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Original article

Synergistic effect of pretreatment, packaging and cobalt-60 gamma irradiation on nutrition and shelf life of pine nuts

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Summary. Pakistan is among the major producers of pine nuts but its export value is low which is attributed towards prevailing poor post-harvest conditions such as improper handling and storage and unhygienic conditions from field to the market. In this context, the present study was designed to evaluate the combinatorial effect of pretreatment, packaging and gamma irradiation on pine nut shelf life through evaluation of its microbial quality, sensory attributes and proximate analysis. Shelled pine nuts were pretreated (dried) under hygienic condition by two drying methods, solar drying (42°C) and cabinet drying (55°C). Two packaging strategies were undertaken in which samples were packed in inner aluminium and outer polythene packing whereas the other with only polythene packing. All the samples were irradiated with 7kGy radiation. Total bacterial load, Gram negative Enterobactereacae lactose and non-lactose fermenters and fungal count was observed in the range of log1 to none in all samples from initial till last day which showed the positive influence of drying treatment on samples. Insect infestation was not detected in any sample which showed that all combination of treatments were successful in eliminating the infestation. Oxidative stress when evaluated showed that oven dried control sample with aluminium and polythene packing showed high iodine value (78.8 $mg.g^{-1}$) and low Thiobarbituric acid value (TBA) (0.006 $mg.ml^{-1}$) whereas organoleptic properties showed good texture, colour and taste upto 20th day of storage. Conclusively, aluminium packing with polyethylene was effective with oven dried samples whereas if radiation is to be used then low dose with only polyethylene packing would be sufficient for long term preservation and to prevent post-harvest losses.

Key words: insect infestation, microbial analysis, organoleptic properties, oxidative stress, *Pinus gerardiana* (Pine nuts), sensory evaluation.

Introduction

The economy of any agriculture country depends largely upon its crop yield. The more the better, however certain circumstances affects the yield badly apart from the quality and quantity of it produced. Several stages underlay the process of moving the crop from field to the fork. Inadequate storage and transport conditions leads to massive post-harvest losses faced by the farmers and eventually severely disturbs the country's economy. Pakistan is an agricultural country and its economy is run by the export of its fresh produce and is among the major producers of pine nuts however its export value is very low and it is due to not meeting the international food export standards. The poor quality of pine nuts is attributed to poor prevailing post-harvest conditions. On one side these factors are the cause of decline in quantity of the product that reaches the market while on the other hand affecting the quarantine attributes of the remaining ones.

Improper storage conditions develop rancidity in nuts and contamination from microbes along with their toxins (1). Number of studies has reported pine nuts contamination with *Salmonella* and *Escherichia coli* during processing (2, 3). Fungal mold can grow upon food when they are kept under parameter that allows sufficient moisture for their multiplication and produces various metabolites (4, 5). Food gets contaminated by fungal spores through wind, insects and rain (4). Fungal contamination and enzymatic degradation of structural components of the nuts causes reduction of grain quality, nutritional value, germination ability and increase in free fatty acid content (6, 7). The common toxic species belongs to the genera *Aspergillus*, *Penicillium* and *Fusarium* including molds such as *Scopulariopsis* and *Sporendonema* (8, 9). All these factors affect the quality of pine nuts thereby reducing its export value (10).In many developing countries, aflatoxins contamination of food by two fungi known as *Aspergillus flavus* and *Aspergillus parasiticus* are the main cause of both humans and animals illness (11). Now, the worldwide range for AFB_1 and aflatoxin total are 1-20ng.g^{-1} and 0-35ng.g^{-1}, respectively (12).

Dried fruits and nuts are also damaged by insect infestation another reason behind pre and post-harvest losses (3, 13). Pine nuts are known for their high quality of fats and unsaturated fatty acids. Storage stability is affected by fat content, degree of unsaturation and polysaturation, moisture content and temperature (14, 15). Lipid oxidation give rise to undesirable flavors, aromas and changes the nutritional quality of fats and oils which results in the production of unsafe compounds (1). At now, low moisture storage has proven beneficial to control respiration as a post-harvest treatment (16). Low moisture storage inhibits microbial growth and retard oxidative rancidity (17, 18).

