

EFFECT OF REINFORCED ORANGE PEEL FILMS ON QUALITY ATTRIBUTES OF BUTTER DURING STORAGE

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Abstract

Packaging is a useful approach in improving the quality, safety and shelf life of food products. In current research, the orange peel films reinforced with wheat straw cellulose were applied on butter. The butter wrapped with films was compared with unwrapped butter during 30 days of storage at 4 °C. The quality of butter was assessed through physicochemical (pH, acidity, peroxide value, hardness), microbial (coliform, yeast and mold) and sensory analysis. The application of films significantly maintained the quality of butter by preventing chemical oxidation and microbial growth. The films also prevented moisture loss and maintained the hardness of butter. Further, the film wrapping also reduced the rate of pH decrease and acidity increase. The wrapped butter showed higher L*, a* and b* values compared to unwrapped butter. The wrapped butter scored high sensory points (color, texture, aroma and overall acceptability) compared to unwrapped butter throughout the storage period. The quality and safety of butter was significantly maintained during storage with the application of reinforced orange peel films.

Keywords: Biodegradable Packaging, Butter Quality, Bioactive Properties, Shelflife.

INTRODUCTION

Packaging is a fundamental part of food industry that remarkably improves the food handling, storage, preservation and transport. It also protects the food from external contaminants (microbes, oxidants) and other environmental abuses (temperature, humidity and light). Hence, packaging is essential to ensure food safety and to minimize food losses throughout the supply chain [1]. With increase in consumer awareness and preferences, the functions of packaging have been increased beyond its native functions. Considering the food safety and quality perspectives, food packaging has also been employed to enhance the shelf life as well as monitor the quality of food products. It involves incorporation of active and intelligent substances as a part of packaging material which work in response to change in internal environment of the package. Realini and Marcos [2] defined active packaging as material that interacts with surrounding environment of food and extends its shelf life. While, in case of intelligent packaging, an

indicator is used to monitor the condition of packaged food in response to change in surrounding environment i.e. freshness/spoilage, gas composition, temperature etc. [3].

Environmental issues regarding plastic packaging and consumer awareness have triggered the development and application of biodegradable packaging due to their good biocompatibility, biodegradability, edibility and various potential uses [4]. To make biodegradable packaging materials sustainable, scientists and researchers are trying to exploit the potential of agricultural residues in preparing biodegradable packaging. Agricultural residues are good and cheap source of biopolymers and bioactive compounds which are the primary materials for the development of biodegradable films [5]. Every year, plenty of agriculture residues are produced that serve as a source of inexpensive and renewable biopolymers. Among them, citrus waste is found to possess potential to be used in biodegradable films. Citrus is the major fruit crop of Pakistan covering the area of 192230 hectares with 2344 thousand tones annual production [6]. During citrus processing waste is generated in the form of peels, seeds and segment membranes. Peel covers 40-55% of total waste and is one of the most underutilized biowaste. Moreover, orange peel is rich source of valuable bioactive compounds (β -carotene, limonoids, phenolics) and biopolymers including pectin, hemicellulose and cellulose essential for development of packaging films [7].

Butter is a water-in-oil emulsion containing 16% moisture and 80 % fat. It is one of the conventional methods of milk fat preservation. Like other dairy products, butter is also a perishable food item. Microbes and chemical rancidity cause butter spoilage leading to lowering its sensorial and nutritional attributes [8]. In current study it was hypothesized that orange peel films can contribute to improved shelf life of butter owing to the presence of various active compounds in their composition.

2. METHODOLOGY

Orange peels were collected from the local juice processors at Faisalabad, Punjab, Pakistan. The orange peel powder (OPP) was prepared by drying in dehydrator at 65 °C. The dried peels were ground and passed through sieve (mesh No. 80) to obtain powder. The cellulose was extracted from wheat straw (procured from local farms) according to the method described by Wang, Lin [9].

2.1. Preparation of films

The films of OPP were prepared through casting technique by following previous method [10]. 3 g of OPP was added in distilled water to obtain 3% solution and stirred for 15 mins. Then, glycerol (30% of dry weight) as plasticizer was added in the mixture followed by heating at 85 °C for 30 mins with continuous stirring. Subsequently, 12 % wheat straw cellulose (WSC) was added as reinforcement material into mixture and homogenized for 2 mins at 10000 rpm using Ultra-Turrax homogenizer (T18 D, IKA-Germany). After that the mixture was stirred using magnetic stirrer (RHT-340, UK) at 90 °C for 1 hr to obtain homogeneous film forming solution. The solution was casted on petri plates succeeding

to drying in hot air over at 50 °C. Finally, the films were conditioned at room temperature and peeled off.

