

METHOD OF FLAVOUR PROTECTION IN HERBS AND SPICES AND PRODUCTS THEREOF

5 RELATED PATENT APPLICATION

This application claims the priority to and benefit of Indian Patent Application No. 202041016515 filed on April 16, 2020; the disclosure of which are incorporated herein by reference.

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FIELD OF THE INVENTION

The invention relates to method for preparing microbial contamination free spices and food products. More particularly, the invention relates to method for preparing cryogenic grinded and cold pasteurized (gamma irradiated) spices and spice related products which retains essential oils, aroma taste, color and chemical compounds of the products and is free of any microbial contamination. Invention also relates to such microbial contamination free food products and spices thereof.

20 BACKGROUND OF THE INVENTION

Prevention of food spoilage becomes particularly important to deliver safe and healthy products. There are many methods developed since ages to prevent food spoilage. The traditional methods of preservation, such as drying, smoking and salting have been supplemented with pasteurization (by heat), canning (commercial sterilization by heat), refrigeration, freezing and chemical preservatives. However, these traditional methods have shown certain disadvantages. Chemical treatment leaves cancer causing chemical residues and also pre-packed food cannot be treated. Freezing method has not been found suitable for certain food materials including spices and also has been found to be expensive. Drying and smoking cannot meet the hygienic requirements and will also change texture, color and flavour of the food. The use of steam has also been found to be disadvantageous as the packages have to be opened and food material is likely to absorb moisture.

Food irradiation is another technology that has been used to prevent food spoilage. Irradiation has been extensively used in the medical field for sterilizing instruments, dressings etc. Food

irradiation is the process of exposing food to a carefully controlled amount of energy in the form of high-speed particles or rays. The purpose of food irradiation is to improve the quality of the food being irradiated, either from a microbiological, physical, or organoleptic perspective. This technology can also be called as cold pasteurization since gamma irradiation
5 can kill many disease-causing bacteria (like *E. coli*, *Listeria* spp, *Salmonella* spp etc) without causing any heat in the food stuff. This is the most modern, scientific & safest technology as on date to make spices and allied food stuffs free from bacterial contamination, mold, mycotoxins and other pollutants, insect infestation etc. As a result of significant reduction in the total number of bacteria in a food will result in an increase in the shelf life of the food.

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The US Patent No. 6868136 discloses an irradiation apparatus particularly well suited to the irradiation of food at or near the point of consumption and includes an irradiation chamber, one or more ionizing radiation sources such as x-ray tubes, and a rotating food support.

15 The US Patent Publication No. 20030039726 discloses a method of treating a food product which includes packaging the food product in a modified atmosphere, removing oxygen from the modified atmosphere and irradiating the food product, such that the oxidation of the food product is impeded for a predetermined period of time after irradiating the food product.

20 The US Patent Publication No. 20030091708 discloses a method of irradiating food, comprising: grinding the food in an environment having an oxygen level lower than the oxygen level of air, irradiating the food in an environment having an oxygen level lower than the oxygen level of air, and packaging the food in a package in an environment having an oxygen level lower than the oxygen level of air.

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The Indian Patent Application No. 8491/DELNP/2014 discloses a food sterilization and preservation method comprising a water vapor plasma irradiation step of irradiating a food with water vapor plasma and a step of applying a calcined powder of a natural calcium-rich material to the food which has undergone the water vapor plasma irradiation step.

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The Indian Patent Application No. 5/CHE/2013 discloses a method of processing of edible food products to retain the natural aroma, flavor, prevention from biological degradation, microbial contamination and protection from re-infestation of the said food product. The method comprises filling the food product in a three-layered packaging system consisting of

low density polyethylene layer as the innermost layer followed by aluminum layer and then biaxially oriented polyester layer; flushing nitrogen in the filled food product to remove the oxygen; subjecting the food product to gamma irradiation and hermetically sealing the package. The food product in the above disclosed method is selected from the group comprising spices, condiments, infant food, baby and weaning food and any agricultural product.

