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Assessment of total microbial count of tray dried selected leafy greens

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Abstract
This review presents the total microbial count of selected leafy greens dried in tray dryer. Commonly consumed fresh green leafy vegetables namely fenugreek, spinach, dill, coriander and curry leaves were selected for the study and given two pre-treatments that were one of plain water blanching and second of chemical blanching with KMS (0.02 percent) + MgO (1.5 gram) + citric acid (1 percent) + $NaHCO_3$ (1.5 percent) + NaCl (1.5 percent) and dehydrated in tray dryer at the temperature of 55 °C for the time of 7-8 hours. Dehydrated vegetables were packed in polypropylene bags as packaging material and kept for 3 months storage period. Dehydration of vegetables mostly accomplished by convective air drying technology led to reduction in water activity of hygroscopic agricultural products which in turn greatly decreased the microbial growth. No microbial colonies were observed at initial stage before storage in all pre-treatments of those selected dried leafy greens while at final stage after storage period, little growth of colonies were observed due to slight increase in moisture content of dried vegetables during storage due to their hygroscopic nature. Total microbial count of those dehydrated leafy greens ranged from 1.10×10^5 cfu per gram to 1.58×10^5 cfu per gram with lowest and highest counts recorded for fenugreek and dill leaves respectively. The tray drying of vegetables at low temperature (50-60 °C) also demonstrated that an adequate pretreatments given can produce safer dried products.

Keywords: Blanching, tray dryer, dehydration, pretreatment, colony forming unit

1. Introduction
In all communities across the world, fruits and vegetables are an important component of a healthy and balanced diet. They are known to possess non-pathogenic epiphytic microbiota, but pathogens from human, animal, or environmental sources can infect them during growth, harvest, shipping, processing, and handling (Beuchat, 2002; Olsen *et al*., 2000) [2, 13]. Vegetables sold in the open market frequently have a greater microbial burden, which can be harmful to human health. As a result, vegetables may serve as a reservoir for a variety of microorganisms, which will colonise these vegetables and infect vulnerable hosts. Due to their high water content (about 80%), fresh fruits and vegetables are particularly fragile (Kaleta *et al*., 2013; Karam *et al*., 2016) [9, 10]. One of the oldest and most frequent methods of food preservation is to reduce the water activity of the food. Most bacteria, yeasts, and moulds are unable to develop below 0.87, 0.88, and 0.80, respectively, due to reduced water activity (Beuchat *et al*., 2013) [3]. Moreover, removal of water lowers the weight of product, facilitating economical storage, and transport (Brown *et al*., 2008) [4] and thereby offering a wider distribution potential (Jayaraman and Das Gupta 1992, 2014) [7, 8]. Drying not only prevents the growth of spoilage bacteria, but also prevents browning and other moisture-driven degradation processes, maintaining the original material's structure, properties, and nutritional content (Karam *et al*., 2016) [10]. Despite the fact that microbial activity is avoided or slowed in dry foods, if a considerable number of harmful pathogens persist after the drying process, the consumers may be at risk. Microbial growth is hindered once dehydrated, yet vegetative cells and spores can survive for months (Beuchat *et al*., 2013) [3]. Alarm water content of dehydrated vegetables is 14-20%, stating the values equivalent to water activity of 0.7 at which point microbial stability can be expected.
The standard plate count, also known as aerobic plate count or total plate count, is a microbiological test that may be used to determine a product's overall microbiological quality and, as a result, can be used to predict possible deterioration in perishable items. Its purpose is to assess the total number of aerobic organisms present in a given foodstuff. Dried foods with no coliform microbes should have a total count of less than 100,000 per gram. The present review deals with total microbial load of selected dried leafy greens.

2. Materials and Methods

2.1 Study area

The study was carried out in the Department of Food Science and Technology at Post Graduate Institute of Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2020-21.

2.2 Sample collection and preparation

The green leafy vegetables viz, fenugreek, spinach, dill, coriander and curry leaves were procured from the local market of Rahuri. Leafy vegetables were washed thoroughly, sorted and blanched. Pre-treatments of plain water blanching and chemical blanching of KMS (0.02%) + MgO (1.5g) + Citric acid (1%) + $NaHCO_3$ (1.5%) + NaCl (1.5%) were given at the temperature of 45 °C for 30 seconds and treatments named as T_0 and T_1 for fenugreek, T_2 and T_3 for spinach, T_4 and T_5 for dill, T_6 and T_7 for coriander and T_8 and T_9 for curry leaves respectively. Leafy vegetables were dehydrated at the temperature of 55 °C for time of 7-8 hours in tray dryer. After dehydration, vegetable samples were packed in polypropylene bags and stored for 3 months duration for further study. The samples were microbiologically tested for total plate count at initial stage before storage and after 90 days storage period.

2.3 Determination of total microbial count

2.3.1 Media preparation

Nutrient agar media was used to determine total microbial count in dehydrated vegetable samples which was prepared by adding peptone (2.5g), beef extract (1.5g), agar (10g), yeast (1g) in 500 ml distilled water making final volume of half litre. Then the media was autoclaved at 121 °C for 15 minutes and used for further plating of samples.

