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Effect of processing, packaging and storage conditions on bacteria and yeast load of *anardana*

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Abstract

Anardana is mostly used as an acidulent for most of the Indian curries as it impart special taste, flavour and aroma. In traditional Indian system, the pulp-coated arils of pomegranate are dried in the open space and hence dust, dirt and microorganisms deposit over them. The improper packing in gunny bags results in moisture ingress and caking of product causing chemical and microbial colonization in *anardana*. Hence, in present experiment drying and dehydration of Bassein Seedless genotype of pomegranate under different conditions along with selection of suitable packaging material was done to reduce the microbial population and make the product hygienic. Steam blanching (with and without sulphuring) prior to drying, in a cabinet drier gave excellent quality of *anardana* with attractive red colour, firm texture, soft, free flowing with minimum microbial population. Microbial population was not detected in *anardana* exposed to blanching (with and without sulphuring) before drying in a cabinet drier and hence steam blanching before drying was recommended for preparation of *anardana*. From the storage studies, it was found that *anardana* dried under cabinet drier could be safely stored in aluminium pouches and plastic bottles (50% transparent) at low temperature for a period of six months.

Key Words : *anardana, pre-treatments, microbes, drying, packing and storage*

Introduction

The pomegranate (*Punica granatum* L.) has been grown since biblical times for its delicious fruit. It belongs to the family *Punicacea* and is one of the oldest known edible fruits. In India, it is grown as arid and semi-arid fruit crop because it can withstand different soil and climatic stresses. Fruit cracking at maturity stage is one of the major problems in pomegranate leading to huge economic loss. Seeds of these cracked fruits are traditionally utilized by drying to yield a value added by-products known as *anardana*.

Whole pomegranate seeds which are dried and sour are popularly known as *anardana* and used as an acidulant in Indian curries, chutneys etc. in place of tamarind and dried raw mango in North India (Singh and Singh 2003). It is also used in Ayurvedic and Unani system of medicine. The therapeutic properties are reportedly due to presence of betalic acid and usolic acid and different alkaloids i.e. pseudopelletierine, elletierine, isopelletierine and methyl pelletienine and sme other basic compounds (Anonymous, 1969).

In traditional system, the pulp-coated seeds are dried n the open sun and it takes 10 to 15 days for complete rying in addition to contamination and dust deposition. Moreover, the dry product is packed in gunny bags, results in moisture ingress and caking due to high humidity during rainy season. It also results in chemical and microbial degradation of *anardana* (Singh and Sethi, 2003). However much attempts were not made on proper processing of *anardana* and therefore, the present investigation was undertaken to study the effect of processing and storage conditions on microbial population of *anardana*. The selection of suitable packaging material was also undertaken.

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Materials and methods

Pomegranate fruits (variety Bassein seedless) were harvested at the optimum edible stage from horticulture farm of Indian Agriculture Research Institute, New Delhi and transported to laboratory in plastic crates. Pomegranate fruits were taken out at random and peeled, the seeds and remaining portion were stored separately. The entire seeds (aril) were divided into three lots as fresh seeds, steam blanching (5 min) and steam blanching (5 min) followed by sulphuring (5g /kg). These three lots were then divided into two sets. The lots were loaded with pre-standardized tray loads and kept in aluminium trays. One set was exposed to drying under open atmosphere i.e. sun drying and another lot was dried in a cabinet drier (60 ± 5° C). The cross flow cabinet drier (Kilburn make, Model 0248) was used for drying the lot. Airflow of drier was 1.2 - 1.8 m/s. After obtaining required and standard moisture percentage (8 %) in *anardana*, the product from all the six lots was subjected for microbial enumeration. Out of the six lots, the best-processed product viz. *anardana* was packed in different containers viz., P_1= Low density polyethylene (LDPE, 150 gauge), P_2= Polypropylene (PP, 100 gauge), P_3= Bio-oxy polypropylene (BOPP, 150 gauge), P_4= High Molecular Milky (HMM, 150 gauge), P_5 = Aluminum co-extruded pouches (ACEP, 150 gauge), P_6=Plastic bottles (100T, 100% transparent) and P_7= Plastic bottles (50 T, 50% transparent). The *anardana* packed in 7 types of packaging materials was stored for a period of 6 months at room temperature (23±1°C) and cold storage (5±1°C) seperately. At the end of storage period the product in different packing material and under different storage conditions was analyzed for microbial population.