Number of studies have shown the positive influence of dry heat treatment on shelf life of pine nuts where solar tunnel drier proved more fruitful mode for drying nuts as compared to the others (19). Open sun drying results in the reduction of crop yield in high amount every year but remains in use by many farmers as a method of drying which is due to the high cost for commercial dryers (20). The higher color, texture, taste and overall acceptability of nuts dried in tunnel drier revealed its superiority over other drying modes (19). Another drying method frequently used for drying of nuts is the cabinet drying at temperature 55°C. Packaging materials also plays an important role in enhancing the shelf life of pine nuts. The difference between packaging materials can be attributed to their thermal conductance properties which affect the internal decomposition reactions in the products during storage (21).

Food irradiation is used in the betterment of food hygiene, reduction in contamination and increase in shelf-life. Many studies have reported the effect of irradiation in preventing food quality from oxidation, insect and microbial contamination during storage and handling (22, 23). Recently, some countries have limited the irradiation dose above 10kGy for decontamination purposes (24). The aim of the present study was to observe the combine effect of dry heat treatment, packaging type and irradiation in enhancing the shelf life of pine nuts thereby reducing the post-harvest losses.

Materials and methods

Sample Preparation and Pretreatment

Pine nuts were shelled under hygienic conditions and were dried by two drying methods i.e. solar tunnel and cabinet drying (55°C for 160 hr). After drying, the samples were weighed and packed into two types of packaging, aluminium (inner packing) with additional packing of polythene (outside) and the other group with only polythene packing (14, 19).

Irradiation

Pine nut samples were irradiated at dose of 7kGy to find its effect regarding reduction in microbial flora and their toxins from the samples (25).

Microbial Analysis

Enumeration media used was nutrient agar for obtaining total bacterial count for each group of pine nut samples. For lactose fermenters MaCconkey agar was used and for Gram negative enterobacteriaceae non-lactose fermenters *Salmonella Shigella* agar was used. Whereas for obtaining total fungal count Potato dextrose agar was used. Spread plate method was used to obtain colony forming unit per gram (26).

Microflora Characteristics

Cell morphology of the bacterial isolates were studied by Gram staining (26). Whereas for fungus cell morphology was studied by methylene blue staining (27).

Organoleptic properties

Taste, aroma, colour and texture of pine nuts kept under different conditions were analyzed by 9-point hedonic scale (28).

Proximate Analysis

Different tests were performed such as moisture content (%) and lipid profiling to find the quality of samples in each experiment.

Lipid profile

Lipid profile was analysed by measuring the samples peroxide value, acidity value, iodine value and thiobarbituric acid value.

Peroxide Value

Initial rancidity, peroxide value was studied (29).

$$\text{Peroxide value (mg.g}^{-1}) = \frac{(S - B) \times N \text{ sodium thiosulphate} \times 1000}{\text{Weight of sample}}$$

S = titration of sample, B= titration of blank

Acid Value

Free fatty acids, acid value was estimated (30).

$$\text{Acid value (ml.g}^{-1}) = (56.1 \times V \times C)/ m$$

V = volume of Potassium hydroxide (KOH), C = concentration of KOH, m = mass in grams of the test portion, 56.1 = equivalent weight of KOH

Thiobarbituric Acid

For the estimation of conversion of free fatty acids into secondary oxidative products such as aldehydes and ketones, Thiobarbituric acid (TBA) value was estimated (31).

$$\mathbf{\mu M}\ \text{TBARS.g}^{-1}\ \text{fat} = (A - b) / (a \times m \times 1000)$$

A= absorbance of the sample (oil), a= slope of the standard curve, b= intercept of the standard curve, m = amount of the sample (g)

Iodine Value

For the estimation of unsaturation present in sample, iodine value was estimated (29).

$$\text{Iodine value (mg.g}^{-1}) = \frac{\text{mL of blank} - \text{mL of sample} \times \text{N (sodium thiosulphate)} \times 12.69}{\text{Weight of sample in grams}}$$

Insect Infestation

The pine nut samples were checked from time to time for evaluation of different stages of insects with the help of magnifying glass.