2.2. Application of Films

The butter was prepared using traditional method as explained by Shekhara Naik, Pavithra [11]. 25 g of solid butter was wrapped in 10×10 cm² films (see figure 1) and kept at 4 °C for 30 days. The quality of wrapped and unwrapped butter was assessed at ten days intervals during storage. Following parameters were analyzed as quality indicators:

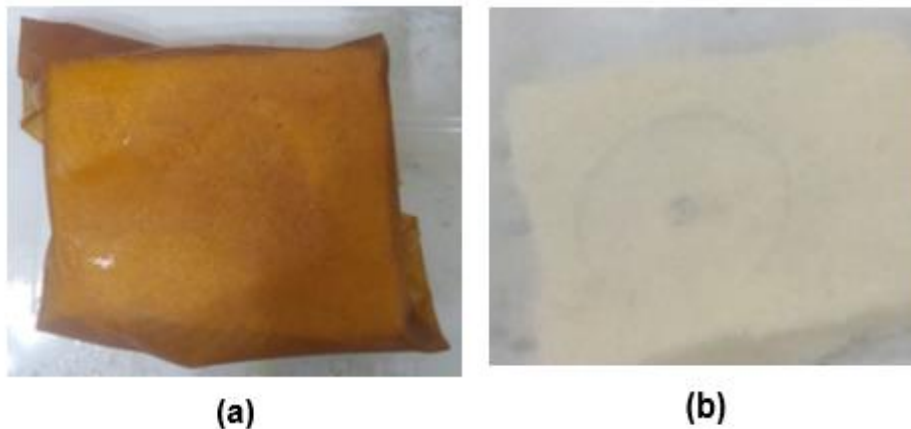


Figure 1: Storage of butter with (a) and without (b) film wrapping

2.2.1. Color

The color of butter was analyzed through colorimeter (Color Tec-PCM, USA) using the protocol explained by Sert and Mercan [12].

2.2.2. Hardness

Hardness of butter was monitored through texture analyzer (TA-XT plus Stable Microsystems, UK) using wire cutting probe [13]. The butter for texture analysis was cut into 5×3 cm pieces. The cutting probe was penetrated 35mm/min into butter with 1mm/s speed. The force (Kg) applied on the better was recorded as butter hardness.

2.2.3. Acidity

The acidity of butter was monitored according to the protocol explained by Sert and Mercan [14]. Firstly, 10 g of butter was mixed with boiling deionized water (90 ml). After mixing, 5 drops of phenolphthalein (1%) were added in mixture as indicator. The titration was carried out with 0.1 N NaOH till the rosy, pink color was obtained. The acidity was measured as % lactic acid and calculated as:

$$Acidity = \frac{Volume\ of\ NaOH\ used \times normality \times 0.009}{weight\ of\ sample} \times 100$$

2.2.4. Peroxide value (PV)

The peroxide value of butter was analyzed according to protocol defined by Pirsá and Asadi [15]. For this, butter (5g) was mixed with 30 mL acetic acid and chloroform (3:2) followed by addition of 0.3 mL saturated potassium iodide solution. After that, distilled water (30mL) and 1% soluble starch solution (0.5 mL) were added. Then, the solution was titrated against 0.01 N sodium thiosulphate. The PV was measured in milli equivalent of iodine per kilogram of fat/oil (mEq/Kg) using below equation:

$$\text{Peroxide value} = \frac{(S - B) \times N \times 1000}{W}$$

Where W is weight of sample, N is normality of sodium thiosulphate, S and B are the volumes of titrant used for sample and blank, respectively.

2.2.5. Total coliform

For coliform count, 1 g of butter was mixed with 9 mL saline solution (8.5g/L) at 40 °C followed by serial dilution in same solution. Then, 1 ml of diluent was poured on violet red bile agar (VRBA) and incubated for 2 days at 37 °C. The colonies were counted using colony counter [16].

2.2.6. Yeast and mold

For yeast and mold, 1 ml of diluent was poured over potato dextrose agar with pH adjusted to 3.5. Then, inoculated plates were incubated for 7 days at 21 °C [17].

2.2.7. Sensory evaluation

Sensory attributes of wrapped and unwrapped butter were investigated using protocol of Hřivná, Machálková [18]. The sensory panel was consisted of 10 postgraduate students including both male and female between age groups of 23-30 years. The panelists were blinded about the samples by coding the samples with random three digits. The samples were analyzed for texture (hardness, melting rate, spreadability, adhesiveness), odor, color and overall acceptability. The water was used for mouth rinse between the analysis. The samples were rated according to 9-point hedonic scale ranging from 1 (extremely like) to 9 (extremely dislike). The sensory evaluation was carried out just after production, day 10, day 20 and day 30.

