8 as the overall acceptability while sample D (control sample) had the least value of acceptability (2.9).

George (2004) explained the role of ginger and other spices as plant that tends to be more aromatic adding flavours and improving organoleptic properties of food substances. The effect of ginger extract on the pH of the wara samples over six-day storage are presented in table 2. A significant decrease ( $P \leq 0.05$ ) in the pH of the samples was observed during storage. Sample D had the highest pH (5.39) while sample C had the least pH (4.37) on the third day of storage. A significant increase ( $P \leq 0.05$ ) in titratable acidity (TA) was observed during the storage of wara samples (table 3) and it ranged between 0.018 and 0.099%. This observation is in accordance with the reports of previous studies (Nout, 1991; Achi and Akubor, 2000). The result of the work of Pazakova *et al.* (1997) and Adesokan *et al.* (2008) showed an increase in titratable acidity as well as decrease in pH values during the storage of yoghurt and nono respectively. Sample D had the highest peroxide value (34.15meq/kg) while sample C had the least peroxide value (22.91meq/kg)

by the third day of storage (Table 4). The increase in POV of wara samples during storage was an indication that peroxidation is associated with spoilage of wara. It may therefore be implied that incorporation of ginger extract into wara samples was responsible for the observed reduction in peroxidation during storage (Kolapo *et al.*, 2007).

The effect of ginger on the total viable, coliform, fungal and LAB counts (logcfu/g) revealed a general increase in microbial population in all the samples during storage. However, the control sample had a significantly higher microbial load than the treated samples. Sample D lasted only three days while samples A and B lasted four days and it was only sample C that lasted the six days of storage. Also the highest count for LAB (7.79logcfu/g) was observed for sample C whereas sample D had the lowest count (7.45logcfu/g) on the third day of storage (figure 1). The increase in LAB counts during storage could be attributed to ginger protease which provides essential growth factors for their replication (Abd El-Aziz *et al.*, 2012). Numerous studies have been published on the antimicrobial activities of plant extract against different types of microbes including food borne pathogens (Smith - Palmer *et al.*, 1998; Hara-kudo *et al.*, 2004; Binshan *et al.*, 2007).

#### 4. CONCLUSION

The results from this study showed that incorporation of 3.2% ethanolic extract of ginger in wara significantly reduced microbial load during storage; improved its acceptability and extended its shelf life by three days.

**Table-1.** Sensory evaluation of wara samples produced with different concentration of ginger extract.

Sample	Appearance	Taste	Texture	Colour	Aroma	Overall Acceptability
A*	3.6 <sup>a**</sup>	2.8 <sup>b</sup>	3.1 <sup>b</sup>	3.3 <sup>b</sup>	3.6 <sup>a</sup>	3.3 <sup>b</sup>
B	3.7 <sup>c</sup>	4.0 <sup>c</sup>	3.8 <sup>c</sup>	3.7 <sup>c</sup>	3.7 <sup>c</sup>	3.8 <sup>c</sup>
C	3.8 <sup>d</sup>	3.8 <sup>d</sup>	4.3 <sup>d</sup>	3.9 <sup>d</sup>	4.0 <sup>d</sup>	4.0 <sup>d</sup>
D	2.8 <sup>e</sup>	2.8 <sup>e</sup>	2.6 <sup>e</sup>	3.1 <sup>e</sup>	2.8 <sup>e</sup>	2.8 <sup>e</sup>

\*\*Values are means (n=3); means with different superscripts are significantly different ( $P \leq 0.05$ ) according to Duncan Multiple Range Test. \*Sample A: wara produced with 1.6% ginger; Sample B: wara produced with 2.4% ginger; Sample C: wara produced with 3.2% ginger and Sample D: wara produced without ginger (control).

