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Assessment of shelf life study on microbial and organoleptic quality of little millet flakes and its products

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Abstract
Little millet is an important food for millions of people and is a major source of calories and the vital component of food security in the developing world important to know the keeping quality of the little millet flakes for consumption, changes and the level of microbial activity that would make the flakes unfit for consumption in longer storage periods. The little millet whole grain flakes were analyzed for various microbiological quality attributes and also the products prepared from little millet flakes were subjected to organoleptic qualities during the storage period. The average total plate count was 2.30 log cfu/g in the little millet flakes. However, there was no coliforms and yeast and molds were observed in the product. The initial overall acceptability of the little millet flakes was 8.18 in metalized polyester. As the storage increased, the overall acceptability was decreased. The overall acceptability observed was 8.10, 8.04, 8.01, 7.92, 7.28 and 6.85 respectively after storage period of 15, 30, 45, 60, 75 and 90 days stored in a metalized packaging material. The initial overall acceptability of the little millet flakes was 8.12 in polypropylene pouch. The overall acceptability observed was 8.08, 8.02, 7.76, 7.56, 7.12 and 6.98 respectively after storage period of 15, 30, 45, 60, 75 and 90 days. Microbial study suggested that the products are of acceptable quality. Storage studies revealed that the product can be stored safely for a period of 3 months.

Keywords: Little millet flakes, shelf life, MP, PP and organoleptic qualities

Introduction
Millets are a group of small seeded species of cereal crops, widely grown around the world for food and fodder. The group includes millets such as little (*Panicum sumatrense* L.), foxtail (*Setaria italica*), kodo (*Paspalum scrobiculatum*), common (*Panicum miliaceum*), barnyard (*Echinochloa frumentacea*), pearl millet (*Pennisetum glaucum* L.) and finger (*Elusine coracana*) millets. Little millet (*Panicum sumatrense* L.) is nutritious and has a significant role in providing nutraceutical components such as phenols, tannins and phytates along with macro and micro-nutrients. It is a fair source of protein (7.70 to 16.50%), fat (2.45 to 9.04%), carbohydrates (62.50 to 76.30%), an excellent source of dietary fiber (15.90 to 18.10%) with good amount of soluble (3.15 to 5.70%) and insoluble fractions (10.20 to 14.95%). Besides, it also contains appreciable amounts of minerals such as iron (9.30 to 20.00 mg/100 g), magnesium (133 mg/100 g) and zinc (3.70 mg/100g) as revealed by several scientists in the field (Hadimani and Malleshi 1993; Ramulu and Rao 1997 and Itagi 2003) [4, 8, 5].
Shelf life is defined as the time span under defined storage conditions within which a food remains acceptable for human consumption in terms of its safety, nutritional attributes, and sensory characteristics (Bell *et al*., 1992, Corradini and Peleg, 2006, van Boekel, 2009) [1, 2, 11]. The progressive deterioration of quality and safety limits a food's shelf life and distribution or storage of foods under inadequate conditions, such as high temperature or relative humidity, accelerate this process (Taoukis *et al*., 1997) [10].
Good storage quality of processed food is an essential attribute to extend their utilization. Various factors like quality of raw foods, preprocessing methods, composition of food, packaging material, and extent of heat application influence the storage quality. The storage quality of processed foods was evaluated by several investigators in terms of sensory characters and chemical components. The flaking process is being used increasingly for the manufacture of snack foods.

In flaking, cereals are cooked at high temperature for a very short time and dried. Starch is gelantinized and proteins may be inactivated, microorganisms are largely destroyed and the product's shelf life is there by extended (Sowbhagya and Ali, 2001) [9].

Materials and methods

Developed little millet flakes were subjected to storage quality evaluation. The samples were packed in unit packs in metallized polyester and polypropylene pouches (MP and PP). Heat sealed and stored at ambient temperature and relative humidity during March to June. The storage quality was evaluated in terms of sensory Quality at pre and post storage with intermittent subjective and objective tests. Sensory evaluation was carried out during storage period of 3 months.

Microbial quality of little millet flakes

Total plate count (TPC)

Total plate counts were enumerated according to the method of FSSAI (2002) [3].

