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Development of Whey Protein Concentrate Edible Membrane with Cinnamon Essential Oil

Idris Aisha[1*] and Yahaya Abdullahi[2]

[1]Department of Biology, Federal University, Dutse, Jigawa State, Nigeria.
[2]Department of Biology, Faculty of Science and Science Education, Kano University of Science and Technology, P.M.B. 3244, Wudil, Kano State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Authors IA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YA managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to develop whey protein edible films, characterize the film for structural and physical properties and food preservation or shelf life improvement studies.
Study Design: Development, characterization and food preservation of edible film.
Place and Duration of Study: Department of Bio Science, lovely professional university India between January 2016 and May 2016.
Methodology: Whey protein concentrate film (prepared by casting method) containing 1.0% - 4.0% of cinnamon essential oil was tested against *Escherichia coli* and *Staphylococcus aureus*. Circular disc of the film were placed on Muller Hinton Agar plates containing bacterial lawn and the zone of inhibition was measured post incubation of 24 hours. The effect of cinnamon essential oil on the film property was studied by measuring the physical, optical and mechanical properties of the film.
Results: Whey protein concentrate edible film containing 4.0% cinnamon essential oil was the most effective against *S. aureus*. None of the whey protein concentrate films incorporated with

**Corresponding author: E-mail: idrisaisha24@gmail.com;*

cinnamon essential oil has antimicrobial activity against *E. coli*. Edible film obtained in present study was found to be effective to control the food spoilage in various food stuffs.
Conclusion: It can be concluded that the film can be used to improve food safety as well as extending the shelf life of food by controlling the release of antimicrobial on food surfaces.

Keywords: Antimicrobial; edible film; essential oil; whey protein concentrate; film properties; food packaging.

1. INTRODUCTION

Edible film can be elucidated as a substance use for packaging food, which contain a thin layer of edible substances and it is placed on the food or between the food substances [1]. Edible films can be prepared from different materials like lipid, polysaccharide, and proteins. These edible films protect the food from moisture, gases and other solutes. It also has certain antimicrobial property.

Antimicrobial edible films provide security to the consumers [2]. It mainly decreases or prevents the growth of microorganisms in food.

The film can contain antimicrobial compounds so that high amount of preservatives on the food surfaces is maintained [3]. Many substances like essential oils, plant extracts, organic acids, inorganic nano particles, chitosan, bacteriocin and fungicides were reported for their antimicrobial activity in food packaging [4]. These substances possess antimicrobial activity. Besides, natural substances, like lysozyme and nisin, have been reported as an excellent preservatives that are added to the edible films and they are safe for human consumption [5].

Essential oils are derived from plants and they contain the plant fragrance, that are extracted by different methods like distillation or solvent extractions. They have wide ranges of applications in food microbiology. Some of the applications are; they can be used as antibacterial agents and therefore prevent food from spoilage by extending the food shelf life [6].

Edible films usually protect the food material from environmental hazards. Little study has been plotted to made theses membranes as well as to and apply them for foods storage and extending the food shelf life. The edible films and membranes have some merits which include edibility, biological compatibility, beautiful appearance, boundary properties, non-harmful nature, non-defilement and having cheaper cost. Also, bio-films and membranes can be incorporated with foods additives which can prevent oxidation as and spoilage of food by micro organisms. Edible films made from starch have been use frequently due to their transparent, lack of smell and taste nature [7]. Plasticizers are important component in the bio-film and they increase the film flexibility as well as its extensibility [1].

There are different methods for preparing edible membranes including casting method, extrusion method, knife-coating and spraying methods etc. [8].

When comparing edible film with normal traditional or synthetic coatings it is reported that edible coatings and films did not replace the traditional or synthetic coating materials but they provide an additional favorable property which can be helpful during food preservation. These edible films can also be used to decrease the expenses as well as the amount of synthetic or traditional packaging that were previously used. These films can keep up moisture, gas, and lipid movement as well as backs other additives and nutrients in the food. For the formulation of this films, there can be polymers, peptides, and organic compounds and they must not affect the color and flavor of food [1]. Edible films are normally use to increase the storage life of food products.

Cinnamon is an essential oil which belongs to Lauraceae, mostly grown in South Asia. Cinnamomum oil is having the greatest antimicrobial activity in the Lauraceae essential oils, which are *Cinnamomum zeylanicum*, *Ravensara anisata* and *Laurus nobilis*. The antimicrobial action of different essential oils on different microorganisms was studied and it was recorded that cinnamon oil was having a high antimicrobial characteristics. Also, Espetia et al. [9] reported that cinnamon can stop the growth of *Escherichia coli* and pathogenic *Listeria innocua* in fruits.

Cinnamon oil can inhibit both bacterial contamination as well as prevent oxidation of

food; and therefore can be applied in food packaging. It can be incorporated in edible films as an alternative to other antibacterial and antioxidants into foods. Developed films with varying cinnamon oil concentrations were studied including the moisture content, thickness, eugenol content, water vapor transmission, antimicrobial properties and *in-vitro* antioxidant activity of the developed films [10]. As the level of the cinnamon oil increased, the eugenol content and antioxidant activity also increased. The film was able to inhibit the tested microorganisms. The film was use to preserve peaches and it increase the peach antioxidant activity, makes the peach odor acceptability and decrease the peach bacterial growth there by making edible film made with cinnamon leaf oil suitable for peach preservation.

The significance of edible food membranes include the ability of the film to keep up moisture, control gas, and lipid movement as well as backs other additives and nutrients in the food. Edible films are also use to increase the storage shelf life of food products and also to respond to buyers needs for natural products and they are also use due to their low environment contamination. Other significance of this edible films include their edibility, their biological compatibility, their beautiful appearance, their boundary properties, their non-toxic nature, their non-polluting nature, their biodegradable nature and their low cost. Polythene cannot be degraded and it contributes to environmental pollution. Due to this, edible food membranes can serve as the best alternative because it provide solution to the above mentioned problems as well as its additional antimicrobial and antioxidant activity.

