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Stability Assessment of Papaya and Ginger Blend Meat Tenderizer

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ABSTRACT

The present study was conducted to determine the shelf life of a meat tenderizer formulated from papaya-ginger (1:1) under room temperature (25°C) for six (6) months. The physiochemical properties: colour, pH, and titratable acidity of the tenderizer were assessed on a monthly basis based on the AOAC 988.13 method, AOAC, 2000 method 981.12, and AOAC, 2000 method 942.15, respectively. Furthermore, the microbial quality of the tenderizer was determined based on the ISO 7218:2007 (E) protocol. Based on the collected data, the studied parameters were determined to be: pH ranged from 3.81 to 6.02; titratable acidity (0.0375% to 0.2325%); colour ranged from 0.3005 to 0.339 for yellow and 0.297 to 0.341 for red. The total microbial count ranged between 1.8×10^2 CFU/ml and 42×10^3 CFU/ml. The ginger-papaya blend meat tenderizer during the storage period was observed to have significant variation in total bacteria count and physico-chemical properties (titratable acidity and content of reducing sugars). Other studied parameters, such as coliform bacteria, were not significantly different during the storage period. It was determined that the developed meat tenderizer can last up to six months from the day of manufacturing.

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1.0 Introduction

The tenderness of meat is one of the major factors affecting consumer acceptability (Burke and Monahan, 2003). The process of tenderization breaks down myofibrillar, cytoskeletal, and costameric proteins to make meat more tender (Hopkins and Bekhit, 2014). According to reports, the traditional and industrial method of marinating tenderises muscle by bringing the marinade and meat into contact through soaking, injection, or tumbling (Bunruk et al., 2013). Additionally, marinating improves colour, boosts juiciness, and decreases off-flavours, giving the meat a higher perceived quality when given to the consumer. Furthermore, producers get a product with a higher yield and a longer shelf life (Starowicz et al., 2022).

Natural products that contain proteolytic enzymes, such as various fruits and vegetables, are referred to as "natural tenderizers" (Parkash et al., 2021). To tenderise tough meat, one can effectively use these proteolytic enzymes from natural products. One of the greatest methods is to tenderise meat using exogenous proteases (Tantamacharik et al., 2018). Generally regarded as safe (GRAS) exogenous protease enzymes from plant sources, such as papain, bromelain, ficin, and zingibain, have received approval from the United States Department of Agriculture (USDA). When used in a mix or alone, these enzymes break down muscle protein (Ikram et al., 2021).

Pawpaw (Carica papaya L.) is a tropical American fruit tree often seen in orange-red, yellow-green, and yellow-orange with a rich orange pulp. People around the world consume the fruit either as fresh fruit and vegetables or as a processed product. Papaya's benefits are due to its high content of vitamins A, B, and C. Besides, papaya contains proteolytic enzymes like papain and chymopapain, which have tenderising, antiviral, antifungal, and antibacterial properties. With these tenderizing and health benefits, papaya has become an advantageous plant (Yap, 2022). Papain, a proteolytic enzyme obtained from pawpaw, is used to enhance the tenderization of meat by working on the structural component of muscle; papain, chymopapain, and papaya peptidase-A sharpen the application (Madhusankha and Thilakarathna, 2021). According to a comparative study done by Ashie et al. (2002), beef treated with papain and stored at 50 °C for two weeks experienced a considerable decrease in shear force. It primarily affects the mucoproteins and collagen of connective tissue, converting collagen suspensions into dense gels (Darvish, 2022).