Microbial enumeration

The samples were subjected to microbiological analysis initially and at the end of storage period i.e. 6 months after processing. The samples were analyzed for the population of bacteria and yeast by using Standard Plate count agar (Harrigan and Mc Cance, 1966) Martin Rose Bengal agar (Martin, 1950) and MGYP (Manitose Glucose Yeast Peptone) medium (CMI, 1968) respectively. Appropriate dilutions (10^{-1} and 10^{-2}) were plated on respective media and incubated at 30°C for 24 to 28 hrs. The results were expressed in terms of Colony forming units/gm of sample. First dilution (10^{-1}) was inoculated in BCP (Bromo Cresol Purple) lactose broth tubes and incubated at 37°C for 48 hrs. and observations were taken for acid and gas production. If tubes recorded positive for acid and gas then it was considered positive for presence of *E. coli*. BCP lactose broth media (Seelay and Denmark, 1970) was used for checking the presence of *E. Coli*.

Statistical analysis

The entire experiment was laid out in complete randomized block design with three replications. Data collected for the experiment for the preparation of *anardana* was subjected to statistical analysis by the analysis of variance technique as suggested by Panse and Sukhatme (1989). Whenever variance value (f value) was found significant, the critical difference value at 5% level of probability was compared for making the comparison among the different treatments.

Results

Drying Methods: Total viable cells (TVC) of microorganisms were enumerated for different pretreatments and drying methods (Table 1). Population of bacteria and yeasts were recorded in all samples in different proportion.

Bacteria: Bacterial population varied from 11.0 to 181.5 colony-forming units (cfu/g) in sun drying samples. Population was maximum in the sample dried under sunlight and minimum in the sun dried sample exposed to steam

Table 1. Bacteria and Yeast population in anardana under different drying conditions.

Drying Methods	Pre-treatment	Bacteria*	Yeasts*
Control		181.50	144.0
Sun drying	Steam blanching	33.50	81.5
	Steam blanching + sulphuring	11.00	20.5
Mean		75.33	82.0
Cabinet drying	Control	120.0	73.50
	Steam blanching	ND	ND
	Steam blanching + sulphuring	ND	ND
Mean		40.0	24.5
Drying methods (DM)	SEm ± CD	SEm ± CD	
	1.92 5.49	1.45 4.15	
Treatment (T)	3.33 9.52	2.51 7.18	
DM x T	4.71 13.46	3.55 10.16	

ND = Not detected, * = values are mean of three replications

blanching alongwith sulphuring. Significant differences in mean bacterial count were observed among sun dried ($7.53x10^2$ cfu/g) and cabinet dried samples ($4x10^2$ cfu/g) and it was not detected in the cabinet dried samples pretreated with steam blanching with and without sulphuring . In this case these pretreatments were the best to destroy all microbes. *Esturaetia coli* was not detected in any of the sample. No acid or gas was produced in any of the treatment, which indicates that the samples were free from *Coliforms*.

Yeasts: Yeasts population varied from $14.4x10^2$ cfu/g in sundried samples to $2.05x10^2$ cfu/g in sun-dried arils exposed to steam blanching alongwith sulphuring. Yeast population was not detected in cabinet dried samples exposed to steam blanching either with or without sulphuring. Mean yeast population ($8.2x10^2$ cfu/g) in sun-dried arils showed significant difference ($2.45x10^2$ cfu/g) from that of cabinet dried arils. The steam blanching and steam blanching alongwith sulphuring differed in comparison to untreated arils.

Storage Studies: Results presented in Table 2 represents the population of bacteria and yeasts influenced by packaging material under different storage conditions. The microbial quantity was assessed after 6 months of storage.

Bacteria: Bacterial population in stored *anardana* samples was maximum ($1x10^2$cfu/g) in package P_3 (BOPP) at ambient temperature (25-30°C) and minimum ($1x10^1$ cfu/g) in P_7 (PB 50% transparent) both at ambient and low temperature (0-7°C). Bacterial population was not detected in P_5 (AP) sample at low temperature. Among different packages P_5, P_6 and P_7 samples recorded a minimum population ($1x10^1$ cfu/g) whereas, P_3 recorded maximum population ($1x10^2$ cfu/g) showing significant difference. At ambient temperature bacterial population ($5.42x10^1$ cfu/g) differed significantly when compared to samples stored at low temperature ($2.42x10^1$ cfu/g) indicating low temperature is useful for preservation of *anardana*. *E.coli* was absent in all the packaging materials and samples stored at ambient temperature and low temperature up to 6 months.