The Indian Patent Application No. 441/DEL/2001 (granted as IN192345) discloses a cryogenic process for the preparation of dry and fine spice powder. The method employs spraying liquid nitrogen over the material for 3 to 15 minutes and maintaining temperature of the cryogen between -20°C to -60°C, the product is transferred to grinding mill and pulverized.

The Indian Patent Application No. 331/DEL/2002 (granted as IN222861) discloses an improved cryogenic method for producing of mixed spice powders and the said process helps in higher retention of etheric oils and also prevention of oxidation and rancidity. The method involves injecting liquid nitrogen at the grinding zone containing raw materials; maintaining the temperature of cryogen between -20°C to -60°C and pulverizing under the controlled temperature.

India is located in a belt of sub-tropical and semi-arid to arid zones, such climatic conditions are conducive for the rapid growth of microorganisms and insects. The safety and quality of the food consumed by the general public is very important in the perspective of maintaining and enhancing general health. A new consciousness is growing all over the world about disease-causing organisms, poisonous substances and impurities. Parallel to this, the degree of excellence which consumers expect from foods is also growing. Exporting countries are thus constrained to maintain quality standards set by stringent food laws and regulations to ensure that foods including spices, are safe, wholesome and produced under sanitary and hygienic conditions.

Thus, there is an ever increasing need to develop newer and improved methods for preventing food spoilage. The present invention employs newer method for preventing spoilage of food including spices.

OBJECTIVE OF THE INVENTION

The primary objective of the invention is to provide a method for preparing microbial contamination free spice powder which retains the aroma, essential oils, taste, color, chemical
5 compounds and antioxidant properties of the spice.

Another objective of the current invention is to provide a microbial free and hygienic food product including spice, rice, nutritional bar or instant mix to the consumer.

10 Another objective of the current invention is to provide cryogenically grinded spices which retains the aroma, essential oils, taste, color and chemical compound (Curcuminoids, Capsaicinoids, Piperine, etc.,) of the product.

Another objective of the current invention is to irradiate the food product including spice, rice,
15 nutritional bar or instant mix to kill the microorganisms like coliforms, bacteria, fungi, yeast & molds, and larvae in the manufactured products.

Another objective is to provide cryogenically grinded spices which have been irradiated to prevent oxidation.
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Another objective is to pack the spices with nitrogen purging to prevent spoilage of spices.

Another objective is prevention of oxidation in grounded spice powders by using Gamma irradiation at 6-14 kGy post Cryogenic grinding.
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Another objective of the present invention is to provide method for grinding spices which involves Cryogenic conditions and Gamma irradiation.

Another objective is recovery of specific chemical compounds and antioxidant activity with
30 Cryogenic conditions and Gamma irradiation.

SUMMARY OF THE INVENTION

Accordingly, there is provided an improved method for preparing grinded spices which involves grinding under cryogenic conditions followed by gamma irradiation.

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In one aspect of the invention there is provided a method for preparing a microbial free spice powder which retains the aroma, essential oils, taste and color, chemical compounds and antioxidant properties of the spice, the method comprising the steps of:

- a. Filling one or more dried raw spice into a feeding hopper,
- 10 b. Grinding the raw spice(s) under cryogenic conditions,
- c. Packaging the grinded powder obtained in step (b) first into primary packaging with nitrogen purging and then into secondary packaging,
- d. Subjecting the packed spice powder to gamma irradiation and
- e. Obtaining microbial free packed spice powder

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In some embodiment of the invention the combination of cryogenic grinding and gamma irradiation are used to produce microbial free spice powder.

In some embodiment, the method according to invention is applicable for preparing Cryogenic spice powder selected from turmeric, pepper, chilli, coriander, rasam powder, garam masala, 20 clove, cinnamon, cumin, cardamom and the like.

In some embodiment, in the claimed invention the grinding under cryogenic conditions involves the following steps:

- 25 a. mixing the raw material with liquid nitrogen at temperature of 20 to 22°C,
- b. passing the mixture through hammer mill to obtain the coarse powder,
- c. grinding the obtained coarse powder at universal mill under liquid nitrogen and at temperature of 5 to 7°C,
- d. grinding the powder obtained in step c) at cryogenic mixer at temperature of -5 to 30 2°C to obtain the fine powder, and
- e. sieving the fine powder through rotary sifter.

