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Assessment of Total Colony Count in Mungbean (*Vigna radiata*) Sprouts Preserved Using Crude Extract of Biopreservatives During Storage

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Abstract

The present investigation was conducted with the aim to determine effective application of natural antimicrobial compounds mungbean sprouts and to assess the microbial quality of treated sprouts in terms of total plate count (TPC). Mungbeans were treated right from the time of germination (pre-germination mode) or its sprouts were treated for 15 min (post-germination mode) with 0.1% sodium benzoate as chemical preservative (Control II) and with various bio-preservatives viz., 7.7% clove, 9.5% cinnamon, 7.9% garlic 7.9% ginger crude extracts. The un-treated mungbean served as control (Control-I). The sprouts were packed in plastic disposable cups and stored in dark at room temperature (20±3°C) conditions and low temperature (7±1°C) conditions. A significant decreased rate of growth in TPC of sprouts during storage was observed under various treatments, however, the effect was lesser in post-germination mode. In pre-germination mode, at both temperature regimes, the minimum total plate count was observed in clove, while all other treatments were showing equal effectiveness. In post-germination mode all the treatments were equally effective in reducing total plate count. In conclusion, 7.7% clove crude extract showed highest effectiveness in pre-germination mode while in post-germination all crude extracts of bio-preservatives showed equal effectiveness at both storage temperatures.

Keywords: Bio-preservatives; Clove; Crude extract; Total plate count.

1. Introduction

Mungbean (*Vigna radiata* L. Wilczek) belongs to family *Leguninoseae* which is grown for its protein rich edible seeds which is comparatively rich in lysine, an amino acid deficient in cereal grains [1]. Sprouting is an inexpensive and simple, an easy method of soaking seeds until they germinate and begin to sprout. Sprouting or germination has been reported to improve vitamin and protein quality of some cereals and legumes coupled with reduction in anti-nutritional factors [2]. As per Patil and Mangaraj [3], mung bean sprouts are comprised of 30 Kcal energy, 5.94 g carbohydrates, 1.8 g dietary fiber, 3.04 g protein per 100 g and rich in vitamin C (13.6 mg), folate (61 µg), riboflavin (0.124 mg), thiamin (0.084 mg) potassium (149 mg), magnesium (21 mg), calcium (13 mg) and iron (0.91 mg). Mung bean sprouts have more health benefits compared to other legumes such as its detoxifying, anti-inflammatory, anti-tumourogenic, cholesterol-lowering and diuretic properties [4].

Mung sprouts are highly perishable and remain in saleable conditions at 0 °C and 95% to 100% relative humidity (RH) for 6-7 days, while exposure to 20 °C for 30 minutes each day can reduce the storage-life by half [5]. As the sprouting process provides suitable physical conditions such as water activity, temperature, pH and nutrients released by sprouting seeds, it promotes the bacterial growth [6] and it is noted that high microbial counts in sprouts are the main reasons of their short shelf life [2]. The Popularity of sprouts has decreased due to the food safety issues due to the possibility of become contaminated with a variety of bacteria, including pathogens [7]. The common microbial load ranges between 10^3 and 10^6 cfu/g, which comprised mainly with *Pseudomonas*, coliforms and lactic acid bacteria [8]. Many studies have done to evaluate the different seed decontamination methods over past years, which includes chemical treatments using single chemical compound or combination of several chemicals [9, 10], natural antimicrobials [11] and physical methods like ozone [12, 13], electrolyzed water [14], UV light [15], irradiation [8, 16] and high pressure processing [17]. The most common preservatives used in preservation are the weak organic acids, like acetic, lactic, benzoic and sorbic acid [18]. However, physical and chemical techniques commonly have adverse changes in organoleptic characteristics and nutrients of the produce. Therefore, the food industry investigates more about the replacement of traditional food preservation techniques by new preservation techniques due to the increased consumer trend for green consumerism and demand for tasty, nutritious, natural and high quality, preservative-free, safe but mildly processed foods with extended shelf-life. An increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives [18, 19]. This demand could be met by using natural antimicrobial systems, such as essential oils of botanicals to preserve foods [20] and most of these essential oils are generally regarded as safe (GRAS) and are acceptable to consumers [21]. Bio-preservation is an emerging concept in the world to prolong the shelf life of perishable fresh produce. Bio-preservatives are a wide range of natural products that can be used to minimize or eliminate the load of pathogenic microorganisms while