2.3. Statistical analysis

The obtained data was analyzed using analysis of variance (ANOVA) through statistix 8.1 for windows. The 2-way ANOVA was used for analyzing the effect of film application and storage time on the quality parameters of films storage study data. The data was presented in mean±SD of three replicates. The difference between mean values of different treatments was evaluated using tukey test at significant level $P < 0.05$ [19].

3. RESULTS AND DISCUSSION

3.1. Color

Color is an influential and promising parameter regarding consumer acceptability. It gives an indication of product freshness that may change during storage as a result of microbial and chemical reactions. It also plays an important role in marketing and sensorial perception. The color of butter was measured in colorimeter that presents L^* , a^* and b^* values. The L^* value indicates the level of lightness. $+a^*$ values show the redness and $-a^*$ values present the greenness of specimen. Whereas, $+b^*$ values indicate the presence of yellowness and $-b^*$ show blueness of the sample [20].

The film application significantly affected the color values of butter as shown in tables 3.1. The color values changed over time in both wrapped (B_1) and unwrapped butter (B_0). The L^* value decreased from 93.64 to 79.51 in unwrapped butter and up to 83.24 in wrapped butter. Similarly, the a^* value decreased with time and more decrease was observed in B_0 (6.87 to -3.54) compared to B_1 (6.87 to 0.11). Moreover, the b^* value increased significantly with time in both butter samples but the B_0 (27.37-41.63) presented more increase as compared to the B_1 (27.37-35.8).

The results clearly show that a more color change was observed in unwrapped butter. The change in color of butter can be attributed to the oxidation of milk chromophores (β -carotenes) and microbial activity (production of lipase enzymes) [21]. The reinforced films owing to their phenolic contents, possess antimicrobial and antioxidant properties. This resulted in less color change in wrapped butter during storage [22].

Amjadi, Emaminia [23] applied the gelatin/chitosan nanofiber composite films reinforced with ZnO nanoparticles on cheese and fish fillets. The authors observed more color change in neat food models (unwrapped) compared to wrapped samples. Similarly, plantago major seed gum composite films were activated with fennel EO and reinforced with nanoclay. The butter was wrapped into neat seed gum films and reinforced activated films at 4 °C for 40 days. The highest color difference was noticed in unwrapped butter compared to wrapped butter [24].

Nor Adilah, Noranizan [25] applied the gelatin and mango peel extract coated polyethylene films on margarine for 28 days at 4 °C and 25 °C. The significant decrease in L^* and a^* values while an increase in b^* values over time was observed. Moreover, higher color change was observed in uncoated films as compared to coated films. Pirsá and Asadi [15] also reported a decrease in color values with storage time and the color changes decreased by the application of PLA/lycopene films.

Table 3.1: Color values of wrapped and unwrapped butter during storage

Days	L^*		a^*		b^*	
	Unwrapped	Wrapped	Unwrapped	Wrapped	Unwrapped	Wrapped
0	93.65±0.05 ^g		6.8±0.05 ^a		27.37±0.06 ^g	
10	87.78±0.2 ^e	90.28±0.04 ^f	2.29±0.05 ^c	4.15±0.02 ^b	32.17±0.5 ^e	29.37±0.03 ^f
20	81.18±0.4 ^b	87.31±0.3 ^d	-2.06±0.03 ^f	2.08±0.03 ^d	36.45±0.2 ^b	33.06±0.5 ^d
30	79.51±0.1	84.58±0.2 ^c	-3.54±0.03 ^g	0.11±0.06 ^e	41.63±0.6 ^a	35.80±0.3 ^c

3.2. Hardness

Hardness is an important textural attribute of a product essential for its acceptance and application. The hardness of butter was measured through texture analyzer using a cutting probe. Hardness of butter was remarkably affected by film application during storage. The hardness of butter increased with the passage of time, but the wrapped butter showed less increase compared to unwrapped butter (see figure 2). The hardness for unwrapped butter was increased with storage time. The increase in butter hardness can be explained by the moisture loss and increase in TSS during storage. Moreover, the less hardness in wrapped butter can be attributed to the moisture barrier properties of films [26].

Aziman, Jawaid [26] applied sage extract antimicrobial films on meat and compared with the control (without film). The hardness increased with time and control samples showed more hardness (N) compared to packed samples. Mahcene, Khelil [27] coated cheese with sodium alginate films activated with EO, which improved the quality of cheese as compared to uncoated. Coated cheese exhibited significantly less hardness than uncoated cheese. Aminian-Dehkordi, Ghaderi-Ghahfarokhi [28] evaluated the potential of savory oil activated sage gum/sodium alginate films for shelf life extension of cheese. The hardness of cheese increased during storage due to moisture loss but the application of films remarkably reduced increase in hardness of cheese.

