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Development of Pectin/Gelatin/Glutathione/Calcium Chloride Crosslinked Hybrid Composite Antioxidant Edible Coatings for the Preservation of Local Butter

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Given the vulnerability of butter to rancidity there continues to be a drive to develop active edible coatings to preserve the characteristics and prolong shelf-life of butter. In this work, inspiration is taken from biomedical and pharmaceutical research and crosslinked pectin-based edible composite coatings are developed for the preservation of butter. Pectin is blended with gelatin and glutathione to induce antioxidant characteristics and crosslinked with calcium chloride to overcome the problem of moisture sensitivity of neat pectin-based coatings. The blended active edible coatings exhibit significant preservation characteristics in coated butter in terms of peroxide, acid, and fatty acid values during the 60 days storage period with 15 days examination intervals in a calcium chloride dose dependent response. Furthermore, the developed coatings exhibit significantly improved antioxidant properties as assessed from β-carotene and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity in coated samples compared to control prolonging the shelf-life of coated butter. For the first time, it is demonstrated that pectin can be crosslinked to prepare edible coatings and extent of crosslinking can directly influence coating performance in food preservation. The obtained results are believed to be significant to the field and have the potential to disrupt the status quo in edible coating research and inspire commercial development of such coatings.

1. Introduction

Food packaging has become critical with globalization of the food industry in particular for fresh perishable food products. The primary aim of a food packaging material is to retain the food quality during transportation, easy to apply and potentially remove, economical and biodegradable where possible.[1] In general, food packaging regulations demand that packaging materials should be non-toxic, fit for purpose, and not contaminate the food through leachates. There has been a drive to explore packaging materials that can enhance properties of packaged foods, which has been termed as active coatings.[2] These active coatings can be made from edible materials, enhance the shelf-life of the packaged food while maintaining its sensory and quality characteristics.[3] In addition, edible coatings preserve food by functioning as a barrier to avoid moisture loss and avoid oxidation mediated degradation of perishable food products.[2] To this end, natural materials are gaining a significant interest as active edible coatings in food packaging to replace fossil fuels-based packaging materials given the environmental and potential health impacts. A range of natural proteins and polysaccharides have been explored to develop active edible coatings for example, chitosan,[4] gelatin,[4b] alginate,[5] starch,[6] cellulose[4c,7] and hyaluronic acid.[4b] The use of natural proteins and polysaccharides in edible coatings has additional advantages including barrier properties (oxygen and

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carbon dioxide permeability), optical properties (transparent, colorless), sensory score (flavorless, tasteless, odorless) and intrinsic antioxidant and antimicrobial properties.[2,8]

Pectin is a natural occurring heteropolysaccharide found in plant cell wall with the main sources being apple, pear, and citrus peel.[9] Pectin has been recognized safe by the Food and Drug Administration (FDA). Pectin has already found applications in food manufacturing industry as a stabilizer, thickening, and gelling agent in beverages, jams, yogurt, fruity milk drinks, and ice cream.[10] Pectin has been explored as an edible coating material with advantages such as good oxygen barrier, good hardness, and adhesiveness.[11] However, the developed coatings have been fragile and pectin being highly hydrophilic, these coatings have been highly sensitive to water and permeable to moisture making their (neat pectin coating) use difficult.[12] To circumvent this limitation, pectin is either chemically modified to introduce hydrophobicity or combined with natural extracts and blended into composite coatings after mixing with natural polysaccharides.[8,9,11,13]

Glutathione is a natural tripeptide known for its antioxidant activity and is considered crucial to human, animal, and plant life. However, innate glutathione in cells have a short life and therefore there has been a growing interest in using it to fortify good to deliver it exogenously.[14] The interest in using glutathione in edible coatings has just started to emerge to take advantage of its potent antioxidant activity in preserving perishable foods thus prolonging their shelf-life.[15]

Butter continues to be an essential diary component of human diet being the second most-derived milk product.[9a] Locally butter is made from milk from a variety of species including cow, sheep, goat, camel, and buffalo. Butter typically comprises 80–83% fat and therefore has a high affinity to lipolysis, oxidation, and microbial spoilage.[16] Rancidity which is the biggest challenge in storing butter compromising its long-term storage further limiting its export around the globe. Rancidity is caused by the oxidation of the fats in butter resulting in undesirable flavour, color change, and nutrient loss.[9a] A range of approaches have been explored over the years to preserve and store butter by resisting its oxidation including the addition of natural oils, salt, and extracts and use of edible coatings.[1,9a,11,16,17] The premise of all these approaches have been to introduce or enhance antioxidant properties of treated butter to subdue radical mediated oxidation of fats. There are very limited studies conducted on the use of pectin-based edible coatings for the preservation of diary product in particular, butter.[9a,11,17i] For example, Candido et al.[11] supplemented pectin with pracaxi oil nanoemulsions to prepare nanocomposite edible coatings and compared them against neat pectin coating for the preservation of butter. The pectin/pracaxi oil nanoemulsions to prepare nanocomposite coatings exhibited significantly enhanced protection of coated butter against oxidation mediated rancidity compared to neat pectin coating. The significantly better preservation performance was attributed to the improved antioxidant and water barrier properties of nanocomposite coating with the incorporation of pracaxi oil nanoemulsion.[11] Recently, Asdagh and Pirsa[9a] prepared pectin/nanoclay (montmorillonite)/*Carum copticum* essential oils/β-carotene (Pec/Clay/CCE/βC) composite films to both stabilize pectin (against water sensitivity) and induce antioxidant properties, and used them as edible coatings for butter preservation. They reported that composite films significantly reduced oxidation of coated butter, exhibited the lowest microbial load, and limit the change in color of coated butter samples. The antimicrobial performance was attributed to the synergistic effect of *Carum copticum* essential oils/β-carotene in composite coatings.[9a] The use of pectin in protective butter coating is still in its infancy with a considerable potential for improvement.

