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Impact of Packaging Materials on Quality of Fresh Cut Pineapple Using Biopreservative to Ensure Safety

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A B S T R A C T

Packaging of a food product is an operation, which aims at the prevention of all kinds of degradation that renders it unsuitable for consumption or of a lower sensorial value. Fresh cut products are experiencing an increasing popularity mainly due to their convenience, freshness and associated health benefits. Pineapple is appreciated for its taste, flavour. Juiciness and has many medicinal properties of bromelin which is found in pineapple regulates and cures many diseases. Pineapple were minimally processed in the form of slices and treated with 0.04mg of nisin packed in thermocol, arecanut sheath, aluminium foil and polypropylene stored at room and refrigeration temperatures. Changes in physiological loss of weight, firmness, TSS, pH, ascorbic acid, β-carotene, total antioxidant activity, sensory quality and microbial growth were evaluated over a time. For all the packaging materials polypropylene allowed conservation of fresh cut pineapples treated with nisin without undesirable changes up to 3 days in room temperature and 12 days in refrigerated temperature in all parameters. Packaging materials used in this study are beneficial to food industry and consumers since they can extend the lag-period and reduce the growth rate of microorganism to prolong shelf life of fresh cut pineapple to maintain food safety.

Introduction

Pineapple (*Ananas comosus*) is the world's most popular non-citrus tropical and subtropical fruit. It has cylindrical shape, square shoulders, an intense orange-yellow shell colour and a medium to large size (1.3 to2.5g), and stands out for its excellent quality and sensory characteristics. The flesh is clear yellow, very sweet, compact and fibrous and has a high ascorbic acid content but low total acidity. Bromelain is a complex mixture of substances that can be extracted from the stem and core fruit of the pineapple and has serious impact on health and medicinal benefits.

It contains high amounts of vitamin C and manganese thiamine, B vitamin, dietary fibre which is important for antioxidant defense and involved in energy production (Anonymous, 2017).

Fresh cut fruits and vegetables with the advantage of health, convenience, high nutrition and flavour while still maintaining freshness, have gained great popularity among customers worldwide. This has led to a global trend of increased consumption and research investment of fresh cut fruits and vegetables in recent years (Oliveira *et al.,* 2015, Siddiq Sogi and Dolan, 2013).

Fresh pineapple possesses a thick inedible peel and a large crown which takes up storage space and results in higher transportation cost (James and Ngarmsak, 2010). Value addition by processing into a ready-to-eat product is an attractive alternative since consumers will spend less time on food preparation (Rocculi *et al.,* 2009). However, fruit peeling and cutting increase metabolic activities such as respiration rate and delocalisation of enzymes and substrates leading to quality deterioration such as browning, softening, off-flavour and microbial growth, resulting in a short shelf life (Montero-Calderon *et al.,* 2008).

In general, traditional preservation methods of fresh cut fruits and vegetables could be broadly classified into three categories namely physical based preservation, chemical based preservation and biopreservation technology (Krasaekoopt and Bhandari, 2010). Cold storage is one of the most commonly used physical based methods to improve shelf life of fresh cut fruits and vegetables. In terms of chemical based preservation technology, a number of natural or synthetic preservatives have been used to prolong the shelf life of fresh cut fruits and vegetable in the past decades (Meireles *et al.,* 2016). However consumers have also become more critical of the use of synthetic additives as their awareness of health and food safety has increased. This has called for the synthetic additive free or natural additive based preservation techniques in recent years.

As for traditional biopreservation with a long history of safe use it refers to the rational utilization of the antimicrobial potential of natural microorganisms and their antibacterial products to extend the shelf life and enhance the safety foods (Stiles, 1996). With the rapid development of biotechnology in the fast few decades, particular attention has been paid to novel biopreservation techniques, including the uses of bacteriophages, bacteriocins and bioprotective microorganisms.

Nisin is good example of commercial success and a good perspective is open to continue the study and development of new bacteriocins and their biotechnological applications (Parada *et al.,* 2007). Nisin possesses a broader antimicrobial spectrum than most other bacteriocins, has been shown to be of no or low toxicity, has proven success as a food preservative, and is produced commercially in the form of so-called "nisin concentrate".