The optimized little millet flakes 1g was diluted in sterile distilled water 9 mL to make primary dilution was 10^{-1}. Transferred primary dilution 1 mL into test tube containing 9 mL of sterile distilled water was 10^{-2} to 10^{-4}. Approximately 15-20 mL of molten and cooled media, standard plate count agar at 45°C was poured to sterile petri-dishes and the plates were rotated clockwise and anti-clockwise directions on the flat surface to have a uniform distribution of medium. One mL aliquot from 10^{-2} to 10^{-4} dilutions was poured on the solidified agar and spread over the entire surface using a sterile bent glass rod. Total plate counts were enumerated after an incubation period of 48±2h at 35˚C. The colonies were counted after the incubation period and the number of cfu/g of sample were calculated by applying the following formula:

$$\text{No. of cfu/g of sample} = \frac{\text{Mean number of cfu} \times \text{Dilution factor}}{\text{Volume of sample}}$$

Coliforms count

The coliform counts were enumerated as per the FSSAI (2002) [3]. Flakes samples and decimal dilutions were prepared as directed under determination of total plate count (section 3.5.1). For coliforms the dilutions were from 10^{0} to 10^{-1}. If the pH of dilution is outside the range of 5.5-7.6, adjust the pH to 7.0 with sterile NaOH or HCL petri plates with 15 to 20 mL of sterile violet red bile agar media was used for enumeration of coliform count. One mL aliquot dilutions were poured on the solidified agar and spread over the entire surface using a sterile bent glass rod and the plates were placed inverted. Coliforms counts were enumerated after an incubation period of 24±4 h at 37˚C. The colonies in cfu/g were counted after the incubation period.

Yeast and Mould Count

The yeast and mould counts were enumerated as per the FSSAI (2002) [3]. Flakes samples and decimal dilutions were prepared as directed under determination of total plate count section 3.5.1. For yeast and moulds the dilutions were from 10^{0} up to 10^{-1} petri plates with 15 to 20 mL sterile potato dextrose agar media were used for enumeration of yeast and mould count. One mL aliquot 10^{-1} dilutions were poured on the solidified agar and spread over the entire surface using a sterile bent glass rod and the plates were placed inverted. Yeast and Mould counts were enumerated after an incubation period of 2 to 5 days at 25 ^{0}C. The colonies in cfu/g were counted after the incubation period.

Sensory evaluation

Semi trained panel of judges comprising students and staff at the University of Agricultural Sciences, Banglore had experience with sensory test on little millet flakes and its products (Avalakki, Roasted masala and muesli) during the storage period.

Results and Discussion

Effect of packaging material on the overall acceptability of little millet flakes during storage

The effect of packaging material on the overall acceptability of little millet flakes and its products during storage was depicted in Table 1. and plate 1. The initial overall acceptability of the little millet flakes was 8.18 in metalized polyester. As the storage increased, the overall acceptability was decreased. The overall acceptability observed was 8.10, 8.04, 8.01, 7.92, 7.28 and 6.85 respectively after storage period of 15, 30, 45, 60, 75 and 90 days stored in a metalized packaging material. The decrease in overall acceptability observed during storage was 8.10, 8.04, 8.01, 7.92, 7.28 and 6.85 after storage period of 15, 30, 45, 60, 75 and 90 days from the initial overall acceptability of 8.18.

The initial overall acceptability of the little millet flakes was 8.12 in polypropylene pouch. As the storage increased, the overall acceptability was decreased. The overall acceptability observed was 8.08, 8.02, 7.76, 7.56, 7.12 and 6.98 respectively after storage period of 15, 30, 45, 60, 75 and 90 days. The overall acceptability for flakes in metalized polyester pouches showed similar trend as millet flakes in polypropylene pouch. The decrease in overall acceptability observed during storage was 8.08, 8.02, 7.76, 7.56, 7.12 and 6.98 respectively after storage period of 15, 30, 45, 60, 75 and 90 days from the initial overall acceptability of 8.12. Among the packaging materials used metalized polyester was found most suitable followed by polypropylene.

Effect of storage on organoleptic quality of Avalakki

The best adjudged Avalakki were stored at room temperature. During storage the results on effect of storage on organoleptic quality of Avalakki was presented in Table 2. It was observed that during storage days the scores of the sensory attributes for Avalakki the overall acceptability was initially 8.20 and 8.16 in MP and PP and it cannot be stored for longer period. The appearance, colour, texture, aroma and taste scores were initially 8.22, 8.14, 8.28, 8.32 and 8.30 which is stored in metalized polyester pouch. Similarly, for PP the appearance, colour, texture, aroma and taste scores were initially 8.20, 8.12, 8.24, 8.30 and 8.28. During storage the sensory scores decreased as the storage period increased. The extent of decrease was significant; however, the products were acceptable throughout the storage period except the Avalakki. Similar study reported that RTC barnyard millet flakes storage life as 3 months (Lenkannavar, 2010) [7] and little millet flakes exhibited storage life of 6 months (Kotagi, 2011) [6].