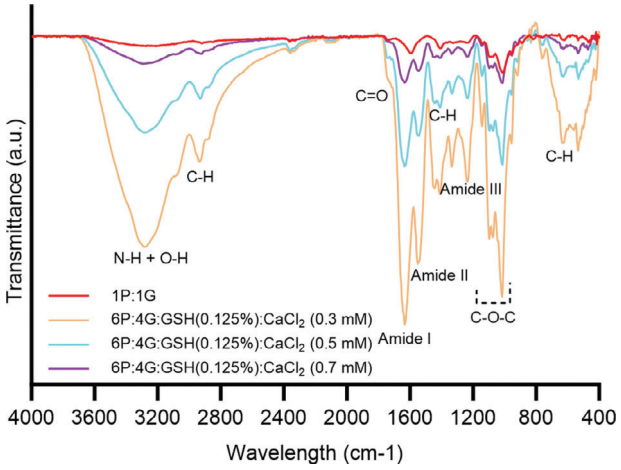

Figure 1. Characterization of composite coatings using FTIR. P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings.

In this study, we prepared composite active edible coatings by blending pectin with gelatin and glutathione. Taking inspiration from pharmacy research in drug development[18] and biomedical science,[19] we crosslinked pectin with calcium chloride to improve its (pectin) intrinsic properties as an innovative approach in edible coatings application. As reported previously,[20] calcium ions (Ca^{2+}) crosslink carboxylate ions in galacturonic acid residues of pectin improving mechanical strength and dramatically slowing down degradation of pectin-based matrix.[20a] The obtained pectin/gelatin/glutathione/calcium chloride hybrid edible coatings in this study were investigated for their physicochemical and food preservation effectiveness of local butter. Glutathione (GSH) was supplemented due to its intrinsic antioxidant properties[14] with a hypothesis that its inclusion will significantly enhance properties of produced edible coatings. We observed a significant preservation and shelf-life improvement of coated butter with the increasing amount of crosslinking over 60 days storage period.

2. Results and Discussion

2.1. Coating Characterization

2.1.1. FTIR

The chemical composition of different constituents in standalone composite edible coatings prepared on Petri dishes was determined using an FTIR. **Figure 1** shows the obtained FTIR data for different coatings. We observed sharp bands at 3270 cm^{-1} with a shoulder at 3084 cm^{-1} attributed to absorption due to stretching

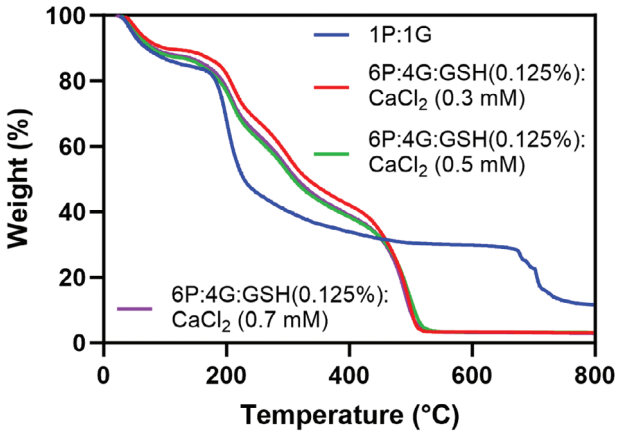

Figure 2. Thermal characterization of composite coatings measured using a TGA. P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings.

of the —OH groups, a sharp band at 2930 cm^{-1} assigned to the —C—H stretching of the —CH_2 groups, a shoulder at 1718 cm^{-1} attributed to the —C═O ester stretching vibration,[23] which could be arising from the contribution of pectin, gelatin, and glutathione. We also observed the three characteristic peptide bands at 1630 cm^{-1} (amide III, combination of —CN stretching and —NH bending vibrations, —CC stretching, and —CH bending), 1545 cm^{-1} (amide II, combination of in-plane —N—H bending, —C—N stretching, and C—C stretching vibrations) and 1230 cm^{-1} (amide I, combination of C—N stretching and N—H in plane and also from C—C stretching and C═O bending vibration).[24] These peptide bands became more prominent in composite pectin/gelatin/GSH coatings compared to the pectin/gelatin (1P:1G) control coating as anticipated due to the presence of peptide linkages in both gelatin and GSH contributing resulting in sharper bands.

The narrow band at 1383 cm^{-1} (shifted to 1418 cm^{-1} in crosslinked samples) can be attributed to the carboxylate groups arising from the presence of polysaccharide (pectin), protein (gelatin), and the peptide (GSH) in composite coatings. The bands between 1096–960 cm^{-1} has been assigned to glycosidic bonds and band at ≈1140 cm^{-1} corresponds to the ring vibration coupled with —C—OH bending vibrations of pectin.[25] The shouldering band observed ≈950 cm^{-1} can be attributed to C—O—C bridge vibrations consider typical to polysaccharides (pectin in this case). Overall, we observed an increase in band intensities with the inclusion of GSH in coatings which can be attributed to the functional groups in polysaccharide GSH which are similar to pectin and gelatin thus having an additive effect on the intensity of signals.