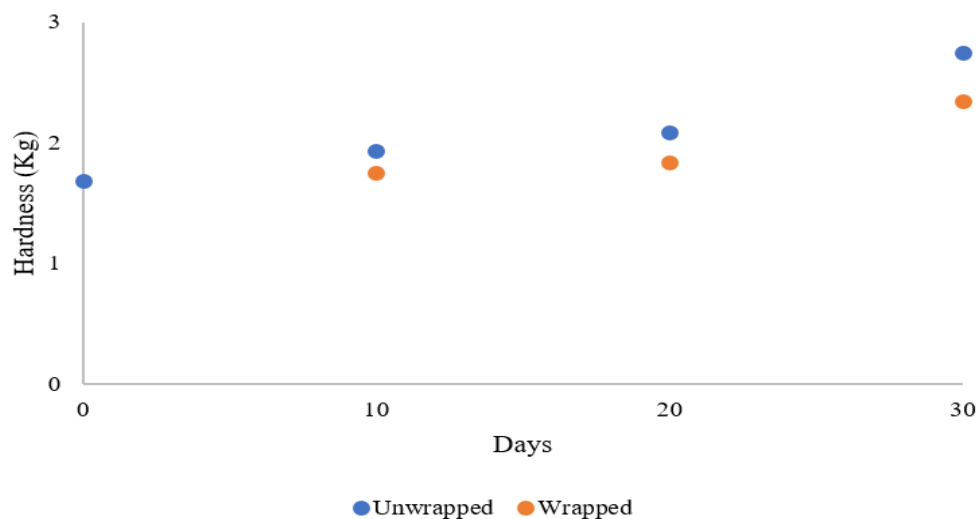


Figure 2: Hardness (kg) of wrapped and unwrapped butter during storage

3.3. pH and Acidity

Acidity has great impact on the texture and flavor of butter. An increase in free fatty acid contents in fats and oils; as a result of breakdown of triglycerides in the presence of water, tends to trigger the acidity of butter. The oxidation and rancidity can increase the acidity which ultimately influences quality and organoleptic properties [24]. pH and acidity are inter-relatable parameters where the increased acidity represents the decrease in pH.

The effect of film wrapping on acidity and pH of butter during storage has been presented in figures 3. The acidity increased whereas the pH decreased for both wrapped and unwrapped butter (respectively) throughout the storage. Moreover, a significant difference between the acidity and pH of wrapped and unwrapped butter was observed. The increase in acidity and decrease in pH can be correlated with free acid production as result of hydrolysis of the fatty acids in butter during oxidation [25].

The wrapped butter recorded less change in pH (6.53-6.08) and acidity (0.02-0.17%) compared to unwrapped (6.53-5.81 and 0.02-1.17%, respectively). These findings can be justified by the antioxidant, anti-microbial and barrier properties of cellulose reinforced orange peel films [22]. The observations were in line with the results of Santacruz [29] who evaluated the effect of starch and *L. acidophilus* films on the shelf life of fresh cheese. The authors reported a high decrease in pH and increase in acidity of coated cheese compared to control cheese during 30 days of storage.

Similarly, Ríos-de-Benito, Escamilla-García [30] also suggested relevant findings who developed chitosan/sodium caseinate films reinforced with silica nanoparticles (SNP) and activated with oregano essential oil. The effect of films application on cheese quality reported that a highest pH decline was observed in uncoated cheese compared to coated throughout the storage period. Additionally, the uncoated cheese presented 60% more increase in acidity compared to other treatments. Other studies on decreasing effect of films application on pH and acidity change include El-Sayed, El-Sayed [31]; Nor Adilah, Noranizan [25] and Nottagh, Hesari [32].