In some embodiment, in the above described method the primary packaging is done in pouches with nitrogen purging and secondary packaging is done aseptically in cartons. In some

embodiment, the material for primary packaging is selected from polyethylene terephthalate, metalized polyethylene terephthalate, polyethylene, polynatural, or biodegradable plastic or food grade plastic. In some embodiment, the secondary packaging is carried in 5 ply carton box.

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In some embodiment, the method for preparing the spice powder according to invention provides the spice powder which is prevented from oxidation and rancidity. In some embodiment, the method for preparing the spice powder according to invention provides the spice powder which has extended shelf life due to prevention of oxidation process of volatile oils and chemical compounds and which is due to microbial elimination.

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In some embodiment, the method according to invention employs subjecting packed Cryogenic spice powders to gamma irradiation of 240 kCi strength for 30-60 minutes. In some other embodiments, the source used for gamma irradiation is Cobalt 60.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a, 1b and 1c shows the layout design.

Figure 2 shows the procedure for preparation according to invention and testing for microbial growth.

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Figure 3 shows the procedure for testing commercial samples for microbial growth.

Figure 4a and 4b shows the test results for microbial contamination of the marketed spices and the spices prepared according to method of invention.

Figure 5a shows the test results for microbial load in the spice product prepared according to method of invention after gamma irradiation treatment.

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Figure 5b: Test results for microbial load in the commercial spice product after gamma irradiation treatment.

Figure 5c: Test results for microbial load in the commercial spice product after gamma irradiation treatment.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to provide cryogenically grinded spices and cold pasteurized food products wherein aroma, flavor, essential oils, chemical compounds, taste of the spice has
5 been maintained and the product is free of any microbial contamination.

In some embodiment, there is provided a hygienic food product including spice, rice, nutritional bar or instant mix to the consumer. In some embodiment, there is provided a method to irradiate the food product including spice, rice, nutritional bar, soup powders or instant mix
10 to kill the microorganisms like coliforms, bacteria, fungi, yeast & molds, and larvae in the manufactured products. In some other embodiment, there is provided a cryogenically grinded spice which have been irradiated to prevent any further oxidation.

In one aspect of the invention there is provided a method for preparing a microbe free spice
15 powder which retains the aroma, essential oils, taste, color, chemical compounds and antioxidant properties of the spice, the method comprising the steps of:

- a. Filling one or more dried raw spice into a feeding hopper,
- b. Grinding the raw spice(s) under cryogenic conditions,
- c. Packaging the grinded powder obtained in step (b) first into primary packaging and then
20 into secondary packaging,
- d. Subjecting the packed spice powder to gamma irradiation and
- e. Obtaining microbial free packed spice powder.

In some embodiment of the invention the combination of cryogenic grinding and gamma
25 irradiation are used to produce microbial free spice powder. The cryogenic conditions during the grinding of the spices when combined with the gamma irradiation has provided significant reduction in microbial contamination.

The method of present invention improves the aroma /flavour retention of cryogenically
30 grinded spices. The claimed method retains the essential oils of the spices and increase the palatability of cryogenically grinded spices.

In some embodiment, the method according to invention is applicable for obtaining spice powder selected from turmeric, pepper, chilli, coriander, rasam powder, garam masala, clove, cinnamon, cumin, cardamom or the like.

- 5 In some embodiment, the method according to invention is also applicable to rice (black, red, unpolished and other varieties of rice), nutria bar, nutritional upma and other food products.

In some embodiment, the gamma irradiation method is used for reducing the microbial contamination. In some embodiment, the product categories which are subjected to gamma
10 irradiation include herbs & spices, trail mix nuts, rice (red, black, unpolished rice) and nutritional bars.

In some embodiment, in the claimed invention the grinding under cryogenic conditions involves the following steps:

- 15 a. mixing the raw material with liquid nitrogen at temperature of 20 to 22°C,
b. passing the mixture through hammer mill to obtain the coarse powder,
c. grinding the obtained coarse powder at universal mill under liquid nitrogen and at temperature of 5 to 7°C,
d. grinding the powder obtained in step c) at cryogenic mixer at temperature of -5 to
20 2°C to obtain the fine powder, and
e. sieving the fine powder through rotary sifter.

