The Pharma Innovation Journal 2021; 10(6): 1290-1292

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(6): 1290-1292

www.thepharmajournal.com
Received: 02-04-2021
Accepted: 06-05-2021

Afreenkousar A Yaligar
M. Sc student Food science and Nutrition, College of Community science, University of agricultural sciences, Dharwad, Karnataka, India

Sarojani J Karakannavar
Professor, Food science and Nutrition, College of Community science, University of agricultural sciences, Dharwad, Karnataka, India

JS Hilli
Professor, Seed science and technology, University of agricultural sciences, Dharwad, Karnataka, India

Corresponding Author:
Sarojani J Karakannavar
Professor, Food science and Nutrition, College of Community science, University of agricultural sciences, Dharwad, Karnataka, India

Microbial study on *Masala Khara-Spicy* chilly powder

Afreenkousar A Yaligar, Sarojani J Karakannavar and JS Hilli

Abstract
The present investigation was carried out to study the effect of preservatives and packaging on the shelf life of *masala Khara*. Masala Khara recipe from Vijayapur district, stored in two different packaging materials High-Density Polyethylene (HDPE 10 x 21cm) and Metalized polyethylene (10 x 16 cm) using two preservatives for up to six months. The preservatives used were Sodium Benzoate and ascorbic acid. They were stored for six months and after every fifteen days interval, analyzed microbial load. Total bacterial count, fungal count, yeast count in *masala Khara* were significantly lowered in metalized packaging compared to HDPE packaging. A similar trend was seen with the addition of preservative Sodium Benzoate significantly lowered microbial count when compared with Ascorbic Acid. Hygienic indicator E-coli was not detected in samples throughout the storage period. Thus *masala Khara* can be stored for up to six months without any undesirable change.

Keywords: Masala Khara, microbial load, Hdpe, metalized polyethylene, sodium benzoate, ascorbic acid

Introduction
In northern parts of Karnataka; the traditionally chilly powder is used in the form of *Masala Khara*. *Masala Khara* is a chill powder mix prepared by using ingredients like onion, ginger, garlic, fenugreek seeds, curry leaves, dry coconut, and coriander seeds. However, masala *Khara* is a traditional chilly powder mix that can be used directly to sambar and subject preparation in the routine cooking process. This will enhance the taste of the prepared dish as well as reduce the cooking time. This *masala Khara* is also used as a side dish along with oil for roties. Hence it is gaining more importance these days; especially the working women prefer using *masala Khara*. It will be prepared during the summertime and stored for up to one year. It is the microbial population in the product which will ultimately determine the storage stability of the product. M*asala Khara* happens to be the moist product, which makes essential the study of microbial quality. Hence long-term storage study was undertaken in two different packaging's *viz*., High-Density Polyethylene (HDPE) and metalized polyethylene using two preservatives.

Materials and Methods
Masala Khara traditional chilly spice mix from Vijayapur of North Karnataka was prepared in the laboratory and was studied for the shelf life quality during six months of storage. *Masala Khara* was stored in two different packaging materials *i.e*., High-Density Polyethylene (HDPE 10 x 21cm) and Metalized polyethylene (10 x 16 cm), and sealed using an electric sealing machine. The sample was stored with two preservatives *viz*., sodium benzoate at the concentration of 600 mg/kg and ascorbic acid (1%) and mixed uniformly at ambient temperature. They were stored for six months and after every fifteen days interval analyzed for pH, moisture, and microbial load. The experiment consisted of the following treatment combinations.

Without Additive
SP_1 A_0 = Sample stored in HDPE pouches
SP_2 A_0 = Sample stored in Metalized polyethylene

With Additive
SP_1A_1= Sample stored in HDPE pouch with Sodium benzoate
SP_1A_2= Sample stored in HDPE pouch with Ascorbic acid
SP_2A_1= Sample stored in Metalized polyethylene pouch with sodium benzoate
SP_2A_2= Sample stored in Metalized polyethylene pouch with Ascorbic Acid

Microbial load in *masala Khara*
The percent moisture was calculated using the formula. The color of the powder or *masala Khara* was noted visually. The pH of the *masala Khara* was recorded using a pH meter. Microbial load in *masala Khara* stored at ambient temperature was analyzed for microbial quality, initial and at an interval of one month up to 6 months before.

