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EFFECT OF POMEGRANATE (*PUNICA GRANATUM*) PEEL EXTRACT (PPE) IN INCREASING THE SHELF-LIFE OF HOME-MADE BUTTER

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ABSTRACT

Pomegranate (*Punica granatum*) fruit is known for its medicinal properties due to its various bio-active polyphenols and flavonoids which exhibit anti-microbial and anti-oxidant properties (Chaturvedula et. al, 2011). The extraction of these bio-active ingredients from fruit peels maximises fruit by-product utilisation and can be used as an alternative to chemical preservatives. These compounds of importance were extracted from powdered dried peels in ethanol using a water bath shaker to obtain pomegranate peel extract (PPE) with concentration of 125mg/ml. The preservative effect of PPE was studied on home-made butter for 15 days at 4°C using various concentrations like 1.25 mg/g, 2.5mg/g and 3.75 mg/g. The result showed the extended shelf life of PPE incorporated samples as compared to the control sample (R) which was found unacceptable on the ninth day and onwards. The rejection of control sample (R) was on the basis of sensory evaluation and increased number of microbial count, i.e., log 2.74 cfu/ml. The sample C (3.75 mg/g) showed better storage life of 15 days without any effect on its sensory attributes. PPE can be used as preservative in home-made butter as result indicates that the concentration of PPE inversely effects the microbial growth and hence there is a sharp decline in microbial count at high concentrations.

1. Introduction

With an environmental-friendly approach, there is an increased attention to devise and adopt suitable methods to utilize wastes as value-added products to reduce the problem of environmental pollution. Fruits and vegetable processing in India generate substantial quantities of waste. About 50% of the total fruit weigh corresponds to the peel, which is an important source of bioactive compounds such as phenolics, ellagitannins, flavonoids, etc. (Sreekumar et al., 2014 ; Ismail et al., 2012). These in turn have wide range of actions which includes anti-oxidants, antibacterial, antimutagenic, cardioprotective, antiviral and antifungal activities (Singh and Immanuel, 2014 ; Bhandari, 2012). Use of waste as a source of polyphenols and antioxidants may have considerable economic benefit to food processors, being cheap, efficient and environmentally sound.

It was reported that pomegranate peel contains antioxidant phenolic compounds like punicalagin, punicalin, ellagic acid, gallic acid, etc (Bharani and Namasivayam, 2016 ; Ibrahium, 2010 ; Murthy et al., 2002 ; Subashini, 2016). These compounds of importance can be extracted from the peels using organic solvents like ethanol which degrades cell wall, disrupts the cytoplasmic membrane, damage membrane proteins and interfere with membrane-integrated enzymes of cells and thus aids in food preservation due to its some preservative efficiency (Subashini, 2016 ; Tiwari et al., 2011).

Keeping in view the characteristics of pomegranate peel extract (PPE) experiments were planned and conducted to establish its use as a

natural food preservative. Home-made butter was selected because like all other dairy products, it is very perishable and prone to rancidity. The bio-active compounds in PPE are detrimental to psychrophilic microbes like *Pseudomonas flourescens* and coliforms like *E. coli* which cause deterioration in home-made butter (Kanatt et al., 2010). Synthetic antioxidants like BHT and BHA used to combat oxidative changes has decreased due to their suspected action as promoters of carcinogens, as well as for consumer rejection of synthetic food additives. Thus, the two basic reasons responsible for the spoilage home-made butter, microbial growth and lipid oxidation, made it ideal for incorporation of PPE in it.

2. Materials and methods

2.1. Materials

Pomegranate fruits were purchased from the fruit market of Sipri, Jhansi and the cream was extracted from Amul gold milk (milk fat-6% and SNF-9%) purchased from local market of Jhansi. Buttermilk from previous batch was used as a starter culture. Chemicals and apparatus were made available in food chemistry laboratory of Institute of food science and technology, Bundelkhand University, Jhansi.

2.2. Methods

2.2.1. Preparation of pomegranate peel extract (PPE)

Fruits were washed properly using distilled water, peeled and their edible portions were carefully separated. The fruit peels were dried in hot oven at 40°C for 48 hours, ground into a fine powder and passed through a 24-mesh sieve. 100g powdered sample was extracted with 800ml ethanol at room temperature for 24 hours in water bath shaker (Shan et al., 2009). The mixture was filtered through a Whatman filter paper No. 2 for removal of peel particles. The filtrate obtained as PPE having concentration of 125mg/g was stored in refrigerator at 4°C for further use (Murthy et al., 2002).

