Original Research Article

PRESERVATION OF STRAWBERRIES USING GUAVA LEAVES EXTRACT.

Abstract

Background: A lot of health hazards have been associated with artificial preservatives including: hyperactivity in children, breathing problems such as asthma and bronchitis, weakening of heart tissues, obesity since some contain fatty acids especially in processed foods and gastrointestinal disorders. Sulfite is a common preservative in fruits and was found to have the following side effects: headaches, allergies, palpitations and cancer [18]. Another down side of artificial preservative is that a lot of time is used in developing and acquiring it thus making it expensive. There is therefore need for a natural preservative.
Objectives: This study was conducted to develop a natural preservative from guava leaves extract that would help increase the shelf-life of strawberries.
Methods: The study was conducted in the food microbiology laboratory, Department of Food Science, Nutrition and Technology, University of Nairobi, Kenya.
Crude extract was obtained by crushing dry guava leaves into 100 g powder and adding 600 ml of boiled water. The mixture was allowed to stand for 1 hr to allow extraction .The extract was then filtered and used to determine its inhibition against fungi. The extract was also serially diluted to obtain 5 different concentrations which were used to determine their effectiveness in increasing the shelf life of the strawberries. The results were recorded and discussed.
Results: The plates with the highest concentration of guava leaves extract had the lowest number of microbial colonies while the one with the lowest concentration on had the highest number of microbial colonies. The control samples had by far a higher number of microbial colonies than all the different concentrations of the extract.
Conclusion: The crude extracts of guava leaves showed inhibition against fungi and increased the shelf life of strawberries. Further studies need to be conducted in order to obtain a purified preservative from the extract.

Keywords: ***Extraction, Preservation, Inhibition, Shelf life, Fungi***

1. INTRODUCTION

Food preservation is the process of treating and handling food to stop or slow down food spoilage , loss of quality, edibility or nutritional value and thus allow for longer food storage for human consumption. There are various methods used in food preservation such as minimal processing, drying/dehydrating, canning, freezing, freeze, fermentation, Immersion in alcohol.and preserving in Salt and Sugar.. When food preservation is compromised, food spoilage will occur and the food quality will decrease from the time of harvest up to the time of consumption. Food spoilage results in quality loss due to physical, chemical, microbiological and enzymatic changes. Economic losses are also experienced due to microbial contamination which causes loss in flavor, off odors, changes in texture, discoloration as well as toxicity by disease causing microorganisms. Food spoilage can be prevented by the following methods: inhibiting microbial growth, irreversible inactivation of microbial cells and mechanical removal of microorganisms in food [2].

Food borne diseases caused by lack of food preservation measures have continuously been a menace to human health and has led to the use of preservatives.

Preservatives increase the longevity of food by protecting the food from deleterious effects of microbial growth and inherent deterioration. They target pathogenic microorganisms [13] and spoilage microorganisms. This has led to formation of artificial preservatives. This is an artificially produced preservative or may be synthetic in nature [9].

A natural preservative is a preservative whose chemical composition has not been interfered with and is not mixed with any synthetic substances [7]. They may be obtained directly from plants or animals. They are therefore inexpensive to acquire yet still meet the high quality standards.
Strawberries are considered as highly perishable foods, lasting 2-3 days and therefore require a preservative to increase their shelf – life. Their spoilage is mainly due to growth of moulds [20]. Rural small scale farmers incur huge losses due to lack of advanced storage facilities as well as long durations of transportation of the strawberries to the consumer.

A lot of health hazards have been associated with artificial preservatives including: hyperactivity in children, breathing problems such as asthma and bronchitis, weakening of heart tissues (which is dangerous to old people) obesity since some contain fatty acids especially in processed foods and gastrointestinal disorders. Sulfite is a common preservative in fruits and was found to have the following side effects: headaches, allergies, palpitations and cancer [18]. Another down side of artificial preservative is that a lot of time is used in developing and acquiring it thus making it expensive.

1.1 Guava Leaves

The guava leaves are available all year round and are commonly known for their diverse health benefits [1]. This is because herbal medicines are considered to be better than conventional medicines due to their minimal side effects. The leaves are therefore taken as supplements in form of capsules and guava leaf tea.

The guava leaves aid losing weight by preventing complex starches from being converted to sugars. They also help in lowering the blood sugar levels without increasing the insulin

production [10]. This is achieved by preventing absorption of sucrose and maltose by the body as well as reducing that alpha glycosidase enzyme activity. In addition, it aids in permanently inactivating bacteria in the digestive system and stop invasion of toxic enzymes by the bacteria [6]. This results in prevention of food poisoning, vomiting and nausea[12]. The production of digestive enzymes is also stimulated. People suffering from bronchitis could use it to open up the lungs, loosen mucus and sooth coughs.