Standard serial dilution plate count technique was followed to determine the microbial load in *masala Khara* [1]. Ten grams of *masala Khara* was transferred to a 90 ml water blank and was then mixed thoroughly to obtain 10^{-1} dilution which was further diluted up to 10^{-2} using 9ml sterile water blanks. One ml each of the aliquot of 10^{-2} dilution was transferred onto sterile Petri plate in duplicate. About 20ml of warm nutrient agar was poured into each Petri plate and gently rotated clockwise and anti-clockwise thrice for proper mixing. On solidification, plates were incubated at 37±1°C for a period of 24-48 hrs in the inverted position and colony counts were recorded. Similarly, fungi, yeast, and Escherichia coli were isolated in the same dilution in Rose Bengal Agar, Malt extracts Glucose peptone agar and *Macconkey* Agar respectively. The observations were averaged from the replications under different dilutions. The microbial load was expressed as the population of microflora per gram of *masala Khara*. Colonies were counted and calculations were done to obtain the CFU (Colony Forming Units) per gram sample by the following formula,

CFU/g = Number of colonies X dilution factor / Weight of sample

Statistical analysis

The data obtained from storage studies such as moisture, pH, and microbiological analysis of the *masala Khara* was statistically analyzed using SPSS-16.0 software package (SPSS Inc. Chicago, IL, USA) (3 factorial analysis) to find out the significant difference during the storage period.

Results and Discussion

A significant difference in moisture content was observed during the storage period. Initially moisture content of Vijayapur *masala Khara* was 26.56 percent. As the storage period advanced the moisture content of *masala Khara* decreased. The mean moisture content of *masala Khara* was 24.56, 21.96, 19.14, 18.10, 17.99, and 17.79 percent after one, two, three, four, five, and six months of storage respectively. Moisture content decreased significantly ($p \leq 0.05$) after two and three months of storage.

Irrespective of additives, moisture contents in HDPE (20.74%) were significantly less than metalized packaging (21.05%). However, in both packaging materials the moisture content decreased during storage. Irrespective of packaging material moisture content was significantly higher in the package without additive *viz*; (A_0 -21.09%) and Ascorbic acid (A_1 -21.4%) than Sodium benzoate (A_2 -20.55%).

The initial pH of Vijayapur *masala Khara* was 4.10 the pH of *masala Khara* was 4.10, 4.19, 4.35, 4.55, 4.58, and 4.60 percent after two, three, four, five, and six months of storage respectively. However increase in pH was significant after three, four, and six months of storage. The mean pH of Vijayapur *masala Khara* in HDPE packaging was significantly higher (4.40) when compared to metalize packaging (4.30). The mean pH in Vijayapur *masala Khara* without preservative was 4.46, with sodium benzoate and ascorbic acid was 4.26 and 4.34 percent respectively. The pH of the *masala Khara* was significantly lower when additives were used in comparison to non-use of additives.

Effect of packaging and preservative on a total bacterial count in storage is presented in Table I. Initially bacterial count was zero in 10^{-2} dilution; it increased to 9.5×10^2 cfu/g at the end of 180 days of storage. The bacterial count observed after one, two, three, four, five, and six months of storage was 0.42, 1.17, 4.08, 5.58, 6.42, and 9.50×10^2 cfu/g respectively. The bacterial count increased significantly after three, four, five, and six months of storage when compared to previous months. After six months maximum count was observed. The bacterial count in *masala Khara* in metalized packaging was significantly lower (2.48×10^2 cfu/g) when compared to HDPE packaging material.(3.47×10^2 cfu/g).The mean bacterial count in *masala Khara* without preservative was 7.42×10^2 cfu/g with sodium benzoate and ascorbic acid was 1.56×10^2 cfu/g and 2.67×10^2 cfu/g respectively. The use of additives reduced bacterial count significantly. Sodium benzoate was significantly more effective in hindering the growth of bacteria when compared to ascorbic acid. And ascorbic acid has antimicrobial and antioxidant properties, which prevents bacterial, mold, and yeast development [2].

Addition of ascorbic acid reduces microbial growth [3]. Statistically, a significant difference was observed in the interaction between additive and packaging (A×P) and packaging and months of storage (P×M), the interaction between Additive and months of storage (A×M), and interaction of Additive, packaging, and months (A×P×M).