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increasing food quality. Among these type of antimicrobials, essential oils of botanical extracts have long been applied as flavouring agents in foods, and due to their content in antimicrobial compounds, they have potential as natural agents for food preservation [21]. Application of bacteriocins and bacteriocin-producing strains as bio-preservatives on the preservation of foods of animal origin has been revealed to greater extent but to a much lesser extent on vegetable foods such as sprouted seeds [22]. *Cinnamomum zeylanicum* (L.), commonly known as cinnamon, is rich in cinnamaldehyde as well as β-caryophyllene, linalool and other terpenes. Earlier studies have shown that cinnamon has good antioxidant and antimicrobial potential [23]. Garlic (*Allium sativum*) is also well known as flavouring agent and contains natural antimicrobial compounds namely, allicin and diallylthiosulphinic acid [24]. In Clove (*Syzygium aromaticum*), eugenol is the responsible chemical compound that accounts 72-90% in essential oil extract along with eugenol acetate (11%), β-caryophyllene (5%) and α-humulene (0.56%), as reported by Thomas [25]. Ginger (*Zingiber officinale*) contains Zingiberene, the active component along with lesser amounts of other sesquiterpenoids [25]. Arora and Kaur [26] revealed that garlic and clove possess antimicrobial activity. The bactericidal effect of garlic extract was apparent within an hour of incubation and 93% killing of *Staphylococcus epidermidis* and *Salmonella typhi* was achieved within 3 hours and yeasts were totally killed in an hour by garlic extract but in 5 hours with clove. Further, Hill, *et al.* [27] revealed that the application of complexes containing essential oil (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) were having antimicrobial properties against the pathogens *Salmonella enteric serovar;Typhimurium LT2* and *Listeria innocua*. All other antimicrobials except free eugenol effectively inhibited bacterial growth. Yeh, *et al.* [28] analyzed bioactive components and antioxidant effect of two various ginger extracts. The antioxidant effect of ethanolic extracts of ginger showed higher effectiveness than aqueous extracts in Trolox equivalent antioxidant capacity and Ferric reducing ability of plasma. Contrarily, aqueous extracts of ginger showed higher effectiveness in free radical scavenging activities and chelating abilities.

Due to the increasing demand for the mungbean sprouts and the use of bio-preservatives, the extraction of crude extract at cost effective way and application of naturally present antimicrobials could be an easy and effective tool for sprout industry. Hence, the present research was undertaken to determine effective application of natural antimicrobial compounds for mungbean sprouts and to assess the microbial quality of treated sprouts in terms of total plate count (TPC).

2. Materials and Methods

The study was carried out at the Centre of Food Science and Technology, CCS Haryana Agricultural University, Hisar, Haryana (India).

2.1. Materials

2.1.1. Raw Materials

(a). For Sprouts: Mung beans var. MH 421 was procured from pulse Section, Department. of Plant Breeding, CCS HAU, Hisar.

(b). For antimicrobial compounds: Available varieties of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were procured from the local market of Hisar.

2.2. Methods

2.2.1. Extraction of Natural Antimicrobial Compounds from Clove, Ginger, Garlic and Cinnamon

a. Preparation of Clove and Cinnamon Extracts (Stock Solution)

Clove and cinnamon were purchased from local market and they were cleaned by washing in potable water. Clove and cinnamon were kept in hot air oven at 60°C for 2 days for drying and then ground into a fine powder. 100 g of each of fine powder were weighed using electronic weighing balance and mixed with 200 mL distilled water. Solution was centrifuged at 6000 rpm for 10 minutes. Supernatant was filtered out and stored under refrigerated conditions (4°C) for further use.

b. Preparation of Ginger and Garlic Extracts (Stock Solution)

Cold water extract of both garlic and ginger was prepared using method described by Akintobi, *et al.* [29] with slight modifications. Garlic and ginger rhizomes were peeled, washed and coarsely minced. Coarsely minced garlic and ginger were kept in hot air oven at 60 °C for 2 days for drying and then ground into a fine powder. Hundred gram of fine powder of each were weighed, mixed with 500 mL distilled water. The Solution was kept for 72 h at room temperature (25±1°C). The solution was filtered out through a cleaned muslin cloth and stored under refrigerated conditions (4°C) for further use.

c. Treatments

The extract concentration giving maximum inhibition of microbial growth (on the basis of total plate count) without adversely affecting germination was regarded as the effective concentration and used for further study. The details of the various extracts and their effective most concentration are given below:

Table-1. Most effective concentrations of crude extracts for application

Sources Of Natural Antimicrobial Extracts	Crude Extract Concentration (% W/V)
Clove	7.7
Cinnamon	9.5
Garlic	7.9
Ginger	7.9

The values in parenthesis are concentrations on g plant material/ mL basis.