2.1.2. TGA

Thermal stability of the coatings was investigated using a TGA particularly to assess any impact of inclusion of $CaCl_2$ in coatings (**Figure 2**). In all samples, the first primary decomposition was observed between room temperature and 100 °C with the weight loss of ≈10–12%, which can be attributed to the loss of absorbed and molecular water. The second main decomposition was observed 180 to ≈300 °C with the loss of ≈35–38% which can be assigned to the degradation of polysaccharide and protein functional groups. Final decomposition loss of ≈25% was observed between 450 and 530 °C which can be attributed to the complete degradation of the carbon containing components, i.e., polysaccharide and protein backbone. The residual ≈5% weight post 600 °C can be due to the $CaCl_2$ in coatings. No observable difference was observed between different coatings except for the control 1P:1G coating.

In the case of control 1P:1G coating, we observed a rapid degradation in the second decomposition phase, it can be attributed to the fully organic nature of the coating with the lack of inorganic component ($CaCl_2$). In the case of $CaCl_2$ containing coatings, we believe the amount of $CaCl_2$ is not significant to cause a prominent change in the weight loss profile. Overall, all coatings exhibited thermal stability at room temperature and no noticeable difference or detrimental effect on the thermal properties was caused by the inclusion of $CaCl_2$ in these edible coatings.

2.1.3. Optical and Electron Microscopy

Coatings prepared on Petri dishes were subjected to optical and electron microscopy imaging to understand their surface properties. Under optical imaging, all coatings exhibited glossiness with a tint of yellow upon drying at ambient temperature as shown in **Figure 3**. All peeled coatings from Petri dishes were quite thin to determine their thickness. We observed no noticeable difference in consistency between the control and $CaCl_2$ containing coatings, and between coatings containing different amounts of $CaCl_2$ indicating that the inclusion of $CaCl_2$ had no considerable effect on film appearance. Next, we conducted electron imaging of these coatings using a scanning electron microscope (SEM). As shown in Figure 3, we observed a rough surface for the control 1P:1G coating with few nanometre size holes, which could have been caused by bubbles during drying, however, the real reason is not clear at present. In the case of GSH and $CaCl_2$ containing coatings, we observed a wavy pattern on the surface of all coatings regardless of $CaCl_2$ loading. The other uneven and heterogeneously spread protruded features on the surface can be attributed to $CaCl_2$ particles. However, no difference in the number of such protruded features was observed with increasing $CaCl_2$ loading. Overall, no defects or cracks were observed on any coating indicating that inclusion of $CaCl_2$ did not cause any detrimental effect on the consistency of coatings.

2.2. Effect of Composite Edible Coatings on Butter

2.2.1. Acid Value

Rancidity in butter is associated with hydrolytic oxidation resulting in off-flavors described as bitter, wintery, butyric, and lipase.[26] **Figure 4** shows the change in acid value in butter coated with different coatings and stored over a period of 60 days. An increase in acid value was observed under all conditions (uncoated control and coated samples). However, a linear increase in acid value was only observed for uncoated control samples reaching

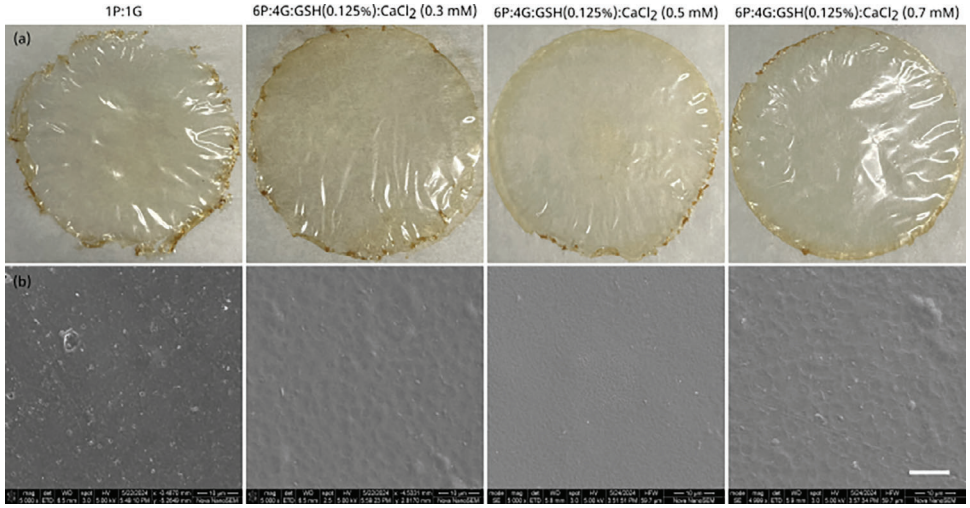

Figure 3. Representative a) optical and b) SEM images of different composite edible films. P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings. Scale bar in (b) 10 µm.

the maximum by ≈3 times at 60 days storage time (≈1.28 mg g^{-1}) than day 0 (0.45 mg g^{-1}). A reduction in the increase in the acid value was observed in all coated samples albeit to different extents. For example, in the case of control 1P:1G coating, a significant increase ($p < 0.01$) was observed at all data points (days of storage), although the increase was significantly lower than uncoated control samples at all data points. In the case of GSH and $CaCl_2$ loaded coatings, a significant reduction in the increase in acid value was observed with increasing amount of $CaCl_2$ loading in coatings compared to uncoated samples and control 1P:1G coatings with the minimum increase observed in 0.7 mM $CaCl_2$ loaded coatings (6P:4G:GSH (0.125%):$CaCl_2$ (0.7 mM)). Given that all GSH and $CaCl_2$ loaded coatings have same amount of GSH, the observed reduction in acid value with increasing storage time can only be attributed to the change in $CaCl_2$ in these coatings. Based on this data, it can be concluded that $CaCl_2$ plays a considerable role in reducing the hydrolytic oxidation of butter thus significantly extending the shelf-life of butter by reducing its rancidification.