The effect of packaging and preservative on total fungal count during storage is presented in Table II. Initially, the fungal count was zero in 10^{-2} dilution which increased to 12.92×10^2 cfu/g at the end of 180 days of storage. The fungal count observed after one, two, three, four, five, and six months of storage was 0.75, 2.50, 6.25, 9.17, 11.25, and 12.92×10^2 cfu/g respectively. The fungal count increased significantly after two, three, four, five, and six months of storage when compared to previous months. The fungal count of *masala Khara* in metalized packaging was significantly lower (5.29×10^2 cfu/g) when compared to HDPE packaging material (6.26×10^2 cfu/g). The mean fungal count in *masala Khara* without preservative was 7.67×10^2 cfu/g whereas sodium benzoate and ascorbic acids were 4.89×10^2 cfu/g and 5.78×10^2 cfu/g respectively. The use of additives reduced fungal count significantly. However, there was no difference between the additives used. Statistically, a significant difference was observed in the interaction between additive and packaging (A×P) and packaging and months of storage (P×M), the interaction between Additive and months of storage (A×M), and interaction of Additive, packaging, and months (A×P×M).

The effect of packaging and preservative on total yeast count in storage is presented in Table III. It is noticed that yeast count in *masala Khara* was increased during storage. Initially, the yeast count was zero in 10^{-2} dilution it increased to 3.17×10^2 cfu/g at the end of 180 days of storage. The yeast count observed after two, three, four, five, and six months of storage was 0.50, 0.50, 0.92, 2.08, 2.92, and 3.17×10^2 cfu/g respectively. The yeast count increased significantly after six months of storage maximum yeast count was observed. The yeast count in *masala Khara* stored in metalized packaging material (1.21×10^2 cfu/g) was significantly lower than samples stored in HDPE packaging (1.57×10^2 cfu/g). The mean yeast count in *masala Khara* without preservative was 2.39×10^2 cfu/g whereas in sodium benzoate and ascorbic acid-treated samples were 0.71×10^2 cfu/g and 1.21×10^2 cfu/g respectively. A significantly higher yeast count was found in samples without preservatives followed by a sample with ascorbic acid and sodium benzoate. Statistically, a significant difference was observed in the interaction between additive and packaging (A×P) and packaging and months of storage (P×M), the interaction between Additive and months of storage (A×M), and interaction of Additive, packaging, and months (A×P×M).

The packaging material and preservatives showed a significant effect on the inhibition of microbial load. The inhibition of microbial load was more in metalized packaging material than HDPE packaging material. It may be due to the permeability of oxygen in HDPE packaging which increases the growth of aerobic microorganisms as presented [4]. Similarly, the inhibition of microbial load was more in the samples with the addition of sodium benzoate and ascorbic acid. Sodium benzoate acts by entering the individual microbial cell and balancing its pH value, increasing overall acidity of food by lowering the intracellular pH of food [5, 6]. During storage, it was observed that there was a significant increase in the microbial load irrespective of packaging and preservatives used. A similar increase in microbial population was also observed when sodium benzoate and ascorbic acid was used [7].

The microbial counts observed in the *masala Khara* in both types of packaging, as well as packages with and without preservative during the storage period, were considered as low (<30CFU/ml) despite being significant, as the count is below 30cfu/ml which is an upper limit. Hence the product was found microbiologically safe and Ecoil was not detected throughout the storage period in any samples. Microbial levels accepted in an industry range from 500 to 100,000 CFU/ml of in ready to use food products. Microbial load such as total bacterial count, total fungal count, and total yeast count had increased in the storage period in all samples. Significantly less growth was observed in samples containing preservatives such as ascorbic acid and sodium benzoate than without preservatives. Less microbial growth (total bacterial count, total fungal count, and total yeast count) was observed in *masala Khara*. Microbial growth increased with increasing storage period. Though significantly higher it was within the permissible level. Hygienic indicator E-Coli was not detected in samples throughout the storage period. The packaging material and preservatives showed a significant effect on the inhibition of microbial load. The inhibition of microbial load was more in metalized packaging material than HDPE packaging material. It may be due to the permeability of oxygen in HDPE packaging which increases the growth of aerobic microorganisms.