In some embodiment, in the above described method the primary packaging is done in pouches with nitrogen purging and secondary packaging is done aseptically in cartons. In some
25 embodiment, the material for primary packaging is selected from polyethylene terephthalate, metalized polyethylene terephthalate, polyethylene, polynatural, or biodegradable plastic or food grade plastic. In some embodiment, the secondary packaging is carried in 5 ply carton box.

30 In some embodiment, there is provided a process of nitrogen packaging of cryogenically grinded spices and condiments. Nitrogen reduces the oxygen content within food packaging and hence prevents the product from deterioration. Thus, packaging with nitrogen gas is efficient, cost-effective way to displace oxygen and moisture. In some embodiment, the

irradiation process and packaging of cryogenically ground spices/ condiments are useful in retention of their sensory properties.

5 In some embodiment, microbial free sterile spice powder packed in fully sterile pouch retains volatile oil content and antioxidant properties. In some embodiment, the spice powder obtained according to method of invention is with 20-30% additional recovery of volatile oils.

10 In some embodiment, the method for preparing the spice powder according to invention provides the spice powder which is prevented from oxidation and rancidity. In some embodiment, the method for preparing the spice powder according to invention provides the spice powder which has extended shelf life due to prevention of oxidation process of volatile oils and chemical compounds and due to microbial elimination.

15 In other embodiment, Gamma Irradiation at 6 to 14 kGy of Cryogenic grounded powders retains 99% of the phenolic compounds and volatile oil of a Spice powder with full sterilization. Other sterilization techniques like fumigation or steam are heat based which reduces volatile oils and other chemical components and results in oxidation of spice powders. Prevention of oxidation in grounded spice powders by using Gamma irradiation at 6-14 kGy post cryogenic grinding prevents microbial oxidation of essential oils and other chemical components.

20 In some embodiment, the method according to invention employs subjecting the packed spice powder to gamma irradiation of 240 kCi strength for 30-60 minutes. In some other embodiments, the source used for gamma irradiation is Cobalt 60.

25 The method of present invention reduces the power consumption and specific energy compared to the ambient temperature grinding. Usually spices are contaminated with the microbial load and cryogenic spices having higher level of volatile oils, oleoresins and chemical compounds which is prone to spoil by microbes in the spices and oxidize the product. The gamma irradiation (cold pasteurization) reduces/ kills the microbes which are present in spices and other food products.

In other embodiment, higher level of volatile oils oleoresins and chemical compounds recovery and protection of the same by 2nd stage Cryogenic grinding & Gamma irradiation respectively.

Process improvement in extension of shelf life in spice powders, by prevention of oxidation process by microbial elimination using Gamma irradiation.

5 This invention also relates to cryogenic grinding and irradiation processing and packaging of food products including spices, rice, nutritional bar or instant mix food for retention of quality with easy serve in a hygienic wholesome way.

10 The method of present invention is particularly advantageous in providing efficient size reduction, improved product flow, better retention of natural flavoring principles, free of microbial contamination and has large potential for commercial exploitation when compared to the conventional heat based methods.

15 EXAMPLES

Example 1: Procedure for producing cryogenic grinding and gamma treated spices:

- a. Cryogenic grinding:** Raw material (RM) were dumped into the feed hopper/dump booth cleaning of RM in cyclone separator and graded in the grader. The RM was mixed with liquid
20 nitrogen and was taken to the cryogenic mixture 1 and temperature maintained at 20 to 22°C. Air Coolers and Moisture separators ensure no heat and moisture add into the system aiding the prevention of any oxygen. Then the mixture was taken to the hammer mill and made into coarse powder. The coarse mixture was further taken to the cryogenic mixture and temperature maintained -5 to 2°C. Then the product was grounded as fine powder at universal mill and
25 further passed through with rotary sifter for sieving and was stored in the batch hopper 1. Air Purging ensures no particles strand for any scope of oxidation. Then the product was mixed at vertical mixer and goes to batch hopper 2 and passed through the metal detector for metal particles and removed and discharged through internal bulk containers (IBC) or bulk bagging.
- 30 **b. Packaging:** The product from IBC was taken in to packing machine for the retail pouches (100g, 200g). The primary packing material was composed of 12 micron PET, 12 micron metalized PET and 90 micron poly natural and secondary packaging material was 5 ply carton box. Bulk bagging (1 to 25 kg) material was composed of Kraft paper 80GSM outer layer and

inner layer composed of HDPE fabric mesh 10×10, HDPE Denier 860 and LDPE lamination 118G.