2.2.2. Peroxide Value

The peroxide value indicates the primary oxidation of butter. Oxidation caused spoilage in lipid (fat) containing foods such as butter.[17i] It is considered to be on the most efficient methods for determining the initial stages of oxidative rancidity.[16] The starting peroxide value for the butter was 0.26 meq O_2/kg fat (value at day 0). The peroxide value increased in all samples with and without coating during the storage period of 60 days ($p < 0.01$) (**Figure 5**). Although no difference in the peroxide value was observed between different samples (uncoated and coated) during the first 15 days of storage. The peroxide value increased significantly in uncoated samples reaching the maximum value of 3.27 meq O_2/kg fat at day 60 of storage.

This was in line with a previous report showing increasing peroxide value with increasing butter storage time.[17a] Samples coated with the 1P:1G coating also exhibited a more linear increase in the peroxide value during the storage period reaching the maximum value of ≈2.87 meq O_2/kg fat. Although the increase in peroxide value was slower than uncoated control samples. Furthermore, difference in peroxide values increased between control 1P:1G coatings and uncoated control samples with storage time reaching significance at day 30 and remained significantly lower until day 60 (than uncoated control samples). In the case of GSH and $CaCl_2$ loaded coatings, a significant reduction in increase in peroxide values was observed with increasing amount of $CaCl_2$ loading in coatings compared to uncoated samples and control 1P:1G coatings. Between GSH and $CaCl_2$ loaded coatings, the increase in peroxide value was more tapered with the difference between different GSH and $CaCl_2$ loaded coatings varied at different storage time periods. However, the difference in peroxide value became significant at day 60 of storage between different GSH and $CaCl_2$ loaded coatings with the minimum value of ≈1.85 meq O_2/kg fat observed for 0.7 mM $CaCl_2$ loaded coatings (6P:4G:GSH (0.125%):$CaCl_2$ (0.7 mM)). It is difficult to make a direct comparison with previous studies because different butter has different peroxide values. Therefore, comparison is made in terms of the impact of treatment strategies on changes in peroxide value with previous studies. The obtained results conform with previous studies showing that the inclusion of antioxidants leads to a significant retardation in the increase in peroxide value in treated butter samples during the storage period (which in itself leads to increase in peroxide value both with or without coating).[9a,17a,i]

2.2.3. Beta (β)carotene

β-carotene is naturally found in butter and associated with its yellow color. β-carotene has a strong antioxidant activity by antagonising autooxidation of natural lipids by peroxyl radicals, trapping free radicals, and quenching singlet oxygen.[17b,27] The reduction in β-carotene is associated with oxidation mediated discoloration of butter.[11] We investigated the effects of different coatings on naturally occurring β-carotene in the butter used

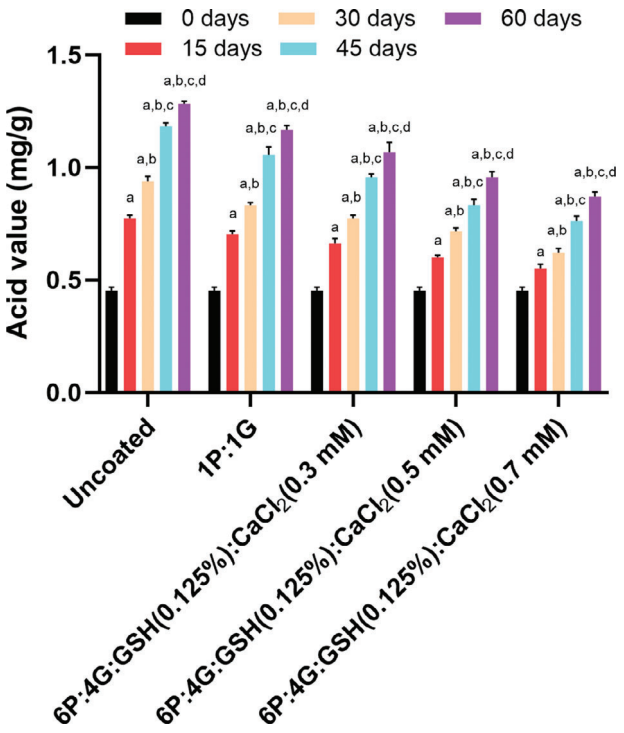

Figure 4. The acid value of butter samples treated with different coatings during the storage period of 60 days. Data are presented as mean ± SD ($n = 3$). P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$). Values with different letters on the top of bars are significantly different ($p < 0.01$) and determined by using a Turkey test in a one-way ANOVA analysis – a, b, c, d, e are relative to untreated and treated butter samples at day 0, 15, 30, 45, and 60 days of storage, respectively.

