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Natural Antimicrobial Edible Film for Preservation of Paneer

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The present study attempts to assess the efficacy of active packaging films incorporating natural antimicrobial agent like cinnamon essential oil (CEO) into sodium alginate- calcium formulations to extend the shelf- life of paneer at refrigeration temperature (4 ± 1 ºC). Paneer, analogous to the western cottage cheese, is characterized by a short shelf- life mainly due to spoilage by psychrotrophs, coliforms, yeasts and molds. Natural methods of preserving paneer are an improvement of food safety since there is growing concern among the population about the chemical nature of sorbates and other chemical preservatives used. Cinnamon essential oil (CEO) has been identified to possess outstanding antioxidant activity as well as high antimicrobial activity against a wide range of spoilage and pathogenic micro-organisms. Paneer samples were left untreated (C), or were treated with alginate- calcium coating incorporating 2.5% cinnamon essential oil (CP). Proximate analyses, microbial and sensory analyses of all the samples were performed at regular intervals for a period of 15 days in order to determine the storage stability of the paneer samples. Cinnamon essential oil in alginate- calcium coating treatment could efficiently maintain the quality of the paneer samples during storage better than that of the control. Edible coating also increased the shelf- life of paneer samples to 13 days from 5- 6 days of the control sample.

Keywords: Edible film, Antimicrobial agent, Paneer, Shelf- life.

INTRODUCTION

Owing to the success of the Operation Flood programme, there has been a phenomenal rise in milk production in India over the past few decades. Milk products like paneer, curd etc. provide a cheap and nutritious food source to variety of vegetarians in the country. Paneer, which is analogous to the western cottage cheese, is a type of soft cheese produced by acid and heat coagulation of milk. The whey produced by this coagulation process is then drained off and the resultant curd is collected without coloring or aging to form paneer. In India, approximately 5% of the produced milk is transformed to paneer[1, 2].

Paneer has a very small shelf- life of a day at ambient temperature and about 5-6 days at refrigeration temperature [3]. Surface growth of micro- organisms are considered to be mainly responsible for the spoilage of paneer samples. Although the heat treatment given to milk destroys all the pathogenic and spoilage micro- organisms, washing and handling of the curd can re- introduce spoilage organisms like psychrotrophs, coliforms, yeasts and moulds. An important defect caused due to microbial spoilage is the formation of a greenish yellow slime on the surface of the cottage cheese along with the release of an off- odor[4]. Cottage cheese curd has a pH ranging from 4.5 to

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4.7 which favors the growth of various gram-negative psychrotrophic bacteria[5].

Various techniques like brining, chilling, vacuum packaging, heat sterilization as well as use of chemical preservatives are practiced to extend the shelf- life of paneer by reducing the surface growth of micro- organisms. Various paneer samples commercially available in the market include chemical preservatives like potassium sorbate (E202) having a maximum acceptable daily intake of 25 mg/kg[6]. Authors have also discussed methods like wrapping paneer in sorbic acid (2 g/ m^2) coated butter paper[7], use of sodium chloride and potassium sorbate for hot (60 ºC for 5 min) and cold (8–10 °C for 6 h) diffusion of paneer cubes followed by microwave drying[8], use of sorbic acid (0.10%) in milk along with irradiation (2.5 KGy) of the product[9] with the objective of extending the shelf- life of paneer. The development of a technique involving natural compounds for the preservation of paneer is highly appreciated since there is an increased demand among the population, throughout the world, for healthier food products devoid of chemical preservatives[10].

Essential oils obtained from plants like cinnamon essential oil (CEO) have been identified to possess outstanding antioxidant activity[11, 12] as well as high antimicrobial activity against a wide range of spoilage and pathogenic micro-organisms[13]. Furthermore, FDA has granted cinnamon essential oil the generally recognized as safe (GRAS) regulatory status[14] which has led to extensive studies on incorporation of the oil in edible coatings and active packaging films of fish and meat products to curb microbial growth[15].

