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Studies on Different Methods to Reduce the Bacterial Load in *Spirulina platensis* powders

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The present study was aimed to enumerate the total bacterial count in *Spirulina platensis* powder and to reduce their bacterial level by different treatments such as storaging in different temperature, N_2 and CO_2 gas packaging, *Lactobacillus* and glucose, tyndalization. While comparing the sample with the treated samples, the bacterial load was found to be reduced in treated samples. Treatments such as CO_2 gas packaging and storage in 5 and 12^{0}C shows increased level.

Key Words: Spirulina, *Lactobacillus*, Tyndalization.

Spirulina is blue green algae containing many nutrients and is beneficial for human health. *Spirulina* has been industrially grown and produced as powder on large scale in artificial outdoor pond (Belay, 2002). The bacterial contamination, especially aerobes are found higher in outdoor cultivation than the indoor cultivation (Mituya et al., 1953). The good quality powder containing bacterial load under permissible limit is outlined by FDA. Hence it is important to reduce the bacteria in final product powders. *Spirulina platensis* sample was taken in powder form from Sanat Product Ltd, Kodairoad. The total plate count of samples was enumerated and it was treated by different methods such as storage in different temperature, N_2 and CO_2 gas packaging, *Lactobacillus* and glucose, tyndalization.

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MATERIALS AND METHODS

Storage in different range of temperature

Spirulina platensis powder from can I was taken as control (untreated sample).The sample was treated under different conditions. In one condition, the sample was stored in refrigerator for a period of five days at 5^0 C and it was represented as T_1a (treated sample), in second condition the sample was stored in cold room for a period of five days at 12^0 C and it was T_1b (treated sample) and in other condition the sample was in freezer for a period of five days at -6^0C and it was T_1c (treated sample). The total plate counts of both untreated and treated sample were determined by standard plate count method. (USFDA-BAM., 2001)

Storage of packed powder with N_2 and CO_2 gas packaging

Spirulina platensis powder from can II was taken as control (untreated sample).The sample was packed with N_2 gas and kept for 24 hrs at room temperature {T_2a (treated sample)} and in other condition the sample was packed with CO_2 gas and kept for 24 hrs at room temperature {T_2b

(treated sample)}. The total plate counts of both untreated and treated sample were determined by standard plate count method. (USFDA-BAM., 2001)

Isolation & Identification of *Lactobacillus* sp

MRS agar media was used to isolate the *Lactobacillus* sp from the curd sample. The isolated *Lactobacillus* sp was identified using gram staining, motility test and biochemical tests such as catalase, oxidase, starch hydrolysis and glucose fermentation based on Bergy's manual of bacteriology., 1994. The isolated *Lactobacillus* was transferred to the 10 ml of MRS broth and incubated at 37^0 C for 18 ± 2 hours.

Treatment with *Lactobacillus* sp

Spirulina platensis powder from can III was taken as control (untreated sample).The sample was inoculated with *Lactobacillus* and glucose by the following procedure. 2500ml of distilled water was taken in 30 litre can.1kg of *Spirulina platensis* powder (can III) and 100gm of glucose was added. A loop full of 18 hrs grown *Lactobacillus* culture was inoculated to the above mixture (cells may be nearly 2×10^8 CFU/ml) and incubated for 24 to 48 hours at 35-40°C. After 48hours the above mixture should be allowed to spray dried. The dried powder thus obtained was represented as T_3 (treated sample). The total plate counts of both untreated and treated sample were determined by standard plate count method (USFDA-BAM, 2001).

Tyndalization

Spirulina platensis powder from can IV was taken as control (untreated sample).100gm of sample was taken in conical flask and heated up to 80°C in water bath for 30 minutes. The heating process was repeated for three days (Torres & Anjel., 1971). The powder thus obtained after three days was represented as T_4 (Treated sample). The total plate counts of both untreated and treated sample were determined by standard plate count method. (USFDA-BAM., 2001)

RESULTS

Treatments

T_1a- Storage at 5° C for 5 days, T_1b- Storage at 12° C for 5 days. T_1c- Storage at -6 °C for 5 days. T_2a- N_2 packed powder; T_2b- CO_2 packed powder at room temperature, T_3- Treatment with *Lactobacillus* and glucose, T_4 - Tyndalization process.

The bacterial load was found to be reduced in treatment T_1c when compared to untreated sample of Can I. But the bacterial load was found to be increased in treatment T_1b than the treatment T_1a when compared to untreated sample of Can I. Selvakumar *et al.*, 2002 reported that there was no growth of bacteria in prawn when stored in frozen temperature of -20^0C. In this case frozen temperature of about - 6^0C showed good inhibitory effect on bacteria, as this temperature does not favour for the growth of bacteria.

The bacterial load was found to be reduced in treatment T_2a when compared to untreated sample of Can II. But the bacterial load was found to be increased in treatment T_2b when compared to untreated sample. Coyne, 1933 in his

Table 1. Studies of different methods to reduce bacterial load in *Spirulina* powder

S. No	Can – (Powder Sample)	Total Plate count of sample (CFU/gm.)	Treatment given to the sample	Total Plate count of the treated sample (CFU/gm)	Bacterial Load % Reduction/ increase
1	I	1.45×10^4	T_1a	2.05×10^4	41 % Increased
			T_1b	2.15×10^4	48 % Increased
			T_1c	1.02×10^4	29 % Reduced
2	II	6.21×10^4	T_2a	4.12×10^4	33 % Reduced
			T_2b	6.85×10^4	10 % Increased
3	III	1.35×10^5	T_3	8.09×10^3	94 % Reduced
4	IV	1.65×10^5	T_4	9.86×10^3	94 % Reduced

study reported that CO_2 inhibit aerobic bacteria, but in case of CO_2 packaging of powder, the bacterial count was found to be increased.

The bacterial load was found to be reduced in treatment T_3 when compared to untreated sample of Can III. Kllanhammer, 1993 in his study with *Lactobacillus* exhibited inhibition of many gram positive bacteria. Sakakibara et al., 2008 inoculated *Lactobacillus* and sugar in *Spirulina to* minimize its odour and taste and also reduce the foreign bacteria. In this present study, it was found that inoculation of *Lactobacillus* have good inhibitory effect on bacteria in *Spirulina* powder. This is because *Lactobacillus* converts the glucose by producing lactic acid which decreases pH from neutral to acidic condition which leads to the inhibition of bacteria.

The bacterial load was found to be reduced in treatment T_4 when compared to untreated sample of Can IV. Torres & Anjel., 1971 reported that the tyndalization process reduces the spores and vegetative bacteria.

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