Table 1: Effect of packaging and preservatives on total bacterial count (cfu×10^2/g) in stored *masala khara* from Vajayapur

Treatment	Storage period (months)							Mean
	0	1	2	3	4	5	6	
$P_1 A_0$	0.00	2.00	4.00	10.50	10.50	12.50	13.50	7.57
$P_1 A_1$	0.00	0.00	0.00	0.50	1.50	2.50	4.50	1.29
$P_1 A_2$	0.00	0.00	0.00	2.00	3.50	3.00	6.50	2.14
P_1 Mean	0.00	0.17	1.00	3.83	5.17	6.00	8.17	**3.47^p**
$P_2 A_0$	0.00	0.50	3.00	9.50	12.00	13.00	13.00	7.29
P_2A_1	0.00	0.00	0.00	0.50	1.50	2.50	8.00	1.79
$P_2 A_2$	0.00	0.00	0.00	1.50	4.50	5.00	11.50	3.21
P_2 Mean	0.00	0.67	1.33	4.33	6.00	6.83	10.83	**2.48^q**
A_0 Mean	0.00	1.25	3.50	10.00	11.25	12.75	13.25	**7.42^x**
A_1 Mean	0.00	0.00	0.00	0.50	1.50	2.50	6.25	**1.56^y**
A_2 Mean	0.00	0.00	0.00	1.75	4.00	4.00	9.00	**2.67^z**
Monthly mean	**0.00^a**	**0.42 a**	**1.17 a**	**4.08 b**	**5.58^c**	**6.42^c**	**9.50 d**	3.88

	'F' value	SEM	CD
A	28.30*	0.23	0.66
P	0.82*	0.19	0.53
M	76.90*	0.35	0.99
A x P	2.15*	0.33	0.92
P x M	25.30*	0.50	1.40
A x M	2.52*	0.62	1.72
A x P x M	1.42*	0.87	2.43

Note:-*Significant at 5% level, NS- Non significant, P_1 = HDPE packaging materials, P_2= Metalized packaging materials A_1 = Sodium Benzoate, A_2 = Ascorbic Acid, M = Month of Storage, a b c: The values with different superscripts in same row are significantly different in storage, p q/ x y: The values with different superscripts in same column are significantly different packaging/additive

Table 2: Effect of packaging and preservatives on total fungal count in stored (cfu×10^2/g) *masala khara* from Vajayapur

Treatment	Storage period (months)							Mean
	0	1	2	3	4	5	6	
$P_1 A_0$	0.00	2.50	8.00	9.00	11.00	15.00	11.00	8.07
$P_1 A_1$	0.00	0.00	0.00	5.00	8.50	12.00	10.00	5.07
$P_1 A_2$	0.00	0.00	0.00	6.50	9.50	11.50	12.00	5.64
P_1 Mean	0.00	0.83	2.67	6.83	9.67	12.83	11.00	**6.26^p**
$P_2 A_0$	0.00	2.00	7.00	7.50	9.50	15.00	10.00	7.29
P_2A_1	0.00	0.00	0.00	3.00	8.00	10.50	10.50	4.71
$P_2 A_2$	0.00	0.00	0.00	5.50	8.50	13.50	14.00	5.93
P_2 Mean	0.00	0.67	1.50	4.67	7.67	12.00	10.50	**5.29^q**
A_0 Mean	0.00	2.25	7.50	8.25	10.25	15.00	10.50	**7.67^x**
A_1 Mean	0.00	0.00	0.00	4.50	8.25	11.25	10.25	**4.89^y**
A_2 Mean	0.00	0.00	0.00	6.00	9.00	12.50	13.00	**5.78^y**
Monthly mean	**0.00^a**	**0.75^a**	**2.50^b**	**6.25^c**	**9.17^d**	**11.25^e**	**12.92^f**	6.11

	'F' value	SEM	CD
A	16.08**	0.35	0.98
P	1.49**	0.28	0.80
M	91.50**	0.54	1.50
A x P	0.58**	0.50	1.38
P x M	3.18**	0.76	2.12
A x M	0.31**	0.93	2.6
A x P x M	0.19**	1.32	3.67