- c. Gamma Irradiation:** Packed finished cryogenic spice products and rice nutritional bar, instant mix etc., was taken for the gamma irradiation and the product was inspected at gamma center as packaging condition and batch/lot number. The product was labeled with Radura label and loaded in to the tote box and exposed for gamma irradiation.

- Cobalt 60 was the source for gamma irradiation. Based on the source strength set the cycle time in the PLC for 6-14 kGy dose. Reference dosimeters were placed along with the product to know the absorbed dose. After completion of treatment dosimeters were evaluated.

Gamma Irradiation Source: Cobalt 60

Strength: 240 kCi

- Time: 30 min

The Table 1 shows the comparison of physical parameters after traditional grinding and cryogenic grinding.

Table 1: Physical Parameters			
S. No	Parameter	Ambient grinding	Cryogenic grinding
1	Energy consumption	High	Low
2	Throughput	Low	High
3	Mill clogging	Frequent	No clogging
4	Volatile losses	Higher	Minimum
5	Grinding of soft material	Not possible	Possible
6	Control on particle size	No control	Effective
7	Fire risk	High	No risk
8	Microbial load	Possible	Does not exist
9	Colour of ground powder	Not original	Original
10	Prevention of oxidation and rancidity	Low	High
11	Power saving	low	High

- Table 2 shows the volatile oil content and Table 3 shows oleoresin after ambient grinding and after cryogenic grinding of various spices.

Table 2: Volatile oil Content

S. No.	Name of the spice	Volatile Oil			
		Raw Material	Ambient Grinding	Cryogenic Grinding	% of Recovery
1	Turmeric	5.5	3.4	5.4	58.8
2	Coriander	0.6	0.4	0.6	50.0
3	Chilli	1.9	0.9	1.6	77.8
4	Black Pepper	3.4	2.5	3.0	20.0
5	Garam Masala	4.5	2.2	4.0	81.8
6	Cardamom	8.5	5.7	8.3	45.6
7	Cloves	17.0	11.9	16.6	39.5
8	Cumin	3.4	2.3	3.2	39.1
9	Ajwain	5.1	4.2	4.8	14.3
10	Oregano	3.2	2.6	3.0	15.4
11	White Pepper	3.3	2.2	3.1	40.9
12	Mace	15.0	9.3	14.2	52.7
13	Piminto	3.2	2.6	3.0	15.4

Table 3: Oleoresin Content					
S. No.	Name of the spice	Oleoresin			
		Raw Material	Ambient Grinding	Cryogenic Grinding	% of Recovery
1	Turmeric	15.0	10.1	13.3	31.7
2	Coriander	20.0	11.1	18.5	66.7
3	Pepper	12.8	7.7	9.4	22.1
4	Fenugreek	8.0	4.6	5.5	19.6
5	Cumin	18.0	11.6	15.0	29.3
6	Ajwain	25.0	19.9	23.0	15.6

Table 4 shows the antioxidant activity after ambient grinding and after cryogenic grinding of various spices.

Table 4: Antioxidant Activity									
S. No.	Name of the spice	Antioxidant activity							
		Total Phenolic Content (mg GAE)		Total Flavonoids C (mg QE)		Antioxidant content (mg BHT E)		Scavenging %	
		Ambient Grinding	Cryogenic Grinding	Ambient Grinding	Cryogenic Grinding	Ambient Grinding	Cryogenic Grinding	Ambient Grinding	Cryogenic Grinding
1	Turmeric	1.6	2.1	15.1	28.0	0.5	1.3	78.0	81.8
2	Coriander	0.8	1.0	6.9	12.1	0.4	0.8	39.0	89.5
3	Cumin	99.0	110.5	27.1	39.4	6.5	8.8	18.9	23.5

4	Fenugreek	4..5	5.7	69.0	79.3	6.0	10.0	48.5	80.7
5	Ajwain	75.3	115.0	343.0	523.0	133.2	145.2	48.0	94.5

Table 5 shows chemical Compound Recovery after ambient grinding and after cryogenic grinding of various spices.