Edible films have been developed using natural products for application on food items with the objective of elongating their shelf- life and lowering environmental contamination. Edible films play an important role in controlling moisture loss, gas transfer and lipid migration in food substances. They can also act as support medium for incorporation of additives as well as nutrients. Though edible coating cannot replace traditional packaging, they provide a hurdle that can be applied for preservation of food. Alginates are salts of alginic acid isolated from brown seaweeds. Owing to their unique colloidal property as well as their ability to form strong gels, they are used in edible coating formulations. They can form insoluble polymers on reaction with multivalent metal cations, for eg., calcium[16]. The efficiency of alginate- calcium coating incorporating cinnamon and nisin to effectively maintain the quality of fresh northern snakehead fish fillets during storage has been studied[17].

Evaluation of different coating materials on the effectiveness of edible coating on mozzarella cheese showed that sodium alginate based edible coating had the best effect on maintaining the physico- chemical properties of the cheese during storage[18]. Studies show that packing of edible coated paneer samples in low density poly-ethylene (LDPE) or laminates can increase their shelf- life up to 40 days[19]. The objective of the current study is to evaluate the efficiency of alginate- calcium coating incorporating cinnamon essential oil to maintain the quality of paneer during storage as well as to assess their storage stability.

MATERIALS AND METHODS

Milk and citric acid required for the preparation of cottage cheese in the laboratory was procured from the local market. Food-grade sodium alginate (Sisco Research Laboratories) was used for the coating formulations. Calcium chloride was used to induce the crosslinking reaction. The antimicrobial agent, cinnamon essential oil (CEO) was purchased from Parry's street, Chennai. CEO was stored in a cool, dry place away from sunlight.

Preparation of paneer

The paneer in the present study is made from milk having 4.5% fat content. The milk is heated up to 82ºC for five minutes which helps in destroying the pathogens, after which, it is cooled to 70°C, then 10% citric acid is added for coagulation. The whey formed is drained out and milk solids are filled in hoops and pressed by weights which are removed after sometime. The pressed paneer is then dipped in chilled water at 4-6ºC. After 1-2 hours, chilled water is drained out from water bath and the paneer is stored at 4-6ºC temperature.

Preparation of antimicrobial edible coating solution

Two grams of sodium alginate was dissolved in 100 ml of sterile distilled water at 70°C to form stock solutions of alginate (2% w/v). This

solution was stirred for 30 min for complete dissolution. Glycerol as plasticizer and $CaCl_2$ to strengthen the film was added at level of 0.3 g/g alginate and 0.05 g/g alginate respectively. Tween 80 at the level of 0.2% (w/v) was added as an emulsifier to aid dissolution of essential oil in film forming solution. Cinnamon essential oil was added to reach a final concentration of 2.5% and the resulting mixture was homogenized at 7000 rpm for 2 mins. Final alginate concentration of 1% w/v in the film-forming mixture was achieved by addition of required amount of distilled water. A magnetic stir plate was used to stir the mixture for 30 min. After cooling to room temperature, the solution was degassed under vacuum for 5 mins.

Coating paneer with antimicrobial edible coating formulation

The pH of the coating solution was adjusted to 7.0 using 1 mol/L NaOH. The edible coating was applied onto the surface of paneer after 2 days of manufacture, under aseptic conditions, by gentle brushing of the paneer surface until the entire surface is covered by the coating. The samples were allowed to stand for some time so that the residual coating could drip off. The samples were left in a temperature and humidity controlled chamber for 8 h at 12ºC (85% relative humidity, RH) until the coating was sufficiently dry. The coated (CP) and the control (C) samples of paneer were stored under refrigeration conditions (4 ± 1 ºC).