Tropical and subtropical regions widely distribute ginger (Zingiber officinale), a perennial herbal plant. Other names of ginger are African ginger, black ginger, cochin ginger, ganjiang, gegibre, Jamaican ginger, and race ginger (Vasala, 2012). It has been used worldwide as a spice and meat tenderizer, as well as in disease prevention and treatment. Thompson et al. (1973) studied ginger rhizomes as a potential new source of the zingiban plant proteolytic enzyme. According to Thompson et al.'s (1973) findings, ginger protease had a multiplicity of times more proteolytic activity on collagen than actomyosin. Meat that was considerably tenderer was produced because of the combination proteolysis of these two muscle proteins. Additionally, it was noted that the shear force value decreased from 4.27 to 2.8 kg/cm3 when sheep meat was cooked with fresh ginger slices. In comparison to other tenderizing agents, zingibain made from ginger rhizome has the benefit of having a higher level of desired proteolytic activity when heated (Bekhit et al., 2014). Furthermore, zingibain can increase the tenderness of meat products as well as their juiciness and improve the flavour of culinary preparations (Naveena et al., 2004). Thus, the present study determined the storage stability of the prepared papaya and ginger blend meat

tenderizer through a six-month storage experiment at room temperature.

2.0 Materials and Methods

2.1. Study Area Description

This study was conducted at the Department of Food Sciences and Agro-Processing Laboratory at Sokoine University of Agriculture (SUA), Morogoro Region (6°, 49'S, 37°, 40'E).

2.2. Study Design

This study employed an experimental research design, analysing samples every month for six months to determine the shelf life of the prepared papaya-ginger meat tenderizer.

2.3. Shelf-Life Determination

The shelf life of samples packed in plastic packages and stored at room temperature for six months was determined by periodic assessments of both microbial load and physiochemical parameters (pH, colour, and titratable acidity). We took samples every month for evaluation.

2.3.1. Laboratory Sample Analysis

As per the analysis requirements, we divided the samples into two portions (named samples A and B) during the laboratory study. Parameters including colour, pH, titratable acidity, and microbial load (mainly coliforms and other bacteria) were analysed.

2.3.1.1. pH Determination

The pH of the sample was measured using JENWAY pH Metre No. 4330. We standardised the pH metre using standard pH buffer 7. We put three equal parts of a homogenised sample (10 ml each) into a 100 ml beaker and used the standard procedure from the AOAC (2000) method (981.12) to measure the electrode potential between the glass and reference electrode.

2.3.1.2. Titratable Acidity (TA) Determination

We pipetted about 10 ml of the sample into a 250-ml beaker and diluted it to 100 ml with distilled water. Next, we added 3 drops of a 1% phenolphthalein solution to 100 ml of the diluted sample. We titrated a solution against a 0.1N NaOH solution, following the standard procedure described by the AOAC (2000) method, until we obtained a faint pink color that persisted for 10 seconds.

Here is how we calculated the acidity percentage:

$$\%\ TA = \frac{1}{10} x\ Eq.wt\ of acidy\ x\ Titre\ volume\ x \frac{100}{Sample\ volume}$$

2.3.1.3. Color Determination

We determined the colour of the samples using a UK-made CHROMA Model 260 colourimeter to determine the colour of the samples. The AOAC 988.13 method's standard procedure guided the colour measurement.

2.4. Microbiological Assessment

According to ISO 7218:2007 (E), we pipetted about 25 ml of the sample into a glass bottle containing 225 ml of sterilised pre-enrichment buffered peptone water (BPW) and mixed it with a frequency agitation for 1 minute. Using a sterile micropipette, we carried out a 10-fold serial dilution from 10-1 to 10-3 into tubes containing 9 ml of the diluent (0.1% BPW). We added about 1 ml of the sample to 9 ml of the diluent to make a 10-1 dilution, and then transferred 1 ml of this dilution to the second tube containing 9 ml of the diluent to make a 10-2 dilution. The procedure was repeated up to 10-3 dilutions for each sample. We used sterile petri dishes for plating, pouring and plating 1 ml from each dilution in duplicate, and preparing two replicates for each dilution. About 12–15 ml of plate count agar (PCA) and MacConkey agar (MCA), which were initially cooled in the water bath to 44–470 °C, were poured into each petri dish as labeled. We carefully rotated the petri dish to allow the inoculum to mix with the media, then left it to cool and solidify on the laminar floor's horizontal surface. We inverted the

petri dishes and placed them in the incubator at 30 °C for 24-72 hours, following the standard procedure as outlined by ISO 4833-1:2013.