The inhibitory effect of nisin is broader than most bacteriocins and extends to a wide variety of gram positive bacteria including spore formers (Abee and Broughton, 2003

Packaging of a food product is an operation, which aims at the prevention of all kinds of degradation that renders it unsuitable for consumption or of a lower sensorial value. Fresh cut products are experiencing an increasing popularity mainly due to their convenience, freshness and associated health benefits. Thus the objective of this study was to evaluate the effects of packaging materials on the physico chemical properties, microbiological and sensory quality of fresh cut pineapple using different concentrations of nisin during refrigeration and room temperature.

Materials and Methods

Materials

Local varieties of pineapples (*Ananas comosus*) were purchased from the market at Madurai city. Pineapple fruit of regular shape and uniform size without any defect were selected. Fruit at maturity stage 5 (about 50% of eyes were orange yellow, half ripe fruit) were used. The stage of maturity was determined based on the Malaysian standard by Federal Agricultural Marketing Authority (FAMA) (Shamsudin *et al.,* 2009.).

Food grade quality of nisin was used in powder form was used as preservative. Packaging materials such as aluminium foil, arecanut sheath, polypropylene, thermocol container were purchased from local market. The containers were of 200g capacity each made up of different kind of material. Cling wrap was used for covering the fresh cut pineapple packed in different containers.

Preparation of fresh cut pineapple sample

Working area, cutting boards, knives, containers and other utensils and surfaces in contact with fruit during processing were washed and sanitized with 200 μLL^{-1} sodium hypochlorite solutions at pH to have a maximum sanitizing effect prior to processing. The selected fruits were peeled and cut superficially in order to remove the eyes, washed in running tap water followed by washing in chlorine solution (200ppm) for 2 minutes to keep surface pH low enough to reduce microbial growth and to improve tissue firmness ; excess water was drained for 2 minutes. Then pineapples were cut with a sharp knife into cubes of 2cm (Fig. 1).

Application of Nisin

Nisin dissolves well in low pH (3-4) condition. Nisin solution (pH 3-4) was prepared by dissolving citric acid solution in different concentrations of 0.04, 0.05 and 0.06mg. The fresh cut pineapples were pre-treated by soaking them in the above solutions for 5 to 10 minutes. Then it was surface dried for one hour and packed in thermocol container and the samples were stored at room and refrigeration temperatures for its storage stability.

Determination of antimicrobial activity of nisin

Isolates of nisin were very separately grown in test tubes without agitation in 10 ml Man Rogosa Sharpe **(**MRS) broth. The broth culture was incubated at 35ºC, for 42 h. After incubation the culture was centrifuged (10,000 rpm for 10 minutes) to obtain the culture supernatant.

The pH of the culture supernatant was adjusted to pH 7 with 1N NaOH and again filtered using membrane filter. Sterile filter paper disc were dipped into the above sterile culture filtrate of LAB and placed on nutrient agar seeded with test microorganisms. The plates are kept at 4ºC for 3 to 4 h to permit diffusion the assay material and then incubated at 37ºC for 24 hours. The disc dipped in uninoculated MRS broth served as control.

Standardization of level of concentrations of nisin

The colour, appearance, firmness, flavour, taste and general acceptance of fresh cut pineapple using different concentrations of nisin was determined by the method described by Alegria *et al.,* (2009). It was evaluated by panel of trained judges using 1-5 scale by using thermocol as storage container.

Standardization of packaging material

Fresh cut pineapple treated with nisin was packed in thermocol container as a common packaging material. For each preservative the best concentration level of 0.04mg of nisin was selected for further studies using the different packaging material such as arecanut sheath, aluminium foil and polypropylene with covering of cling wrap and stored in room and refrigeration temperatures. The untreated pineapple served as control.

Methods

Storage studies

Chemical and physical analyses were done periodically once in a day for fresh cut fruits and vegetables stored at room temperature and on alterative days for the samples stored under refrigeration as per the procedures given below.

Determination of physical characteristics

Weight loss of the fresh cut pineapple sample was determined by comparing the weights of samples during the end of the storage with initial weights by using a digital balance (Electronic balance shimadzu, B.L-120-H) and expressing the results as a percentage (Chien *et al.,* 2007). Firmness of the treated fresh cut pineapple was evaluated during the storage period with a texture analyser (Texture analyzer stable micro system, model: Texture Export Version 1.22) Treated Fresh cut pineapple were placed on the platform horizontally and cut with the speed of 1.0 mm/s and the distance of cut was 10 mm. The firmness of the treated fruits and vegetables was tested during the storage period and reported as peak force of cutting and expressed in Newtons (N) (Rocculi *et al.*, 2009).