Proximate analysis

The fat and the protein content of the coated (CP) as well as the control (C) samples were determined at regular intervals for a period of 15 days (0, 5, 10, 15 day). Fat content of the paneer samples were determined using a Soxhlet apparatus[20]. A Soxhlet apparatus consisting of a one litre capacity round bottom flask as well as a condenser was fed with five grams sample of paneer. N-hexane was used as the solvent for extraction. The extraction was carried out at 68-70ºC for about 6- 8 hours (20- 30 cycles). The solvent in the extract was removed by heating on a water bath at 70ºC. The weight of the recovered extract was measured and expressed as percentage of fat.

The protein content of the paneer samples were determined by Lowry's method[21].

Microbial analysis

Microbial analysis of the paneer sample was carried out by evaluating the total plate count on the 0, 5, 10, 15 day of storage. Paneer sample weighing about ten grams was aseptically withdrawn from the upper surface of the paneer sample and diluted to 1:10 (w/v) in sterile 1% sodium citrate. This solution was blended for 1.5 min at 260 rpm. Decimal dilutions were made using 0.9% saline and 1 ml was plated on plate count agar by pour plate method. Triplicate analysis was performed. The plates were incubated at 37ºC for 48 hours[22].

Sensory analysis

The sensory analysis for paneer samples C and CP was conducted for taste, color, aroma, texture, appearance and overall acceptability. The sensory evaluations were conducted on nine point hedonic scale. A set of 14 panel members including semi- trained and trained members were asked to rate the acceptability of the product on a scale of 9 points ranging from 9 to be "like extremely" to 1 to be "dislike extremely". The scores received by each sample was then averaged and compared with the average score received by other sample[23]. A star chart was developed based on the scores obtained.

Shelf- life assessment

Shelf- life of the control (C) and the coated sample (CP) was determined on the basis of visual appearance. Any one of the following signs was treated as the end point of shelf- life:

1. Sour or bitter taste
2. Slimy or greasy texture
3. Production of rancid or sour or putrid odor
4. Yellow or brown colored patches on the surface of the sample.

RESULTS AND DISCUSSION

Proximate analysis

The fat and the protein content of the control (C) and the coated (CP) sample were assayed on 0^{th}, 5^{th}, 10^{th}, 15^{th} day of storage. The fat (%, w/w) and protein (%, w/w) content of the samples during storage have been presented in table I & II respectively. From the data, it can be seen that the fat and protein content do not significantly change during the storage period.

Microbial analysis

The total plate count of the paneer samples was determined on 0^{th}, 5^{th}, 10^{th}, 15^{th} days of

storage of sample (Table III). Figure 1 depicts the results of the microbial analysis. It was seen that coating of the paneer samples had a significant effect on the total plate count of the samples. While the 0th day count remained approximately same for both the samples, C and CP had microbial count $\log_{10}$5.65±0.08 cfu/g and $\log_{10}$4.18±0.10 cfu/g respectively on 5th day. The microbial count of the control (C) was beyond acceptable levels on the 10th day following spoilage on the 7th day, whereas, the coated sample was yet acceptable on the 10th day with a microbial count of $\log_{10}$5.39±0.04 cfu/g.

Sensory analysis

Sensory evaluation was carried out using 9 point hedonic scale (Table IV). A diagram depicting the results has been presented in Fig. 2. Sensory analysis of the control (C) and the test samples (CP) i.e. paneer samples coated with alginate- calcium coating incorporating 2.5% cinnamon essential oil was conducted and the subjects were asked to score the samples based on taste, color, texture, appearance, aroma and overall acceptability. The sensory results showed that there is no such significant difference between the control and incorporated samples. In fact, the test samples were accepted slightly better than the control samples. The flavor associated with the cinnamon essential oil as well as the texture and appearance of the coated paneer samples were reasonably accepted by the subjects.