Table 1: Effect of storage on organoleptic quality little millet flakes

Parameters	Duration of storage (days)							
	Packaging material	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Appearance	MP	8.10	8.08	8.02	7.98	7.95	7.5	6.21
	PP	8.08	8.04	8.0	7.95	7.90	7.42	6.19
Colour	MP	8.09	8.05	8.02	7.99	7.88	7.68	6.98
	PP	8.04	8.02	7.98	7.80	7.75	7.5	6.45
Texture	MP	8.14	8.12	8.04	8.01	7.95	7.54	6.34
	PP	8.12	8.10	8.05	7.90	7.80	7.40	6.22
Aroma	MP	8.16	8.10	8.04	7.86	7.75	7.60	6.52
	PP	8.13	8.07	8.02	7.80	7.60	7.43	6.28
Taste	MP	8.20	8.16	8.10	8.0	7.85	7.69	6.32
	PP	8.17	8.15	8.10	7.95	7.80	7.42	6.21
Overall acceptability	MP	8.18	8.10	8.04	8.01	7.92	7.28	6.85
	PP	8.12	8.08	8.02	7.76	7.56	7.12	6.98

Plate 1: Shelf life study of optimized flakes using metalized polyester and polypropylene

Plate 2: Shelf life study of prepared products using metalized polyester and polypropylene

Table 2: Effect of storage on organoleptic quality of Avalakki

Parameters	Duration of storage (days)							
	Packaging material	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Appearance	MP	8.22	The shelf life of Avalakki is only 8 hours					
	PP	8.20						
Colour	MP	8.14						
	PP	8.12						
Texture	MP	8.28						
	PP	8.24						
Aroma	MP	8.32						
	PP	8.30						
Taste	MP	8.30						
	PP	8.28						
Overall acceptability	MP	8.20						
	PP	8.16						

Effect of storage on organoleptic quality of Roasted masala

The best adjudged roasted masala product was stored at room temperature. During storage the results on effect of storage on organoleptic quality of roasted masala product was presented in Table 3 and plate 2. It was observed that during storage days the scores of the sensory attributes for roasted masala the appearance was initially 8.86 and 8.75 in MP and PP and decreased to 6.98 and 6.76. The colour, texture, aroma, taste and overall acceptability scores were initially 8.87, 8.77, 8.89, 8.86 and 8.77; and decreased to 6.55, 6.87, 6.74, 6.34 and 6.20 which is stored in metalized polyester pouch to 90 days storage period. Similarly, for PP The colour, texture, aroma, taste and overall acceptability scores were initially 8.74, 8.72, 8.84, 8.79 and 8.64; and decreased to 6.43, 6.54, 6.14, 6.21 and 6.12 to 90 days storage period. During storage the sensory scores decreased as the storage period increased. The extent of decrease was significant; however, the products were acceptable throughout the storage period.

Table 3: Effect of storage on organoleptic quality of Roasted masala product

Parameters	Duration of storage (days)							
	Packaging material	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Appearance	MP	8.86	8.75	8.60	8.10	8.0	7.86	6.98
	PP	8.75	8.64	8.52	8.0	7.95	7.52	6.76
Colour	MP	8.87	8.77	8.65	7.95	7.85	7.58	6.55
	PP	8.74	8.70	8.62	7.86	7.78	7.24	6.43
Texture	MP	8.77	8.69	8.52	8.0	7.75	7.37	6.87
	PP	8.72	8.65	8.50	7.95	7.64	7.21	6.54
Aroma	MP	8.89	8.83	8.75	8.15	8.02	7.86	6.74
	PP	8.84	8.80	8.70	8.12	8.0	7.23	6.14
Taste	MP	8.86	8.82	8.76	8.16	8.03	7.45	6.34
	PP	8.79	8.74	8.64	8.05	7.98	7.12	6.21
Overall acceptability	MP	8.77	8.70	8.65	7.98	7.56	7.29	6.20
	PP	8.64	8.62	8.40	7.84	7.42	7.10	6.12

Effect of storage on organoleptic quality of Muesli

The best adjudged muesli product were stored at room temperature. During storage the results on effect of storage on organoleptic quality of muesli product was presented in Table 4 and plate 2. It was observed that during storage days the scores of the sensory attributes for roasted masala the appearance was initially 8.81 and 8.72 in MP and PP and decreased to 6.15 and 6.24. The colour, texture, aroma, taste and overall acceptability scores were initially 8.82, 8.74, 8.82, 8.84 and 8.75; and decreased to 6.42, 6.23, 6.34, 6.55 and 6.89 which is stored in metalized polyester pouch to 90 days storage period. Similarly, for PP The colour, texture, aroma, taste and overall acceptability scores were initially 8.70, 8.69, 8.80, 8.74 and 8.62; and decreased to 6.51, 6.12, 6.29, 6.48 and 6.77 to 90 days storage period. During storage the sensory scores decreased as the storage period increased. The extent of decrease was significant; however, the products were acceptable throughout the storage period.