Only handful of information is available in terms of natural antimicrobials incorporated in edible films. There is an increase for the demand of more natural foods that have high antimicrobial property. These motivated us to explore different way of improving production of edible bio-films so that the quality, freshness and safety of the food can be maintained.

Keeping in mind the above said problem and motivation we had proposed the present work with various objectives which are development of whey protein edible films, characterization of the film for structural and physical properties and food preservation or shelf life improvement studies.

2. MATERIALS AND METHODS

2.1 Materials

Whey protein concentrate (80%) was bought from a food industry with the name Davisco Foods International Inc, USA. Gelatin was obtained from molychem, India. Cinnamon essential oil was obtained from Surabhy's natural essential oils, India. Glycerol, and tween 20 where obtained from LobaChemePvt. Ltd, India. Nutrient broth and Mueller Hinton agar were obtained at HiMedia laboratories.

2.2 Culture Preparation

Escherichia coli and *Staphylococcus aureus* cultures were obtained from the culture collections of a University in India. These strains were revived, and selected using Eosin Methylen blue for *E. coli*, and mannitol containing agar for *S. aureus.* Overnight cultures of *Escherichia coli* and *Staphylococcus aureus* were grown in nutrient broth at 37ºC in an incubator aerobically.

2.3 Determination of the Antimicrobial Activity of Cinnamon Essential Oil (CEO)

The antimicrobial activity of cinnamon essential oil against the selected microorganisms was studied using the agar diffusion method. Organism strains were cultured overnight in nutrient broth at 37ºC. A total of 15 uL of the essential oil was poured into 7.9 mm diameter Mueller-Hinton agar wells which were previously sealed with Agar agar. These plates had been inoculated using a sterile swab and all petri plates were maintained at 37ºC for 24 h. After incubation, the zone of inhibition was measured, as described by [11].

2.4 Whey protein isolate-based film preparation

Whey protein concentrated solution (8% w/v) was prepared using the following series; whey protein powder plus gelatin and adjusted pH of 8.0, and heating in a water bath at 90^{o}C for 30 min. Glycerol was added in the ratio 1:1. Later, Tween 20, at a level of 0.2% v/v, was added as an emulsifier to help dissolution in the film-forming solution. After 30 min of stirring, essential oils at 1%, 2%, 3% and 4% v/v concentration was added to the film-forming

product. The solution was incited at room temperature for 30 min using a magnetic bead stirrer. Ten grams of the solutions was casted on 90 mm petri plate and dried for 72 h at 37ºC. Dried films were peeled from the plates and stored in a chamber at ambient temperature [11].

2.5 Film Characterization

The films were characterized for Film thickness and disc weigh, Fourier Transform Infrared (FTIR) Analysis, Film transparency (UV characterization), Microscopic structure and moisture content.

2.5.1 Film thickness and disc weight

The film thickness was measured with a digital micro meter (digital calliper) with sensention of 0.0001 mm. The measurements was taken at ten random locations of the peeled films. Also, circular ‘‘discs’’ were removed from the edible films using a well borer of diameter 10.00 mm. Disc weights were measured at four random location of film on each circular film and the average disc weight was recorded.

Film thickness: $\frac{\boldsymbol{total\ thickness\ at\ various\ places}}{\boldsymbol{total\ number\ of\ places}}$

Film weight: $\frac{\boldsymbol{weight\ of\ 4\ disc\ of\ 10mm\ diameter}}{\boldsymbol{4}}$

2.5.2 Fourier transform infrared (FTIR) analysis

Fourier transform infrared spectroscopy (FTIR) analysis was performed using SHIMADZU, JAPAN model spectrum. The FTIR spectral measurement was done in the transmittance state. The measurement was done in the mid-infrared range from 4000 to 500 cm^{-1} with air as the background reference [12].

2.5.3 Film transparency (UV characterization)

The light barrier property of the film was measured by cutting a small rectangular section of the film (4 X 0.5 cm) and characterized using spectrophotometer and the absorbance at A550 was recorded [13]. Transparency of the films was calculated by:

Transparency: $\frac{\text{wavelenght A550}}{\text{mean thickness}}$

2.5.4 Moisture content analysis

A small section of the films was cut and measured and then dried at 100ºC for17 hour. The weight lost by the film samples was calculated, and moisture content was calculated using the equation below:

Moisture content= $\frac{\text{mi}-\text{mf}}{\text{mi}} \boldsymbol{x} \frac{\boldsymbol{100\%}}{\boldsymbol{1}}$

Where *mi* initial weight and *mf* is final weight.

2.5.5 Microscopic structure whey protein concentrates film

The microstructure of whey protein concentrate based film was evaluated by cutting small section of the film and observed under a light microscope. The Images were saved with a computer and use for the qualitative evaluation of the film.

2.6 Resolution of Anti-microbial Effect Of Whey Protein Concentrates Film

The zone of inhibition assay on Mueller Hinton agar was used for resolution of the antimicrobial effects of the films against *E. coli* and *S. aureus*. The films were cut into 10.00 mm diameter disks with a borer and then placed on the plates containing the inoculums of the tested organisms. Then, the plates were incubated in chamber at 37ºC for 24 h. After incubation, the zone of inhibition was measured with a calliper [11].

2.7 Spoilage Assay

Chicken salami meat and bread were cut into small sections and wrapped with the films containing 4% Cinnamon essential oil. Smaller section without film was sealed in polythene bag, and another section leave in open air environment. All samples were kept at room temperature and observed for the presence of visible spoilage and colour change. The pH of the polythene packed chicken, whey protein concentrate packed chicken, and control chicken was recorded after the assay was completed using a pH meter. Total aerobic plate count of the polythene packed chicken, whey protein concentrate packed chicken, and control chicken was also determined after the assay was completed by serial dilution method as described by various researchers and plated on nutrient agar.