Statistical Analysis

All the experiments were performed in triplicates. One way ANOVA, Duncan multiple range test was applied on the values to find the significance difference at $p \leq 0.05$.

Results and Discussion

Total bacterial count of control samples (solar dried and oven dried) for both packaged groups i.e. with or without aluminiumwaslog1 for up to 20th days of storage (Fig. 1a, b). The growth was acceptable for total bacterial count according to the international food export standards (32). Heat pretreatment given to the samples has reduced the moisture content considerably that prevented bacterial growth on pine nut samples. Low moisture content increases the shelf life of samples whereas excessive moisture content supports microbial activity (33). Both methods of packaging that is aluminium with polythene packaging and polythene alone were successful in eliminating the microbial growth. Gamma irradiated (7kGy) and control samples showed no sign of microbial contamination of any type for the entire storage period which is assumed to be due to shelling process done under hygienic conditions.

Extremely less fungal growth (log 1) was observed in control samples of both oven and solar

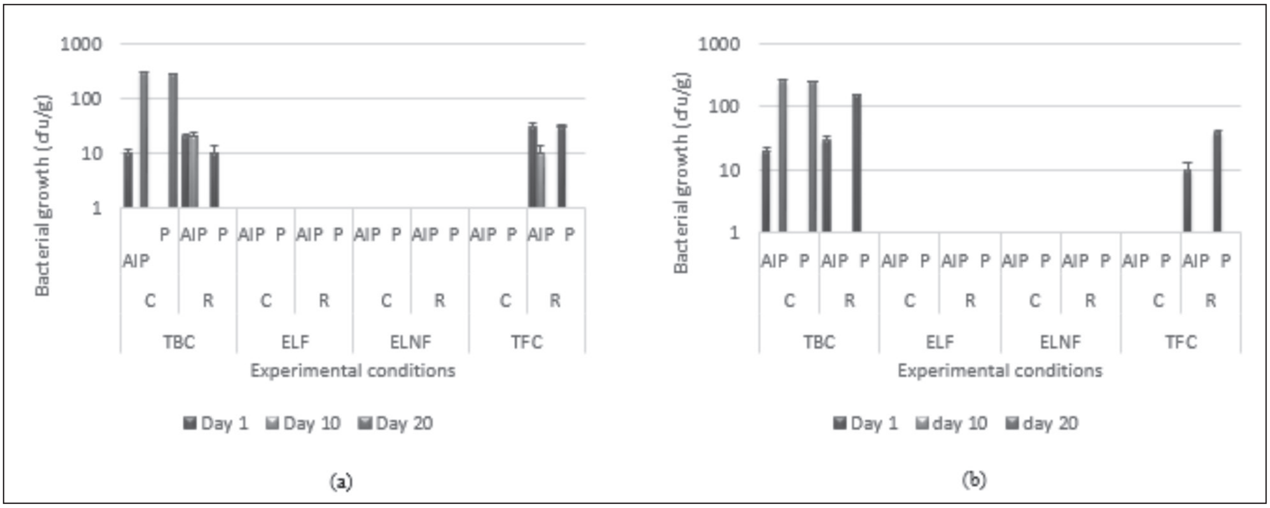

Fig. 1. Microbial evaluation of Pine nut samples at different experimental conditions (a) oven dried (b) solar dried.

