ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 09, Issue, 10, pp.8955-8960, October, 2018

RESEARCH ARTICLE

DEVELOPMENT OF EDIBLE FILMS BASED ON WHEY PROTEIN CONCENTRATE

[1]Harneet Kaur Mehndiratta, [2,*]Anshu Sibbal Chatli and [1]Prerna Singh

[1]Department of Biotechnology, Guru Nanak Girls College, Model Town, Ludhiana 141002, Punjab, India
[2]Department of Microbiology, Guru Nanak Girls College, Model Town, Ludhiana 141002, Punjab, India

ARTICLE INFO

Article History:
Received 17th July, 2018
Received in revised form
25th August, 2018
Accepted 20th September, 2018
Published online 30th October, 2018

Key words:
Whey Protein Concentrate,
Essential oils, peppermint oil,
Cinnamaldehyde, Clove oil,
Lemongrass oil, Antimicrobial activity,
E.coli, B.cereus, S.flexineri.

ABSTRACT

The aim of this project was to develop an eco-friendly biodegradable film for the packaging of food materials for the safety purposes. The present study was conducted for the development of whey protein based edible film. The processing conditions were optimized and 6% level of WPC was selected with the incorporation of 3% glycerol as a plasticizer and 0.3% of sodium alginate as a binder. These levels were pre-standardized on the basis of biomechanical characteristics viz. thickness, penetrability, moisture, WVTR, density, *L**, *a** and *b** values. Four different essential oils were selected on the basis of their antimicrobial activity. 0.5% level of cinnamaldehyde, lemongrass oil, peppermint oil and clove oil was added into the formulation of WPC in order to increase the antimicrobial efficacy of WPC based edible film. The antimicrobial activity was checked against three different pathogenic microorganisms viz. *Escherichia coli*, *Bacillus cereus* and *Shigella flexineri* by well diffusion method. This antimicrobial efficacy was then assessed on the basis of zone of inhibition.

Citation: ***Harneet Kaur Mehndiratta, Anshu Sibbal Chatli and Prerna Singh*, 2018.** "Development of edible films based on whey protein concentrate", *Asian Journal of Science and Technology*, 09, (10), 8955-8960.

INTRODUCTION

The packaging films that degrade naturally are known as biodegradable films. These films are developed from the natural products, which can be easily decomposed by the microbes without having any ill effect on environment. However, plastic bags did not get decomposed for years in the environment and led to soil, water and air pollution. On burning, plastic material produces various toxic compounds and gases leading to air pollution. This has led to the development of biodegradable films for environment and food safety. The biodegradable films can be developed from various sources such as proteins, polysaccharides, starches, lipids and composites. These films are also known as bio-based plastics and exhibit various properties viz. edibility, moisture and gas barrier, flexibility etc. Protein is one of the most promising material considered for the preparation of biodegradable films. Proteins have high nutritional value and excellent functional attributes. The commonly used proteins for the development of films include soy protein, milk protein, maize protein, gluten protein etc (Luhovyy *et al.,* 2007). Packaging of food materials is an essential process to avoid contamination and deteriorative changes caused by microbes. So, the packaging of food particles should be in such a way which preserves and improves their quality.

Corresponding author: **Anshu Sibbal Chatli,**
Department of Microbiology, Guru Nanak Girls College, Model Town, Ludhiana 141002, Punjab, India

Whey proteins have typical gelling properties, which can be utilized to develop films with mechanical and gas barriers properties. These biodegradable films can act as a delivery vehicle for carrying various bioactive compounds such as nutrients, minerals, antioxidants, antimicrobial, preservatives etc. These bioactive compounds improve the functional attributes of the films, which can be utilized for the extension of storage life of food products and to combat the food safety issues. These biodegradable films also exhibit antimicrobial properties. The antimicrobial property can be enhanced with the addition of essential oils having antimicrobial activities for example, lemongrass oil, clove oil, cinnamaldehyde, peppermint oil etc. These essential oils in selected quantities can be added for the formulation of biodegradable films to increase the antimicrobial properties. These essential oils also affect the structural and physical properties of these edible films (Bahram *et al.,* 2014). The presence of these oils in selected amount may imply some impact on several properties like tensile strength, thickness, permeability, density, penetrability etc. In view of the above discussion, the present study was conducted with the following objectives:

- To develop whey protein concentrate based biodegradable films and their quality evaluation.
- To incorporate selected levels of different essential oils in the biodegradable films and their quality evaluation.
- To measure the antimicrobial activity of developed films against food pathogens.