2.8 Degradation Assay

A small section of whey protein based films was weighed and placed on moist soil in order to determine the degradation assay. The films were observed every day for any change in appearance, colour, or size. This assay was performed for ten days.

2.9 Statistical Analysis

Two observations were performed at each concentration (1%, 2%, 3%, and 4%) of essential oil incorporated to Whey Protein concentrate films. The level of significance of the incorporated essential oils depending on the area of inhibition was calculated using T test at 5% level of significance. All the experimental means were given as Mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Assay

Increasing levels of cinnamon essential oil was tested against *E. coli* and *S. aureus* and it was observed that the essential oil can inhibit all the tested organisms (Table 1 and Fig. 1). Increasing levels of cinnamon essential oils was incorporated to whey protein concentrate films and the films were tested against the microorganisms for the zone of inhibition (Table 2). Films containing 1%, 2%, 3% and 4% cinnamon oil were having antimicrobial activity against *S. aureus* but not against *E. coli* but cinnamon oil alone without whey protein was able to inhibit *E. coli*. The highest zone of inhibition was recorded at 4% level against *S. aureus*. As the concentration of cinnamon essential oil increased, the zone of inhibition also increased arithmetically for *S. aureus*, while an increase in concentration of cinnamon essential oil was not effective for *E. coli*. The zone of inhibition obtained in this study showed that more activity occurs in oil solution than in the film disk but this is not statistically significant at 5% level of significance. These increasing activity of the oil solution than the edible film also correlates with Bahram et al. [14] who reported that cinnamon oil was having greater diffusion rate than the whey protein isolated film which was incorporated with the cinnamon oil. Sample pictures of the inhibitory activity of Whey Protein Isolate based films incorporated with 1%, 2%, 3% and 4% cinnamon essential oil against all the tested microorganisms were represented in Fig. 2.

Babuskin et al. [15] also reported an increase in the activity of cinnamon essential oil on corn starch edible membranes. The activity of the oil was also reported to be similar at both 3 and 4% respectively and Babuskin et al. [15] recommended that corn starch film should be incorporated with 3% of cinnamon essential oil because of the effective activity recorded. The minimum inhibitory concentration and minimum lethal concentration of whey protein isolate film incorporated with antimicrobials was found to be relying on the type of the tested organism because different microorganism are inhibited by different antimicrobials [7].

The result obtained in this research is similar to those procured by Cagri et al. [5]. The Gram-positive bacteria *Bacillus subtilis* was reported as the most sensitive bacteria at a level of 1.5% of whey protein isolate and cinnamon based film, having a region of inhibition as 37.25 mm.

Sanches-Gonzalez et al. [16] reported that Gram-negative bacteria are less sensitive to cinnamon essential oil than Gram-positive bacteria and these can be the reason why *E. coli* was not showing any region of inhibition in this current study. Also, many researchers have reported that essential oils in general are having

Table 1. Antimicrobial activity of cinnamon essential oil and whey protein concentrate film

Antimicrobial	Activity	Percentage	Inhibition zone diameter (mm)	
			S. aureus	***E. coli***
Cinnamon EO	+	1%	16.84±3.18	13.62±1.49
		2%	20.89±3.22	17.77±2.01
		3%	22.4±2.64	19.18±0.93
		4%	24.19±2.91	21.41±0.93
Whey protein concentrate and cinnamon Essential Oil	+	1%	0±0	-
		2%	10.32±0.32	-
		3%	15.32±0.33	-
		4%	16.93±0.96	-