Determination of chemical constituents

The pH of the samples was estimated by the method described by Hurt and Fischer (1971). Five grams of the sample was mixed well using a glass rod in 50ml of distilled water and the pH of the suspension was determined using pH meter. Total soluble solids were measured by using hand refractrometer and expressed as degree Brix (Ranganna, 1995). Ascorbic acid was estimated following the procedure of Mahadevan and Sridhar (1986).The amount of ascorbic acid present in the sample was calculated and expressed as mg per 100g.

The β carotene of the sample was estimated colorimetrically as described by Raghuramulu *et al.,* (1983) expressed as microgram per 100g of the sample. Antioxidant assay was measured using UV-Scan spectrophotometer at 593 nm spectrophotometrically (Benzie and Strain, 1996).

Microbiological examination

The microbial load (bacteria, fungi and yeast) of the stored sample were enumerated at regular intervals by the method described by Istavankiss (1984). All microbiological analysis was carried out in triplicate and the results were expressed as $\log_{10}$ colony forming units per grams ($\log_{10}$ CFU/g).

Organoleptic evaluation

The fresh cut pineapples were standardized based on the method described by Alegria *et al.,* (2009) using 1-5 scale. It was evaluated by panel of trained judges.

Statistical analysis

The data obtained were subjected to statistical analysis to find out the impact of treatments, storage period, different packaging materials, and temperatures on the quality of the products during storage. Four factorial completely randomized designs were applied for the analysis (Gomez and Gomez, 1984).

Results and Discussion

Assessment of antimicrobial activity of nisin

The antimicrobial activity of nisin was assessed by studying the area of inhibition caused by isolates on test organisms viz., *Escherichia coli, Salmonella sp, Streptococcus sp, Enterococcus sp and Bacillus sp.* Inhibition growth of pathogenic bacteria by, nisin was presented in Table 1.

Nisin showed maximum inhibition on *Streptococcus sp* (14.0mm) followed by *Escherichia coli* (13.5mm) *Bacillus sp* (12.0mm), *Salmonella sp* (11.6mm), *Enterococcus sp* (11.3mm). Nisin is first bacteriocin produced by fermentation using the bacterium *Lactococcus lactis*. Nisin is essentially non-toxic to humans and does not lead to cross- resistance with medical antibiotics. It is commercially produced under brand name – Nisaplin. It is inhibitory to many *Micrococcus, Clostridium, Bacillus* and *Staphylococcus* etc. (Chandra *et al.,* 2008).

Standardization of level of concentrations of nisin for fresh cut pineapple

Mean scores obtained for the fresh cut pineapple treated with different types of biopreservatives packed in thermocol container are given in the Table 2. Fresh cut pineapple treated with 0.04mg of nisin was highly acceptable by the judges with the score value of 1.0 for colour, appearance, firmness and flavour; 1.3 for taste and 1.2 for general acceptance. The taste and general acceptance of the fresh cut pineapple treated with 0.05mg and 0.06mg of nisin was not acceptable to the judges because it imparted slight bitter taste. The flavour was not liked by the judges. Fruit phenolics have attracted a great interest recently as potential natural antimicrobial agents that could be used to extend the shelf life of value added fruit and vegetable products. The antimicrobial activity of catechin, chlorogenic acid phloridzin 1,5, 10 and 25 mM concentrations was assessed against three marker pathogenic bacteria, one probiotic bacterium, two yeasts and one food spoilage fungus (Muthuswamy and Rupasinghe, 2007).

Physico chemical characteristics of fresh cut pineapple during storage

Physiological loss of weight (%)

As seen in the Figure 2 the fresh cut pineapple treated with nisin had maximum shelf life of 12 days in refrigeration temperature with the minimum loss of 0.86 % in arecanut sheath container 0.80% in aluminium foil container 0.75% in polypropylene container when compared to control samples. At room temperature fresh cut pineapple had shelf life of 3 days and recorded weight of 1.82 % in arecanut sheath, 1.70% in aluminium container and polypropylene container. The weight loss of nisin treated samples was significantly lower than untreated samples.

Rojas-Grati *et al.,* (2007) observed that the incorporation of lemongrass into alginate-apple puree edible film did not significantly affect water vapor permeability.

They noted that it could be related to the main components of this essential oil, which is not lipid and mostly contains terpene-like compounds.