MATERIALS AND METHODS

Preparation of Whey Protein Concentrate based Edible Film (WEF): WEF was prepared by dissolving 6% WPC powder and 0.3% sodium alginate in 100ml of distilled water and glycerol (3%) as plasticizer. The components were mixed well by heating them to 35-40^{0}C. The mixture was homogenized with the homogenizer (S22 digital ultra TURRAX, Germany) followed by heating on the magnetic stirrer Hot plate (Macro Scientific Works, India) at temperature of 90-95^{0}C for 25 minutes. The pH of the mixture was adjusted to 8.5-9.0 or more than 9.0 with the addition of 10% NaOH. Thereafter, mixture was filtered through the cheese cloth to remove undissolved material. The mixture solution 30 ml was casted on the plastic petri plates of diameter (15cm) and 80ml on the rectangular dishes of size (10×15cm) and dried for 7 hour at 35±2^{0}C. The dried films were peeled off gently and stored in a chamber at 50% relative humidity and 25^{0}C temperature until evaluation. The films were analyzed on the basis of extensibility, penetrability, color profile (*L*, a*, b**), water vapor transmission rate (WVTR

Analytical techniques

pH: The pH of mixture was determined as per the method described by (Trout *et al.,* 1992) using digital pH meter equipped with a combined glass electrode in the homogenized mixture.

Microbiological analysis

Revival and Maintenance of Pure Cultures: The freeze dried cultures of various pathogenic and spoilage organisms viz. *Escherichia coli, Bacillus cereus, Shigella flexineri* were provided by GADVASU, Ludhiana. Cultures were maintained at refrigeration temperature by subculturing the required bacterial population obtained by serial dilution using sterile peptone water. Stock cultures were prepared and maintained in cryovials at -20^{0}C by regular passaging.

Optimization of Inoculation Dose: The dose rate of the inoculums was standardized on the basis of cell number in the inoculums (Pranoto *et al.,* 2005). The dose rate of the above mentioned microbial cultures was optimized in the range of 10^5-10^6 cfu/ml.

Well Diffusion Method: Formulation Solutions of the WEF were prepared and incorporated with the known levels of cinnamaldehyde, lemon grass oil, peppermint oil and clove oil. About 10mm of diameter developed WEF were cut by using sterile cork borer and placed on solid media; Nutrient agar for *E. coli* and *Bacillus cereus* agar. The media was previously inoculated with 0.1ml of inoculums of containing indicator microorganisms in the range of 10^5-10^6 cfu/ml. The plates were then incubated at 37^{0}C for 24 hours. The diameters of inhibitory zone surrounding the WEF discs were measured with the help of digital vernier calipers.

Processing Quality Characteristics of WEF

Color Profile Analysis: Color profile was measured using Lovibond Tintometer (Lovibond RT-300, Reflectance Tintometer, United Kingdom) set at 2^{0}C of cool white light (D65) are known as *L*, a*, b** values. *L** value denotes (brightness 100), or lightness (0), *a** (0+ redness/-greenness), *b** (+ yellowness/-blueness) values were recorded on WPC film. The instrument was calibrated using light trap (back hole) and white tile provided with the instrument. The instrument was directly put on the surface of 3 different WPC film and values were recorded.

Water Vapour Transmission Rate: Water vapour transmission rate was measured using a modified ASTM 96-00 method (ASTM 2000). The film was sealed on a modified test cell containing 15mL of distilled water. The test cell was then kept in a dessicators containing pre-dehydrated silica gel. Silica gels were dried at 180^{0}C for these measurements. The whole assembly was kept at 25^{0}C and weight loss of the test cell was measured after storage for 24 hour. WVTR of the film was calculated according to the equation of $WVTR = \Delta W/(\Delta t \times A)$ where ΔW is the weight loss of total cell, Δt is the time of storage, and A is the area of exposed film.