2.2.2. Preparation of home-made butter

Homemade butter samples were prepared by following the standard method using ripened cream (Deosarkar and Khedkar, 2016). Cream was collected in a clean and sanitized container and stored in refrigerated conditions at 4°C till use. It was then inoculated with buttermilk obtained from previously made butter and allowed to ripen overnight. The ripened cream was churned for the separation of butter from buttermilk at 9-11°C using chilled water. During churning the butter was emerged and was separated from butter milk using sieve. The butter granules were washed using chilled water (18-20°C) to remove adhering buttermilk. The butter granules were pressed by hand to convert into ball shape mass (De, 2013).

2.2.3. Sampling

The prepared PPE was added by thorough mixing, in each 50g sample of home-made butter taken with varying concentration. In this manner four samples were prepared (i) SAMPLE A-1.25mg/g, (ii) SAMPLE B-2.5mg/g, (iii) SAMPLE C-3.75mg/g and (iv) CONTROL (R)-sample with no PPE added. Sample R was kept with the PPE incorporated samples to examine its effect on butter.

All samples were stored in refrigerated conditions until the tests were conducted.

2.2.4. Physicochemical analysis

All the tests were conducted in triplicates.

The physicochemical analysis was conducted using the standard AOAC methods to ensure that the product developed has constituent fractions in accordance to the FSSAI standards with moisture not more than 16% and milk fat not less than 80%. pH was calculated as per the instructions mentioned in the testing kit of the pH meter.

2.2.5. Microbial analysis

The microbial tests were conducted for the enumerating the total bacterial count, yeast and mould, and coliforms using pour plate technique on the 0th day, 3rd day, 6th day, 9th day, 12th day, and15th day (Buch et al., 2014).

Standard Plate Count (SPC) method was used with Plate Count agar of CBH® JO 0479 to determine the population of viable bacteria in the milk product (butter) as per the recommended methods for testing dairy products in Microbiological Spoilage Of Dairy Products. The petri-plates with plate count agar were incubated for 48 hours at 35-37°C (Ledenbach and Marshall, 2009 ; Nwogu et al., 2012).

Potato Dextrose Agar (PDA) of CDH® JO 0013 was used as a culture media to enumerate the yeast and mould colonies of butter samples stored in a refrigerator (Ledenbach and Marshall, 2009). It was reported that butter is a good substrate for the growth of pathogenic fungus. Potato dextrose agar petri-plates were incubated for 120 hours in an incubator at 22-25°C (Ledenbach and Marshall, 2009 ; Nwogu et al., 2012).

E. coli count was determined using MacConkey agar using pour plate technique and incubated at 37°C for 24 hours (Ahmed et al., 2016).

The number of colonies present in the particular test sample were determined using the formula:

CFU/ml = CFU* dilution factor

Dilution factor =1/ diluent (Aneja, 2018)

2.2.6. Sensory evaluation

It was carried out on the basis of 9-point hedonic scale rating by fifteen partially trained panellist using score cards. The sensory evaluation was carried on as per the schedule of Analysis evaluation of storage study, that is, 0^{th} day, 3^{rd} day, 6^{th} day, 9^{th} day, 12^{th} day and 15^{th} day. The samples were coded in three figures including control sample.

2.2.7. Statistical analysis

All experiments were conducted in triplicates and the calculated mean was recorded for statistical data analysis through 'Analysis Of Variance- Two Way Classification' at 5% level of significance. MS Excel (Windows 10) was used for this analysis.

3. Results and discussion

3.1. Chemical composition

Proximate analysis conducted on the home-made butter in control sample R ensured the FSSAI standard. The fat and moisture content were 83% and 16% respectively which is in accordance to the FSSAI standards. Ash content was 1% and protein content was negligible (non-significant).

3.2. Microbial analysis

3.2.1. Effect of PPE on SPC

The graphical representation Figure 3.1 showed the effect of PPE on SPC of home-made butter. The control sample denoted as R represent a linear and continuous increase in CFU/ml. Lactic acid bacteria dominated made the sample highly unacceptable on the 9^{th} day onwards. In sample A (1.25mg/g), B (2.5mg/g) and C (3.75mg/g) the microbial growth showed a linear pattern but the number of microbial counts observed were less as compared to the sample (R). The graph also indicates the increased effect of concentrations of PPE on microbial growth i.e. as the concentration of PPE increases from sample A (1.25mg/g) to sample B (2.5mg/g) and sample C (3.75mg/g) the number of colonies appear to decrease from 3^{rd} day to 6^{th} day and from 6^{th} day to 12^{th} day. Sample C (3.75mg/g) showed the minimum colonies of standard plate count and hence the reduced sourness due to the inhibitory effect of PPE on lactic acid bacteria (Nikfallah et al., 2014).