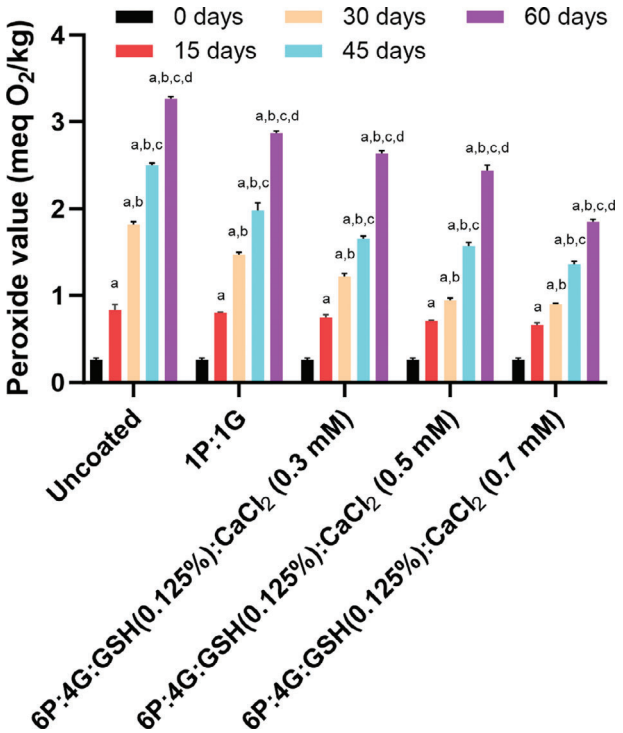

Figure 5. The peroxide value of butter samples treated with different coatings during the storage period of 60 days. Data are presented as mean ± SD ($n = 3$). P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$). Values with different letters on the top of bars are significantly different ($p < 0.01$) and determined by using a Turkey test in a one-way ANOVA analysis – a, b, c, d, e are relative to untreated and treated butter samples at day 0, 15, 30, 45, and 60 days of storage, respectively.

in this study. The starting β-carotene value of uncoated butter was ≈3.46 mg/100 g of butter (**Figure 6**). A significant reduction in β-carotene was observed in uncoated butter samples from ≈3.46 to ≈1.76 mg/100 g by day 60 of storage (total reduction of 1.7 mg/100 g) ($p < 0.01$). This dramatic reduction is indicative of oxidation and rancidity of butter during storage. In the case of control 1P:1G coating, some reduction in β-carotene was observed during storage reaching the minimum value of ≈2.55 mg/100 g butter, a total reduction of ≈0.91 mg/100 g butter. The improved performance of 1P:1G coating compared to uncoated samples can be attributed to the combination of i) intrinsic antioxidant properties of pectin due to the free galacturonic acid groups which can scavenge free radicals,[11,28] and ii) presence of glycine, proline, and hydroxyproline amino acids in gelatin known for their free radical scavenging ability.

The reduction in β-carotene amount was significantly curtailed when butter samples were coated with GSH and $CaCl_2$ loaded coatings with minimum reduction observed in 0.7 mM $CaCl_2$ loaded coatings (total reduction of ≈0.34 mg/100 g) followed by 0.5 mM $CaCl_2$ loaded coatings (total reduction of ≈0.5 mg/100 g) and 0.3 mM $CaCl_2$ loaded coatings (total reduction of ≈0.7 mg/100 g). Compared to control 1P:1G coated and uncoated samples, GSH and $CaCl_2$ loaded coatings exhibited significantly better performance in terms of hindering reduction in natural β-carotene amount in coated butter samples indicating protective effect of GSH and $CaCl_2$ in edible coatings. Due to the same amount of GSH in all GSH and $CaCl_2$ loaded coatings, the difference observed amongst them has to be attributed to the $CaCl_2$ in these coatings. The supplementation of β-carotene in butter has been reported to improve the oxidation stability of butter.[27] The obtained results conform with previous studies showing that the inclusion of antioxidant constituents in active coatings can significantly improve the retention of β-carotene in coated butter.[9a,11]

2.2.4. Antioxidant Capacity DPPH Radical Scavenging Activity Assay

The DPPH (2,2′-diphenyl-1-picrylhydrazyl radical) is a standard colorimetric assay, which can be used to determine the antioxidant effect of edible coatings as shown previously.[4a,29] A typical DPPH assay involves reaction of DPPH radicals with the antioxidant species in the coating resulting in a formation of yellow color from starting violet color.

The determination of the intensity of the changes color leads to the quantification of the antioxidant efficiency of a test sample, i.e., higher the intensity higher the antioxidant activity and lower the amount of free radical formation. The DPPH activity of different coatings over the storage period of 60 days is shown

