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Microbial Decontamination Process for *Salmonella abony* on *Moringa oleifera* Leaves Powder by Heat Sterilization and Their Impact on the Phytochemical Parameters

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ABSTRACT

Sterilization is a process that eliminate or kills the Microorganism and transmitting agents on the surface, dry heat sterilization is lethal as it can eliminate microbes by coagulating the proteins causing oxidative free radical damage leading to death. *Moringa oleifera* is greenish white hairy leaves, having a variety of proteins, vitamins and minerals. The microbial contamination of the raw material may cause deterioration and reduces the stability of the raw materials and causes toxicity to the users, so sterilization of raw material. The purpose of the study is to decontaminate the *Salmonella abony* from leaves of *Moringa Oleifera leaves* by dry heat sterilization method and to optimize the time and temperature for complete destruction of *Salmonella abony* in the given sample and comparison of the protein contents of decontaminated sample with control group. Different time and temperature were tested for decontamination on moringa oleifera salmonella spiked sample, the result showed that among the six treated samples, the sample exposed to 90°C gave complete elimination of salmonella abony and there was a slight variation in protein concentration between the treated samples. It has been concluded that 90°C for 120 minutes was ideal temperature for complete destruction of *salmonella abony.*

INTRODUCTION

Salmonella species are anaerobic gram-negative rod shaped nonspore forming bacteria belonging to family Enterobacteriaceae. Salmonella is a motile with peritrichous flagella, non-flagellated and non-motile strains.[1] They are mesophilic, with optimum growth temperature between 35° and 37°C. They have growth range of temperature of 5° to 46°C. *Salmonella typhi* is a pathogen that invades and inhabits in organs such as spleen, liver, bloodstream and lymphatic tissue. Many process are used for the sterilization such as use of sterilant, antiseptic, chemical disinfectants, sterilization by UV, sanitizing agents and sporicidal agents, the best method of sterilization is heat treatment for thermostable substances which can be sterilized by hot air oven, which is safe, cost effective, less time consuming.

METHODS AND MATERIALS:

Moringa oleifera powder sample is prepared by drying the fresh fully matured leaves at 50℃ and grinding and passing through the 40 mesh. *Salmonella abony* is a non-pathogenic surrogate organism used in this study. The diluted *Salmonella* culture is mixed with *Moringa oleifera* powder and left for 12 hours. The sample is heat treated for 60°C, 75°C and 120°C for 90 minutes and 90°C for 60, 90 120 minutes, the samples are subjected for microbial analysis, the qualitative analysis is carried out by TLC and protein analysis was carried out.

Microbial Analysis: 10 grams of both control and heat-treated samples is transferred to 90 ml of Soya bean casein digest medium and incubated at 35°C for 1 day. Second day 0.1 ml is transferred from the first pre enrichment medium to the Rappaport Vassiliadis Salmonella Enrichment Broth and incubated at 35°C for 1 day. Third day the sample present in Rappaport Vassiliadis Salmonella Enrichment Broth is streaked onto XDL plates and incubated for 24 hours at 35°C. Biochemical tests are carried out for confirmation for the presence of *salmonella*.

Protein Analysis: Protein analysis is carried out by *Kjeldahl* method.

$$\textbf{\% Nitrogen} = \frac{(\text{ml standard acid- ml of blank})* \text{normality of NaOH}*1.4007*100}{\text{exact normality}*\text{weight of the sample in milligrams}}$$

% **of protein**=Nitrogen content * 6.25 **NaOH=** Sodium Hydroxide

TLC analysis: Sample preparation for TLC is done by taking 1 gram of *Moringa oleifera* in 10 ml of methanol, subjected to sonification, heating, and centrifugation, 1 ml of the centrifuged supernatant liquid is collected and subjected to TLC by using mobile phase as toluene: methanol: acetone: formic acid in the ratio 50:30:15:25 v/v/v/v. Anisaldehyde is used as spraying reagents and densitometric scanning is performed using CAMAG TLC 3 scanner.

RESULTS:

Figure 1, 2, 3: The densitometric scanning after the development of TLC plates showed nearly 18 bands the TLC of both control and treated sample showed similar bands determining there was no degradation of phytochemical constituents.

Figure 4: showed the presence of *Salmonella* in all the Petri plates containing samples of control, 60°C, 75°C, but absent in 90°C for 60, 90 120 minutes 120°C for 90 minutes heat treated samples.

Figure 5: was the confirmatory test for the presence of *salmonella.*

Table 1: shows the presence of *salmonella* abony in control 60°C, 75C° samples and absent in 90°C and 120°C sample at various time and temperature.

Table 2: shows the amount of nitrogen content and protein content in control and treated sample, there was a reduction of nearly 4% in comparison with control but there was a slight variation between the treated samples.

