Antioxidant, Antibacterial Analysis of Pectin Isolated from Banana Peel and its Application in Edible Coating of Freshly Made Mozzarella Cheese

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to evaluate the antibacterial, antioxidant activity of pectin extracted from banana peel. Antibacterial activity was investigated against Staphylococcus aureus, Escherichia coli, and Salmonella Enteritidis. The well diffusion method was used to assess the antibacterial effect of the pectin extract on microorganisms. The extract showed maximum activity against Staphylococcus aureus (19.6 mm). The total phenolic content and flavonoid content in the examined extract found to be 3883.6 mgGA/g and 903.03 mg QE/gm on a dry matter basis. Antioxidant activity is analyzed using in vitro Standard spectrophotometer methods. Pectin extract increases DPPH scavenging activity up to 75 µg/ml of concentration. The innovation in food packaging by the use of pectin-based edible coatings is reviewed in this paper. Thereafter, coating of pectin was done in mozzarella cheese and its shelf life was studied at 1, 7, 14, 21, and 28 days of storage at 5˚C. It was analyzed that pectin coating over mozzarella cheese increases their shelf life from 7 to 21 days. Thus, pectin is a natural polysaccharide that attracts interest for maintaining and improving the quality of cheese. Also, it minimizes the waste that occurs from non-biodegradable...
Keywords: Antioxidant; antibacterial; shelf life; edible coating; pectin.

1. INTRODUCTION

Fruits and vegetable byproducts are not waste, as they are often considered, but they have many applications and can be used to extract a variety of value-added products. Pectin is a value-added ingredient that can be derived from a variety of fruit peels, such as banana peels, citrus fruit peels, and so on [1]. The food industry accounts for the potential application of pectin in several ways such as a thickening agent [2], as a stabilizer and emulsifier [3], and also as a gelling agent [4]. Pectin is considered to be the substance present in the greatest proportion in the byproducts of many fruits, including apple, mango, and banana, etc. Pectin is also a strong source of antibacterial and antioxidant compounds.

In this present era of food technology, the edible coating is gaining popularity and value. The idea of edible coating is revolutionary as it helps to provide a semi-permeable barrier to gases and water vapor and also it aids in controlling water loss from the products [5]. The protective functionality of edible coating can be upgraded by the addition of few more ingredients such as antioxidants, antimicrobial, etc [6]. There has been huge damage caused to the environment due to the use of polymer obtained from non-renewable and non-biodegradable resources. When we focus on available data the Municipal solid waste generated in the USA is 19.3 million tonnes [7]. The most common method for packaging waste disposal is landflling which is accompanied by recycling, composting, and incineration. Polysaccharides are the most suitable substances for packaging material it complies with all environmental concerns and can be metabolized by the human body, making them perfectly suitable for use as an edible coating. Polysaccharides are substance present in abundance and also has a relatively low cost for production. Pectin is one of the most significant among polysaccharides with increasing demand.

Considering the market size of pectin which accounts for 1 billion dollars in 2019 and with an expected growth of 6.5% CAGR with an estimation to reach 1.5 billion by the year 2025. This is an indication of consumer and food industries joining hands to achieve better environmental conditions. The cheese industry being evolved as a global business requires proper research focusing upon few points such as increasing shelf life of cheese, product safety, and quality. Traditionally for cheese preservation, a coating and packaging are used acting as an individual packaging material. There has been no study done on Pectin being used as an edible coating over mozzarella cheese [8,9]. The shelf life of Mozzarella cheese is approximately 5 to 7 days and consistent efforts are being made in direction of increasing its shelf life. Using pectin as an edible coating can increase the shelf life of mozzarella cheese and very few amounts of data are available subjecting to fresh cheese.

Studies have been done considering the use of coating on several types of cheese. A modified environment is created by the coating which is quite similar to the modified controlled atmospheric storage conditions. The edible coating applied can preserve the product during the period it reaches from the production point to the consumer [10]. This is quite essential to focus on study promoting scientific research and development in the field of proper utilization of waste filling in the puzzling gap of environment issue and consumer end requirements.