Penetrability: Penetrability was determined by simulating the conditions to measure the force required to pierce the edible film. The probe used had a diameter of 5mm and suitable sample size of edible film was subjected to run at 30mm/min with a displacement of 20mm and load cell of 100 N. Penetrability were calculated automatically by the preloaded software in the texture analyzer (TMS-PRO, Food Technology Corporation, USA) from the force-time pilot.

Extensibility: Extensibility was determined by stimulating the conditions to measure the ability to extend a system and the level of effort required to implement the extension. Extensibility also varies with the addition of new agents such as essential oils. The extensibility was calculated automatically by the preloaded software in the texture analyzer (TMS-PRO, food technology corporation, USA) from the force-time.

Transmittance: Transmittance of WPC films was determined by placing the film strips (3cm × 1cm) in the cuvette containing water and was measured the percentage transmittance at fixed wavelength of 600 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India).

Statistical Analysis: Data was analyzed statistically, on SPSS-16 (SPSS Inc., Chicago IL, USA) software package as per standard methods (Snedecor and Cochran, 1994). Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times to have total six number of observations (n=6). The mean values were reported along with standard error. The statistical significance was estimated at 5% level ($p<0.05$) and evaluated with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The objectives of this study were to optimize the processing technologies using pre standardized formulation for the development of WPC based biodegradable or edible films. The developed films were incorporated with selected levels of essential oils viz. lemongrass oil, clove oils, cinnamon oil and peppermint oil and thereafter elucidation of antimicrobial efficacy of developed films against *E. coli, B. cereus* and *S. flexineri.* The above mentioned objectives achieved by conducting two different experiments. The present chapter details the results obtained from these experiments. These results are represented with a support of statistically analyzed Tables (1-4) and Figures.

Flow diagram showing the development of whey protein concentrate based edible film

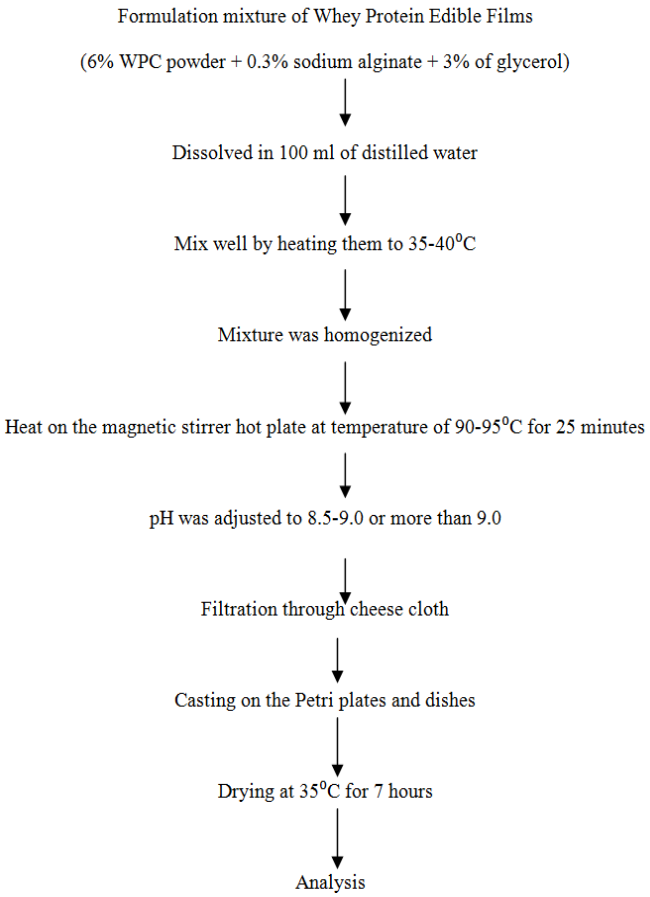


Table 1. Physical parameters of WPC based edible films (Mean ± S.E)

Physiological parameters	Value
Film thickness(µm)	210 ± 4.28
Penetrability(N)	7.78 ± 0.54
Water vapour transmission rate (WVTR) (g/m²/24h)	0.00187 ± 0.53
Extensibility(N)	26.44 ± 0.59
*L**(lightness)	35.05 ± 1.16
*a**(redness)	1.10 ± 0.09
*b**(yellowness)	10.33 ± 0.32

n = 6, *Mean ± S.E. (Standard error)

Film thickness: The thickness of film increased with the incorporation of sodium alginate (0.3%) in the formulation. This might be due to increase in viscosity of the film forming solution with the incorporation of sodium alginate. The average film thickness was measured as 210 µm ± 4.48.

