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Microbial Load of *Spinach* (*Spinacia album*) and *Bathua* Leaves (*Chinopodium album*) Before and After Dehydration

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ABSTRACT

Green leafy vegetables are important for improving the acceptability of the meal, because of the innumerable shades of colour, flavour and texture they contribute. In India leafy vegetables or greens from many plants have been used in the diet from ancient times. Either cultivated or wild types are consumed almost every day by a section of people. It is also recognized that these leafy vegetables are easily available and are relatively cheap. Dehydration is one process which increases the storage period of green leafy vegetables and makes them available throughout the year even in off season. Most of the food products are subject to bacterial spoilage. Temperature and packaging are the two most important factors that influence the type of microbial growth and spoilage of food during storage. The Study conducted to determine the microbial load of spinach (Spinacia album) and bathua leaves (Chinopodium album) before and after dehydration. Only the fresh vegetables were collected. They were then washed with the help of clean water so as to remove the dirt and other disease causing adhering pesticides. Dehydrated leaves were stored at room temperature in double sealed polythene bags for different time intervals. The microbial load of fresh spinach and bathua leaves were found to be high which may be due to their high level in soil. Dehydrated leaves stored upto two months was found to be safe for consumption as the counts were within permitted limits.

Keywords: microbial load, Dehydrated Green Leafy vegetables, Packaging material

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INTRODUCTION

Green leafy vegetables are important for improving the acceptability of the meal, because of the innumerable shades of colour, flavour and texture they contribute. In India leafy vegetables or greens from many plants have been used in the diet from ancient times. Either cultivated or wild types are consumed almost every day by a section of people. It is also recognized that these leafy vegetables are easily available and are relatively cheap. Although foods from animal origin are ideal for obtaining most of the nutrients, they are not generally possible for the rural population to consume, due to economic constraints. Recent developments in vegetable-production technology have improved the production of this food item. However, efforts to prevent the losses between harvesting and consumption are in progress. Vegetables are highly seasonal and are usually available in plenty at a particular season of the year [8]. The surplus is generally thrown away as waste or as cattle feed. Since they are cheap and within the reach of common man the leafy vegetables can be used as a valuable source of micronutrients. However; techniques like dehydration need to be adopted for prolonged storage and to make them available when not in season.

Dehydration is one process which increases the storage period of green leafy vegetables and makes them available throughout the year even in off season. Water removal from vegetables by drying is applied to increase its shelf life, and for easy transport and storage by volume reduction or weight reduction. Dehydration techniques result in concentration of nutrients (three or five fold) especially micro-nutrients. Dehydrated leafy vegetables in the concentrated form can be added in small quantities in various food preparations in order to increase their nutritive value. The products too can't be stored for a certain period and used as and when required; However it should be insured that the vegetable and the

products are wholesome and safe. Micronutrient deficiency can be minimized up to certain extent by supplementing the products enriched with dehydrated leaf concentrates.
Most of the food products are subject to bacterial spoilage. Temperature and packaging are the two most important factors that influence the type of microbial growth and spoilage of food during storage.

MATERIAL AND METHODS

The Present investigation entitled "Microbial load of spinach (Spinacia album) and bathua leaves (*Chinopodium album*) before and after dehydration" conducted in Sam Higginbottom University of Agriculture, Technology and Sciences, formerly Allahabad Agricultural Institute. Collection of fresh spinach and Bathua leaves and sample preparation for microbial analysis of fresh leaves: Green leaves of spinach and bathua required for the experiments were collected from the local market. Only the fresh vegetables were collected. They were then washed with the help of clean water so as to remove the dirt and other disease causing adhering pesticides. Fresh vegetables were chopped into small pieces with a sterile knife under aseptic condition thereafter, 25g of chopped vegetable- samples were added to 225 ml peptone water (0.1%) and blended in a rotary shaker at 220 rpm for 5 minutes.
Dehydration (drying) and storage of spinach and Bathua leaves and sample preparation for microbial analysis :
The procured vegetables namely spinach and *bathua* leaves were tray dried at controlled temperature that is 50-60°C for (3-4h).

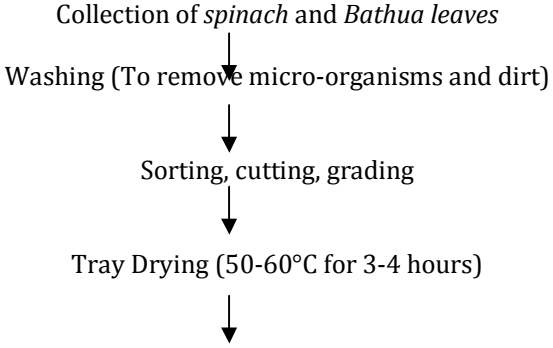

Fig 1: Flow diagram showing steps in dehydration of vegetables

The dehydrated leaves were packed in double sealed polythene bags and were stored at room temperature in a room, away from the sunlight. The duration of storage ranged from zero to 60 days the sample preparation for microbial analysis was done of five different time intervals, viz, zero day, 15 days, 30 days, 45 days and 60 days. For this, one gram of dehydrated vegetable sample was weighed in a sterile petridish and was blended in 9 ml of *Ringer solution* using shaker for 5 minutes.

Microbial analysis of (i) Fresh leaves (ii) dehydrated leaves and (iii) snack items :

The methods of microbial analysis were identical for fresh and dehydrated spinach and *Bathua* leaves as well as for snack items. These included the following:

(i) Standard plate count (SPC)
(ii) Yeast and mold count (YMC)
(iii) Presumptive coliform test (PCT)

Standard plate count: Using the pour plate method . For this, one ml of the prepared sample were serially to 10^{-7} and 1 ml of each dilution was transferred into petridish in triplicate. The Melted *nutrient Agar medium* (appendix II) was poured and the content were mixed the media was allowed to solidify. Then the plates were incubated in inverted position at 37°C±1°C for 24 to 48 hrs. After incubation the plates with 30-300 colonies were counted and the average value were be multiplied with the dilution factor and interpreted in *cfu/g* of samples [1, 2].