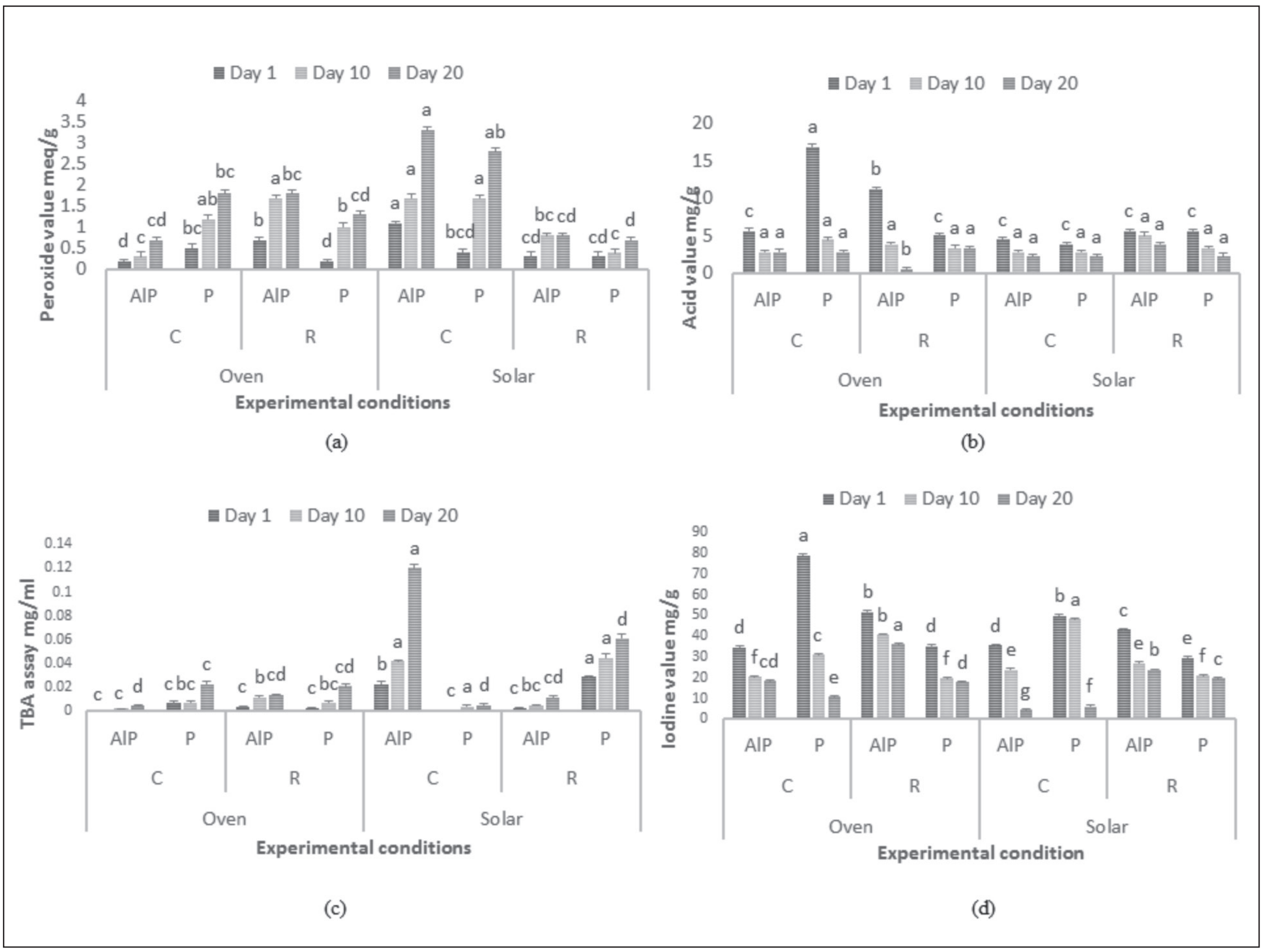

Fig. 2. Lipid profile of pine nut samples at different experimental conditions observed during 20th day of storage period (a) Peroxide value (b) Acid value (c) TBA value (d) Iodine value. Letters on bars show significant difference ($P \leq 0.05$) at different conditions.

dried experiments with both packaging types (Fig. 1a, b). The growth was under the accepted limits for fungal contamination according to the international food export standards (32). Dominant species were *Aspergillus niger* and *Penicillium* sp. Previous studies reported many fungal species such as *Aspergillus, Penicillium*, *Cladosporium*, *Tricoderma*, *Fusarium* from the dry nut samples (34, 35). Current findings indicated that the present strategies were successful in preventing food contamination to a greater extent during storage. Gamma treated (7kGy) samples showed no fungal growth during the entire study period. So, together pretreatment, packaging and irradiation were capable of reducing the fungal growth in pine nut samples during storage. The insect infestation was not detected in all samples. Many insects such as *Cydiacolorana*, *Eucosmabobana*, *Leptoglossus occidentalis* have been reported on pine nuts in other studies (36). So, all combination of treatments were fruitful in preventing insect infestation.