1.2 Guava leaves extract

An extract is a concentrated preparation of a vegetable or animal drug obtained by removing the active constituents there from with a suitable solvent [19], evaporating all or nearly all the solvent and adjusting the residual mass or powder to a prescribed standard. Extracts are prepared in three forms, semi liquid or of syrup consistency, pill form or solid and as dry powder. As the name suggests, guava leaves extract are obtained from the leaves of a guava tree.

The following constituents make the guava leaves extract a suitable natural preservative:

1.2.1 Antioxidants

These are substances that reduce damage due to oxygen such as that caused by free radicals. Free radicals cause spoilage of food, damage cells and reduce the quality of certain materials [8]. When in radical form, they are stable and do not allow further reactions to take place. They therefore increase the shelf life of fruits and vegetables by decreasing oxidation [4]. They inhibit spore germination of plant pathogen and against fungal pathogens of man [11]. They can be enzymes or non-enzymatic for example vitamin C, Vitamin E and beta carotene. In plants, they protect and preserve the plants physical and metabolic integrity as well as their heredity through seeds. They delay decay without adding tastes or odors to food or modify appearance [5].

1.2.2 Flavonoids

These are a group of phytonutrients [6]. Together with carotenoids they are responsible for the colors of the guava fruit[16]. They are powerful antioxidants and exhibit direct antibacterial activity [8]. Flavonoids exhibited antibacterial action against spoilage and food borne pathogenic microorganisms such as *Geobacillus stearothermophilus, Brochothrix thermosphacta, E. coli 0157:H7, Listeria monocytogenes, Pseudomonas fluorescens, Salmonella enterica, Staphylococcus aureus* and *Vibro cholerae* [17]. Flavonoids also show antifungal activity against yeast .

1.2.3 Tannins

These are polyphenolic compounds. They show antibacterial activities and their effectiveness depend on their concentration [15]. The antibacterial activity increases with increase in levels of tannin. They inhibit growth of *Escherichia coli, Pseudomonas aureginosa, Staphylococcus aureus, Aspergillus niger* and *Candida albicans* [3].

The aim of this study was to develop a natural preservative from guava leaves extract to increase the shelf life of strawberries. The study was conducted in the food microbiology laboratory, Department of Food Science, Nutrition and Technology, University of Nairobi.

2.0 MATERIALS AND METHODS

2.1 Sample preparation and extraction procedure

About Two hundred and fifty grams (250 g) guava leaves were randomly picked from Pama gardens in Nanyuki, Laikipia County, Kenya. The leaves were allowed to dry and ground into 100 g powder.600 ml hot water was poured into the container containing the powdered leaves and allowed to settle for 1 hr to allow extraction. The extract was filtered using whatman's paper No.1 to get the filterate which acted as a stock.

Photograph 1: Represents guava leaves powder during extraction of the extract.

Photograph 2: Represents filtration of the extract

2.2 Determining inhibition of the crude extract against fungi

Mueller Hinton agar was poured into a petri dish and allowed to solidify. A layer of fungi was spread on the agar. Holes were drilled into the agar and 0.1 ml of the crude extract, was poured into the holes. A thin layer of the media was poured on top to prevent the extract from moving beyond the holes.

The plates were incubated aerobically at 30 ^{0}C and the hallo regions were observed and measured after 72 hrs to determine the presence or absence of inhibition.

2.3 Determining the effectiveness of the crude extract in increasing the shelf life of strawberries.

Serial dilution of 1 ml in 9 ml of distilled water was done up to 5 serial dilutions

Fresh strawberry fruits were obtained from Kawangware market, Kenya and after two days they were dipped in guava leaves extract of different concentrations. 1 g of strawberry fruit from each concentration was serially diluted with sterile 0.85% NaCl. 4 decimal dilution of the sample were done. After homogenization, the last 3 dilution were plated on potato dextrose agar plates. The plates were incubated aerobically at 30 ^{0}C. Microbial colonies were observed, counted and recorded after 48 hrs. The procedure was repeated for 3 consecutive days.

Photograph 3: strawberries dipped in extract of different concentrations.

2.4 Control

For the control experiment, sample dilution without the extract was inoculated. The colonies were observed, counted and recorded after 48 hrs.

2.5 Shelf life evaluation

The shelf life of the strawberries was estimated using a line of best fit from the results obtained from determining the effectiveness of the guava leaves extract in increasing the shelf life of the strawberries. The end of shelf life is marked when microbial profile is beyond the acceptable limit of fungal growth, 4 $\log_{10}$ cfu/g.

3.0 RESULTS AND DISCUSSION

3.1 Results for determining inhibition of the crude extract against fungi.

The hallo regions were observed and had average diameters of 2.1 cm. Hallo regions of iameters 2.1 cm were observed hence there was evidence of inhibition of the crude extract against fungi.

Mueller Hinton agar was used because it contains starch which absorbs metabolic wastes produced by the fungi. Therefore, the inhibition that occurred was due to the extract and not the metabolic wastes. The inhibition of fungi by the extract can be accredited to the phytochemicals present in the extract [17].