- The study focuses on examining the in-vitro antibacterial, the antioxidant activity of pectin
- Application of pectin in Edible coating over mozzarella cheese and its microbial shelf-life study analyzed on 1, 7, 14, 21, and 28 days.

2. MATERIAL & METHODS

2.1 Sample Preparation

Pectin was isolated from the peel of a banana by using the acid extraction method [1]. Briefly 40 gm of homogenized banana peel was taken in 500 ml of a previously sterilized beaker, add 500 ml of distilled water (1:10). To this mixture, add...
21 ml of 0.5 N Hydrochloric acid (pH 2.5). Continuously stir the mixture for 2.5 hours at 90˚C with the help of a glass rod in a hot plate. The suspension is then allowed to cool down at normal room temperature and is filtered through a muslin cloth (1mm mesh size). To the filtrate, add the same amount of ethanol (absolute) and left overnight for precipitation of pectin. The next day, discard the supernatant and centrifuge at 5000 rpm for 15 min at temperature 5˚C to obtained pectin. The flocculant was then skimmed off and the resulted pectin was dehydrated using a hot air oven to achieve a constant weight. A sealed container is used to store dried pectin to prevent moisture absorption.

Dried pectin samples obtained were thereafter kept in a sterilized dark bottle at ambient temperature (27±2˚C) until needed for use.

2.2 Source of Microorganisms for Antibacterial Analysis

Escherichia Coli, Salmonella Enteritidis, Staphylococcus Aureus were used to test the antibacterial activity of pectin. These cultures were obtained from the laboratory of Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow (INDIA). They were subcultured in a Petri plate with nutrient agar at 4 degrees Celsius [11].

2.2.1 Antibacterial analysis of pectin

The antibacterial analysis of a pectin extract was assessed by the well diffusion method [11], which involved inoculating the organism into a previously sterile nutrient agar, autoclave at 121 degrees celsius for 15 minutes. After 24 hours, the inoculum was put in 5 ml of nutrient broth and incubated at 37±1 degrees celsius for 2 hours to act as a fresh inoculum. Using a flame sterilized cork borer, a 10 mm well was created in nutrient agar, containing 10^7 CFU/ml of test organism was well dispersed using a cotton swab. The wells in the inoculated plates were then filled with 100 μL of pectin aqueous extract. Incubate the plates at 37±1 degrees celsius for one day. The inhibition zone against each organism is measured in millimeters. [12]

2.3 Antioxidant Activity of Pectin

Pectin has good antioxidant properties. It can be determined by its a) flavonoid content (TFC), b) phenolic content (TPC), c) Scavenging activity (DPPH), and d) iron-reducing activity (FRAP).

2.3.1 Aqueous extract preparation

10 ml of distilled water diluted with one gram of pectin in the ratio of 1:10 [13]. A magnetic stirrer was used to stir the mixture and rest for 48 hours. After 48 hours, filtered the mixture through Whatman No 2 filter paper in a 250 ml Erlenmeyer flask and put the resulting filtrate in the refrigerator until use.

2.3.2 Determination of total phenolics content of pectin

The total phenol of pectin extract was measured using the colorimetry assay process [14]. Folin reagent used in this method and as the stock solution gallic acid was used. Preparation of gallic acid (1 mg/ml) was made by combining 10 mg of gallic acid with 10 ml of methanol. Several concentrations of gallic acid (25, 50, 75, 100 μg/ml) were prepared in methanol and pectin extracts. Add 2-3 ml of Sodium carbonate (20% Na_2CO_3) to 1 ml of the Folin reagent at each concentration, using distilled water to increase the volume up to 10 milliliters. The mixture could be kept at room temperature for two hours, and the optical density was measured at 750 nm using a UV Visible spectrophotometer. Pectin extracts are expressed in units of (mg GA/g) [15].