**Values are given as mean ± standard deviation*

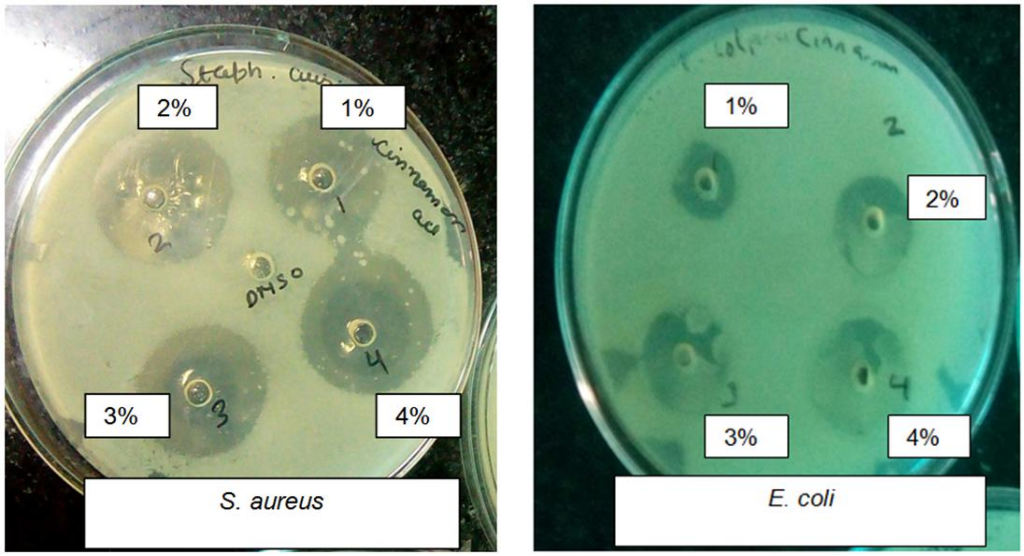

Fig. 1. Cinnamon essential oil solution antimicrobial activity

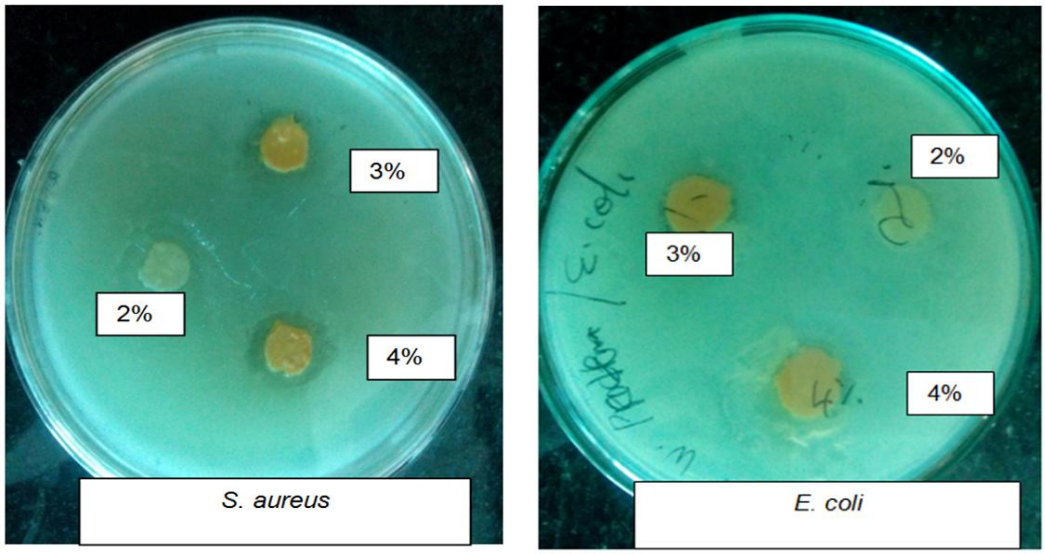

Fig. 2. Antimicrobial activity of whey protein concentrated edible film

more antimicrobial activity against Gram-positive bacteria than against Gram- negative bacteria probably due to the presence of impermeable outer membrane of the latter [6].

The highest antimicrobial dispersing activity was obtained in the oil solution compared to the whey based edible film. The reason might be due to the structure and arrangement of the molecules in the biopolymers. As protein are having nonlinear, and folded structure, the dispersion of antimicrobial component i.e. cinnamon oil is less. On the other hand, the oil solution dispersion is not bound by any membrane and therefore it release more antimicrobial component to the culture surface. Further work should be required to optimize the maximal antimicrobial dispersion in protein based edible membrane.

3.2 Film Characterization

3.2.1 Film thickness

It was recorded that the mean thickness of whey protein concentrate based film incorporated with Cinnamon Essential oil is 0.19±0.06 mm thick. The result shows that the addition of cinnamon essential oil does not affect the thickness of the

film (Table 2). All the films developed were pliable and easily removed from the glass surface. Ayala-Zavala et al. [10], reported that the addition of cinnamon oil increase pectin film thickness while Baharan et al. [14] reported that the incorporation of cinnamon oil did not affect the whey protein film. Rubilar et al. [13] reported that whey protein concentrate films were very thin and flexible and therefore ideal for use as packaging material. There was significant difference in the film thickness depending on the antimicrobial incorporated in the film [9] but this did not coincides with the observations recorded by Ramos et al. [7] who stated that addition of antimicrobials do not affect the film thickness. Seydim and Sarikus [11] reported that the mean thickness of whey protein isolate film was 0.2458 mm which is slightly higher to that obtained in this current study. The thickness of gelato-chitosan films was recorded to be between 0.27 and 0.29 mm [12].

3.2.2 Film disc weight

Whey protein concentrate based film incorporated with Cinnamon Essential oil is having a mean weight of 0.0275±0.01 g (Table 2). Seydim and Sarikus [11] reported that the mean weight of whey protein isolate film was 0.0149 g which is slightly lower than that obtained in these study. There was significant difference in the film weight depending on the antimicrobial incorporated in the film [9].

3.2.3 Moisture content

Whey protein concentrate based edible membranes would be used for packaging of food in order to prevent product spoilage. These packaging films should uphold moisture levels within the packaged food. Due to these, the need to know the water solubility and moisture content of the film is extremely crucial for food packaging usage. Whey protein film was having 5.90±0.85% as the moisture content (Table 2). The edible film have very low moisture content (<10%). This shows that even during storage time, the physical properties of the film would be affected at a very low level because the water loss would be very less since it is already having very less moisture content. This low moisture content might be due to the cinnamon oil present because it is hydrophobic in nature.

3.2.4 Film transparency (UV characterization)

It was observed that the edible films were having low transparency (Table 3). UV light oxidizes food especially bakery product and due to the low transparency of this films, they can protect the food from more than 90% of the UV light. This agrees with many researches that reported the antioxidant nature of edible films. It was observed that whey protein concentrate with 1% and 4% cinnamon oil have the least transparency while whey protein isolate with 2% cinnamon oil have the highest transparency. The appearance of films is dependent upon its ability to separate and assimilate light waves in the visible region of the UV spectrum. Separation and assimilation determines the “transparency” or “opacity” as well as the “chromaticness” which means the blueness, greenness, redness or yellowness of a film. A transparent film allows the light to passes through it with a minimum reflection and assimilation. Opaque film affects the light separation as well as the film transparency [17].

Table 2. Characterization of whey protein concentrate edible membrane incorporated with Cinnamon Essential oil

Characterization	Mean value of film with CEO	Mean value of film without CEO
Film disc weight	0.0275±0.01 g	0.0250±0.03 g
Film mean thickness	0.19±0.06 mm	0.19±0.06 mm
Film moisture content	5.90±0.85%	8.90±0.05%

Table 3. Film transparency (UV characterization)

WPC (%)	Mean thickness	UV A550	Transparency
1%	0.13±0.03	0.1828±0.0005	1.364
2%	0.25±0.01	1.1882±0.035	4.678
3%	0.25±0.02	0.6342±0.0005	2.496
4%	0.13±0.03	0.1834±0.0011	1.369

**Values are given as mean ± standard deviation*
**WPC whey protein concentrate plus cinnamon essential oil*

The internal and surface film microstructure plays an important role in optical properties of the film. Ramos et al. [7] reported that the transparency of whey protein film incorporated with antimicrobials ranges from 1.35 to 3.09% while the transparency of whey film in this study ranges from 1.3 to 4.6%. This might be due to the different method of film casting employed by various researchers. The film transparency is very crucial because it can affect the appearance of packaged products.