3.2.2. Yeast and Mould Result

Figure 3.2 shows the effect of pomegranate peel extract (PPE) on yeast and mould in home-made butter samples during 15 days storage period at 4°C. The four lines in the figure 3.2, representing control sample R, sample A (1.25mg/g), sample B (2.5 mg/g) and sample C (3.75mg/g) showed an elevation from 0-100 colonies on the 0^{th} day of storage period. Few fungal colonies were reported on 0^{th} day samples without any spore formation. As the storage time increases from 3^{rd} to 15^{th} day the lines representing sample B (2.5 mg/g) and sample C (3.75mg/g) showed a very gradual increase from log 1.7781 to 2.3802 cfu/ml and 1.4771 to 2.2552

respectively, on the 15th day of storage. On the contrary, the lines representing control (R) and sample A (1.25mg/g) showed a sharp rise in the colonies to log 2.7242 cfu/ml and log 2.6020 cfu/ml respectively on the 15th day of storage.

Like SPC, yeast and mould colonies also indicate retarded growth due to effect of PPE but the ratio of decrease in colonies as compared to SPC was lower.

Table 3.1.SPC in cfu/ml.

DAYS ↓	**Microbial count (SPC) in cfu/ml.			
	CONTROL R (0 mg/g)	SAMPLE A (1.25 mg/g)	SAMPLE B (2.5 mg/g)	SAMPLE C (3.75 mg/g)
0th	2.3010	2.1461	1.8450	1.6020
3rd	2.4149	2.2552	1.9542	1.8450
6th	2.6020	2.5185	2.2041	2
9th	2.7403	2.6127	2.3010	2.079
12th	2.7853	2.6270	2.4149	2.2304
15th	2.8920	2.7160	2.4913	2.2787

*P<0.05 (Significant). **Above values are in log base 10.

Table 3.2.- Yeast & mould in cfu/ml.

DAYS ↓	**Microbial count (Yeast & Mold) in cfu/ml.			
	CONTROL R (0 mg/g)	SAMPLE A (1.25 mg/g)	SAMPLE B (2.5 mg/g)	SAMPLE C (3.75 mg/g)
0th	1.9030	1.6989	1.4771	1
3rd	2.0791	1.9030	1.7781	1.4771
6th	2.3222	2.2041	2.9542	1.7781
9th	2.5185	2.2787	2.0791	1.9542
12th	2.6127	2.5185	2.2552	2.1760
15th	2.7242	2.6020	2.3802	2.2552

*P<0.05 (Significant). **Above values are in log base 10.

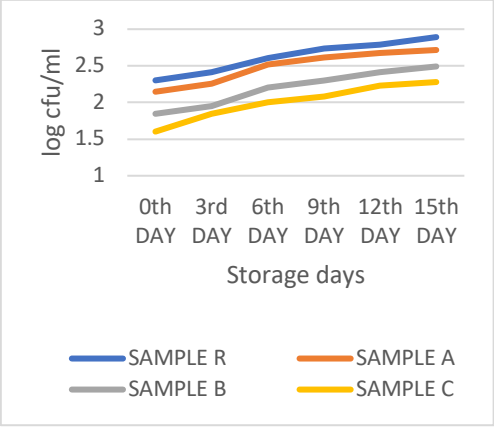

Figure 3.1.Effect of PPE on SPC

E. coli count remained absent in all the 4 samples, i.e., CONTROL and extract incorporated samples A (1.25mg/g), B (2.5mg/g) and C (3.75mg/g), on all days of test. The results, of investigation done to prove efficiency of pomegranate peel extract (PPE) to supress the growth of microbial colonies supported the work of Nikfallah et al., 2014, and Braga et al., 2004, who also stated in their work that PPE had antimicrobial activity against microorganisms like S. aureus, Klebsiella, L. acidophilus, L. subtilis and E. coli. The reduction in the number of colonies was due to the presence of polyphenols in PPE having antibacterial and antioxidant properties, caused the reduction in the number of microbial colonies (Bhandari, 2012 ; Bharani and Namasivayam, 2016 ; Subashini, 2016 ; Bopitiya and Madhujith , 2014).