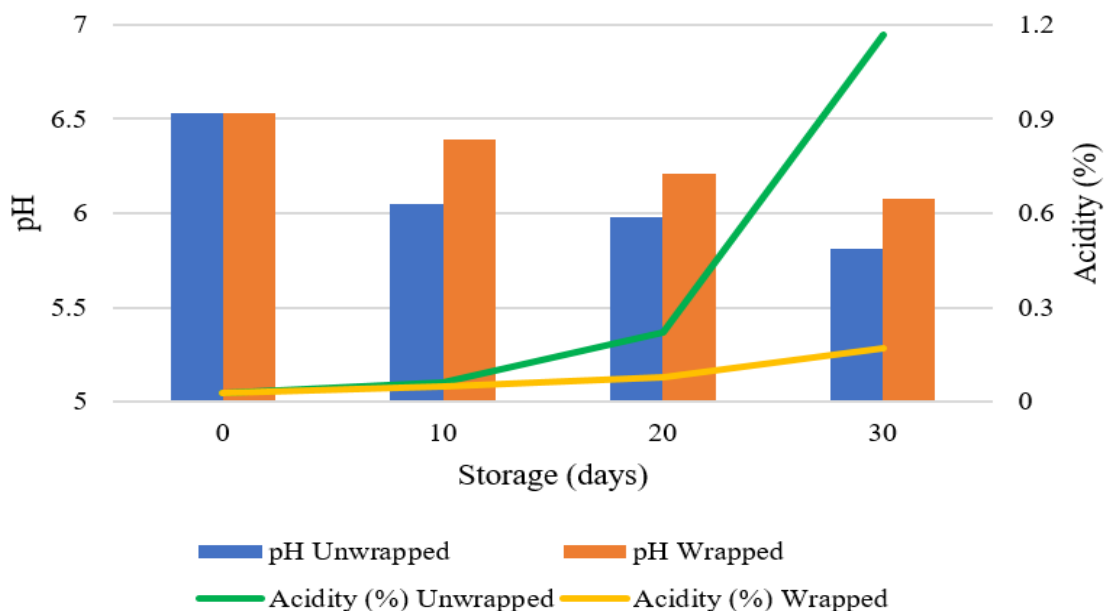


Figure 3: Acidity (%) and pH values of wrapped and unwrapped butter during storage

3.4. Peroxide value (PV)

Oxidation is a quality destructive process which leads to spoilage in lipid containing products like cream, cheese and butter. Oxygen and heat exposure trigger oxidation. Peroxide value is a prominent chemical method for monitoring oxidative deterioration of lipids [33, 34].

Butter contains 82% fat and is more susceptible to oxidation which leads to flavor, nutrient and color loss. The PV of butter was measured using sodium thiosulfate titration method and expressed as milli equivalent peroxide/Kg of lipid. The film application had significant effect on peroxide value of butter (see figure 4). Both butter samples showed an increase in PV but wrapped butter showed less PV in contrast to unwrapped butter at the end of storage. The PV of wrapped and unwrapped butter increased from 0.09 to 1.28 and 2.06, respectively showing that the films significantly prevented the oxidation/ spoilage of butter. This can be attributed to the presence of bioactive compounds in orange peel films that inhibit the oxidation of butter [35]. These results comply with the results of Rossi-Márquez, Helguera [36] who applied the whey protein-pectin activated films on peanut to extend its shelf life. The authors demonstrated that the PV increased with storage time but the coated peanuts exhibited low PV than uncoated during 50 days of storage. Kazemian-Bazkiaee, Ebrahimi [37] also observed a similar trend while applying β -glucan and chitosan coating on roasted peanuts.

Fasihnia, Peighamardoust [38] demonstrated that application of activated PP (polypropylene) films improved the oxidative stability of donuts during storage. The donuts with active films showed less increase in PV value than control. Moreover, Khezrian and Shahbazi [39] also evaluated the potential of CMC and chitosan films loaded with natural extract on quality of camel meat during storage. Decrease in PV was noted by the application of activated films as compared to unpacked meat.