It is the lipid oxidation that changes the taste and aroma of pine nuts (37). The factors affecting the lipid oxidation are light, temperature, moisture and exposure to oxygen (38, 39). Different tests such as iodine value, peroxide value, thiobarbituric acid assay (TBRAS) and acid value were performed to check the rancidity in pine nut samples during storage. The peroxide value was less in oven dried samples as compared to solar samples. This increase in value might be due to the effect of solar heating and high oxygen concentration that caused unsaturated fatty acid formation. However radiated solar dried samples had less peroxide value as compared to non-radiated samples. Previously it has been reported that food irradiation increases the antioxidant property of a food generating radical scavengers that actually prevent radical generation (40). Same phenomenon occurred as radiated pine nut samples had less peroxide value and therefore at much better condition than the non-radiated solar dried samples. Peroxide value is the initial indicator of rancidity in nuts during storage (41). So, irradiation had positive impact on pine nuts during storage. Effect of packaging material when studied revealed that for oven dried samples aluminium with polyethylene packing was more suitable as it stopped the light from peroxidation of lipids whereas for radiated samples only polythene packing was effective presenting the positive long term effect of radiation on pine nuts storage (Fig. 2a). TBA value for oven dried non radiated samples with aluminum and polyethylene packing was less as compared to other experimental groups (Fig. 2c). This combination prevented the conversion of unsaturated fatty acids into aldehydes and ketones and rapid lipid oxidation (19). Degree of unsaturation was higher in oven dried samples as compared to solar dried samples. Whereas all radiated samples had high iodine value indicating less oxidation than the non-radiated samples (Fig. 2d). Iodine value of samples packed in aluminium and polythene packing was higher as compared to samples only packed in polyethylene packing. Thus if pine nuts were oven dried and packed in aluminium with polythene packing their shelf life can be increased by preventing lipid oxidation. Whereas if samples are gamma irradiated (oven dried) then only polythene packing can be used without effecting the shelf life of pine nuts. Decrease in iodine value indicates increase lipid oxidation due to degradation of unsaturated fatty acids after formation of hydroperoxides (42, 43).

The solar dried samples showed reduce acid value which showed less breakdown of unsaturated fatty acids (Fig. 2b). The higher acidity value means higher amount of free fatty acids which decreases the sample quality rapidly as seen in oven dried samples (44). According to AOCS, the acceptable acidity value for all oils should be 0.6mg.ml^{-1}whereas all the samples had above the limit. Decline in acidity value with time indicated conversion of free fatty acids into secondary oxidative products.

The oven dried samples of both packaging materials (aluminium plus polythene packing and polythene packing alone) showed smooth and shiny texture upto 11th days of storage but gives moderate change in colour and taste due to lipid oxidation (Fig. 3a, c). The radiated oven and solar dried sample of both packaging material showed extremely unpleasant taste during the entire period of study (Fig. 3b, d, f, h). Conclusively, it can be said that solar drying method did not proved an effective strategy for long term preservation of pine nut samples. Aluminium packing with polyethylene was effective with oven