2.3.3 Analysis of total flavonoid in pectin extract

The total concentrations of flavonoids in pectin were measured by the Colorimetric assay technique [14]. The standard solution of quercetin contains 4 milligrams of quercetin diluted with 1 ml of methanol reagent of various concentrations (0.25,0.5,0.75,1 mg/ml). Dilute 0.3 ml Sodium carbonate (5% Na_2CO_3) in 4-5 ml of distilled water in addition to 1 ml of the Folin reagent at each concentration, using distilled water to increase the volume up to 10 milliliters. The mixture could be kept at room temperature for 5-6 minutes. Thereafter, 0.3 ml of aluminum chloride (10% AlCl_3) was added and again rest for few minutes. Finally, 2 ml of 1N Sodium hydroxide (NaOH) was added to the mixture which was then diluted up to 10 ml of volume. The optical density at 510 nm was measured using a spectrophotometer, and the measurements were calculated using a standard curve. [16].

It is expressed in units of (mgQE/g) [17].
2.3.4 Free radical scavenging activity (DPPH)

The antioxidant potential of DPPH is calculated using a spectrophotometer [18] with small modifications [19]. In methanol, the color of DPPH is dark blue. In its reduced form, the antioxidant compound changes color from purple to yellow, allowing DPPH to gain electrons. DPPH shows strong absorption at 517 nm, determined by 1,1-diphenyl-2-pyridyl hydroxylase (DPPH). DPPH was prepared in a 0.1 mmol/L in methanol solution.1 ml of this solution can be combined with 3-5 ml of pectin extract at varying concentrations (0, 25, 50, 75, 100, 150 µg/ml). A control sample of 1 ml of methanol was prepared and incubated in the darkroom for 30 minutes at ambient temperature. A UV Visible spectrophotometer was used to measure absorbance at a wavelength of 517 nm. To calibrate the UV spectrophotometer, methanol is used as a blank. Reduction in the absorption value, Shows high activity in scavenging free radicals [20]. It was measured as a percentage of DPPH Scavenging by using the following formula given below:

\[
\text{%DPPH Scavenging activity} = \frac{OD_{control} - OD_{Sample} \times 100}{OD_{control}}
\]

OD=Optical density

Note: The test tube was covered with brown paper as DPPH is very sensitive to light.

2.3.5 FRAP assay

The reducing and antioxidant capacity of iron is determined by [21] and with slight modifications [22]. The following three reagents were used to prepare for FRAP analysis:- Reagents A include: 10 mM TPTZ solution in 40 mM HCl, Reagents B include: 300 mM acetate buffer, Reagent C include: 20 mM FeCl₃.6H₂O. The reaction was conducted by combining all three reagents in a 10:1:1 ratio.1.8 mL of working solution was combined with 200 mL of pectin extract and kept at room temperature for 4-5 minutes for the FRAP assay. At a wavelength of 595 nm, a UV Visible spectrophotometer was used to determine the optical density.

2.4 FTIR Analysis of Pectin

The functional group of pectin is determined by analysis known as FTIR (Fourier Transform Infrared Spectroscopy). FTIR analysis was performed at the Instrument Research Center of Babasaheb Bhimrao Ambedkar University in Lucknow (UP). The spectral range is 4000-500 cm⁻¹. Sample with particle size <0.15 mm was employed for analysis.

2.5 Application of Pectin in Edible Coating of Mozzarella Cheese

2.5.1 Preparation of Mozzarella cheese

Whole milk or skimmed milk was utilized for the preparation of fresh mozzarella cheese at the university laboratory. Pasteurization of milk was done to avoid pathogenic microorganisms and also for prevention from acidification due to acetic and lactic acid. Preparation of mozzarella cheese was done in Food Science laboratory Babasaheb bhimrao Ambedkar University Lucknow by using the direct acidification method as follows-