Following incubation, we counted the number of colonies on the plate as well as the number of colonies forming a unit per mL. We plated at least two critical dilutions consecutively, considering 15–300 colonies for recording. We converted the countable colonies into CFU/ml of the sample and expressed the results using the following equation:

$$\text{CFU/ml} = \frac{number of colonies formed}{dilution \times ml}$$

2.5. Statistical Data Analysis

The collected data was entered into Microsoft excel and then loaded to SPSS version 20.0 for statistical analysis. Analysis of Variance (ANOVA) was used to determine whether there is a statistically significant difference that occurs between means in storage stability of meat tenderizer in every month. Mean was separated by Turkey's Honest Significant difference (P<0.05). Results were expressed as mean ± SD and presented in tabular and graphical forms.

3.0 Results

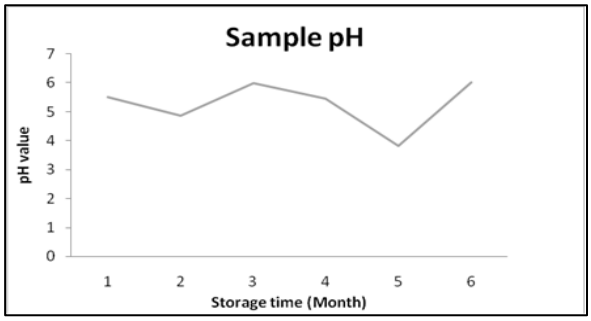
3.1. Physico-Chemical Analysis

3.1.1. pH

We entered the collected data into Microsoft Excel and then loaded it into SPSS version 20.0 for statistical analysis. Analysis of Variance (ANOVA) was used to determine whether there is a statistically significant difference that occurs between means in the storage stability of meat tenderizer every month. The mean was separated by Turkey's Honest Significant difference (P<0.05). Results were expressed as mean ± SD and presented in tabular and graphical forms.

Figure 1

pH of the Papaya-Ginger Meat Tenderizer

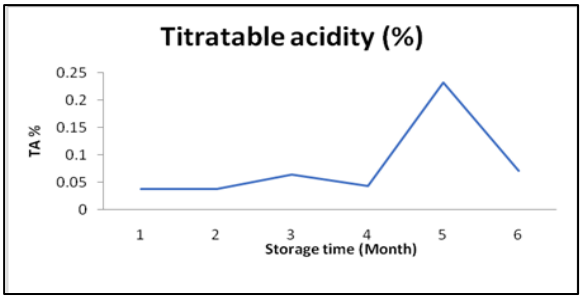


3.1.2. Titratable Acidity (TA)

The titratable acidity of papaya-ginger meat tenderizer changed from 0.0375 to 0.2325% during six months of storage (Figure 2). There was a significant increase (P<0.05) with storage time (Table 3). The meat tenderizer showed an increase in titratable acidity from 0.0375 to 0.06375% after 3 months of storage. Titratable acidity for meat tenderizer decreased from 0.06375 to 0.04375% after 4 months, increased to 0.2325% during 5 months of storage, and decreased to 0.07125% towards the end of the storage period at 6 months.

Figure 2

Titratable Acidity Papaya-Ginger Meat Tenderizer

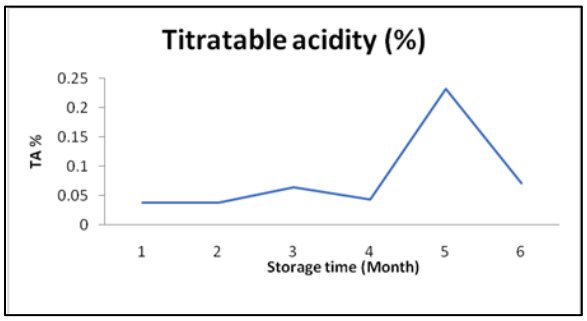


3.1.3. Color

During six months of storage, the color of papaya-ginger meat tenderizer changed from 0.339 to 0.3005 (Figure 3) and 0.341 to 0.297 (Figure 3) for yellow and red, respectively. With storage time, there was a significant decrease in color (P<0.05) (Table 3). A decrease in yellow colour was found in the meat tenderizer from 0.339 to 0.306 for the