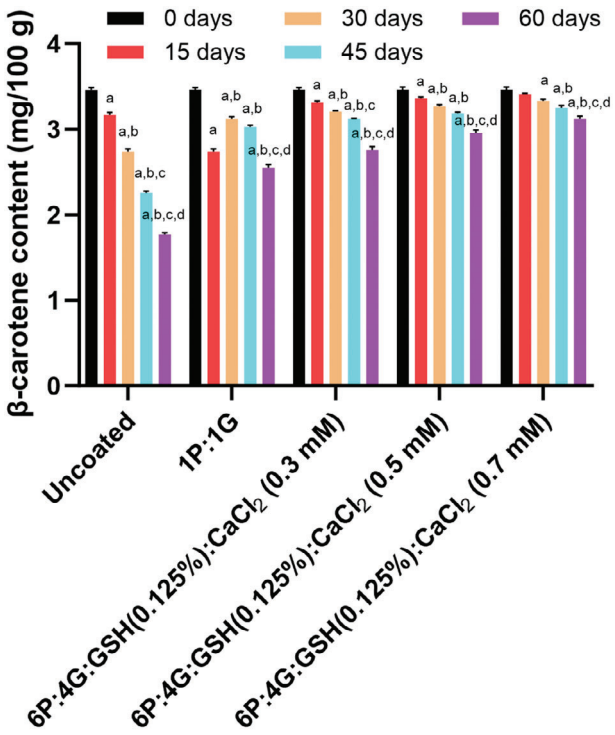

Figure 6. The β-carotene content of butter samples treated with different coatings during the storage period of 60 days. Data are presented as mean ± SD ($n = 3$). P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$). Values with different letters on the top of bars are significantly different ($p < 0.01$) and determined by using a Turkey test in a one-way ANOVA analysis – a, b, c, d, e are relative to untreated and treated butter samples at day 0, 15, 30, 45, and 60 days of storage, respectively.

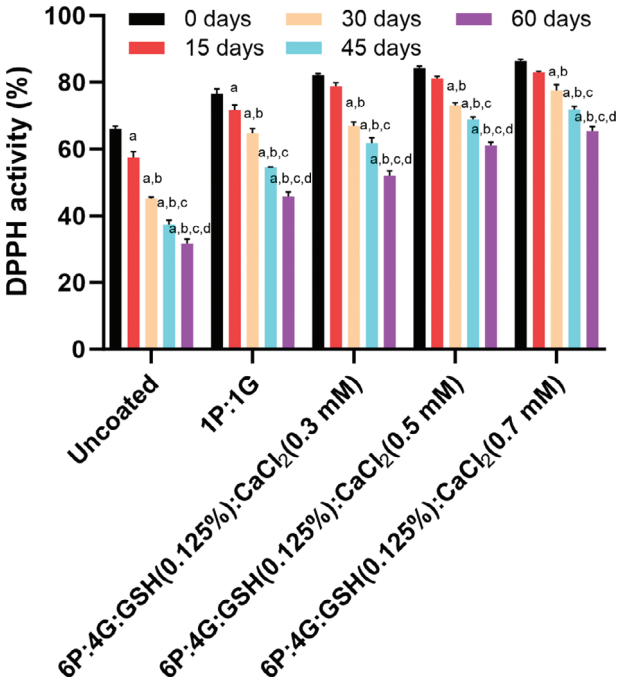

Figure 7. The total antioxidant (DPPH) activity of different coatings during the storage period of 60 days. Data are presented as mean ± SD ($n = 3$). P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$). Values with different letters on the top of bars are significantly different ($p < 0.01$) and determined by using a Turkey test in a one-way ANOVA analysis – a, b, c, d, e are relative to untreated and treated butter samples at day 0, 15, 30, 45, and 60 days of storage, respectively.

in **Figure 7**. In the case of uncoated samples, a dramatic reduction in DPPH activity ($p < 0.01$) was observed during the 60 days storage period reaching the minimum value of ≈31.7% by day 60. Samples coated with the 1P:1G coating exhibited a significantly lower reduction in DPPH activity than uncoated control samples at all time points reaching the minimum value of ≈45.7% at day 60 of storage. The improvement in DHHP response in 1P:1G coatings compared to uncoated control can be attributed to the hydroxyl groups in pectin which can act as proton donors thus exhibiting antioxidant response.[11,28] In the case of GSH and $CaCl_2$ loaded coatings, the extent of reduction in DHHP activity reduced with increasing amount of $CaCl_2$ in coatings where 0.7 mM $CaCl_2$ loaded coatings (6P:4G:GSH (0.125%):$CaCl_2$ (0.7 mM)) exhibited minimum reduction in the activity followed by 0.5 and 0.3 mM $CaCl_2$ loaded coatings, respectively. Compared to day 0, at day 60 of storage, 6P:4G:GSH (0.125%):$CaCl_2$ (0.7 mM) coatings exhibited the total reduction in DHHP activity of 21%, followed by 23% in 6P:4G:GSH (0.125%):$CaCl_2$ (0.5 mM) coatings and 31% in 6P:4G:GSH (0.125%):$CaCl_2$ (0.3 mM) coating. The inclusion of antioxidant GSH further enhanced the total protective response by reducing the concentration of free radical production. The reduction in the concentration of free radicals has been reported to directly influences the oxidation of fatty acids, hydroperoxides and thus restricts the production of side products in butter, thus preserving it against unpleasant taste and consequently increasing its shelf-life.[9a] Our results are in agreement with previous reports.[1,17i] For example, chitosan/sandalwood essential oil coated butter samples retains the DPPH activity during storage compared to uncoated control which was attributed to the antioxidant properties of the essential oil.[1]

2.2.5. Images of Coated Butter

The physical appearance of uncoated and coated butter samples during storage is shown in **Figure 8**. We observed a gradual degradation and decay of uncoated control butter samples which became mouldy and pale by day 60. No such clear decay was observed in coated samples. In the case of coated samples, we do observe a retention of color in butter samples which corroborates the β-carotene data, which has been reported to be associated with the color of butter. Although a marginal paleness in color was observed in control 1P:1G coated butter samples, which is also supported by the β-carotene data that was reduced during the storage period. Just by visual inspection no noticeable difference was observed in composite coated samples (6P:4G:GSH (0.125%):$CaCl_2$) independent of the amount of $CaCl_2$. Taken together, it is postulated that developed composite edible coatings preserved butter from decomposition during storage thus prolonging their shelf-life.