1 Litre of pasteurized and standardized buffalo milk was taken with 4% fat and 9% solid not fat (SNF) respectively. The milk was allowed to cool overnight at a temperature less than 10°C. The next day, milk was warmed at 20 °C, and when milk temperature reaches at 30°C, 2.4% (w/v) acetic acid (CH₃COOH) was added and mixed thoroughly to achieve a PH of 5.6. Acidification is followed by coagulation, cutting off the coagulum into a relatively large-sized curd and minimal cooking. The large curd size, low cooking temperature, and short cooking time decrease syneresis and contribute to the high moisture content. The curd obtained was then stretched manually in lukewarm water for about 3-4 minutes. The whey was separated with the help of a strainer. A round block was formed by curd which was then immersed in cold water at 4°C to 5°C for 15 minutes. The block obtained was then placed on stainless steel wire mesh for the next 1 hour to extract extra whey. This block was then cut into small cubes and was placed in clean and sterilized plastic bags at a temperature of 5°C. Freshly made mozzarella cheese is given in Fig. (1). The yield of mozzarella cheese was measured by using the Van Slyke equation [23] modified by [24] as follows-

\[
\text{Yield (gram)} = \frac{[(0.85 \times \text{% milk fat}) + (\text{% milk casein} - 0.1)] \times 1.13}{1 - (\text{% cheese moisture} \div 100)}
\]
2.5.2 The edible coating on cheese

Different methods such as spraying, brushing, dipping, and electrostatic spraying can be used in the edible coating of cheese [25]. Here the method of brushing was applied for pectin coating over mozzarella cheese. Very few studies have been carried out on the coating of pectin. Suspension of Coating was prepared by dissolving 3 gm of pectin. 2.81 gm of starch in 50 ml of distilled water followed by 2.11 gm of glycerol (plasticizer), 1.25 gm polyvinyl alcohol, and 1% of citric acid and stir for about 15 minutes on a magnetic stirrer at 90˚C, while at 80 degrees celsius corn starch was gelatinized. After 15 minutes, the solution is allowed to degas. A transparent suspension was obtained. Thereafter with the help of a brush, the coating was applied to cheese cubes and kept a coated cheese in the refrigerator at 4˚C for its shelf life study. Test design of different composition are given in Table 1:

<table>
<thead>
<tr>
<th>Coating</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>PECTIN (gm)</td>
<td>0.5</td>
<td>3</td>
<td>0.5</td>
<td>3</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>PVA (gm)</td>
<td>3</td>
<td>1.25</td>
<td>2.81</td>
<td>3.75</td>
<td>2.81</td>
<td>3.75</td>
</tr>
<tr>
<td>STARCH (gm)</td>
<td>1</td>
<td>2.83</td>
<td>2.81</td>
<td>1.25</td>
<td>2.81</td>
<td>2.81</td>
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<tr>
<td>Glycerol (gm)</td>
<td>2</td>
<td>2.11</td>
<td>1.87</td>
<td>2.5</td>
<td>1.87</td>
<td>2.11</td>
</tr>
<tr>
<td>The citric acid (%)</td>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Distilled water (ml)</td>
<td>30</td>
<td>50</td>
<td>30</td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Stirring time (min)</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

2.5.3 Microbiological shelf life study of coated and uncoated cheese

Cheese (coated and uncoated) is stored at the temperature of 5°C for up to 28 days. The cheese samples were analyzed on 0, 7, 14, 21, and 28 days of storage. The total number of yeast, mold, and bacteria is determined using standard methods [26]. Yeast and mold are measured using Chloramphenicol Yeast Agar (YGCA) media; dissolve 40 grams of media in 1000 milliliter of distilled water and heated until fully dissolved. Sterilization was performed by autoclaving at 121°C and 15 pounds of pressure for 15 minutes. For the determination of coliforms, MacConkey agar was used, it was prepared by suspending 49.53 grams of the medium in 1000 ml of distilled water. To make the BPW solution, dissolve 20 grams of BPW in 1000 ml distilled water. Cheese samples (5 grams) were homogenized for 1 minute using 45 milliliters of buffered peptone water solution. Further decimal dilutions were done with the same diluent using the serial dilution method.1 milliliter of solution and 15-20 ml of MacConkey agar media were taken and poured into sterilized Petri plates using pour plate method. This was incubated at 37°C for one day. For yeast and mold, 1 ml from the diluent was taken in four Petri plates and 20-25 ml of yeast glucose chloramphenicol agar (YGCA) media was poured and incubated at 25°C for 3-5 day. Fig. 2 shows the media prepared for the shelf life study of coated and uncoated cheese.
3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content

