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]. Other down sides of artificial preservative include use of a lot of time 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 crude extract was obtained by crushing dry guava leaves into 100g powder and adding 600ml of boiled water. The mixture was allowed to stand for 1hr 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 different concentrations of the extract.
Conclusion: The crude extracts of guava leaves showed inhibition against fungi and have the potential to increase the shelf life of strawberries. Further studies need to be conducted in order to obtain a purified preservative from the extract.

*Keywords: **Strawberries, guava, extraction, leaves, Preservation***

1. INTRODUCTION

Food preservation is the conservation of food in a safe manner for future consumption. Methods of food preservation include: drying, cooling and canning. When food preservation is compromised, food spoilage 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. This is why preservatives are used.

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 cheap 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]. Other down sides of artificial preservative include use of a lot of time 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, cause damage of 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 *Bacillus stearothermophilus, Brochothrix thermosphacta, E. coli 0157:H7, Listeria monocytogens, Pseudomonas fluorescens, Salmonella enterica, Staphylococcus aureus* and *Vibro cholera* [17]. Flavonoids also show antifungal activity against yeast .

1.2.3Tannins

This 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 *Escheria coli, Pseudomonas aureginaa, Staphylococcus aurous, 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.

2.0 MATERIALS AND METHODS

2.1 Sample preparation and extraction procedure

About 250g guava leaves were randomly picked from a farm in Nanyuki, Laikipia County, Kenya. The leaves were allowed to dry and ground into 100g powder.600ml hot water was poured into the container containing the powdered leaves and allowed to settle for 1hr to allow extraction. The extract was then filtered using a filter paper.

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 0.1ml 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 30^0C and the hallo regions were observed and measured after 72hrs 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 1ml in 9ml 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. 1g 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 30^0C. Microbial colonies were observed, counted and recorded after 48hrs. 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 48hrs.

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, 10000cfu/g.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

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

The hallo regions were observed and had average diameters of 2.1 cm.

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

Table 1. Microbial colonies counted in cfu/g for the different extract concentrations in 3 days.

DILUTION	DAY1(CFU/g)	DAY 2(CFU/g)	DAY 3(CFU/g)
1	$1.35*10^2$	$1.6*10^2$	$1.9*10^2$
2	$2.6*10^2$	$2.25*10^2$	$6.1*10^2$
3	$4.9*10^2$	$4.5*10^2$	$10.1*10^2$
4	$5.55*10^2$	$5.4*10^2$	$7.35*10^2$
5	$5.7*10^2$	$7.1*10^2$	$9.2*10^2$

3.1.3 Results for control sample.

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

DAY 1(CFU/g)	DAY 2(CFU/g)	DAY 3(CFU/g)
$11.1*10^2$	$12.3*10^2$	$13.1*10^2$

3.1.4 Results for shelf life evaluation

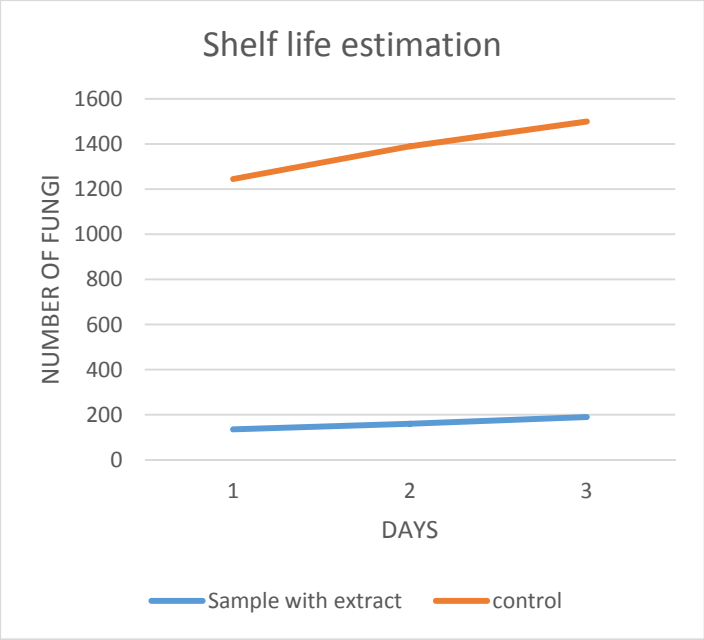

Fig 2. Graphical representation of results for the shelf life evaluation

From the graph above it is clear that the shelf life of the strawberries was extended by more than 3days.

3.2 DISCUSSION

Hallo regions of diameters 2.1cm 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.

Dilution of the sample was done to decrease the number of microbes to a limit that can be counted.

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.
The presence of fungal growth in the plates indicates that some fungi were resistant to the guava leaves extract.
The control samples had by far a higher number of microbial colonies than all different concentrations of the extract .This shows that the extract was effective in inhibiting fungal growth [21] on the strawberries.

4.0 CONCLUSION AND RECOMMENDATION.

4.1 CONCLUSION

1. The crude extracts of guava leaves have inhibition against fungi. However some of the fungi showed resistant.

2. The crude extracts of guava leave have the potential to increase the shelf life of strawberries.

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.