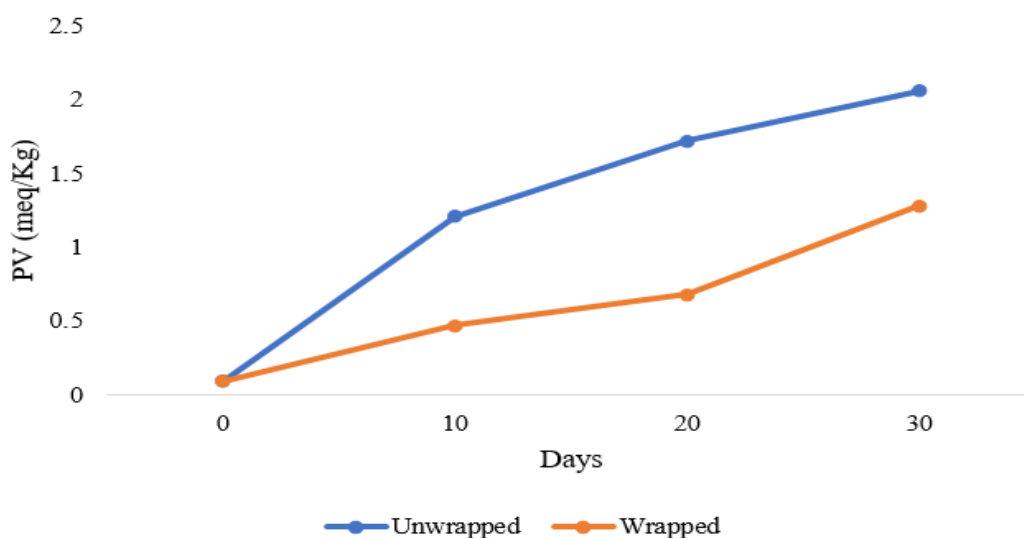
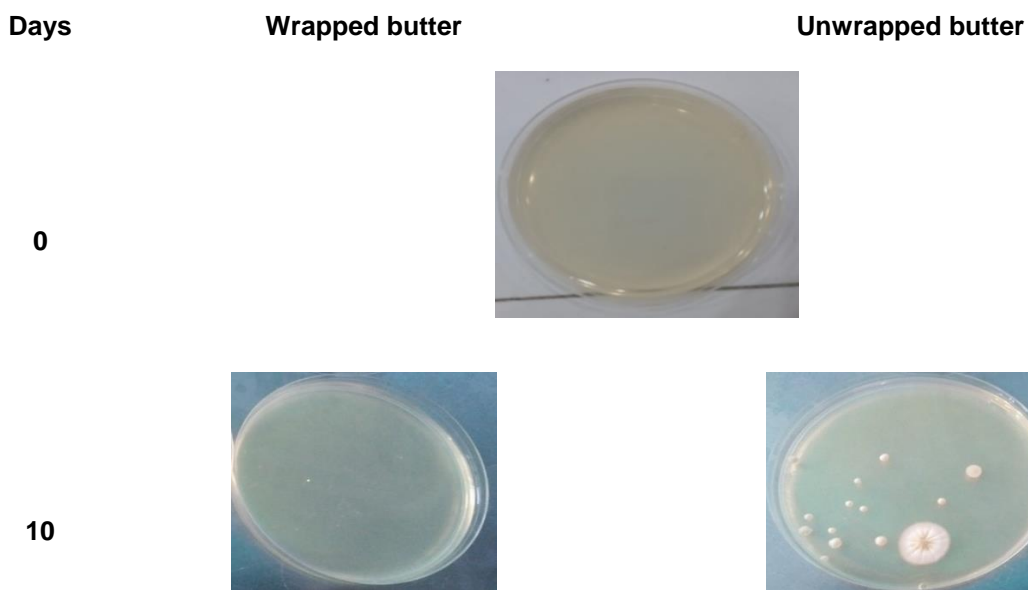


Figure 4: Peroxide value of butter wrapped and unwrapped butter during storage

3.5. Microbial analysis

Coliform bacteria are known as indicator organisms for the sanitary quality of a food product during its processing. Although these are not pathogenic but create suitable environment for the growth of pathogenic microbes. The common coliform bacteria are from the genus *Klebsiella* and *Enterobacter* [40]. There was no coliform found in both butter samples throughout the storage period. This might be due to hygienic conditions during preparation and storage of butter. Similar observation has also been reported by Sharma, Bhat [41] who developed and applied maltodextrin- alginate based active films on meat nuggets. They also observed no coliform in all treatments during storage. Moreover, Esmaeili, Cheraghi [42] also reported the absence of coliform in sausages packed in garlic EO loaded chitosan/whey protein films. Mahajan, Kumar [43] applied *Aloe vera* added carrageenan films on *kulfi* (a traditional Indian ice cream) and did not detect coliform in all treatments throughout the storage.

The application of film had significant effect on yeast and mold count. The films protected the butter from microbial attack. The yeast and mold growth increased significantly with the time but wrapped butter showed less increase (as also shown in figure 5 & 6) thus reflecting the antimicrobial potential of films. The yeast and mold count increased from zero to 2.7×10^{-4} CFU/g for unwrapped butter while 0.9×10^{-4} CFU/g for wrapped. [7]. Sami, Almatrafi [44] reported comparable results during storage study of silicon dioxide coated cantaloupe fresh cuts. The uncoated cuts showed higher yeast and mold unlike the coated cuts. Suwanamornlert, Kerddonfag [45] wrapped bread in polypropylene and PLA active packaging films. They found a decrease in yeast and mold count by the application of activated films as compared to polypropylene. Zhang, Liu [46] also described a decline in yeast and mold count in meat by the application of ginger EO activated sodium alginate films.