Table 5: Chemical Compound Recovery					
Spice Chemical compound Recovery Ambient and Cryogenic grinding					
S. No.	Spice Name	Parameter	Ambient	Cryogenic	Recovery
			g/100g		%
1	Chilli	Capsaicinoids	0.26	0.29	11.5
2	Turmeric	Curcuminoids	4.4	6..0	36.3
3	Black Pepper	Piperine	5.0	6.2	24.0

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Example 2: Effect of Gamma irradiation on cryogenic grinded spices:

The cryogenically grinded spices as prepared according to method described in Example 1 was tested before and after gamma irradiation for microbial contamination and results have been shown in Table 6.

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Table 6: Microbial Content of Foods Spices Before and after Gamma Treatment									
Microbial Parameters									
Spices Before and after Gamma Treatment									
S. No.	Parameters	Before Gamma				After Gamma			
		Chilli	Coriander	Pepper	Turmeric	Chilli	Coriander	Pepper	Turmeric
1	Total Plate count, cfu/g	573	530	2400	796	0	0	0	0
2	Yeast and Mould count, cfu/g	320	1012	100	0	0	0	0	0
3	<i>E. Coli</i> , cfu/g	4	3	1	3	0	0	0	0
4	<i>Pseudomonas</i> spp, /g	0	0	0	0	0	0	0	0
5	<i>Staphylococcus Aureus</i>	0	3	0	0	0	0	0	0
6	<i>Salmonella</i> spp, cfu/25g	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
7	<i>Aspergillus</i> spp, cfu/g	1080	15	30	110	0	0	0	0

As can be seen from the results of the above given Table 6, for the cryogenic grinded spices there was significant decrease in microbial contamination after gamma irradiation.

EXAMPLE 3: Evaluation of microbial contamination for spices prepared according to invention (cryogenic grinding and gamma irradiation) and the marketed spices after gamma irradiation:

- 5 Usually spices and its products contains microbial contaminants. We tested the spice samples (cryogenic grinded according to invention and marketed spice samples) after gamma irradiation treatment for microbial contaminants. The protocol followed for the spices prepared according to invention involving both cryogenic grinding and gamma irradiation were according to process flow diagram given in Figure 2 and for marketed samples according to process flow
10 diagram in Figure 3.

Finished product of spice powder samples were opened in sterile condition under biosafety cabinet and weighed one gram of sample in eppendorf. Then aliquoted 10 ml of normal saline in the test tubes, and added the one gram of sample into the tube. Mixed the sample with sterile
15 tip and vortex and leave to settle. Labeled the media plates and then took 100 µl of sample and added into the media plate. Then by using sterile L rods spread on the media, incubated the media plates in the calibrated 35 °C incubator for 48 hours for bacterial pathogens and 25 °C incubator for 3 to 5 days for fungal and other pathogens. Observed the plates and recorded the growth.

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Tested for pathogens- *E.coli*, *coliform*, *staphylococcus aureus*, *pseudomonas aeruginosa*, *salmonella* spp, *Aspergillus* spp, yeast and molds.

1. *Staphylococcus* spp - Mannitol salt agar
2. *Pseudomonas* spp - Cetrimide agar
- 25 3. *E.coli*- MacConkey agar
4. Coliform- Eosin methylene blue agar
5. *Salmonella* spp -Xylose lysine deoxycholate agar
6. *Yeast molds & Aspergillus* spp - Sabouraud dextrose agar

- 30 The results obtained have been shown in Figures 4a, 4b, 5a, 5b and 5c. It was found that the microbial contaminates were reduced in the market samples, however no contaminants were observed in the spices prepared according to invention. The study showed the way to reduce the microbial load and prevention of oxidation in the products.