As control treatments, sterilized water (Control I) and 0.1% sodium benzoate (Control II) were used. A preliminary study was also conducted with the effective concentration of extract and its mode of application to find out the shelf life of sprouts on the basis of overall organoleptic acceptability.

2.3. Mode of Application of Natural Antimicrobial Extracts

i. Through Germination Medium (Pre-Germination Mode)

Raw seeds of mung bean were sorted to remove foreign material and damaged/aborted seeds and then washed under running tap water. Washed seeds were divided into 6 lots of equal amount and soaked (1:5 w/v) for overnight (10 h) in respective antimicrobial solutions in a glass container. The Soaked seeds were then put in a sprout maker (Novelle Plast, Delhi) for 24 h at 25±1°C and kept in dark.

ii. By Dipping 24 H Sprouts for 15 Min. In The Respective Solutions (Post-Germination Mode)

Raw seeds were sorted to remove foreign material and damaged/aborted seeds and then washed under running tap water. Washed seeds were soaked for overnight (10 h) in potable water in a glass container. Soaked (10 h) mung bean seeds were put in a sprout maker (Novelle Plast, Delhi) for 24 h at 25±1°C and kept in dark. Sprouted mung beans were divided into 6 lots of equal amounts and dipped for 15 minutes separately in respective antimicrobial solutions.

iii. Packaging and Storage

The sprouts from each treatment were packaged in plastic disposable cups (~200 mL volume) and wrapped with 5% perforated cling films. Water soaked filter paper was placed along the inner sides of plastic cups to maintain high humidity inside. There were ~100 g sprouts/pack and the packs were stored in dark at room temperature (20±3°C) conditions and low temperature (7±1°C) conditions maintained in B.O.D. incubator. There were 3 replicates per treatment.

iv. Detection and Enumeration of Total Plate Count (TPC)

Sprouts treated with different antimicrobial and control samples were enumerated for the total plate count using serial dilution technique followed by pour plate method (all the manipulations were done in laminar flow chamber under aseptic conditions).

v. Extraction of Sample

Ten g of sprouts were dipped in 100 mL of distilled water for 1 h and then water samples were diluted by serial dilution technique. 1 mL of aliquot was diluted 10^6 times and was poured on sterilized petri plates. After pouring of plate count agar medium to petri plates containing sample, the plates were allowed to set at room temperature. The plates were inverted and incubated at 30±1 °C for 36 h for growth. After incubation period, the colonies were counted using the colony counter. Results were expressed as $\log_{10}$ cfu/g.

No. of organisms (Log_{10} CFU/ml) = Number of colonies per plate x dilution factor

3. Results and Discussion

3.1. Effect of Different Treatments on Total Plate Count of Sprouted Mung Bean during Storage

The data on total plate count of mung bean sprouts under various treatments during storage is presented in Table 2. The various preservatives when applied through germination medium (pre-germination mode), resulted significant decreased in total plate count of sprouts. At room temperature, the total plate count under various treatments significantly increased from mean value of 7.50 $\log_{10}$ CFU/g at 0-day to 7.85 $\log_{10}$ CFU/g at 72 h of storage. Amongst the various treatments, minimum total plate count was observed in clove, while it was equally reduced by other treatments. At low temperature storage conditions, the total plate count under various treatments significantly increased from mean value of 7.50 $\log_{10}$ CFU/g at 0-day to 7.65 $\log_{10}$ CFU/g by 120 h of storage. All the preservative treatments, throughout the storage treatment, resulted in reduced total plate count compared to untreated mung beans. Maximum reduction in total plate count was observed in clove treatment, while it was equally reduced by other treatments. Interactions between treatment and storage were significant at room temperature and non-significant at low temperature conditions.