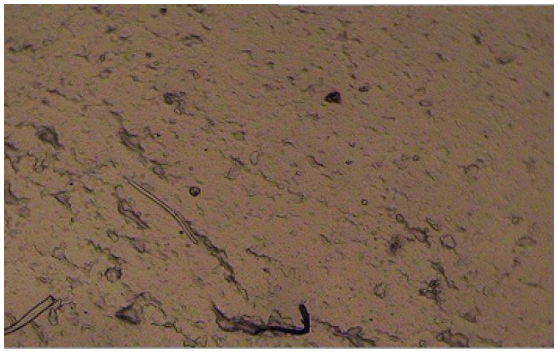

Fig. 3. Microscopic structure of whey protein concentrate based films

3.2.5 Microscopic structure of pectin and whey protein concentrate based films

Whey protein concentrate film (Fig. 3) was observed as to be homogenous but all the particles become nearly equally distributed only after filtration through muslin cloth. These make the whey protein isolate film to be smooth but it is not easily peel-able.

3.2.6 Fourier transform infrared (FTIR) analysis

The FTIR has been used to study the interaction between film and antimicrobial agents incorporated. The infrared spectroscopy spectrum displayed characteristic of cinnamon oil and whey protein concentrate films as shown in Fig. 4a, and 4b from 500-4000 cm^{-1}. The peaks observed are 1969.39 and 2139.13.

3.3 Spoilage Assay

3.3.1 Bread Spoilage assay

Bread sample was stored for a period of 6 days and during storage, open bread sample was the first to spoil after 3 days (Fig. 5a, b, and c). Polythene bread spoiled after 4 days (table 4) while whey protein concentrate packaged bread maintain its freshness up to the end of the study, it was not containing any fungal growth while other form of storage were contaminated with lots of fungi. These show that cinnamon is having antimicrobial activity against fungi as reported by various workers. Tan et al. [18] reported apparent fungal growth on the surface of the control bread sample after 3 days of incubation at 24ºC which coincides with the findings of these work. Schou et al. [19] recorded that the hardening of the bread was reduced in the edible film packaged bread compared to the unwrapped samples.

3.3.2 Chicken salami

The Whey protein packaged chicken salami was observed to maintain its freshness throughout the period of storage. Opened chicken changed colour within 24 hours of experiment while polythene chicken completely rots after 3 days (Table 5 and Fig. 6a, b, and c).

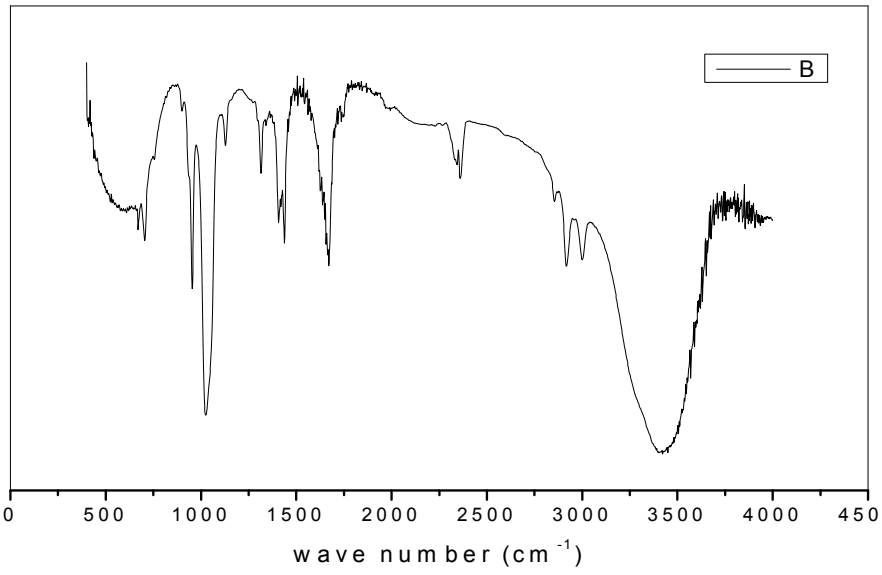

Fig. 4a. FTIR spectra of cinnamon oil without whey protein concentrate

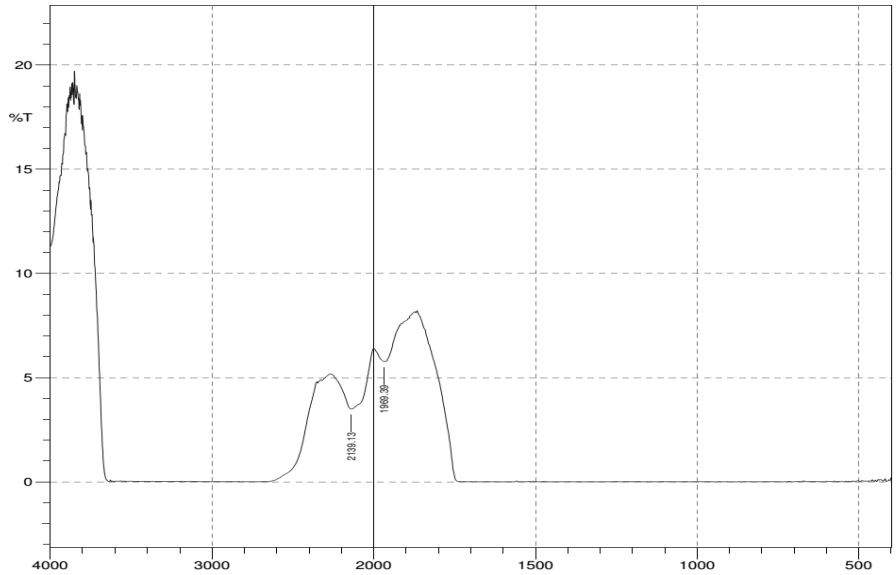

Fig. 4b. FTIR spectra of whey protein concentrate films incorporated with 4% cinnamon essential oil