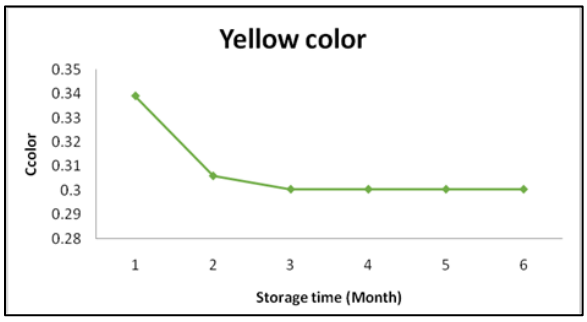
first two months, and then the colour remained constant with a value of 0.3005 towards the end of the storage period at 6 months.

Figure 3
Color of the Papaya-Ginger Meat Tenderizer



The decrease in red colour was found in meat tenderizer from 0.341 to 0.30025 for the first two months, and then the colour remained constant with a value of 0.297 towards the end of the storage period at 6 months.

Figure 4
Red Color of the Papaya-Ginger Meat Tenderizer



3.2. Microbiological Quality
3.2.1. Total Plate Count
The microbial quality of papaya-ginger blend meat tenderizer changed from 42 × 10^3to1.8 × 10^2 CFU/ml during six months of storage (Table 1). There was a significant decrease (P<0.05) with storage time (Table 3). A decrease in total bacteria was found in meat tenderizer from 1.9 × 10^3 CFU/ml to 0 CFU/ml during the first five months of storage. The total bacteria count for a meat tenderizer decreased from 1.9 × 10^3 CFU/ml to 0 CFU/ml after 5 months and increased to 1.6 × 10^2 CFU/ml towards the end of the storage period at 6 months.

Table1
Results for Plate Count (CFU/ml) of the Papaya-Ginger Meat Tenderizer

	Month					
Sample	Jan	Feb	Mar	April	May	June
Meat tenderizer	1.9 × 10^3	42 × 10^3	26× 10^2	13.5× 10^2	Nil	1.8 × 10^2

3.2.2. Coliforms
In the meat tenderizer, there was no indication of coliform growth, as the colonies were 0 (Table 2). The results from Table 2 below of the microbial analysis revealed that coliform was absent from the samples throughout the period of storage. This is because coliforms belong to the enterobacterial group and are mainly of faecal origin, which indicated that the water used for processing and analysis was potable (Reitter et al., 2021).

Table 2
Results for Coliforms (CFU/ml) of Papaya-Ginger Meat Tenderizer

Sample	**Month**					
	Jan	Feb	March	April	May	June
Meat tenderizer	Nil	Nil	Nil	Nil	Nil	Nil

4.0 Discussion

We packed the papaya-ginger blend meat tenderizer in a plastic container and stored it at ambient temperature for 6 months to determine its shelf life. The results show that there is a significant difference (P<0.05) with storage time (Table 4). Physico-chemical parameters, including pH, titratable acidity, and colour, showed significant differences (P<0.05) with storage time

(Table 4). Microbial quality, including total microbial colour, showed a significant difference at $P<0.05$, while coliforms showed a non-significant difference at $P<0.05$ with storage time (Table 4).

4.1. pH

Native yeast, which has been known to survive low pH and cause food spoilage (Borren and Tian, 2020), may be the cause of the pH decrease. Kujero et al. (2020) recommended a pH range of 5.0–6.3 for the sample pH ranges to achieve prolonged shelf stability in products of this nature. This study indicates that a consistent decrease in pH over the storage period is crucial for maintaining the product's quality and tartness (Yusop, 2010). Low pH could enhance the stability of bioactive compounds during storage, thus extending shelf life (Mohd Azmiet al., 2023). Naveena et al.'s (2013) study observed a pH change from 4.5 to 4.9 during the six-month storage of therapeutic ready-to-serve (RTS) made from aloevera, anola, and ginger blends. The storage conditions affect the changes in chemical properties (AOAC, 1995).