Penetrability: The penetrability (N) determines the integral strength of the films and is a measure of the resistance of the films against puncturing. The penetrability of WPC films was recorded as 7.78 N with the area of 29.18 mJ. Similar finding have also been reported in protein based edible films (Singh, 2015) and (Nur Hanani *et al.,* 2012).

Water vapour transmission rate (WVTR): WVTR (g/m²/t) is an indicator of moisture transfer between food and atmosphere, which ultimately determines the deteriorative changes. The average value of WVTR of the developed WPC film was 0.00187 g/m²/24h. Hydrophilic plasticizer like glycerol improves the water vapour permeability as they provide higher amount of polar group in the film.

Extensibility : Elongation of the film is an indicator of the extensibility and flexibility of the film. The extensibility of film depends upon the glycerol content in film forming solution. Since plasticizers are hydrophilic, their addition to protein films will also increase their sensitivity, and at the same time weaken the barrier behaviour towards other gases and vapours due to the enhanced segmental movement of the polymer chains (Vieira *et al.,* 2011). The extensibility of WEF measured as 26.44 N.

Colour Profile Analysis (L*, a* and b* values): The colour profile of the packaging film influences the overall acceptability of the product wrapped within them. The colour of packaging films depend on the type of base material i.e. WPC and plasticizer (glycerol) and their concentration level. As glycerol is colourless so it may have diluted the overall colour of film. Hence, more the plasticizer in films lighters the colour of the films (Sobral *et al.,* 2005).

Physicochemical characteristics of the edible films incorporated with different essential oils: The effects of different essential oils on physicochemical characteristics of edible films are depicted in Table 2. Thickness of film increased with the incorporation of oil in the WPC film formulation, irrespective of type of oil. The thickness (µm) was measured highest for lemongrass oil incorporated films (0.349 ± 0.065) and lowest in cinnamaldehyde incorporated films (0.349 ± 0.077) amongst the treated group. However, film thickness was significantly (P<0.05) higher in all the essential oil incorporated films than control.

Effect on Colour Profile Analysis of WPC edible film with the incorporation of essential oils: The colour profile (*L*, a*, b**) varied significantly (P<0.05) with the incorporation of essential oils in the WPC films formulation (Table 3). Lightness (*L**) was measured lowest (49.79±0.014) with clove oil whereas highest with peppermint oil (51.71±0.09) amongst the treated group. Similarly the yellowness (*b**) value was measured highest (3.98±0.08) for the lemongrass oil incorporated films. The *b** value was comparable in peppermint oil and cinnamaldehyde incorporated WPC films and *b** was measured lowest in these films. Redness (*a**) was measured lowest for cinnamaldehyde and highest for peppermint oil incorporated WPC films amongst treat group. However the *a** value was significantly (P<0.05) higher in control than all the WPC films incorporated with essential oils.

3.8 Inhibitory effect of different essential oils in Whey protein Edible films against selected pathogens by well diffusion method (Mean ± S.E). Table 4 clearly revealed that antimicrobial efficacy of developed antimicrobial films was significantly (P<0.05) higher than control irrespective of type of essential oil. The efficacy of lemongrass oil developed edible films is maximum against *B. cereus* (24.61±0.63) and *E. coli* (22.94±0.78). The antimicrobial efficacy was comparable for peppermint oil, clove oil and cinnamaldehyde against *E. coli* and *Bacillus cereus,* however it was significantly (P<0.05) lower than WPC films with lemongrass oil. The diameter of zone of inhibition was comparable for peppermint oil and cinnamaldehyde against *S. flexineri* and it was the highest with lemongrass oil. (Kim *et al.,* 2008) also tested cinnamaldehyde against different strains of *E. coli* to determine the minimum inhibitory concentration and documented that 0.5% concentration of cinnamaldehyde lead to 90% inhibition of *E. coli*. The results of our experiment are in agreement with their findings.