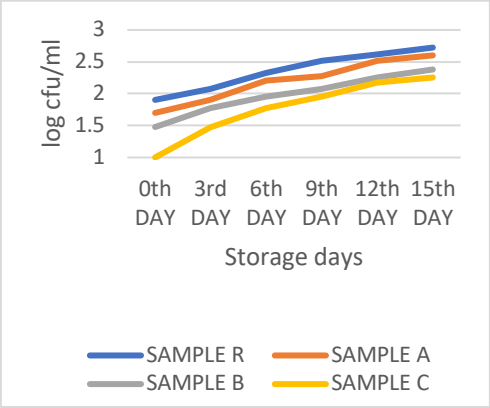

Figure 3.2.Effect of PPE on Yeast & Mould

3.3. Statistical analysis

These interprets that in both the cases there is a significant effect ($p < 0.05$) of varying concentration, i.e., 1.25mg/g, 2.5mg/g and 3.75mg/g of PPE incorporation in home-made butter samples upon microbial count and the days of storage.

Table 3.3. Statistical analysis of microbial enumeration

Analysis Of Variance (ANOVA) – 2 way without replication			
Microbial test	P-value	F value	F_{cri} value
SPC	$P_1<0.05$	12.7447	2.9091
	$P_2<0.05$	27.9878	3.2878
Yeast & Mold	$P_1<0.05$	19.1358	2.9012
	$P_2<0.05$	16.5667	3.2873

*5% level of significance

3.4. Sensory analysis

Figure 3.3 represents the overall acceptability (O.A.A.) of control sample (R), sample A (1.25mg/g), sample B (2.5 mg/g) and sample C (3.75mg/g), obtained by calculating the means of scores given by panellists upon various food attributes. Pomegranate peel extract (PPE) was found to be the most acceptable among extracts of other fruit peels (Murthy et al., 2002).

On the 0^{th} day of storage control sample (R) was found to be most acceptable.

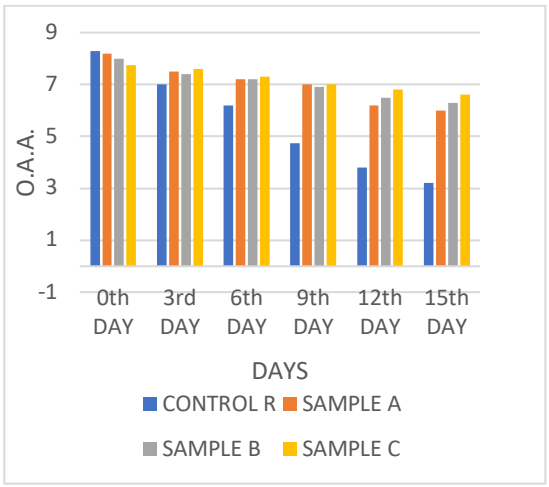

Figure 3.3.Sensory Analysis

The variation in the acceptability of the samples was attributed to the antioxidant activity of pomegranate peel extract (PPE) which supress lipid oxidation, thereby the extract incorporated samples do not turn sour and rancid. The antioxidant activity of PPE was capable of supressing oxidation in fats and oils (Bopitiya and Madhujith, 2014). The results also favour the work of Gandhi et al., 2013, which showed the capability of phenolics extracted from plant parts (using organic solvents like ethanol) in supressing oxidation in similar dairy products like ghee which is clarified butter fat.

4. Conclusions

The present work was conducted to establish the hypothesis that pomegranate peel extract (PPE) can be used as a preservative in home-made butter, to replace synthetic ones in the market. Results gathered after conducting the various tests demonstrated that the pomegranate peel extract (PPE) was capable of extending the shelf life of home-made butter by supressing the microbial growth. PPE incorporated samples had a pleasant taste which does not mask the sensory quality of home-made butter, thereby increasing its consumer acceptance.

5. References

Ahmed, S. S. J., Abdalla, M. O. M., Rahamatalla S. A., (2016). Microbiological Quality of Cows' Milk Butter Processed in Khartoum State,

Sudan. *British Microbiology Research Journal*, 11 (1), 1-10.

Aneja, K. R. (2018) Modern Food Microbiology.(1st ed.). Detection, Identification and Analysis of Foodborne Pathogens. (Chapter 4) pp. 448-449. MEDTECH.