The phenol content in fruits is an important constituent, as they inactivate free radicals or preventing hydrogen peroxide from decomposing into free radicals. The total content of phenol in the pectin extract is expressed in gallic acid equivalent. The total phenolic content can be determined from the standard curve equation. It was found that the pectin extract contained 3883.6 mgGAE/g phenolic compounds.

According to [27], the TPC of acid orange pectin extracted under optimal conditions was 39.95±3.13 mg GAE/g pectin. As per my knowledge, very few studies have been considered to evaluate the Total Phenolic Content of pectin, but several studies have been conducted on various fruits and vegetable peels. According to [28] Eggplant peel pectin contains a high phenolic compound (161.10 mgGAE/g) pectin as compared to Eggplant calyx pectin contain a low phenolic compound (15.59 GAE/g pectin) due to the presence of a large concentration of a phenolic compound in eggplant peel [29] [30]. According to [31] the total phenolic content of orange pectin (38 µg/ml) is more than apple pectin (20 µg/ml).

3.2 Total Flavonoid Content

Very few experiments have been conducted to evaluate the TFC of pectin. TFC of pectin extract was determined by the standard equation where it was found to be 903.03 mg QE/gm pectin.

Previous literature [32] has shown that 39.01 to 389.33 mg CEQ/100 gram on a dry matter basis from various samples of banana pulp and peel powder, their research shows that the TFC of green fruits is higher than that Obtained from ripe and also contain higher TFC than pulp, so the variation between TPC and TFC of different plant materials can be influenced by a variety of factors such as different chemical composition, soil, condition, and maturity of a plant [33].

3.3 DPPH Activity Analysis

DPPH is the most convenient way to determine the antioxidant property of a sample. Because DPPH free radicals are scavenged by antioxidant compounds, the color of the sample changes from purple to yellow (nirmala). Fig. 2 shows the graph between concentration (µg/ml) and antioxidant activity (%) of extract.

By using a spectrophotometer the optical density of a sample and the optical density of the control can be calculated to determine DPPH behavior in a sample. According to [34], If DPPH value is below 50µg/ml it has a very strong antioxidant property, if it lies between 50-100µg/ml it has strong antioxidant property, 101-150µg/ml has normal antioxidant property and if it is above 150µg/ml it has weak antioxidant property. The antioxidant activity of pectin extract at different concentrations (0,25,50,75,100 and 150µg/ml) was evaluated and the results obtained were illustrated in Fig. 2. According to these results, pectin concentration increases up to 75µg/ml. Afterward, the activity of antioxidants was constant. At 50µg/ml highest antioxidant activity was achieved.

A previous study by [28] describes that the antioxidant activity of eggplant peel pectin solution was significantly greater than eggplant calyx pectin due to higher galacturonic acid and TPC of eggplant peel pectin [35]. Another study by [27] illustrated that increased in sour orange peel concentration up to 25 mg/ml then it was fixed and it is very close to ascorbic acid antioxidant activity.
Fig. 3. DPPH activity of pectin extract
*At the concentration of 75µg/ml, the antioxidant activity of pectin was maximum, thereafter it shows constant activity.

Fig. 4. FTIR spectrum of pectin extract
*peaks at 3419.1 cm⁻¹, 2925.3 cm⁻¹, 1573.3 cm⁻¹, 1418.7 cm⁻¹, 1316 cm⁻¹, 1155 cm⁻¹, 1024 cm⁻¹, 642.4 cm⁻¹.