Yeasts: In stored *anardana* samples, yeasts population was quiet low in comparison to population of bacteria. It ranged from $5x10^1$cfu/g in P_1 package at ambient temperature in comparison to $1x10^1$ cfu/g in package P_6 stored at ambient temperature and in packages, P_2, P_4, P_6 stored at low temperature. Yeasts were not detected in P_5 and P_7 packages both at ambient and low temperatures. Amongst various packaging materials, P_5 and P_7 packages were considered best, as yeasts could not colonize in *anardana* packed. Out of different storage temperatures, low temperature ($1x10^1$ cfu/g) was best in comparison to ambient temperature ($2.14x10^1$ cfu/g) for the mean values indicating safe for storage of *anardana*.

Discussion

Microbes counts i.e. the total viable count of bacteria, and yeast were minimum for the treated samples (both sulphured and unsulphured) dried under controlled conditions that is, in a cabinet drier. Further samples pre-treated with sulphur di-oxide proved more useful as SO_2 combines with free moisture to form sulphorus acid, which is very effective against mould, yeast and bacteria (Mc bean *et al.*, 1971; Tandan *et al.*; 1983 and Dziezak, 1986).

Cabinet drying proved to be the best for eliminating microflora from *anardana* as drying occurs under controlled conditions of temperature and relative humidity (Singh and Sethi, 2003). The total count were less due to

Table 2. Population of bacteria and yeasts as influenced by packaging material under different storage conditions

Packaging Material	Bacteria (cfu/g) RT	LT	Mean	Yeasts (cfu/g) RT	LT	Mean
P_1	70	40	55	50	20	35
P_2	80	20	50	30	10	20
P_3	130	70	100	20	20	20
P_4	60	20	40	40	10	25
P_5	20	ND	10	ND	ND	ND
P_6	10	10	10	10	10	10
P_7	10	10	10	ND	ND	ND
Mean	54.28	24.28	21.42	10.00		
		SEm ±	CD	SEm ±	CD	
Packaging material (P)		0.81	2.31	0.44	1.26	
Storage temperature (T)		1.51	4.31	0.83	2.37	
P x T		2.13	6.09	1.17	3.35	

ND = Not detected
RT = Room temperature
LT = Low temperature

P_1 = Low density polyethylene
P_2 = Polypropelene
P_3 = Bio-opeque polypropelene
P_5 = Aluminium pouches
P_6 = Plastic bottles (100% transparent)
P_7 = Plastic bottles (50% transparent)

lowering of available water, as reported by Frazier (1967) who indicated that at 0.93 aw bacterial spores can not germinate while the common species of yeasts and moulds are suppressed at aw of 0.85 and 0.80 respectively. Microbial counts were minimum for all the samples (dried in cabinet drier) as compared to sundried samples as the samples are dried by artificially produced heat under controlled conditions for temperature, humidity and airflow. Drying in a cross flow dehydrator yielded better quality *anardana* (Singh and Sethi, 2003), apple rings (Sharma *et al.*, 1996) and guava, papaya and apple cheeses (Rai, 2001) than open sun drying .

Microbial population after six month of storage was more in samples stored at ambient temperature in comparison to the samples stored at low temperature. Plastic bottles proved to be the best for storage both at ambient temperature and low temperature. The results are in accordance with the findings of Pruthi and Saxena (1984) who indicated that 200 gauge polythene bags were best for storage of *anardana* in comparison to tin cans and glass bottles as moisture retention was minimum in polythene bags giving minimum chances for microbes to survive.

The results are also in close proximity with our observations recorded that as in dried guava pulp (Kalra and Rewati, 1981) stored guava pulp (Tandan et. al. 1983) osmo-vac dehydrated apple rings (Sharma, 1996) dehydrated papaya slices (Ahmed and Chaudhary, 1998) and *anardana* (Singh and Sethi, 2003). All these works had concluded that microbial count was minimum at low temperature irrespective of packaging material during storage.

This study concluded that quality of *anardana* can be improved by pre-treatments i.e. steam blanching and sulphuring. Drying under controlled conditions as well as packaging in aluminium pouches and semi-transparent bottles provide additional advantage in maintaining the quality of *anardana* for prolonged period up to six months. However, this technique can be adopted for large-scale process at industry and farmers level after further refinement if, required.

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