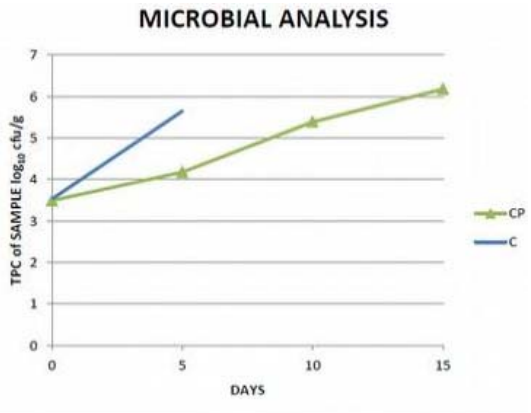

Fig.1. Graph showing microbial analysis

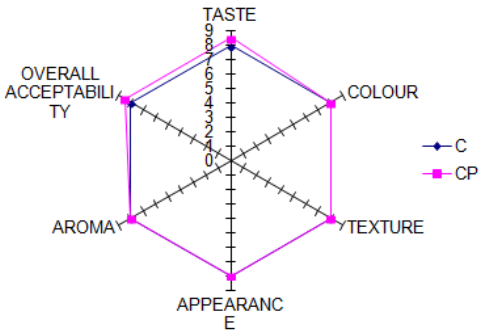

Fig. 2. Sensory analysis diagram

Table 1. Effect Of Coating On Fat Content Of Paneer During Storage

Days	Fat(%) (w/w)	
	C	CP
0	21.82±0.30	22.44±0.26
5	22.24±0.41	22.98±0.29
10	-	23.48±0.11
15	-	23.89±0.53

Table 2. Effect Of Coating On Protein Content Of Paneer During Storage

Days	Protein(%) (w/w)	
	C	CP
0	18.38±0.06	18.24±0.11
5	18.69±0.33	18.78±0.02
10	-	19.32±0.25
15	-	19.48±0.33

Table 3. Effect Of Coating On Total Plate Count Of Paneer During Storage

Days	TPC (log10cfu/g)	
	C	CP
0	3.53±0.08	3.49±0.06
5	5.65±0.08	4.18±0.10
10	NA	5.39±0.04
15	-	6.18±0.10

Table 4. Sensory Analysis Chart

Attributes	Samples	
	C	CP
Taste	8	8.5
Colour	8	8
Texture	8	8
Appearance	8	8
Aroma	8	8
Overall acceptability	8	8.5

Shelf- life assessment

The shelf- life of the samples was analyzed based on visual appearance and detrimental changes in color, texture, taste and odor were seen as the end- point of the shelf- life. The control sample (C) showed starting signs of spoilage on the 6th day with the release of a slightly rancid odor. This was followed by the appearance of a yellow patch on the surface of the sample on the 7th day. The microbial count of the control on the 7th day was enumerated as $\log_{10}$6.19±0.76 cfu/g. Therefore, the shelf- life of the control sample was accepted as 5 days. The coated paneer sample (CP) produced a slightly putrid odor on the 14th day of storage. Visual examination on the 15th day revealed a greasy and slimy texture of the paneer which was not an acceptable sign of storage. The microbial count on the 15th day was $\log_{10}$6.18±0.10 cfu/g which was beyond acceptable as per standards for milk and milk products. Therefore, the shelf-life of the coated samples (CP) was considered to be 13 days.

CONCLUSION

The present study aimed to evaluate the efficacy of antimicrobial edible coating to maintain the quality of paneer during storage as well as to extend their shelf- life since paneer is a highly perishable product. It can be concluded that the sodium alginate- calcium edible coating incorporating 2.5% cinnamon essential oil caters to this need effectively. The edible coating of paneer successfully extended the shelf- life of the product to 13 days compared to 5 days of the product while maintaining the quality of the product. There was no significant difference in the fat and protein content of the test sample as compared to the control. Also, sensory analysis of the test samples showed that test sample was well accepted among the subjects with the flavor of the cinnamon with the paneer slightly increasing the sensory score of the sample as compared to the control. This study also signifies that if the edible coating is coupled with traditional packaging materials like LDPE and laminates, the shelf- life of the product can be further increased. Therefore, this edible coating has the potential to be developed as a novel, innovative and promising technique towards extending the shelf- life of paneer. Further studies on the topic can lead to its successful application on many other food products.

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