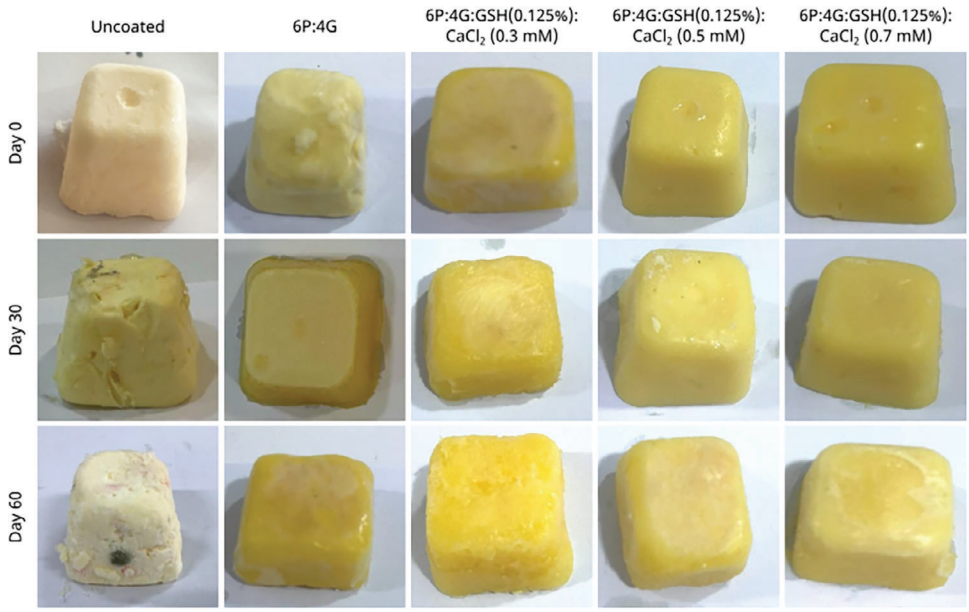

Figure 8. The representative optical images of uncoated control and coated butter samples during storage (0, 30, and 60 days after coating). Gray mold and partial discoloration in uncoated control butter sample particularly at day 60 indicate fouling. P stands for pectin, G for gelatin, GSH for glutathione, and $CaCl_2$ for calcium chloride. All ratios are weight ratios between the different components used to prepare coatings.

3. Conclusion

The short shelf-life of dairy products is a major limiting factor in the globalization of this industry. Despite advance refrigeration facilities leading to considerable improvement in the shelf-life of dairy products such as butter, there is still a limitation on their shelf-life. In the case of butter, despite refrigeration, it is prone to spoilage mediated by the oxidation of fat leading to rancidity, which renders it (butter) unusable. Most strategies cater to this challenge by incorporating materials (natural extracts and additives) to prolong the shelf-life of butter by limiting rancidification. However, this can change the favor profile of the product. Alternatively, edible coatings have started to gain interest as a preservation strategy. In this study, composite edible coatings comprising pectin/gelatin/GSH were developed for the preservation of butter over a 60 day storage period. In order to improve the intrinsic properties of pectin, we crosslinked it with calcium chloride at different concentrations and studied its (amount of calcium chloride) influence on preservation characteristics of produced coatings. The analytical characterization using FTIR revealed the presence of pectin/gelatin/glutathione in coatings and the inclusion of calcium chloride significantly enhanced the thermal properties (as adjudged from TGA) of composite coatings without any detrimental impact on their consistency based on imaging. We observed a significant improvement in coating performance in terms of acid value, peroxide value, and free fatty acid. Comparison between different coatings revealed a calcium chloride dose dependence on their performance (0.7 mM $CaCl_2$ containing coatings exhibiting best performance) indicating a significant improvement in preservation of coated butter compared to uncoated and the control coating. Furthermore, the inclusion of antioxidant GSH lead to a significant improvement in the overall preservation of coated butter samples by reducing the production of (rancidity causing) free radicals as adjudged from the retention of β-carotene and minimal reduction in DPPH activity during the 60 days storage period in composite coatings compared to the uncoated control. We project further exploration of in situ crosslinking approaches in natural polysaccharides-based in particular pectin-based edible coatings potentially leading to the development of commercial coatings.

4. Experimental Section

Materials: Gelatin (from bovine) was purchased from Merck (Germany), glutathione (GSH) was purchased from Avonchem (UK), pectin (from apple), calcium chloride ($CaCl_2$, 93% purity), glacial acetic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium hydroxide, sodium thiosulphate, starch, potassium iodide, methanol, chloroform, acetone, hexane, petroleum ether were obtained from Sigma–Aldrich (Germany), glycerol used as a plasticizer was purchased from Merck (Inc. Corp. Whitehouse Station, NJ, USA). All commercial chemicals used in this work were analytical grade. The packaging properties of the developed edible coatings were investigated on local butter purchased from Basrah city markets (Basrah, Iraq).