Yeast and mold count **:** one ml of each dilution of the sample up to 10^4 was transferred in sterilized petriplates in triplicates. Melted potato Dextrose Agar medium (Appendix 2) was poured into each petriplate, mixed well and allowed to solidify. Then the plates were incubated at 25°C ± 1°C for 3-4 days. The fungal colonies appearing after incubation period were counted and multiplied with dilution each factor and interpreted as *cfu/g of* samples [4].

Presumptive coliform test **:** To check the condition prevailing in the fresh leaves and in the dehydrated leaves and their products, PCT was performed using *Mac Conkey's* broth (Appendix 2) for this 1 ml of each dilution was inoculated in *Mac Conkey's broth* tube with inverted Durham's tube in triplicate. Then the

tubes were incubated at 37°C ±1°C for 24 to 48 hrs, for production of acid if any the indication used was change in colour of the medium from purple to yellow. The preventive evident of coliform in the product was of gas into Durham's tube [5-6].

RESULT AND DISCUSSION

Microbial load of fresh *spinach* and *bathua* leaves –

Standard plate count (SPC) of fresh *spinach* and *bathua* leaves:

The total SPC of *spinach* and *bathua* leaves ranged between 144 to 172 x 10^7 cfu/g and 158 to 192 x 10^7 cfu/g respectively whereas the mean counts were 158 x 10^7 cfu/g and 172.66 x 10^7 cfu/g respectively in the two vegetable samples. (Table 4.1). The quality of the two green leafy vegetables (*spinach* and *bathua* leaves) was comparable to the findings of Pingulkar *et al* 2001 who also reported that the SPC of *bathua* was found to be higher as compared to *spinach*. This could be due to contamination during storage and handling procedure.

Yeast and mold count of fresh *spinach* and *bathua* leaves:

The Yeast and Mold count ranged between 2 to 6x10^3 cfu/g and 3 to 8 x 10^3 cfu/g respectively. Average counts were 4.0 x 10^3 cfu/g and 5.5 x 10^3 cfu/g respectively. (Table 1).

Result of presumptive coliform test of fresh *spinach* and *bathua* leaves:

The coliform test indicated positive result in *spinach* and *bathua* leaves samples. Therefore it may be presumed that the organisms are usually found at high level in soil. (Table 1)

Table 1 Microbial load of fresh *spinach* and *bathua* leaves.

Types of vegetables	SPCx10^7 cfu/g		Yeast and mold x 10^3 cfu/g		Presumptive coliform test	
	Range	Average	Range	Average	Positive	Negative
Spinach	144-172	158	2-6	4.0	Positive	-
Bathua	158-192	172.66	3-8	5.5	Positive	-

Microbial load of dehydrated *spinach* and *bathua* leaves:

The Standard plate count of dehydrated *spinach* and *bathua* leaves:

When the total viable count of *spinach* and *bathua* leaves were observed in different time intervals (Zero to 60 days), stored at room temperature (22^0C $\pm$ 0.5^0C) and packed in double polythene bags the counts were found to range between 21 to 60 x 10^3 cfu/g and 23 to 78 x 10^3 cfu/g respectively. In the present investigation, it was found, that the bacterial population increased during storage of *spinach* and *bathua* leaves. During the storage period the growth was slow and then, it increased and the maximum growth was observed on 60^{th} day. (Figure 2).

The finding on the quality of dehydrated *spinach* and *bathua* leaves was comparable to the total viable count in dehydrated vegetables was 34 to 84 cfu/g which is within permissible limit and can be considered safe for consumption [10].

Yeast and mold count of dehydrated *spinach* and *bathua* leaves in different time intervals at room temperature :This ranged between 0 to 9 x 10^3 cfu/g and 0 to 14 x 10^3 cfu/g respectively (Table 2). Storage had no significant effect upon dehydrated *spinach* and *bathua* leaves. This confirms the finding of Vaidehi and Sunanda [9] who reported that dehydrated leaves could be safely stored in polythene bags for six months without any fungal infection and also there occurred no changes in colour, flavour or odour.

Result of presumptive coliform test of dehydrated *spinach* and *bathua* leaves: The coliform test was negative in dehydrated *spinach* and *bathua* leaves during the entire period of storage. (Table 2)

Table 2- Standard plate count (SPC X 10^3 *cfu*/g) of dehydrated *spinach* and *bathua* leaves in double sealed polythene at room temperature:

Types of vegetables	Zero days	15 days	30 days	45 days	60days
Spinach	21	32	40	56	60
Bathua	23	38	46	63	78

Table 3- Yeast and mold count (YMC X 10^3 *cfu*/g) of dehydrated *spinach* and *bathua* leaves in double sealed polythene at room temperature:

Types of vegetables	**Zero days**	15 days	30 days	45 days	60days
Spinach	0	2	4	7	9
Bathua	0	3	6	10	14

Table 4- Presumptive coliform test of dehydrated *spinach* and *bathua* leaves in double sealed polythene at room temperature:

Types of vegetables	Zero days	15 days	30 days	45 days	60days
Spinach	-ve	-ve	-ve	-ve	-ve
Bathua	-ve	-ve	-ve	-ve	-ve

CONCLUSION

The microbial load of fresh *spinach* and *bathua* leaves is high which may be due to their high level in soil as well as due to contamination during storage. Dehydrated leaves stored upto two months in double sealed polythene bags at room temperature can be considered safe for consumption.

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