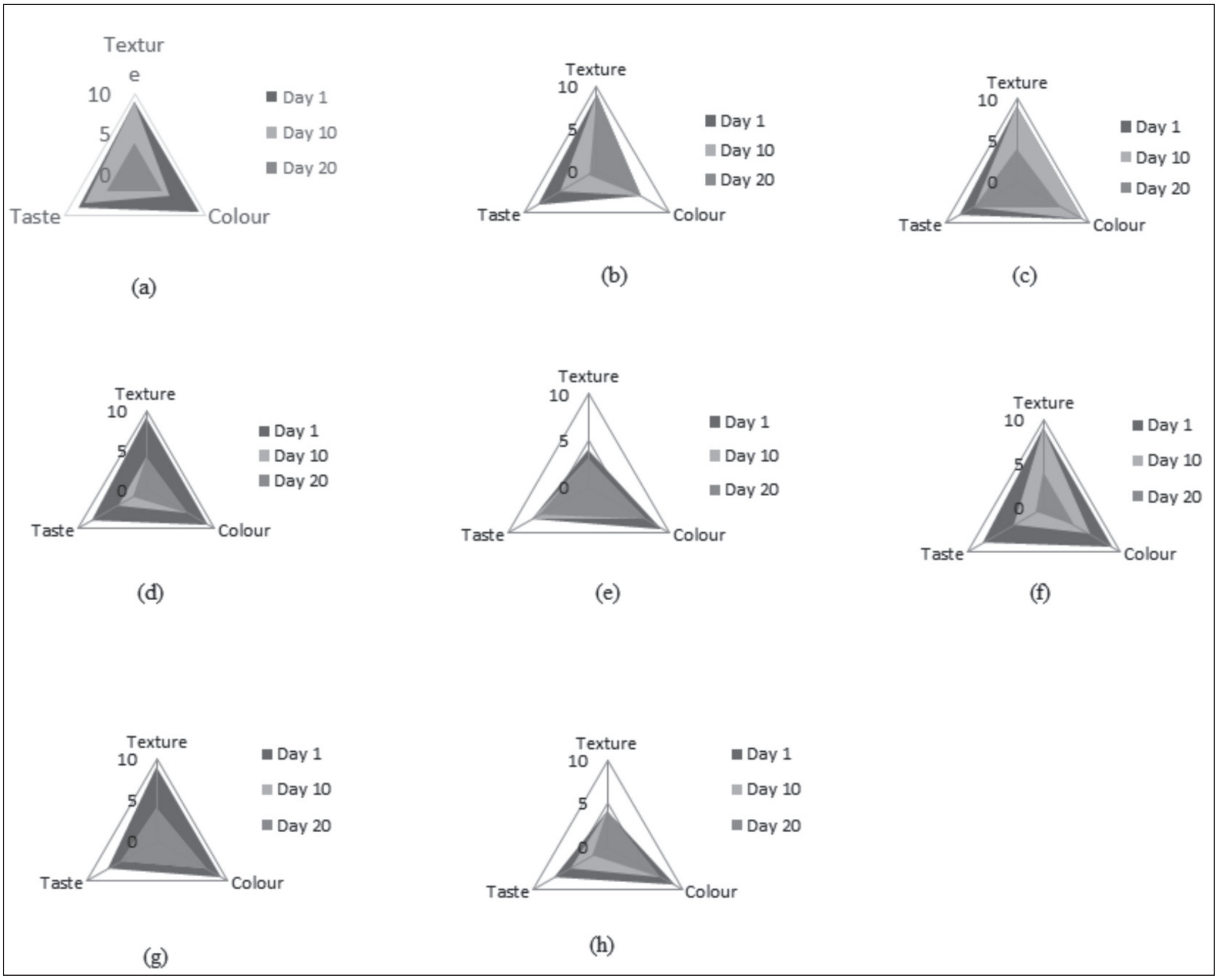

Fig. 3. Sensory evaluation of pine nuts at different experimental conditions for up to 20th day of storage period (a) oven dried pine nuts stored in polythene bag with aluminium packing (b) oven dried radiated pine nuts stored in polythene bag plus aluminium packing (c) oven dried pine nuts stored in polythene bag (d) oven dried radiated pine nuts stored in polythene bag (e) solar dried pine nuts stored in polythene bag with aluminium packing (f) solar dried radiated pine nuts stored in polythene bag plus aluminium packing (g) solar dried pine nuts stored in polythene bag (h) solar dried radiated pine nuts stored in polythene bag.

dried samples whereas if radiation is to be used then low dose with only polyethylene packing would be sufficient for long term preservation.

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