Coating Preparation: The pectin: gelatin: glutathione: calcium chloride ($CaCl_2$) coatings were prepared with different concentration of $CaCl_2$. First, pectin (1% wt./v) was dissolved in distilled water at 40 °C for 30 min, simultaneously, gelatin (1% wt./v) was dissolved in hot water at 50 °C for 20 min. The two solutions were mixed, and glycerol (20% wt./wt.) was added to the mixture to plasticize the coatings and homogenized for 15 min, followed by 0.125% GSH. Finally, $CaCl_2$ at concentrations of 0.3, 0.5, and 0.7 mM was added to crosslink the solution and obtain the film. The entire solution was aliquoted into two halves, one was used to prepare standalone films by drying the mixed solution in 15 mL Petri dishes, and the other half was used to coat butter samples. The final coatings were abbreviated as 6P:4G:GSH (0.125%):$CaCl_2$ (mM) where 6P and 4G represent the ratio of pectin and gelatin. Pectin and gelatin (6P:4G) coating without GSH and $CaCl_2$ was prepared as a control.

Fourier Transform Infrared Spectrometer (FTIR): The standalone coatings were used for FTIR analysis using a Bruker ATR-FTIR (Germany) under the wavelength range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}, on average 32 scans were measured.

Thermogravimetric Analysis (TGA): To assess the thermal properties of coatings, standalone coatings were subjected to TGA using a TA Instruments TGA Q5000. Approximately 2–5 mg of sample was loaded onto a platinum sample holder, which was heated at a temperature ramp of room temperature (20–30 °C) to 800 °C with a 10 °C min^{-1} heating rate under an air atmosphere with a flow rate of 25 mL min^{-1}.

Scanning Electron Microscopy (SEM): The surface profile of coatings was studied using a SEM (FEI Nova NanoSEM 450 FE-SEM) operating at an accelerating voltage of 5 kV. To this end, platinum was sputter coated onto standalone coatings prior to imaging.

Coating of Butter Samples: Butter was melted and poured into silicon molds (2×2 cm^2) to obtain uniform-shaped samples. In the interim, coating mixtures were cooled at 4 °C. Once butter samples were solidified, coating mixture was applied on all sides multiple times using a silicon brush to entire consistent coating and placed on a metal clip to dry under cooling. Uncoated butter samples were prepared as controls. All uncoated and coated butter samples were stored in glass containers in dark in a freezer (−4 °C) for the experimental duration of 60 days. The detailed analysis on the impact of different coatings was conducted at different time points (0, 15, 30, 45, and 60 days).

Peroxide Value (PV): To determine the peroxide value, uncoated and coated butter samples were dissolved in a solution of chloroform and glacial acetic acid in a ratio of 40:60 mL. To this solution, a saturated solution of potassium iodide (1 mL) was added, stirred for 1 min and stored for 5 min in dark. Following which, water (25 mL) and starch reagent (1 mL) were added to the solution and titrated against sodium thiosulfate (0.01 N), and the peroxide value (PV) was calculated using Equation (1):

$$PV \left(meq.\frac{O_2}{kg}\text{butter}\right) = \frac{(S-B)\,xNx1000}{\text{smple weight (kg)}} \quad (1)$$

where, S is the volume of thiosulphate (ml) used in the coated sample, B is the volume of thiosulphate (ml) consumed for the control sample, N is the normality of sodium thiosulphate solution, and the weight of the butter sample (in g).

Acid Value (AV): The acid value was determined using a previously published method with modifications.[21] Briefly, melted butter sample (1 g) was added to a flask containing ethanol (15 mL), chloroform (15 mL), and two drops of phenolphthalein indicator, this mixture was then titrated against sodium hydroxide (0.1 N) until the purple red color appeared. AV was calculated using Equation (2):

$$AV = \frac{(a-B)\ x\ N\ x\ M}{W} \quad (2)$$

where, a is the consumption of titration NaOH solution volume in sample butter (mL); B is the used volume of NaOH titration solution in blank sample; N is the normality of NaOH solution; W is the weight of the butter sample, and M is sodium hydroxide molar mass (g mol^{-1}).

Determination of β-carotene: One gram of coated and uncoated butter samples were separately mixed with a solution of acetone:hexane (2:3, v/v) and stirred to obtain a homogeneous solution in an ice bath. The solutions were then centrifuged at 5000 rpm for 10 min and the supernatant was subjected to UV/Vis spectroscopy where the maximum absorption was recorded at 453, 505, 645, and 663 nm. The amount of β-carotene was calculated using Equation (3):

$$\beta\text{-carotene}\left(\frac{\text{mg}}{100}\text{mL}\right) = 0.216A_{(663)} - 1.22A_{(645)} - 0.304A_{(505)} + 0.452A_{(453)} \quad (3)$$

where, A is measured absorbance at specific wavelengths

Antioxidant Activity: To determine the antioxidant activity, a 2,2-diphenyl-1-picryhydrazyl (DPPH) assay was used on both uncoated and coated butter samples. DPPH is a standard assay to determine the antioxidant activity of materials.[4b,22] Briefly, 1 g of butter samples were dissolved in petroleum ether (10 mL) and 1 mL of this solution was mixed with 1 mL of DPPH (0.01 mM). The mixed solutions were then incubated for 30 min in dark at room temperature followed by absorption measurement at 517 nm to quantify the color change in mixtures.

The amount of antioxidant DPPH activity was calculated using Equation (4):

$$\text{DPPH activity } (\%) = \left[\frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}}\right] \text{x } 100 \quad (4)$$

Statistics: The results for coating performance on butter samples are expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA). Significance was evaluated using a Turkey analysis and set at 99% confidence ($p < 0.01$).

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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antioxidant, butter coating, calcium chloride, composite coatings, edible coatings, food packaging, gelatin, glutathione, pectin

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