4.2. Titratable Acidity (TA)

The increase in acidity may be due to the conversion of some amount of sugar to acids (Mohd Azmi et al., 2023). Bekhit et al. (2014) obtained similar results, reporting an increase in acidity on storage time in beverages such as fruit-flavoured drinks prepared by blending fruits, including mangoes and papaya, to make juice. Ramachandran and Nagarajan (2014) conducted a study on the storage stability of an aloe gel-papaya functional beverage blend, which revealed a change in TA from 0.27% to 0.33% over the storage period. This increase in TA with time was due to the decomposition of fermentable substrates, especially carbohydrates in the fruit. Again, a storage stability study of papaya toffee and leather by Dongre et al. (2019) showed a decrease in TA from 1.19% to 1.10% during the last three months of storage. Furthermore, Kumar's (2013) study revealed a decrease in TA from 0.36% to 0.3% of blended therapeutic RTS as storage time increased.

4.3. Colour

During the storage stability study of the aloe-gel papaya functional beverage blend (Ramachandran and Nagarajan, 2014), researchers found similar results for the yellow colour, which changed from 9.3 to 7.83 and then remained almost constant; similarly, the red colour remained constant throughout the storage time, with a value of 1.3. Ranganna (1986) also observed a decreasing trend, with the colour values changing from 9.23 to 7.83 in Sample A and from 11.9 to 9.3 in Sample B from the first to the last day of storage, respectively. Ahmed et al. (2004) also reported a consistent colour during storage for ginger paste. Singh et al. (2014) conducted a study on the storage stability of ginger paste, revealing a similar result in terms of colour, with values ranging from 66 to 68 during the eight-week storage at ambient temperature. In addition, a study done by Manisha (2017) showed similar results of colour changes from 7.81 to 7.69 that remained constant during storage stability of appetized ginger plum leather stored at 26.1oC for six months.

4.4. Microbiological Quality

4.4.1. Total Plate Count

The acceptable limit of the total plate count is 5×10^5 CFU/ml for herbs and spices and for foods that need further cooking before consumption (Tshabalala et al., 2021). Additionally, a study by Pali (2023) found that blending juice with spices like ginger resulted in a minimum decrease in the microbial population. This might be due to the inhibitory effect of spices on microorganisms. Deka (2001) observed no bacterial growth in spice-mixed fruit juice beverages. Verma (2019) has used spiced extract to prolong the quality of juices and reduce their spoilage.

4.4.2. Coliforms

Mikami et al. (2021) reported the same results of zero coliform growth during storage for pomegranate sauce (which uses ginger as a spice during its manufacture) and for the storage of ginger paste. According to the study's findings, adding ginger was effective in reducing the microbial load and other physicochemical parameters. The presence of essential oil from ginger, known to contain health-promoting bioactive components that offer consumers health benefits (Aneja et al., 2014; Baskaran et al., 2010), may be the reason for this.

5.0 Conclusion and Recommendations

This study looked at how papaya-ginger meat tenderizer changed over six months when it was stored at room temperature. It found that the total number of bacteria and physicochemical properties like pH, titratable acidity, and colour changed a lot. Other parameters, such as coliform bacteria, did not show significant differences during storage ($P<0.05$). However, variations in titratable acidity and reducing sugar content were significant at $P<0.05$. The storage period did not affect colonies or color, which remained constant throughout. We need to conduct further studies using various advanced packaging techniques to extend the shelf life of the ginger-papaya meat tenderizer. Therefore, based on the results obtained, it can be concluded that meat tenderizer is suitable for human consumption, and its shelf life is six months.

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