Table-2. Effect of bio-preservatives on total plate count (log_{10} CFU/g) of mung bean sprouts during storage

Treatment	Storage Duration (H)					
	Room Temperature			Cold Temperature		
	0	72	Mean	0	120	Mean
Pre-germination						
Control I	7.70	8.00	7.85	7.70	7.95	7.83
Control II	7.52	7.86	7.69	7.52	7.72	7.62
Clove	7.40	7.67	7.54	7.40	7.31	7.36
Cinnamon	7.44	7.84	7.64	7.44	7.56	7.50
Garlic	7.39	7.89	7.64	7.39	7.70	7.55
Ginger	7.53	7.84	7.69	7.53	7.68	7.61
Mean	7.50	7.85		7.50	7.65	
CD at 5%	T =0.12 S =0.07 TxS= 0.16			T = 0.12 S =0.07 TxS=NS		
Post-germination						
Control I	7.90	8.01	7.96	7.9	8.02	7.96
Control II	7.67	7.84	7.76	7.67	7.76	7.72
Clove	7.64	7.83	7.74	7.64	7.79	7.72
Cinnamon	7.64	7.84	7.74	7.64	7.84	7.74
Garlic	7.68	7.91	7.70	7.68	7.90	7.69
Ginger	7.64	7.77	7.71	7.64	7.88	7.76
Mean	7.66	7.87		7.66	7.87	
CD at 5%	T =0.1 S =0.1 TxS= NS			T = 0.1 S =0.1 TxS=0.2		

Control I (Distilled water); Control II (Sodium benzoate); T=Treatment; S=Storage; NS=Non-significant

The various preservatives when applied by soaking treatments (post-germination mode) were lesser effective in reducing total plate count than pre-germination mode. At room temperature, the total plate count under various treatments significantly increased from mean value of 7.66 log_{10} CFU/g at 0-day to 7.87 log_{10} CFU/g at 72 h of storage. All the treatments were equally effective in reducing the plate count. At low temperature storage conditions, the total plate count under various treatments significantly increased from mean value of 7.66 log_{10} CFU/g at 0-day to 7.87 log_{10} CFU/g at 120 h of storage. All the treatments were equally effective in reducing the plate count. Interactions between treatment and storage were non-significant at room temperature and significant at low temperature conditions.

The growth of microbes, as evident by total plate count (TPC) was found to increase with increasing storage period, both under room and low temperature conditions (Table 2). In both the modes of application, the significant decreased rate of growth in total plate count of sprouts during storage was observed under various treatments; however, the effect was lesser in post-germination of application. The microbial load of sprouts by 72 h at room temperature and 120 h at low temperature storage conditions was within the safe limit of consumption. In pre-germination mode, at both temperature regimes, the minimum total plate count was observed in clove, while all other treatments were showing equal effectiveness. In post-germination mode all the treatments were equally effective in reducing total plate count. The reduced rate of growth in total plate count might be due to the antimicrobial activity of bio-preservatives. The effect was more pronounced at low temperature conditions because the low temperature acted as an additional hurdle. Essential oils, their active components and the phenolic compounds are well known natural preservatives [21]. The results of the present investigation for various treatments are in conformity of the findings of Van, *et al.* [30], where it was observed that cabbage (*Brassica oleracea*) seeds treated with essential oils of thyme, oregano, cinnamon and clove, plant extract of biosept, and organic acids like ascorbic and propionic acid, showed reduction in bacterial counts significantly compared to untreated seeds and water control, except biosept and oregano oil at lower concentrations. Similar inhibitory activity of biopreservatives against microorganisms was shown by other workers [31-33]. Bari, *et al.* [22] reported pediocin and nisin applications in combination with organic acids caused a significant reduction of native microflora and inoculated populations of *L. monocytogenes* on fresh cut cabbage, broccoli and mungbean sprouts.

4. Conclusion

It can be concluded from the results of the present investigation that 7.7% clove, 9.5% cinnamon, 7.9% garlic and 7.9% ginger crude extracts can effectively be used as bio-preservative for mung beans sprouts, as these treatments could reduce the total plate counts as effectively as the chemical preservative sodium benzoate. The effect was more pronounced at low temperature conditions and pre-germination mode of application. Application of clove extract as bio-preservative was most effective in reducing microbial load, however, it adversely affected colour and appearance score. Since cinnamon extract slightly improved overall acceptability of sprouts, it can be recommended that both clove and cinnamon extracts can used as bio-preservatives. Mungbean sprouts remained acceptable in all the treatments till 48 h at room temperature and for 96 h at low temperature storage conditions.

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