Table 4. Bread spoilage assay

Storage form	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Open bread	Normal	Normal	Spoiled	Spoiled	Spoiled	Spoiled
Polythene bread	Normal	Normal	Normal	Spoiled	Spoiled	Spoiled
Pectin packed bread	Normal	Normal	Normal	Normal	Normal	Normal

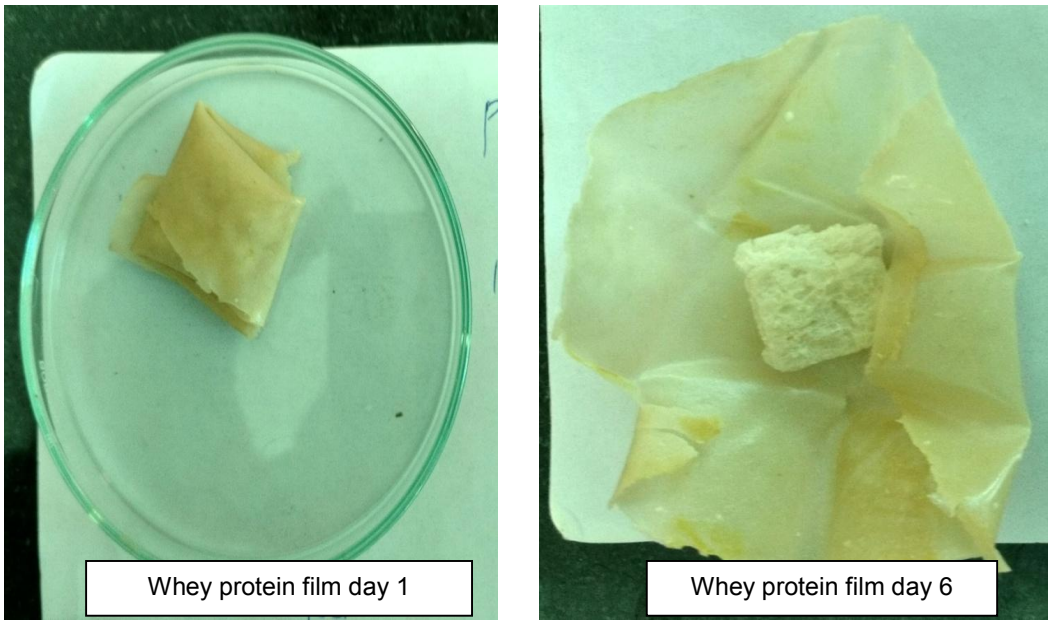

Fig. 5a. Bread spoilage assay (Whey protein packed bread)

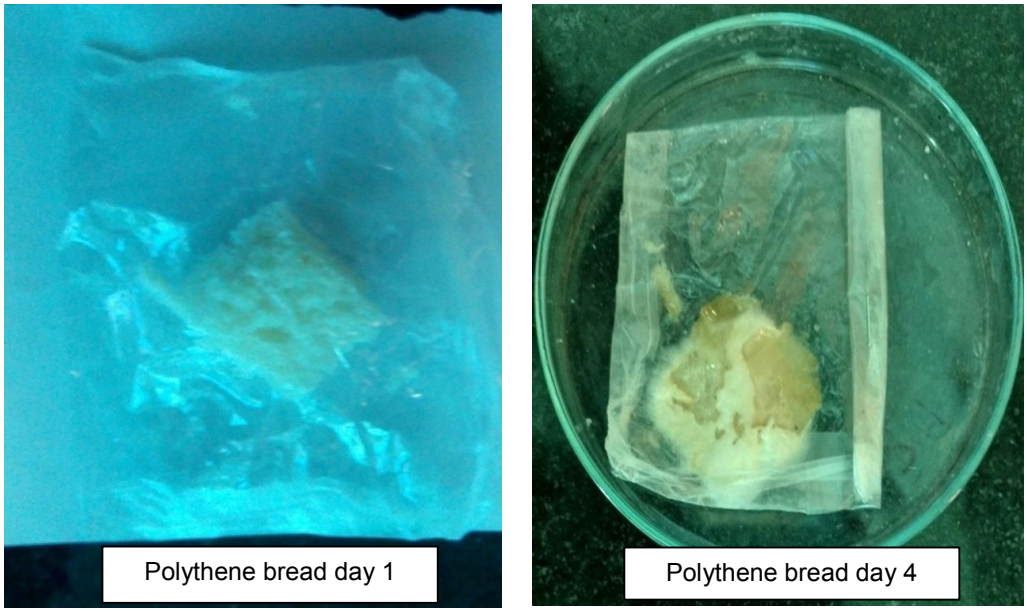

Fig. 5b. Bread spoilage assay (Polythene bread)