Firmness

The firmness of (N) of fresh cut pineapple treated with nisin is given in graphical representation in Figure 3. The initial firmness of fresh cut pineapple was 19.36 Newton and the final firmness values ranged from 22.28 to 25.56 Newton in control samples stored in different packaging materials. The shelf life of fresh cut pineapple stored in room temperature and refrigeration temperature was 2 days and 8 days respectively. The final firmness content nisin treated sample ranged from 22.12 to 25.30 Newton with the shelf life of 3 days at room temperature and 12 days at refrigeration temperature stored in different packaging materials.

Table.1 Inhibition of growth of pathogenic bacteria by nisin

Test organisms	Area of Inhibition (mm)
	Nisin
Escherichia coli	13.5
Salmonella sp	11.6
Streptococcus sp	14.0
Enterococcus sp	11.3
Bacillus sp	12.0

Table.2 Mean scores obtained for standardization level of concentration of nisin

Concentration level of nisin	Mean scores obtained for the various sensory parameters (1-5 scale)					
	Color	Appearance	Firmness	Flavor	Taste	General acceptance
0.04mg	**1.0**	**1.0**	**1.0**	**1.0**	**1.3**	**1.2**
0.05mg	1.0	1.0	1.0	1.5	2.2	2.5
0.06mg	1.0	1.0	1.0	1.8	2.5	2.8

Color	1-Very dark yellow	2- Dark yellow	3-Yellow	4-Light yellow	5-Very light yellow
Appearance	1-Excellent	2-Very good	3-Good	4-Fair	5-Poor
Firmness	1-very firm	2-Firm	3-Moderate	4-soft	5-Very soft
Flavour	1-Very intense	2-Intense	3-Moderate	4-Low	5-Absent
Taste	1-Excellent	2-Very good	3-Good	4-Fair	5-Poor
General acceptance	1-Excellent freshly cut	2-Good	3-Limit of marketability	4-fair limit of usability	5-Poor usable

Table.3 Nutrient content of fresh cut pineapple treated with nisin during storage

Nutrients	Control				Nisin			
	Room Temp.		Refrigeration temp.		Room Temp.		Refrigeration temp.	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ARECANUT SHEATH								
pH	3.95	4.32 (2)	3.95	4.22(8)	3.95	4.22 (3)	3.95	4.10 (2)
Total soluble solids	15.00	15.50 (2)	15.00	15.80 (8)	15.00	15.20 (3)	15.00	15.60 (12)
Ascorbic acid (mg%)	35.00	22.75 (2)	35.00	26.25 (8)	35.00	25.62 (3)	35.00	28.60 (12)
β-carotene (μg/100g)	15.00	9.50 (2)	15.00	10.75 (8)	15.00	12.42 (3)	15.00	13.65 (12)
Total antioxidant activity (μg/g)	19.00	12.00 (2)	19.00	13.00(8)	19.00	12.30 (3)	19.00	14.10 (10)
ALUMINIUM FOIL								
pH	3.95	4.34 (2)	3.95	4.20 (8)	3.95	4.20 (3)	3.95	4.08 (12)
Total soluble solids	15.00	15.50 (2)	15.00	15.80(8)	15.00	15.20 (3)	15.00	15.60 (12)
Ascorbic acid (mg%)	35.00	22.84 (2)	35.00	26.54 (8)	35.00	22.95 (2)	35.00	26.67 (8)
β-carotene (μg/100g)	15.00	9.56 (2)	15.00	10.86 (8)	15.00	12.60 (3)	15.00	13.70 (12)
Total antioxidant activity (μg/g)	19.00	12.10 (2)	19.00	13.50 (8)	19.00	12.35 (3)	19.00	15.50 (12)
POLYPROPYLENE CONTAINER								
pH	3.95	4.36 (2)	3.95	4.20 (8)	3.95	4.18 (3)	3.95	4.06 (12)
Total soluble solids	15.00	15.50 (2)	15.00	15.80 (8)	15.00	15.20 (3)	15.00	15.60 (12)
Ascorbic acid (mg%)	35.00	22.95 (2)	35.00	26.67	35.00	25.82 (3)	35.00	28.90 (12)
β-carotene (μg/100g)	15.00	9.67 (2)	15.00	10.90 (8)	15.00	12.69 (3)	15.00	13.78 (8)
Total antioxidant activity (μg/g)	19.00	12.15 (2)	19.00	13.60 (8)	19.00	12.46 (3)	19.00	16.00 (12)

(Figures in parenthesis indicate the storage days)

Table.4 Microbial analysis of fresh cut pineapple treated with nisin during storage