Bhandari, P. R., (2012). Pomegranate (Punica granatum L). Ancient seeds for modern cure? Review of potential therapeutic applications. *International Journal of Nutrition, Pharmacology, Neurological Diseases*. 2 (3), 171-184.

Bharani, R. S. A., Namasivayam, S. K. R., (2016). Pomegranate (Punica Granatum L.) Peel Extract- A Study On Potential Source Of Pharmacological Activities. *International Journal of Pharma and Bio Sciences*. 7(4), B282 – B290.

Bopitiya, D., Madhujith, T., (2015). Efficacy of Pomegranate (Punica granatum L.) Peel Extracts in Supressing Oxidation of White Coconut Oil Used for Deep Frying. *Tropical Agricultural Research*. 25 (3), 298-306.

Braga, L.C., Shupp, J.W., Cummings, C., Jett, M., Takahashi, J.A., Carmo, L.S., Chartone-Souza, E., Nascimento, A. M., (2005). Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. *Journal of Ethnopharmacology*. 96(1-2), 335-339.

Buch, S., Pinto, S., Aparnathi. K.D., (2012). Evaluation of efficacy of turmeric as a preservative in paneer. *Journal of Food Science Technology*. 51(11), 3226-3234.

Deosarkar, S.S., Khedkar, C.D., Kalyankar, S. D., (2016). Butter: Manufacture. *Encyclopaedia of Food and Health*. Oxford: Academic Press. Vol. 1, pp. 529-534.

Gandhi, K., Arora, S., Pawar, N., Kumar A., (2013). Effect of Vidarikand (Extracts) on Oxidative Stability of Ghee: A Comparative Study. *Research & Reviews: Journal of Dairy Science and Technology*. 2(1), 1-11.

Nwogu, I., Chinyerum, G., Elenwo, Nkem, E., (2012). Fungal Contamination of Butter Stored In The Refrigerator. *Journal of Science*, 1(3), 59-62.

Ibrahium, M. I., (2010). Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents. *World Journal of Agricultural Sciences*, 6(4), 338-344.

Ismail, T., Sestili, P., Akhtar, S., (2012). Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. *Journal of Ethnopharmacology*, 143(2), 397-405.

Kanatt, S. R., Chander, R., Sharma, A., (2010). Antioxidants and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. *International Journal of Food science and Technology,* 45(2), 216-222.

Ledenbach, L. H., Marshall, R. T. (2009). Compendium of the Microbiological Spoilage of Foods and Beverages. Sperber William H., Doyle, Michael P. (Eds.) Microbiological Spoilage of Dairy Products, (pp. 41-67), Springer-Verlag New York.

Murthy, K. N. C., Jayaprakasha, G. K., Singh R. P., (2002). Studies on Antioxidant Activity of Pomegranate *(Punica granatum)* Peel Extract Using In Vivo Models. *Journal of Agricultural and Food Chemistry*, 50(17), 4791-4795.

Nikfallah, F., Venugopa, A., Tejani, H., Lakshmikantha, H. T., (2014). Evaluation of the Antibacterial Activity in Pomegranate Peels and Arils by using Ethanolic Extract against S. Mutans and L. Acidophilus. *Global Journal Of Medical Research: J Dentistry And Otolaryngology,*14(2),1-7.

Prakash, C. V. S., Prakash, I., (2011). Bioactive Chemical Constituents from Pomegranate (*Punica granatum*) Juice, Seed and Peel-A Review. *International Journal of Research in Chemistry and Environment*, 1(1), 1-18.

Shan, B., Cai Y. Z., Brooks J. D., Corke, H., (2009). Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. *Journal of the Science of Food and Agriculture*, 89(11), 1879-1885.

Singh, S., Immanuel, G., (2014). Extraction of Antioxidants from Fruit Peels and its Utilization in Paneer. *Journal of Food Processing & Technology*, 5(7), 1-5.

Sreekumar, S., Sithul, H., Muraleedharan, P., Azeez J. M., Sreeharshan, S., (2014). Pomegranate Fruit as a Rich Source of Biologically Active Compounds. *BioMed Research International*, 2014, 1-12.

Subashini, K., (2016). Review of Phytochemical Screening for Pomegranate Peel Extract Using Crude, Aqueous, Ethanol and Chloroform. *International Journal of Engineering and Computing*, 6(4), 3329-3332.

Sukumar De, 2013. Outlines of Dairy Technology. Oxford University Press. pp. 145-159.

Tiwari P., Kumar B., Kaur M., Kaur G., Kaur H., (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, 1(1), 98-106.