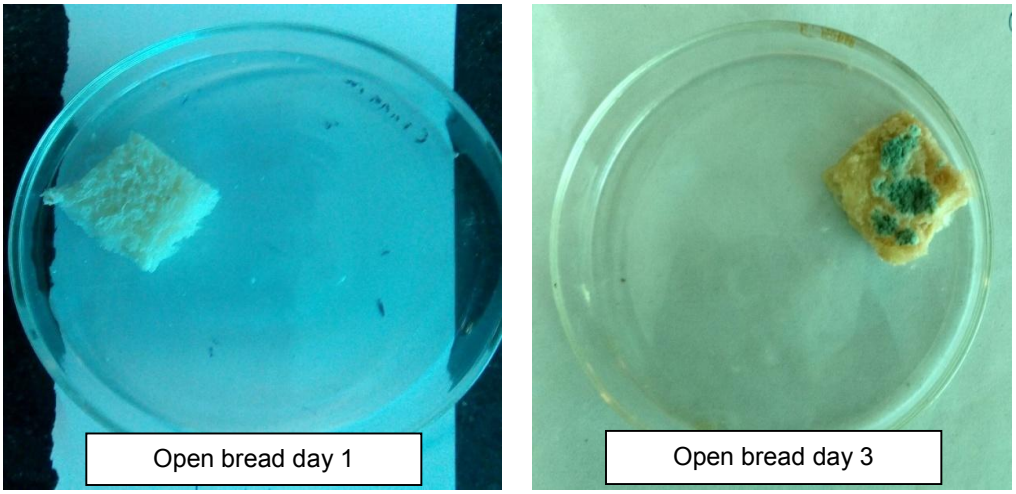

Fig. 5c. Bread spoilage assay (open bread)

The effect of whey protein films incorporated with 4% cinnamon essential oil on the pH of the chicken during storage is represented in Fig. 7. The initial pH of the chicken sample was recorded as 5.01±0.09 and the whey protein packed chicken pH falls to 4.86 after 6 days. These might be due to the fact that microorganisms that cause meat spoilage mostly increase the pH of the meat by producing some metabolites. Due to the absent of this alkaline metabolites in the whey protein packaged chicken, the pH become lower in the whey protein packaged chicken than the polythene packaged chicken. These results coincide with the reports of Babuskin et al. [15] who reported that the pH of cinnamon based edible membrane coated meat was lower than that of the control.

The total aerobic plate assay of polythene chicken was 2.88 X 10^{10}log cfu/g of chicken at dilution factor of 10^{-7}. These shows that the polythene chicken is spoiled with many bacteria compared to the whey protein packed chicken which show only 5.00 X 10^{9} log cfu/g at dilution factor of 10^{-4} and 1 X 10^{7} at dilution factor of 10^{-6} as represented in Table 6. Cinnamon oil film was

found to reduce lactic acid bacteria counts by 0.55 log cfu/g [15]. The food borne pathogen (*Listeria monocytogens*) was inhibited using composite films to 1.5 log CFU/cm2 of *Listeria* within a day after incorporation with the film while in control samples, the bacteria reached over 6.5 log CFU/cm2 [20].

3.4 Degradation Assay

It was observe that whey protein films were degraded by soil microorganisms within 10 days (Fig. 8). This is an added advantage for their application because they are biodegradable polymers which can be degraded by bacterial and fungal enzymes [4]. Fungal growths were clearly visible on the surface of whey protein isolate film after 6 days of incubation. This leads to loss of films weight. Junior et al. [21] reported that fungal growths were clearly visible on the surface of the film after 21 days of incubation. This is a welcome development because of the fact that other synthetic polymers are non-degradable and they cause environmental pollution.

Table 5. Chicken salami spoilage assay

Storage form	Day 1	Day 2	Day 3	Day 6
Open plate chicken	Normal	Red	Dry	Spoiled
Closed polythene chicken	Normal	Slimy	Rotten	Spoiled
Whey protein packed chicken	Normal	Normal	Normal	Normal

Table 6. Total aerobic plate count

Chicken salami	Dilution factor	Visible growth	Colonies	Colony forming unit
Polythene	10^{-4}	Yes	TNTC	TNTC
	10^{-5}	Yes	TNTC	TNTC
	10^{-6}	Yes	TNTC	TNTC
	10^{-7}	Yes	288	2.88×10^{10}
Whey protein packaged	10^{-4}	Yes	50	5×10^{9}
	10^{-5}	Yes	2	2×10^{6}
	10^{-6}	Yes	1	1×10^{7}
	10^{-7}	No	0	0
Control	10^{-4}	Yes	216	2.16×10^{7}
	10^{-5}	Yes	91	9.1×10^{7}
	10^{-6}	Yes	14	1.4×10^{8}
	10^{-7}	Yes	10	1.0×10^{9}

** TNTC: too numerous to count*

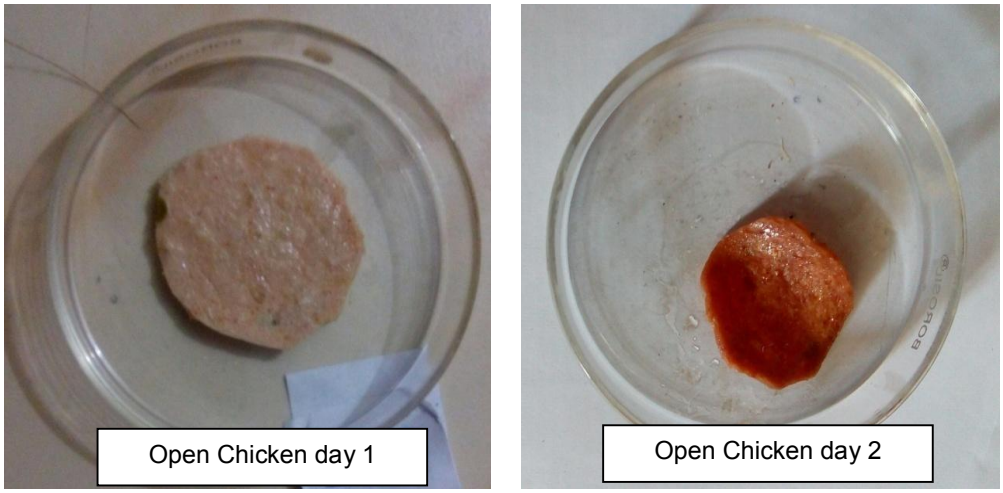

Fig. 6a. Chicken spoilage assay (open chicken)