Nutrients	Control				Nisin			
	Room Temp.		Refrigeration temp.		Room Temp.		Refrigeration temp.	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ARECANUT SHEATH								
Bacteria (10^6cfu/g)	4.00	14.00 (2)	4.00	8.50 (8)	3.00	12.16 (3)	3.00	7.25 (12)
Fungi (x 10^3cfu/g)	4.00	8.50 (2)	4.00	9.30 (8)	2.00	5.20 (3)	2.00	6.40 (12)
ALUMINIUM FOIL								
Bacteria 10^6CFU/g	4.00	14.00 (2)	4.00	8.35 (8)	3.00	12.00 (3)	3.00	7.20 (12)
Fungi (x 10^3cfu/g)	4.00	8.20 (2)	4.00	9.00 (8)	2.00	5.00 (3)	2.00	6.20(12)
POLYPROPYLENE CONTAINER								
Bacteria 10^6CFU/g	4.00	14.00(2)	4.00	8.35 (8)	3.00	12.00 (3)	3.00	7.00 (12)
Fungi (x 10^3cfu/g)	4.00	8.00 (2)	4.00	9.00 (8)	2.00	5.00 (3)	2.00	6.00 (12)

(Figures in parenthesis indicate the storage days)

Fig.1 Experimental process followed to obtain fresh cut pineapple

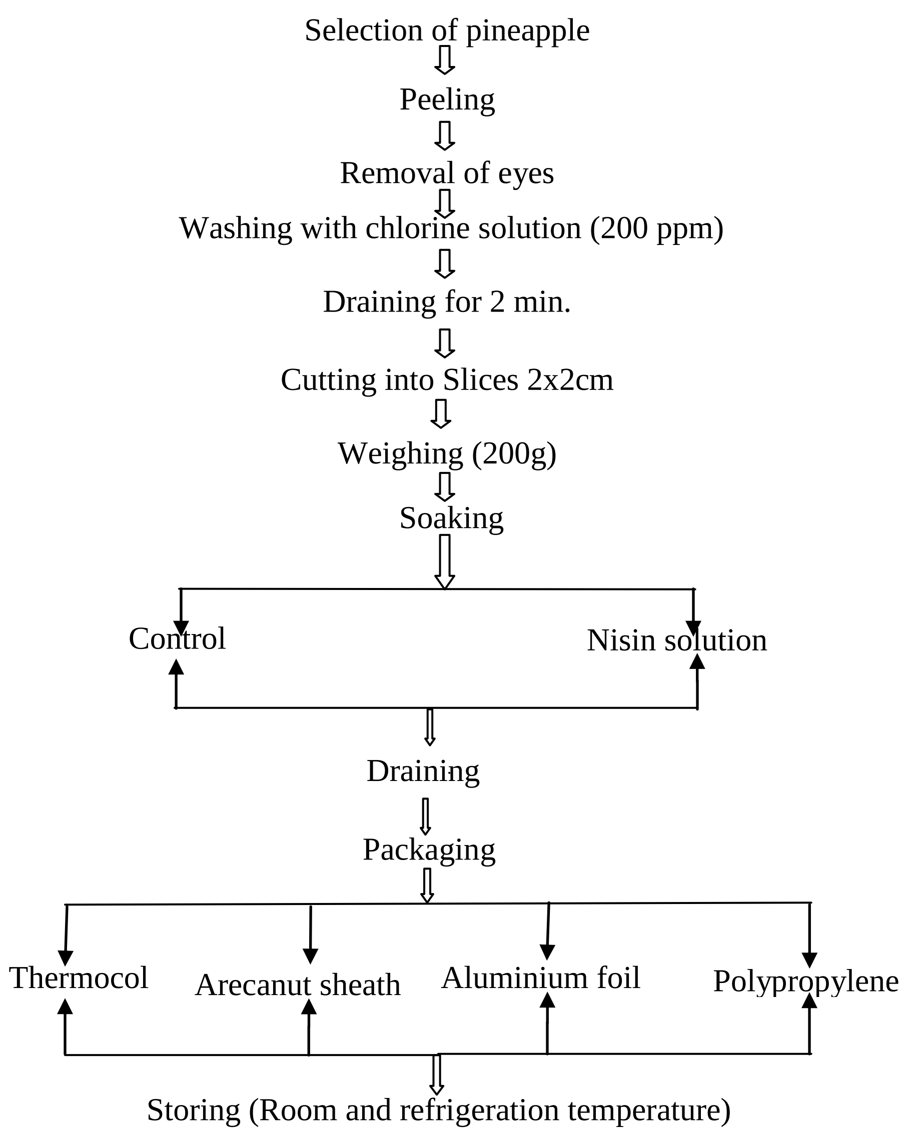


Fig.2 Physiological loss of weight of treated fresh cut pineapple during storage

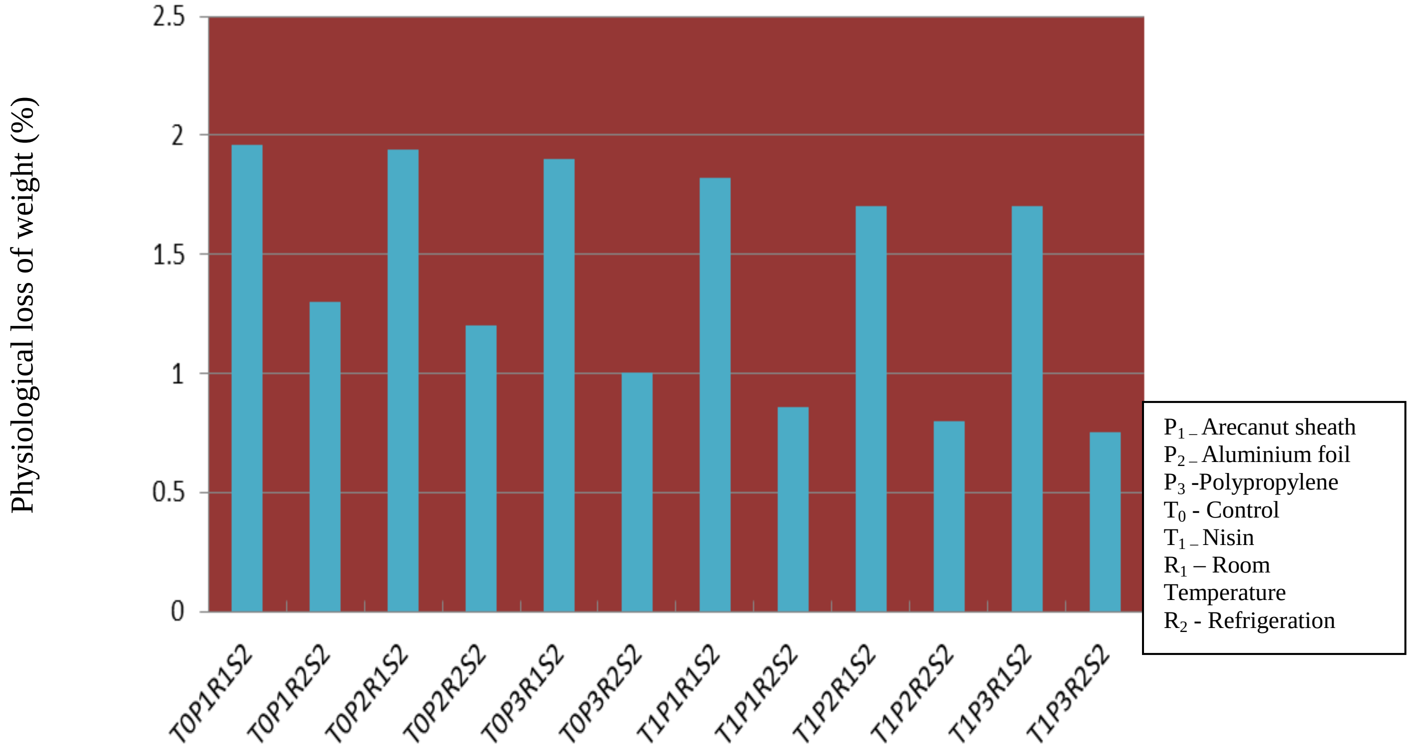
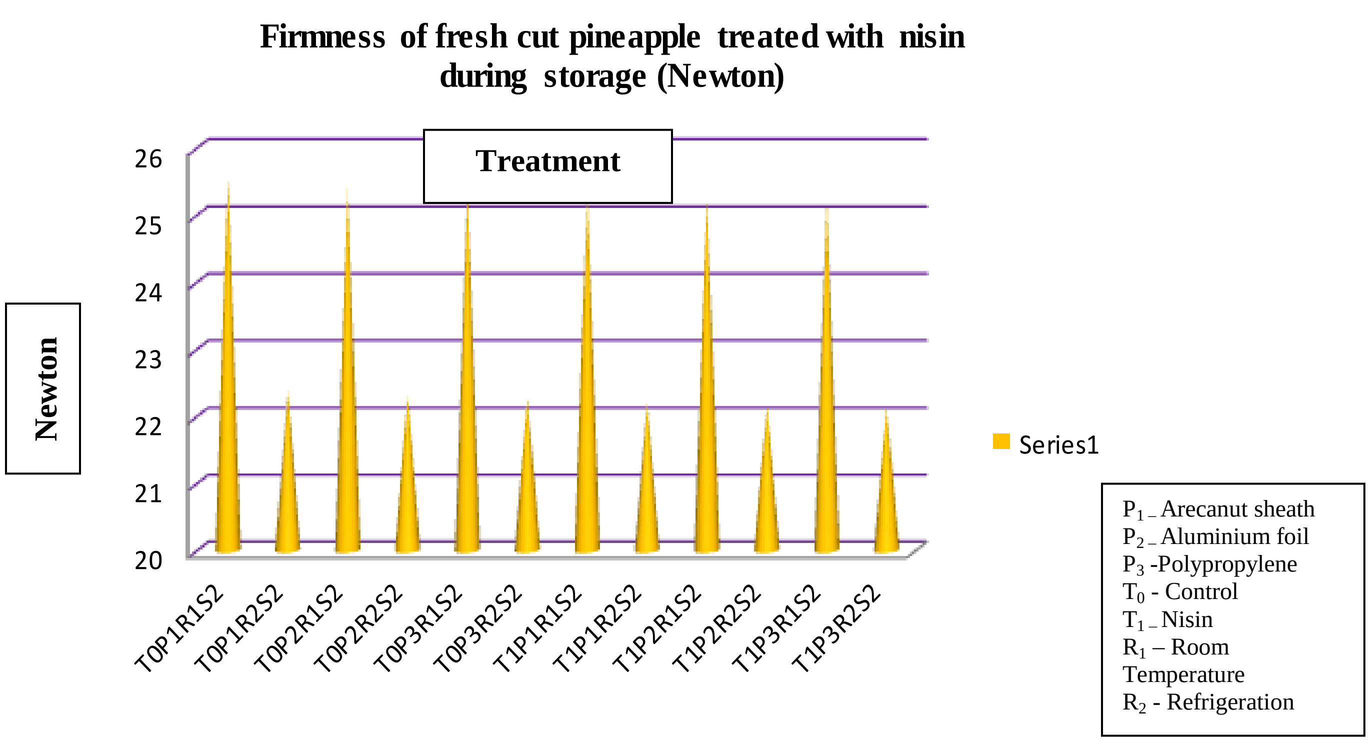