**Table-2.** Effect of ginger extract on pH of wara samples during storage

Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	6.45 ± 0.01 <sup>aa</sup>	5.37 ± 0.03 <sup>ab</sup>	4.95 ± 0.06 <sup>ac</sup>	4.57 ± 0.07 <sup>ad</sup>	4.40 ± 0.31 <sup>ae</sup>	Nd	Nd
B	6.41 ± 0.04 <sup>ba</sup>	5.30 ± 0.01 <sup>bb</sup>	4.82 ± 0.12 <sup>bc</sup>	4.49 ± 0.63 <sup>bd</sup>	4.37 ± 0.03 <sup>be</sup>	Nd	Nd
C	6.35 ± 0.01 <sup>ca</sup>	5.16 ± 0.04 <sup>cb</sup>	4.74 ± 0.03 <sup>cc</sup>	4.37 ± 0.32 <sup>cd</sup>	4.30 ± 0.32 <sup>ce</sup>	4.27 ± 0.02 <sup>cf</sup>	4.17 ± 0.02 <sup>cg</sup>
D	6.55 ± 0.02 <sup>da</sup>	6.32 ± 0.11 <sup>db</sup>	5.48 ± 0.01 <sup>dc</sup>	5.39 ± 0.03 <sup>dd</sup>	Nd	Nd	Nd

Values are means (n=3) ± standard deviation (SD). Means with different superscript are significantly different along the rows and columns. Sample codes are as stated in table 1. Nd means not determined.

**Table-3.** Effect of ginger extract on TA (mg/100g) of wara samples during storage

Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	0.027 ± 0.002 <sup>aa</sup>	0.036 ± 0.001 <sup>ab</sup>	0.045 ± 0.019 <sup>ac</sup>	0.063 ± 0.116 <sup>ad</sup>	0.081 ± 0.033 <sup>ae</sup>	Nd	Nd
B	0.027 ± 0.003 <sup>ba</sup>	0.036 ± 0.004 <sup>bb</sup>	0.054 ± 0.012 <sup>bc</sup>	0.072 ± 0.031 <sup>bd</sup>	0.081 ± 0.004 <sup>be</sup>	Nd	Nd
C	0.036 ± 0.001 <sup>ca</sup>	0.045 ± 0.003 <sup>cb</sup>	0.063 ± 0.004 <sup>cc</sup>	0.081 ± 0.002 <sup>cd</sup>	0.090 ± 0.001 <sup>ce</sup>	0.099 ± 0.011 <sup>cf</sup>	0.099 ± 0.002 <sup>cg</sup>
D	0.018 ± 0.001 <sup>da</sup>	0.027 ± 0.002 <sup>db</sup>	0.032 ± 0.006 <sup>dc</sup>	0.045 ± 0.043 <sup>dd</sup>	Nd	Nd	Nd

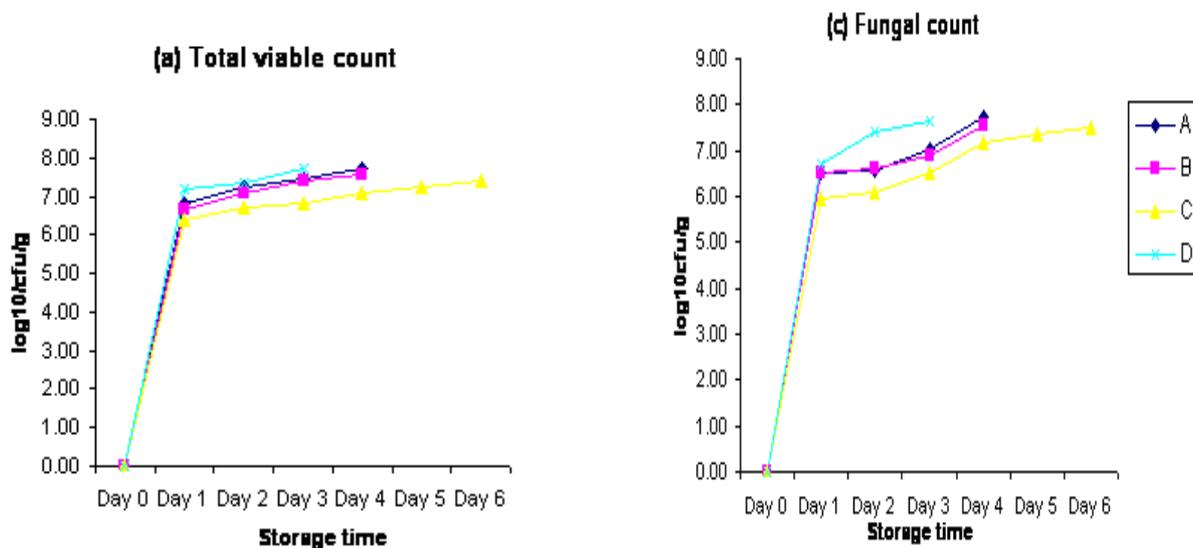
Values are means (n=3) ± standard deviation (SD). Means with different superscript are significantly different along the rows and columns. Sample codes are as stated in table 1.