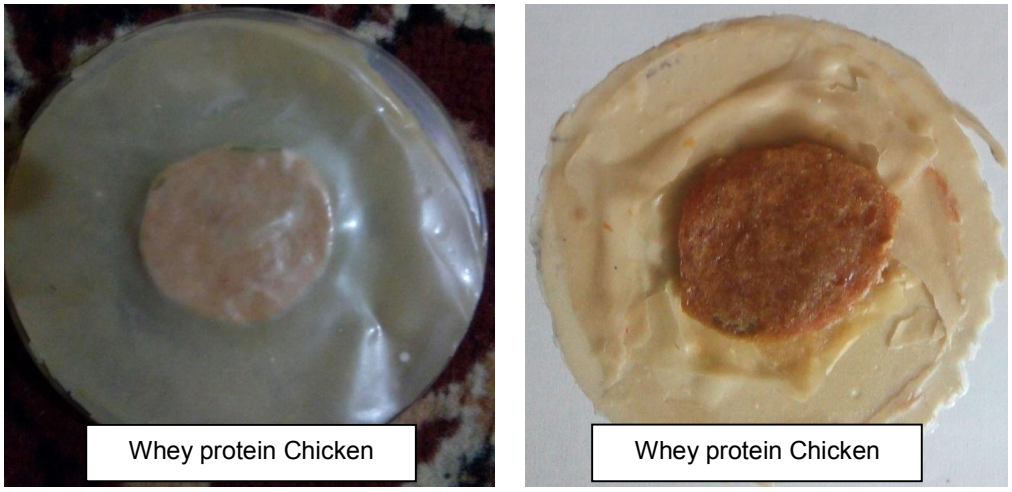

Fig. 6b. Chicken spoilage assay (Whey protein chicken)

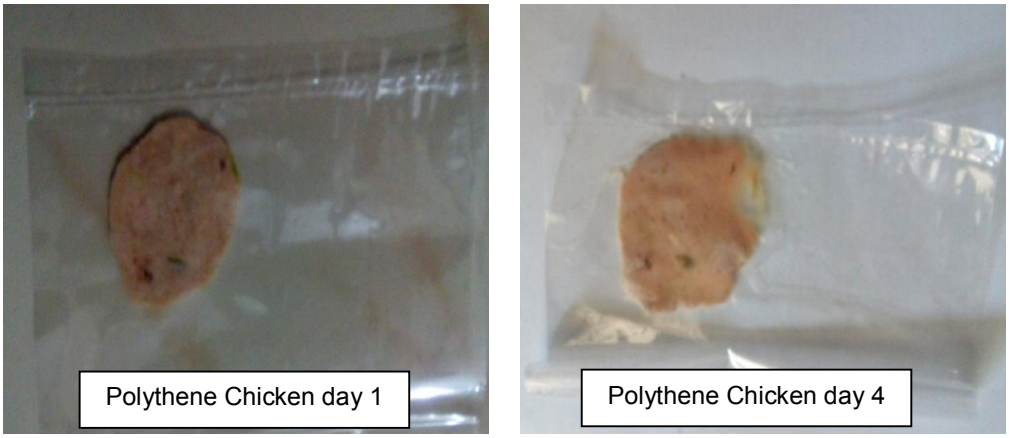

Fig. 6c. Chicken spoilage assay (polythene chicken)

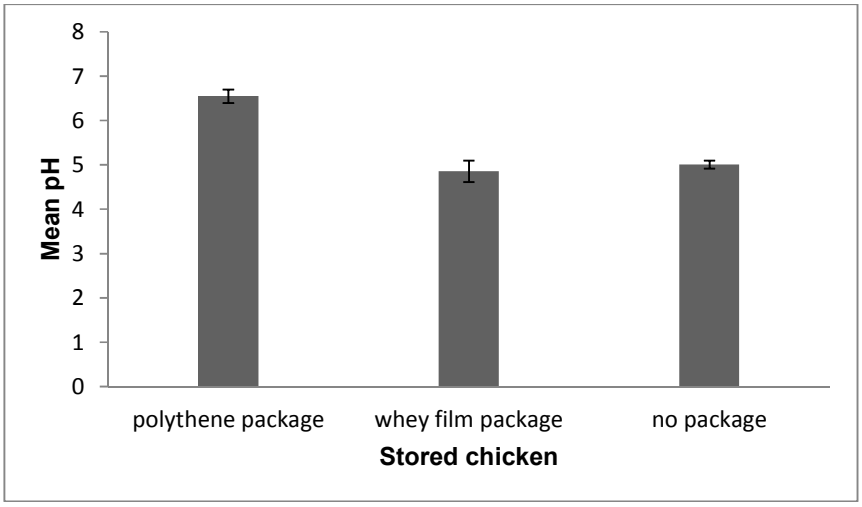

Fig. 7. pH change of chicken after 6 days storage

Fig. 8. Degradation assay of whey protein concentrate

4. CONCLUSION

The application of whey protein concentrate based film could maintain the quality of bread, and chicken meat. Furthermore, the films developed in this study are a suitable alternative to the present day commercial coating due to the merits they possess i.e. their degradability as well as their antimicrobial activity. Edible films need some modifications in order to achieve their purposes as well as controlling the diffusion of antimicrobial from the film to the food, so that the bioavailability of the antimicrobial will be enough throughout the period of storage. More research for the understanding of the antimicrobial behavior of cinnamon essential oil incorporated in whey protein concentrate need to be done. Also, further work is required in order to optimize the maximal antimicrobial dispersion in whey protein based edible membrane.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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