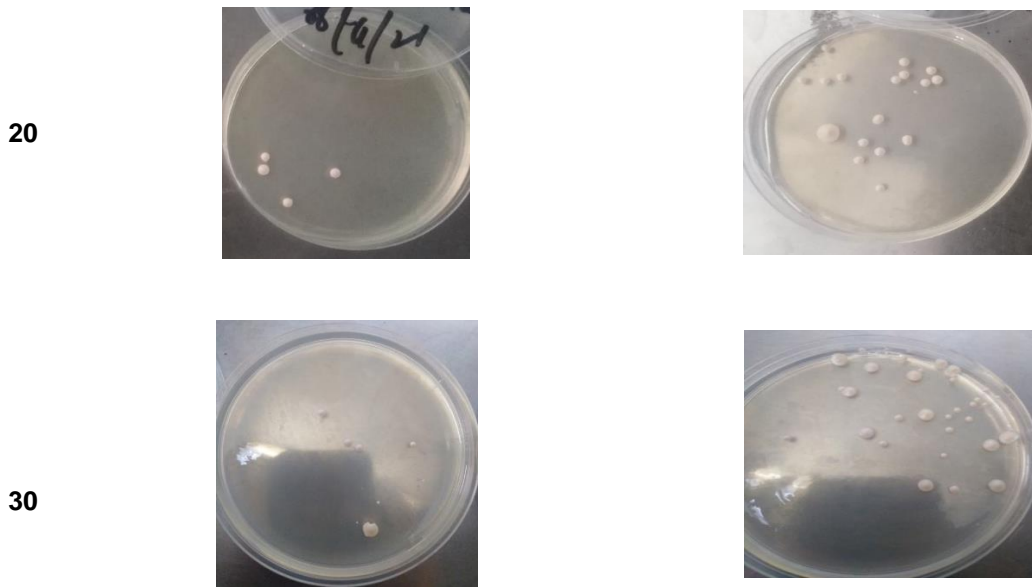


Figure 5: Yeast and mold count (CFU/g) of wrapped and unwrapped butter during storage

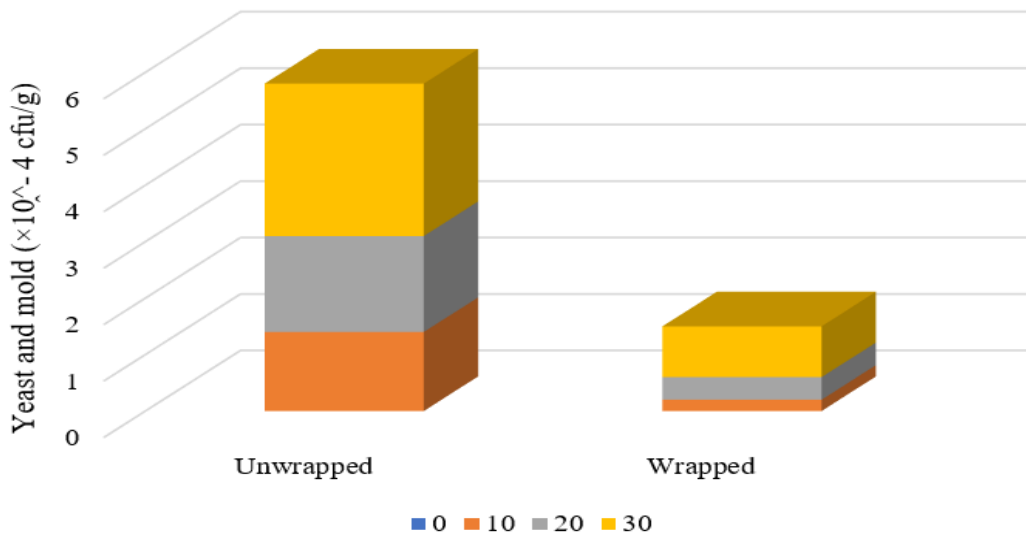


Figure 6: Graphical representation of yeast and mold count ($\times 10^{-4}$ CFU/g) of wrapped and unwrapped butter during storage

3.6. Sensory Evaluation

Organoleptic properties of a product is a prominent and important aspect with which the product competes with other products on the shelf. These attributes include taste, aroma, texture, appearance, color and general acceptability. These can be lost during

stouage as a result of micobial and chemical spoilage. Hence, the sensorial properties are also indicator for the product quality and freshness. To preserve the sensory characeristics of a product, is one of the important function of packaging accomplished by inhibiting the transport of gases, volatiles, moisture, light and microbes between food and enviornment [47].

The sensory properties of butter (aroma, color, texture and overall acceptability) were examined using 9 point hedonic scale. Color is the primary indicator of freshness and consumer acceptance. If the color of a product is not attractive, the consumer may not experience the other organoleptic properties like taste and flavour. The application of films on butter indicated a significant effect on color acceptability. The color scores decreased with time, while, wrapped butter scored more points as compare to the unwrapped. Color scores for wrapped and unwrapped butter decreased during storage. This can be attributed to the oxidative rancidity and microbial deterioriation during storage [22].