Fig.3 Firmness of fresh cut pineapple treated with nisin during storage (Newton)



Results obtained in this study showed that the nisin treated samples could significantly maintain the firmness to untreated samples during 12 days of storage. Similar results were observed by Ferrer and Harper, (2005). They confirmed that the firmness in control group of fresh cut pineapple at 6 and 12 days was significantly different from methyl jasmonate treatments. In both methyl jasmonate treatments, the fruits maintained a better fresh like texture. After 12 days, the control fruits last 66% of their initial firmness, while methyl jasmonate fruits had only an 11% and 16% loss in firmness for vapor and dipping treatments respectively.

Nutrient content of fresh cut pineapple treated with nisin during storage

The nutrient content of fresh cut pineapple treated with nisin during storage is given in Table 3. The initial pH of control samples was 3.95 and the final pH values ranged from 4.20 to 4.36 for control samples stored in different packaging materials. A minimum reduction of pH was observed in nisin treated fresh cut pineapple during storage period. King and Bolin (1989) observed a reduction of pH in case of fruits having a pH of 4.5 and above also functioned as an effective hurdle in improving the shelf stability of minimally processed products.

Initially the TSS content of control samples and treated fresh cut pineapple was 15.00^o Brix in room and refrigeration temperature in all the packaging material. Fresh cut pineapple treated with nisin had the TSS content of 15.20º Brix after 3 days storage in room temperature which was packed in all the packaging material. Similarly in refrigerated temperature there was minimum decrease in the nisin treated fresh cut pineapple with value of 15.60ºBrix in all the packaging material after 12 days of storage respectively. Generally, the sugar content increases after harvesting but it decreases with increase of the storage period due to its utilization during respiration as an energy source. Similar trend was observed in this study.