Note:-*Significant at 5% level, NS- Non significant, P_1 = HDPE packaging materials, P_2= Metalized packaging materials A_1 = Sodium Benzoate, A_2 = Ascorbic Acid, M = Month of Storage, a b c: The values with different superscripts in same row are significantly different in storage, p q/ x y: The values with different superscripts in same column are significantly different packaging / additive

Table 3: Effect of packaging and preservatives on total yeast count in (cfu×10^2/g) stored *masala khara* from Vajayapur

Treatment	Storage period (months)							Mean
	0	1	2	3	4	5	6	
$P_1 A_0$	0.00	1.50	1.50	2.00	3.50	4.50	5.50	2.64
$P_1 A_1$	0.00	0.00	0.00	0.00	1.50	2.00	2.50	0.86
$P_1 A_2$	0.00	0.00	0.00	0.50	2.00	3.00	3.00	1.21
P_1 Mean	0.00	0.50	0.50	0.83	2.33	3.17	3.67	**1.57^p**
$P_2 A_0$	0.00	1.50	1.50	2.50	2.50	3.50	3.50	2.14
P_2A_1	0.00	0.00	0.00	0.00	1.00	1.50	1.50	0.57
$P_2 A_2$	0.00	0.00	0.00	0.50	2.00	3.00	3.00	1.21
P_2 Mean	0.00	0.50	0.50	1.00	1.83	2.67	2.67	**1.20^q**
A_0 Mean	0.00	1.50	1.50	2.25	3.00	4.00	4.50	**2.39^x**
A_1 Mean	0.00	0.00	0.00	0.00	1.25	1.75	2.00	**0.71^y**
A_2 Mean	0.00	0.00	0.00	0.50	2.00	3.00	3.00	**1.21^z**
Monthly mean	**0.00 a**	**0.50^a b**	**0.50^a b**	**0.92 b**	**2.08 c**	**2.92 d**	**3.17 d**	1.44

	'F' value	SEM	CD
A	4.20*	0.12	0.34
P	3.27*	0.10	0.28
M	44.00*	0.19	0.53
A x P	1.00*	0.17	0.49
P x M	2.00*	0.27	0.75
A x M	1.19*	0.33	0.91
A x P x M	0.43*	0.46	1.30

Note:-*Significant at 5% level, NS- Non significant, P_1 = HDPE packaging materials, P_2= Metalized packaging materials A_1 = Sodium Benzoate, A_2 = Ascorbic Acid, M = Month of Storage, a b c: The values with different superscripts in same row are significantly different in storage, p q/ x y: The values with different superscripts in same column are significantly different packaging / additive

Conclusion

Thus it can be concluded that the use of *masala Khara* spicy chilly powder is very common as convenience food in different districts of north Karnataka for regular cooking in different preparations. Storing masala *Khara* in metalized packaging and addition of sodium benzoate significantly reduces the microbial colony compared to HDPE and Ascorbic acid respectively and microbial colonies were within the permissible limits. During storage moisture and sensory score decreased, pH and microbial colony increased (total fungal, total bacterial, and total yeast count). Hence naturally *masala Khara* can be stored for up to 6 months. The addition of preservatives and good packaging enhances the storage period and provides additional benefits to extend shelf life.

Acknowledgment

This research was carried out by MHSC students from the College of Community Science, University of Agricultural Sciences Dharwad.

References

1. Lakshmanan M, Jayaraman K, Jayaraman J, Gnanam A. Laboratory experiments in microbiology and molecular biology. Higginbotham Pvt. Ltd. Madras 1971.
2. (http://www.wikipedia.com).
3. Beirne Alison. Some effects of modified-atmosphere packaging and vacuum packaging in combination with antioxidants on quality and storage life of chilled potato strips. Int. J Food Sci. Tech 1987;22(5):515-523.
4. Ramesh MN, Wolf W, Tevini D, Jung G. Influence of processing parameters on the drying of spice paprika. J Food Engineering 2006;49:63-72.
5. Boylan SL, Acott KA, Labuza TP. *Septoccous. aureus* – challenge study in an intermediate moisture food. J Food Sci 1976;41:918.
6. Bosund I. The action of benzoic and salicylic acids on the metabolism of microorganism. Adv. Food Res 1962, 11.
7. Sheth M, Nandwana V. The Storage stability of commercial mango pickle in oil. Indian Food Packer 2004, 73-77.