REFERENCES

1. Barbalho, S.M., Farinazzi-Machado, F.M.V., de Alvares Goulart, R., Brunnati, A.C.S., Otoboni, A.M. and Ottoboni, B., 2012. Psidium guajava (Guava): A plant of multipurpose medicinal applications. *Med Aromat Plants*, *1*(104), pp.2167-0412.
2. Barth, M., Hankinson, T. R., Zhuang, H., Breidt, F. (2010). Microbial Spoilage of Fruits and Vegetables. *Food Microbiology and Food Safety*. Pg.135–183
3. Biswas, B., Rogers, K., McLaughlin, F., Daniels, D. and Yadav, A., 2013. Antimicrobial activities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. *International journal of microbiology*, 2013.
4. Braga, T.V., das Dores, R.G.R., Ramos, C.S., Evangelista, F.C.G., da Silva Tinoco, L.M., de Pilla Varotti, F., das Graças Carvalho, M. and de Paula Sabino, A., 2014. Antioxidant, antibacterial and antitumor activity of ethanolic extract of the Psidium guajava leaves. *American Journal of Plant Sciences*, *5*(23), p.3492.
5. Carocho, M., Barreiro, M.F., Morales, P. and Ferreira, I.C., 2014. Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), pp.377-399.
6. Choudhury, S., Sharan, L. and Sinha, M.P., 2012. Phytochemical and antimicrobial screening of Psidium guajava L. leaf extracts against clinically important gastrointestinal pathogens. *J. Natural Prod. Plant Resour*, 2(4), pp.524-529.
7. Cleveland, J., Montville, T.J., Nes, I.F. and Chikindas, M.L., 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *International journal of food microbiology*, 71(1), pp.1-20.
8. Cushnie, T.T. and Lamb, A.J., 2011. Recent advances in understanding the antibacterial properties of flavonoids. *International journal of antimicrobial agents*, 38(2), pp.99-107.
9. Davidson, P.M., Taylor, T.M. and Schmidt, S.E., 2013. Chemical preservatives and natural antimicrobial compounds. In *Food microbiology* (pp. 765-801). *American Society of Microbiology*.
10. Deguchi, Y., Osada, K., Uchida, K., Kimura, H., Yoshikawa, M., Kudo, T., Yasui, H. and Watanuki, M., 1998. Effects of extract of guava leaves on the development of diabetes in the db/db mouse and on the postprandial blood glucose of human subjects. *Journal of the Agricultural Chemical Society of Japan (Japan).*
11. Fernandes, M.R.V., Dias, A.L.T., Carvalho, R.R., Souza, C.R.F. and Oliveira, W.P., 2014. Antioxidant and antimicrobial activities of Psidium guajava L. spray dried extracts. *Industrial Crops and Products*, *60*, pp.39-44.

12. Gonçalves, F.A., Andrade Neto, M., Bezerra, J.N., Macrae, A., Sousa, O.V.D., Fonteles-Filho, A.A. and Vieira, R.H., 2008. Antibacterial activity of GUAVA, Psidium guajava Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob shrimp, Xiphopenaeus kroyeri (Heller). *Revista do Instituto de Medicina Tropical de São Paulo*, *50*(1), pp.11-15.
13. Joseph, B. and Priya, M., 2011. Review on nutritional, medicinal and pharmacological properties of guava (Psidium guajava Linn.). *International Journal of pharma and bio sciences*, *2*(1), pp.53-69.
14. Mahfuzul Hoque, M.D., Bari, M.L., Inatsu, Y., Juneja, V.K. and Kawamoto, S., 2007. Antibacterial activity of guava (Psidium guajava L.) and neem (Azadirachta indica A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne pathogens and disease*, *4*(4), pp.481-488.
15. Mailoa, M.N., Mahendradatta, M., Laga, A. and Djide, N., 2013. Tannin extract of guava leaves (Psidium guajava L) variation with concentration organic solvents. *International Journal of Scientific & Technology Research*, *2*(9), pp.106-110.
16. Mercadante, A.Z., Steck, A. and Pfander, H., 1999. Carotenoids from Guava (Psidium g uajava L.): Isolation and Structure Elucidation. *Journal of Agricultural and Food Chemistry*, *47*(1), pp.145-151.
17. Rattanachaikunsopon, P. and Phumkhachorn, P., 2010. Contents and antibacterial activity of flavonoids extracted from leaves of Psidium guajava. *Journal of Medicinal Plants Research*, 4(5), pp.393-396.
18. Sanjay Sharma, 2015, *international journal of science and research publications*, vol 5, pg1
19. Seo, J., Lee, S., Elam, M.L., Johnson, S.A., Kang, J. and Arjmandi, B.H., 2014. Study to find the best extraction solvent for use with guava leaves (Psidium guajava L.) for high antioxidant efficacy. *Food science & nutrition*, *2*(2), pp.174-180.
20. Van-Kan, J. A. L. (2006). Licensed to kill: the lifestyle of a necrotrophic plant pathogen. *Trends in Plant Science*. **11**, Pg.247–253
21. Wu, J.W., Hsieh, C.L., Wang, H.Y. and Chen, H.Y., 2009. Inhibitory effects of guava (Psidium guajava L.) leaf extracts and its active compounds on the glycation process of protein. *Food chemistry*, 113(1), pp.78-84.