2.3.2 Standard plate count method

One gram of dehydrated vegetable sample is weighed and transferred to 10 ml sterilised distilled water containing test tube. Shake the contents of the test tube thoroughly and this will be 10^{-1} dilution. Then transfer 1 ml of 10^{-1} dilution with the help of separate sterile pipette to next 9 ml containing sterile water containing test tube and shake the contents thoroughly and this will give 10^{-2} dilution. Similarly prepare dilutions of 10^{-3}, 10^{-4} and 10^{-5} and shake all the dilutions thoroughly. Pipet out 1 ml of each dilution into separate, triplicate, appropriately marked Petri plates. Add 10-20 ml of cooled nutrient agar media into each Petri plates and then mix sample dilutions thoroughly and uniformly and allow the agar to solidify, cover them with lid properly and sterilise their ends with flame of spirit lamp. This process is carried out aseptically under laminar air flow cabinet. Invert the petri plates. Allow them to incubate for 48± 2 hours at room temperature (37 °C). After incubation, count triplicate plates of each dilution having visible colonies. When only one dilution is in appropriate range, compute average count per gram for dilution report as aerobic plate count per gram.

3. Results and Discussion

Dehydrated vegetables might have high bacterial counts, and that for certain microorganisms there were limiting moisture contents below which marked growth on vegetables would not take place (Prescott, 1920) [14]. The results pertaining total microbial count of tray dried selected leafy greens viz., fenugreek, spinach, dill, coriander and curry leaves are discussed under this topic.

3.1 Microbial count of dehydrated green leafy vegetables

The data given in Table 1, Table 2, Table 3, Table 4 and Table 5 gives total plate count of those selected dehydrated green leafy vegetables respectively. It indicates that the microbial count in fenugreek was noticed 1.10×10^5 cfu/gm in control while in treatment 1.09×10^5 cfu/gm after 90 days storage. The microbial count in spinach was noticed 1.26×10^5 cfu/gm in control while in treatment 1.24×10^5 cfu/gm after 90 days storage. The microbial count in dill was noticed 1.58×10^5 cfu/gm in control while in treatment 1.55×10^5 cfu/gm after 90 days storage. The microbial count in coriander was noticed 1.46×10^5 cfu/gm in control while in treatment 1.46×10^5 cfu/gm after 90 days storage. The microbial count in curry leaves was noticed 1.22×10^5 cfu/gm in control while in treatment 1.21×10^5 cfu/gm after 90 days of storage at room temperature. No visible microbial growth was observed in vegetable samples at initial days of storage period. It was also observed among the pretreatments given, chemical blanching helped to prevent microbial growth slightly better as compared to plain water blanching due to antimicrobial properties of salt used in chemical blanching complemented the antibacterial state achieved through removal of water during tray drying. It can be also illustrated that polypropylene packaging helps to control microbial growth. The values of standard plate count obtained for all dehydrated leafy greens are within acceptable limits given by FSSAI, (2006) [5]; Abadias *et al.*, (2008) [1] and KEBS, (1992) [11]. Dehydrated fruits and vegetables products should not contain more than 40,000 per gram as per PFA rules, (1956) [12].

Table 1: Microbial count of dehydrated fenugreek

Leafy Vegetable	Treatment	(Standard plate count×10^5 cfu/g)	
		Storage period (days)	
		0	90
Fenugreek	T_0	-	1.10
	T_1	-	1.09

All values are mean of three replications. – Not detected
T_0 - Plain water blanching (Control), T_1- Chemical blanching

Table 2: Microbial count of dehydrated spinach

Leafy Vegetable	Treatment	(Standard plate count×10^5 cfu/g)	
		Storage period (days)	
		0	90
Spinach	T_2	-	1.26
	T_3	-	1.24

All values are mean of three replications – Not detected
T_2- Plain water blanching (Control), T_3 - Chemical blanching

Table 3: Microbial count of dehydrated dill

Leafy Vegetable	Treatment	(Standard plate count×10^5 cfu/g)	
		Storage period (days)	
		0	90
Dill	T_4	-	1.58
	T_5	-	1.55

All values are mean of three replications – Not detected
T_4- Plain water blanching (Control), T_5 - Chemical blanching

Table 4: Microbial count of dehydrated coriander

Leafy Vegetable	Treatment	(Standard plate count×10^5 cfu/g)	
		Storage period (days)	
		0	90
Coriander	T_6	-	1.46
	T_7	-	1.46

All values are mean of three replications – Not detected
T_6- Plain water blanching (Control), T_7 - Chemical blanching

Table 5: Microbial count of dehydrated curry leaves

Leafy Vegetable	Treatment	(Standard plate count×10^5 cfu/g)	
		Storage period (days)	
		0	90
Curry leaves	T_8	-	1.22
	T_9	-	1.21

All values are mean of three replications – Not detected
T_8- Plain water (Control), T_9 - Chemical blanching

4. Conclusion
Microbiological control is very important in food industry to prevent food poisoning and other health hazards. In present study, total microbial count of five tray dried leafy greens was assessed and found to be within acceptable limits of microbiological safety standards given by various standard organizations like FSSAI, 2006 and PFA Rules, 1956. Therefore, it reveals that tray drying of vegetables provides improved shelf life and microbiological safety. It is also concluded that the packaging material polypropylene found better in providing microbial protection to product.

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