Aroma is one of the most effetive parameters regarding the product acceptance. The films application had significant impact on aroma of butter. The aroma of butter decreased with storage time due to oxidation of butter that gerenerates alchohols, aldyhydres, organic acids and ketones [48]. The wrapped butter exhibited less decrease in aroma acceptability than unwrapped. The less decrease of aroma in wrapped butter can be attributed to the barrier and bioactive properties of packaging film [42].

Texture of butter was also noticeably affected by the films application as expressed in figure. A loss in textured was observed in both samples during 30 days of storage. The unwrapped butter was reported with a decline in texture points from 8.6 ± 0.04 to 6.26 ± 0.02 , whereas the wrapped butter decreased up to 7.17 ± 0.05 at the end of storage (day 30). Similarly, the overall acceptability was also improved by the films application as shown in figure 7. The highest score was obtained by the wrapped butter 7.12 ± 0.04 on day 30 compared to unwrapped butter (6.06 ± 0.03). Kazemian-Bazkiaee, Ebrahimi [37] studied the effect of chitosan and β -glucan coatings on quality of peanut. The authors suggested that the coated peanuts showed better sensory and quality parameters compared to uncoated.

Furthermore, Amiri, Aminzare [49] also recorded an improvement in sensory properties of beef patties during storage by the application of EO fortified corn starch films. Pirsá and Shamusí [50] fabricated and applied polypyrolle-ZnO modified bacterial cellulose films on chicken meat. They recorded a significant decline in sensorial quality of meat with time. The wrapped meat was seen with better sensory properties. Several other studies concluded the role of films application on improving sensory quality of food products during storage including Santacruz [29]; Nottagh, Hesari [32]; Mushtaq, Gani [51] and Youssef, Assem [52].

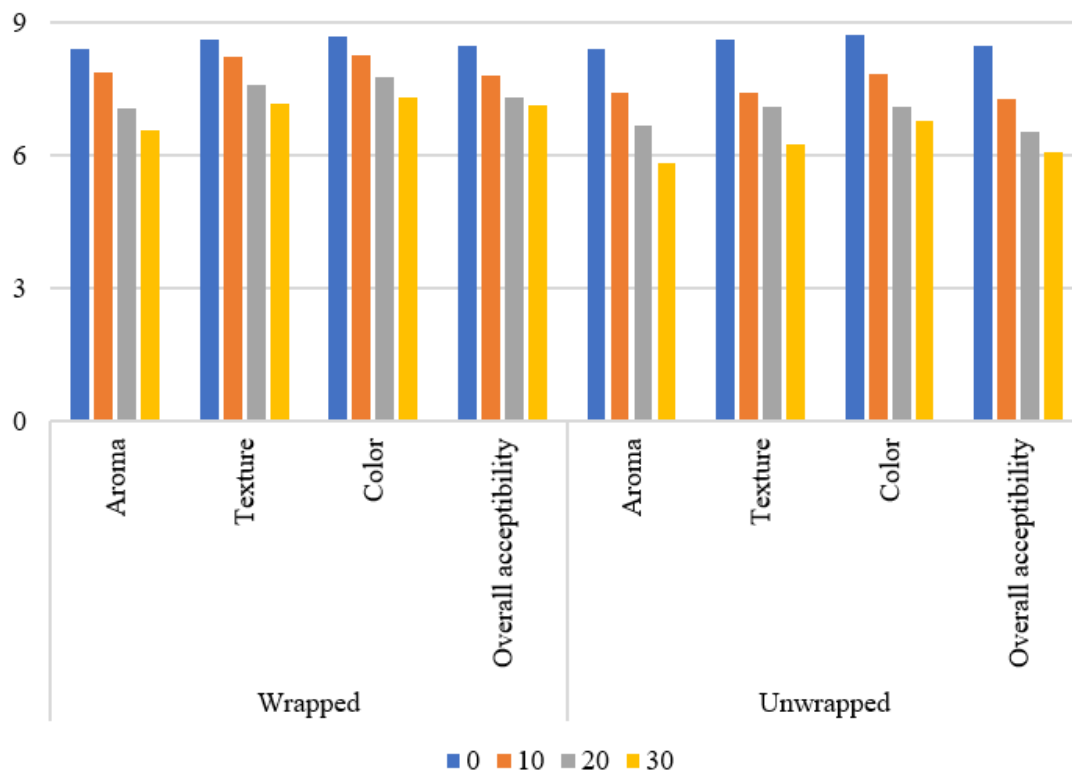


Figure 7: Sensory attributes of wrapped and unwrapped butter during storage

CONCLUSION

Orange peels were utilized for the preparation of active films that were further applied on butter to enhance and evaluate the quality of butter during storage. During storage, the application of film proved effective in preserving the quality attributes of butter including physicochemical (*i.e.* hardness, color, peroxide value, acidity and pH), microbial and sensory, compared to unwrapped butter.

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