3.2 Results for determining the effectiveness of the crude extract in increasing the shelf life of strawberries..

Table 1 shows Microbial colonies counted and expressed in $\log_{10}$ of cfu/g for the samples treated with different extract concentrations in 3 days. Dilution of the sample was done to decrease the number of microbes to a limit that can be counted. Dilution 1 had the highest concentration of the extract while dilution 5 had the lowest concentration of the extract.

Table 1. Microbial colonies counted in expressed $\log_{10}$ of cfu/g for the samples treated with different extract concentrations in 3 days.

DILUTIONS	**MICROBIAL COUNT IN LOG BASE 10 FOR 3 DAYS**		
	DAY1 $\log_{10}$(CFU/g)	**DAY 2 $\log_{10}$ (CFU/g)**	DAY 3 **$\log_{10}$(**(CFU/g)
1	2.13	2.20	2.28
2	2.42	2.35	2.79
3	2.69	2.65	3.0
4	2.74	2.73	2.87
5	2.76	2.85	2.96

The plates with the highest concentration of guava leaves extract had the lowest number of microbial colonies while the one with the lowest concentration on had the highest number of microbial colonies. Therefore, the extract was more effective in inhibiting fungal growth on strawberries when used at a higher concentration. This difference was because, under different concentrations, the phytochemical compounds present in the extract show different effectiveness in inhibiting fungi [15].The higher their concentration, the more effective they are in inhibiting fungi.

The presence of fungal growth in the plates indicates that some fungi were resistant to the guava leaves extract.

3.3 Results for control sample.

The control sample had by far a higher number of microbial colonies than all different concentrations of the extract (Table 2). .This difference is due to the absence of the crude extract in the control sample. This shows that the extract was effective in inhibiting fungal growth on the strawberries.

Table 2. Number of microbial colonies recorded in log base 10, cfu/g from the control experiment in 3 days.

The microbial count in this table has been given in log base 10.

DAY 1 (CFU/g)	DAY 2 (CFU/g)	DAY 3 (CFU/g)
3.05	3.09	3.12

3.1.4 Results for shelf life evaluation

Figure 1 represents the average microbial count of the treated samples against the number of days the experiment was conducted. It also represents the microbial count of the control sample against the number of days. The average shelf life of strawberries at room temperature was 3 days and the strawberry fruits in this study were treated with the crude extract 2 days after they were obtained in the market. From the figure, it is clear that the shelf life of the strawberries was extended by more than 3 days because acceptable limit of fungal growth on strawberries ($4\log_{10}$ cfu/g) had not been reached by the 3rd day.

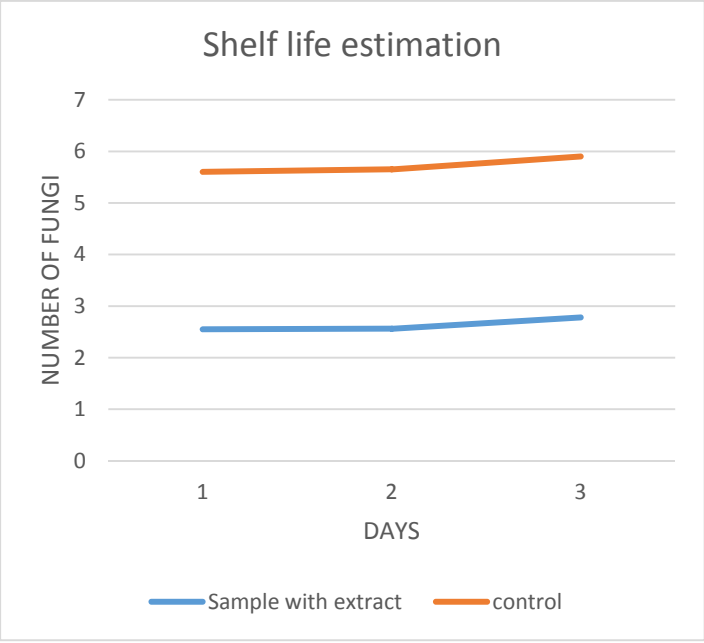

Fig 1. Graphical representation of results for the shelf life evaluation

4.0 CONCLUSION AND RECOMMENDATION.

4.1 CONCLUSION

The crude extract of guava leaves was effective in inhibiting the growth of fungi that causes spoilage in strawberry fruits. The crude extract also increased the shelf life of the strawberries. It could therefore be a suitable natural strawberry fruit preservative.

4.2 RECOMMENDATION

Further studies need to be conducted in order to:

1. Identify the components in the crude extracts causing inhibition against fungi.
2. Purify the crude extracts to obtain a preservative.
3. Identify the fungi that was resistant to the guava leaves extract.
4. Identify the minimum inhibition concentration.

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