3.4 FRAP Analysis

Pectin extract exhibit a Ferric reduction capacity of 0.843 µmol at 593 nm. A previous study by [19] describes Fe³⁺ reducing the activity of different antioxidants such as BHA, BHT, Tocopherol, Tralox, Malvin, etc out of which BHA and BHT showing the highest Fe³⁺ reducing activities of 2.344 and 2.430 while ID-8 showing least Fe³⁺ activity of 0.615, while other literature of [36] describe the results obtained from unripe banana peels was 14.0±1.4 by ANOVA method indicates that a good amount of antioxidant property.

3.5 FTIR Analysis of Pectin

The infrared spectrum of pectin is shown in Fig. 3. Therefore, the peak at 1316 cm⁻¹ determines the presence of uronic acid. A peak at 1024.1 cm⁻¹ reflects arabinofuranose [37]. The peak at 2925 cm⁻¹ represents intermolecular and intramolecular hydrogen Glycoside CH-CH₂ and CH₃ compounds [38].

At 3419 cm⁻¹ O-H stretching takes place and the intensity that occurs is very broad and strong. Weak C=O stretching occurs at 1155 cm⁻¹. At 1418 cm⁻¹ weaker symmetric stretching of COO band occurs whereas 1573 cm⁻¹ shows asymmetrical stretching of COO⁻, at 642.4 C-N stretching takes place.

3.6 Antibacterial Analysis of Pectin

The results of the antibacterial analysis of the pectin extract against the test organism given in Fig. 4 shows that the diameter of the inhibition
region for Escherichia coli is 15 mm, for Staphylococcus aureus is 19.6 mm, and for Salmonella Enteritidis is 12 mm, it seems that the inhibition region for staphylococcus was the largest. The literature of [39] stated that the antibacterial analysis of acylated pectin was significantly increased due to the presence of gallic acid. According to [31] found that apple pectin extract and orange pectin extract possessed antibacterial activity of diameter 17mm and 15mm against E. coli respectively.

3.7 Yield and Shelf Life Study of Mozzarella Cheese (Coated and Uncoated)

The yield of cheese obtained from 1 liter of milk was about 250 grams. On the first day, there was no growth of yeast and mold, coliform on coated and uncoated cheese, on the day seventh there was a growth of $2.3 \times 10^2$ CFU/gm yeast, and mold count occur over uncoated cheese whereas it was observed that no growth occur over coated cheese. On the fourteenth day, the yeast and mold growth count over uncoated cheese was $3.5 \times 10^4$ CFU/gm and no growth of yeast and mold was observed on coated cheese. In all the days no growth of coliform was observed. On day 21, $2.3 \times 10^2$ CFU/gm of yeast and mold growth occur over coated cheese and on the 28th day, $4.5 \times 10^4$ CFU/gm of yeast and mold were observed in uncoated cheese. At last, it concluded that pectin coating over cheese extends its shelf life compared to uncoated.

4. CONCLUSION

The overall conclusion of this study is that the pectin aqueous extract has good antioxidant activity and antibacterial properties against Escherichia coli is 15 mm, for Staphylococcus aureus is 19.6 mm, and for Salmonella Enteritidis is 12 mm. Pectin extract can scavenge DPPH free radicals up to 75 µg/mL of concentration. Furthermore, the applications of pectin over mozzarella cheese significantly increases the shelf life from 7 to 21 days at 5 degrees celsius. Recently, a lot of work has gone into developing new edible pectin-based coating formulations for food preservation, better nutritional efficiency, and longer shelf life. Overall much work remains to be undertaken, although some of the most important improvements are yet to be accomplished by experiments or implemented on a wide scale.

The composition, structure, processing, and characteristics of pectin in edible coating for food packaging must be better understood on a laboratory and industrial scale.

DECLARATION

The authors declare that they have no known competitive financial interests or personal relationships that may affect the work reported in this paper.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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