Table 4: Effect of storage on organoleptic quality of Muesli product

Parameters	Duration of storage (days)							
	Packaging material	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Appearance	MP	8.81	8.70	8.52	7.86	7.54	7.35	6.15
	PP	8.72	8.60	8.45	7.75	7.46	7.24	6.24
Colour	MP	8.82	8.69	8.58	7.56	7.42	7.21	6.42
	PP	8.70	8.65	8.60	7.45	7.36	7.11	6.51
Texture	MP	8.74	8.67	8.50	7.45	7.24	7.08	6.23
	PP	8.69	8.60	8.45	7.36	7.12	7.0	6.12
Aroma	MP	8.82	8.79	8.70	7.88	7.65	7.49	6.34
	PP	8.80	8.75	8.68	7.76	7.58	7.28	6.29
Taste	MP	8.84	8.78	8.72	7.78	7.68	7.45	6.55
	PP	8.74	8.69	8.61	7.64	7.52	7.36	6.48
Overall acceptability	MP	8.75	8.70	8.60	7.75	7.63	7.47	6.89
	PP	8.62	8.59	8.38	7.42	7.25	7.12	6.77

Microbial quality of optimized flakes and its products during storage

The best preferred optimized flakes and its products were stored at room temperature and analyzed for microbial quality during storage. Results of microbial quality of the optimized flakes and its products flakes during storage were presented in Table 5. and 6. In optimized flakes the total plate count was gradually increased from initial value

Table 5: Microbiological quality of developed little millet flakes and Avalakki

Microbial parameter	Duration of storage (days)							
	Packaging material	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Optimized flakes								
Total plate count (log cfu/g)	MP	2.3010	2.3979	3.4771	3.5440	3.6020	3.7403	3.7781
	PP	3.3222	3.4149	3.4913	3.5910	3.6627	3.7860	3.8129
Coliforms (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Yeast and moulds (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Avalakki/poha								
Total plate count (log cfu/g)	MP	3.9542	It cannot be stored for longer period					
	PP	3.977						
Coliforms (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Yeast and moulds (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 6: Microbiological quality of Roasted masala and Muesli

Microbial parameter	**Duration of storage (days)**							
	Packaging material	**Initial**	**15 days**	**30 days**	**45 days**	**60 days**	**75 days**	**90 days**
Roasted masala								
Total plate count (log cfu/g)	MP	3.3979	3.4941	3.6294	3.7481	3.8095	3.8721	3.9777
	PP	3.4623	3.5514	3.6608	3.7745	3.8438	3.8970	3.9956
Coliforms (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Yeast and moulds (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Muesli								
Total plate count (log cfu/g)	MP	3.2985	3.3428	3.5681	3.7125	3.8104	3.8745	3.9428
	PP	3.3246	3.4289	3.6452	3.8176	3.8524	3.8929	3.9865
Coliforms (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Yeast and moulds (log cfu/g)	MP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	PP	Nil	Nil	Nil	Nil	Nil	Nil	Nil

of 2.3010 to 3.7781 log cfu/g as storage days increased to 90 days which is stored in metalized polyester. Similarly, the optimized flakes which is stored in polypropylene was initially increase from 3.4623 to 3.9956 log cfu/g to 90 days. In Avalakki the total plate count was 3.9542 and 3.977 log cfu/g stored in MP and PP pouches. Avalakki is not stored after 6 hours of preparation. There were no coliforms and yeast and molds were observed during storage of both optimized flake and Avalakki.

In roasted masala product the total plate count was gradually increased from initial value of 3.3979 to 3.9777 log cfu/g as storage days increased to 90 days which is stored in metalized polyester. Similarly, roasted masala product which is stored in polypropylene was initially increased from 3.4623 to 3.9956 log cfu/g to 90 days. In muesli the total plate count was 3.2985 to 3.9428 and 3.3246 to 3.9865 log cfu/g stored in MP and PP pouches to 90 days. There were no coliforms and yeast and molds were observed during storage of both roasted masala and Muesli.

Conclusion

The investigation to assess storage stability was conducted. The organoleptic characteristics and microbial load of little millet flakes and its products were analyzed during the storage period. Microbial analysis has indicated acceptable quality of the products. Storage studies revealed that the product can be safely for a duration of 3 months. The sensory characteristics of the stored product were drastically decreased upon further storage. Thus, the shelf-life of the product interpreted as 3 months.

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