Vitamin C is nutritionally an important nutrient and highly susceptible to decompose during storage. It was reported that temperature, oxygen, concentration of secondary metabolites, presence of metals (iron, copper) influence the vitamin content of final product. According to Nunes *et al.*, (1998), the increase of ascorbic acid concentration on a fresh weight basis at higher temperature may be due to water loss during storage rather than to actual increase in ascorbic acid content. Similar changing trends of ascorbic acid in fresh cut pineapple treated with nisin packed in polypropylene was 25.82 mg per cent at room temperature with a shelf life of 3 days. 28.90 mg per cent in refrigeration temperature with the shelf life of 12 days.

The initial β-carotene content of fresh cut pineapple in all the treatment was 15.00 µg/100 grams. The retention of β-carotene in fresh cut pineapple at refrigeration temperature was higher when compared to the fresh cut pineapple stored at room temperature. The fresh cut pineapple treated with nisin packed in polypropylene container had a longer shelf life at room and refrigeration temperature. The total carotenoids content of pineapple cubes was decrease during storage at 5ºC after 3 days of storage. In total carotenoids of fresh cut pineapples resulting in a 25% a reduction relative to the whole fruit (Gil *et al.*, 2006).

The initial total antioxidant activity was 19.00µg/g in control and all the treatments. In the case of fresh cut pineapple treated nisin the total antioxidant activity ranged from 12.30 to 16.00µg/g stored in differed packaging material of at room and refrigerated temperature after 3 days and 12 days of storage respectively. Lopes *et al.*, (2017) confirmed that pulse light could substantially impact quality criteria of fresh cut mangoes. They indicated that 4 pulses of 0.7 J cm^{-2} were the most effective for increasing the antioxidant activity after 7 days of storage at 6^oC. Beirne and Kenny, (2009) have been expressed about possible losses of antioxidants in fresh-cut produce as a result of processing and storage.

Microbial characteristics of fresh cut pineapple treated with nisin during storage

The number of colony forming units (cfu) of bacteria observed for fresh cut pineapple treated with biopreservatives and chemical preservatives stored under room and refrigerated conditions in different packaging materials with reference to storage periods given in Table 4

The bacterial population was decreased during the storage period for samples treated with biopreservatives compared to control samples. The bacterial count was increased and the values were $7.00x10^{-6}$ in nisin treated after 12 days of refrigerated temperature packed in polypropylene. Sharma and Gautam (2007) investigated that the potent bacteriocins of *Lactobacillus brevis* and *Bacillus mycoides* were evaluated to control pathogens/spoilage causing bacteria in different food samples viz. apple juice, cheese and milk. The application of bacteriocin as food preservative has shown very encouraging results.

Microbial load- Fungi $x10^{-3}$ cfu/g

The fungal population was least in fresh cut pineapple treated with nisin than control sample, at room and refrigeration temperature. Comparing the effect of packaging material polypropylene was found to be better than arecanut sheath and aluminium foil container. The final fungal population in fresh cut pineapple treated stored at refrigeration temperature was $6.00x10^{-3}$ packed in polypropylene container after 12 days of storage. According to the institute of Food Science and Technology (IFST) 10^6CFU/g is considered the limit of acceptance of fruit based products during the study of shelf life (Bierhals, 2011). Fresh cut fruit have a large area of cut surface with high moisture conditions and a rich source of nutrients, which provides a good environment for growth of microorganisms (Oms- Oliu *et al.*, 2010). Results obtained in this study showed the total plate count of bacterial and fungi increased during storage.

Sensory analysis

The quality attributes like appearance, flavour, taste and over all acceptability values exhibited maximum score for fresh cut pineapple treated nisin whereas decreasing trend was observed in control samples at the end of storage. The effects of packaging material slightly affect the organoleptic characteristics of fresh cut pineapple.

In conclusion, nisin may provide a novel, safe alternative end effective hurdle which combined with control samples such as pH and temperature, can maximize protection from food borne pathogens on fresh cut products.

Nisin treated fresh cut pineapple reduced weight loss, nutrient loss, bacterial and fungal while maintained the firmness, sensory characteristics of fresh cut pineapple during low temperature storage. Packaging materials used in this study are beneficial to food industry and consumers since they can extend the lag-period and reduce the growth rate of microorganism to prolong shelf life of fresh cut pineapple to maintain food safety.

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