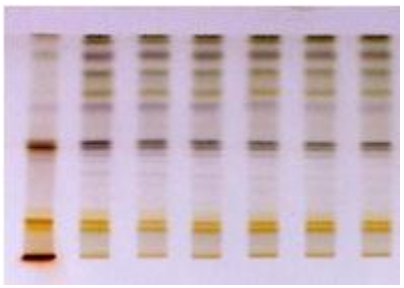

Figure No. 1: TLC chromatogram of *Moringa Oleifera* at Visible Region

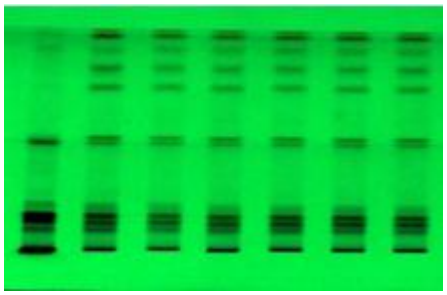

Figure No. 2: TLC chromatogram of *Moringa Oleifera* at at 254 nm

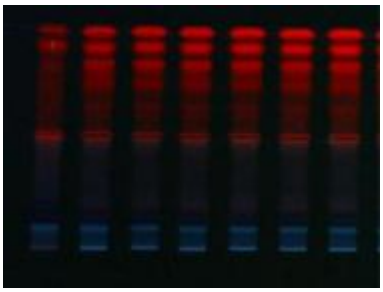

Figure No. 3: TLC chromatogram Of *Moringa Oleifera at* 366 nm

Figure No. 4: Microbial Analysis of *Moringa Oleifera*

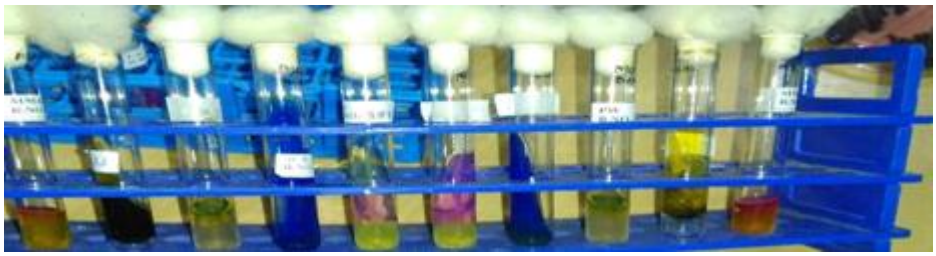

Figure No. 5: Biochemical Tests of *Moringa Oleifera*

Table No. 1: Microbial Analysis of *Moringa oleifera*

Biochemical tests	Negative	Positive	60	75	90		120	
			90 min	90 min	60 min	90 min	120 min	90 min
Mannitol mortality media	Red	Yellow	+	+	-	-	-	-
Triple sugar ion	Yellow	Black	+	+	-	-	-	-
Peptone water	White	Red	+	+	-	-	-	-
Citrates	Green	Blue	+	+	-	-	-	-
Urease	Yellow	Pink	+	+	-	-	-	-

Table No. 2: Protein analysis of *Moringa Oleifera.*

Samples	Nitrogen content	Protein content
Control	3.9 %	24.37 %
60 ℃, for 90 minutes	3.25%	20.34 %
75 ℃, for 90 minutes	3.22%	20.125 %
90 ℃, for 60 minutes	3.20 %	20 %
90 ℃, for 90 minutes	3.13 %	19.56 %
90 ℃ for 120 minutes	3.03 %	18.92 %
120℃ for 90 minutes	2.65 %	16.66%

DISCUSSION

Many products such as Drugs, Veterinary Feed, Food, Beverages, Spices, flavors, Animal Drugs, Dietary Supplements, cosmetic, Cereals, Packed and Tinned Food etc. have been recalled by FDA due to the presence of *Salmonella*. Process effective decontamination of

herbs and spices is of prime importance. Use of sterilant, sporicidal agents, and chemicals may cause hazards to the users thereby making heat sterilization the best method. In the study, *Moringa oleifera* spiked sample is used showed the presence of *Salmonella* at 60°C, 75°C for 90 minutes but at 90°C 60, 90 120 minutes and 120°C for 90 minutes showed the complete absence of salmonella. The protein content reduced to 4% but there was a slight difference in protein content between the treated samples.

CONCLUSION

The microbial analysis result showed that the absence of *Salmonella abony* at 90°C there was no change in the phytochemical parameter of control and the heat-treated sample. TLC is found to be similar in treated and control samples of leaves of *Moringa Oleifera*. Protein analysis showed very less difference between treated samples. Based on the experimental data, it is found that the ideal temperature required sterilizing and for complete destruction of *Salmonella abony* is at 90℃ for 120 min. There was no change in the organoleptic character between the control and treated sample. This process can be easily extended to adopt commercially to destroy the *Salmonella* species in herbs, medicinal plants, and powders.

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