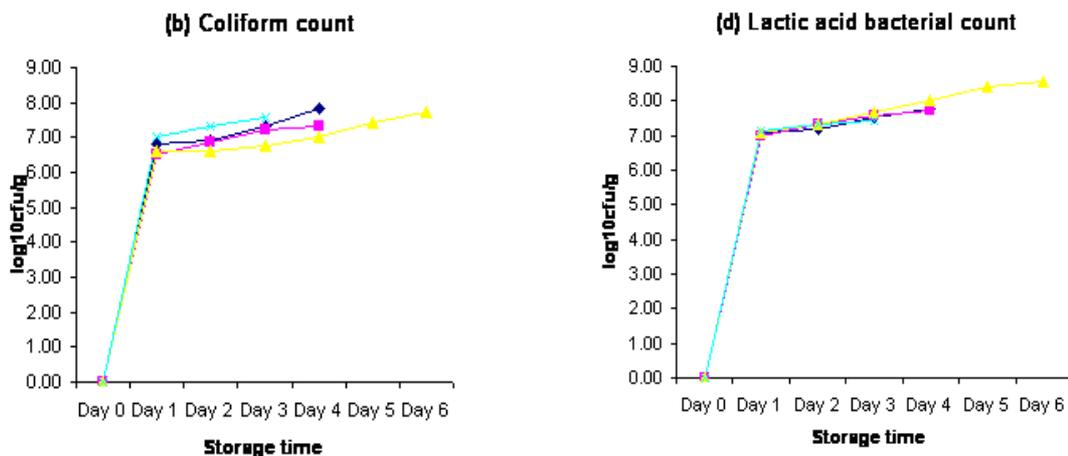
**Table-4.** Effect of ginger extract on peroxide value (meq/kg) of wara samples during storage

Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	23.10 ± 0.10 <sup>aa</sup>	22.91 ± 0.16 <sup>ab</sup>	23.21 ± 0.16 <sup>ac</sup>	23.52 ± 0.16 <sup>ad</sup>	23.78 ± 0.11 <sup>ae</sup>	Nd	Nd
B	20.15 ± 0.19 <sup>ba</sup>	22.75 ± 0.03 <sup>bb</sup>	22.79 ± 0.33 <sup>bc</sup>	22.99 ± 0.38 <sup>bd</sup>	23.14 ± 0.26 <sup>be</sup>	Nd	Nd
C	21.10 ± 0.16 <sup>ca</sup>	21.58 ± 0.51 <sup>cb</sup>	22.61 ± 0.21 <sup>cc</sup>	22.91 ± 0.32 <sup>cd</sup>	23.02 ± 0.44 <sup>ce</sup>	23.41 ± 0.11 <sup>cf</sup>	25.33 ± 0.51 <sup>cg</sup>
D	21.20 ± 0.11 <sup>da</sup>	24.36 ± 0.69 <sup>db</sup>	28.21 ± 0.41 <sup>dc</sup>	34.15 ± 0.6 <sup>dd</sup>	nd	Nd	Nd

Values are means (n=3) ± standard deviation (SD). Means with different superscript are significantly different along the rows and columns. Sample codes are as stated in table 1.

**Figure-1.** Effect of ginger extract on microbial count during storage of wara samples.





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