Table 2. Physicochemical characteristics of WPC films incorporated with different essential oils

Parameter	Control	WPC+ Peppermint oil	WPC+ Cinnamaldehyde	WPC+ Clove oil	WPC+ Lemongrass oil
Essential oil conc.(v/v)	0	0.5	0.5	0.5	0.5
Thickness(mm)	0.342±0.062[a]	0.356±0.048[b]	0.349±0.077[b]	0.366±0.090[b]	0.374±0.065[b]
Moisture content (%)	49.60±0.72[b]	44.68±0.37[a]	45.43±0.68[a]	44.97±0.49[a]	44.79±0.60[a]
Solubility in water (%)	77.25±0.91[b]	75.45±1.24[a]	75.95±0.82[a]	76.08±0.96[a]	74.91±0.96[a]

n= 6 Mean ± S.E with different superscript differ significantly (P<0.05)

Table 3. Effect on Colour Profile Analysis of WPC edible films with the incorporation of essential oils

Parameter	Control	WPC+ Peppermint oil	WPC+ Cinnamaldehyde	WPC+ Clove oil	WPC+ Lemon- grass oil
Essential oil conc. (v/v %)	0	0.5	0.5	0.5	0.5
L * values	52.59±0.04[d]	51.71±0.09[c]	50.85±0.10[b]	49.79±014[a]	50.21±0.12[b]
a * values	7.84±0.09[d]	6.71±0.17[c]	5.84±0.13[a]	6.62±0.11[c]	6.09±0.08[b]
b * values	3.50±0.14[b]	2.91±0.13[a]	2.86±0.16[a]	3.67±0.13[b]	3.98±008[c]

n = 6 Mean ± S.E with different superscript differ significantly (P<0.05)

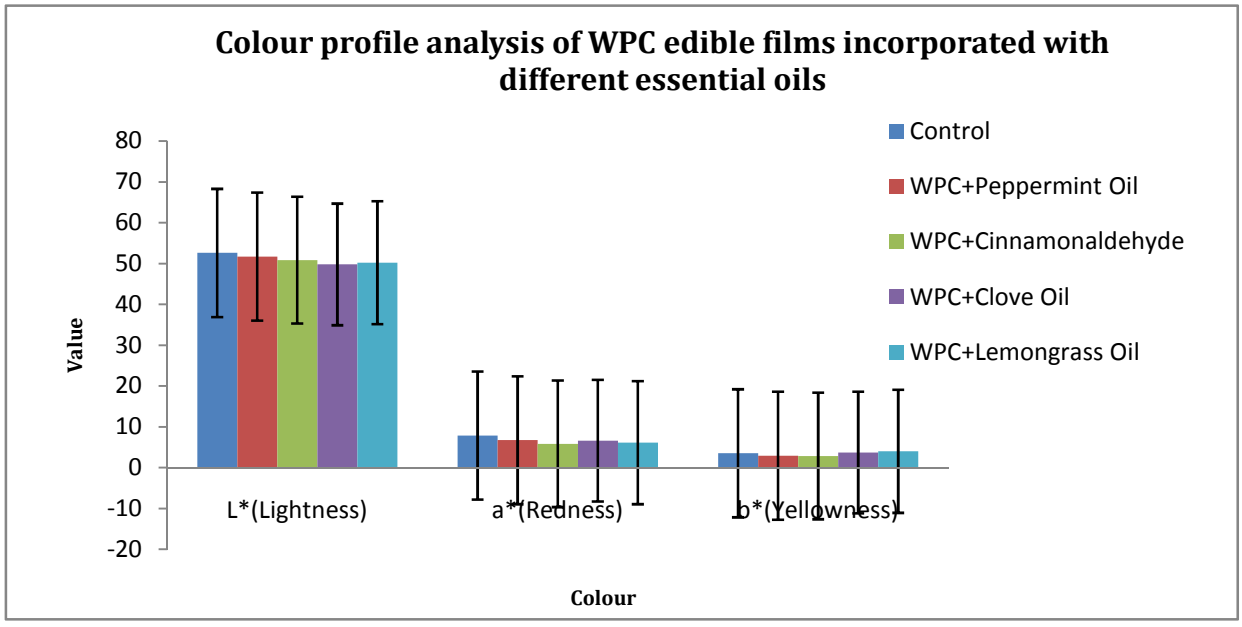
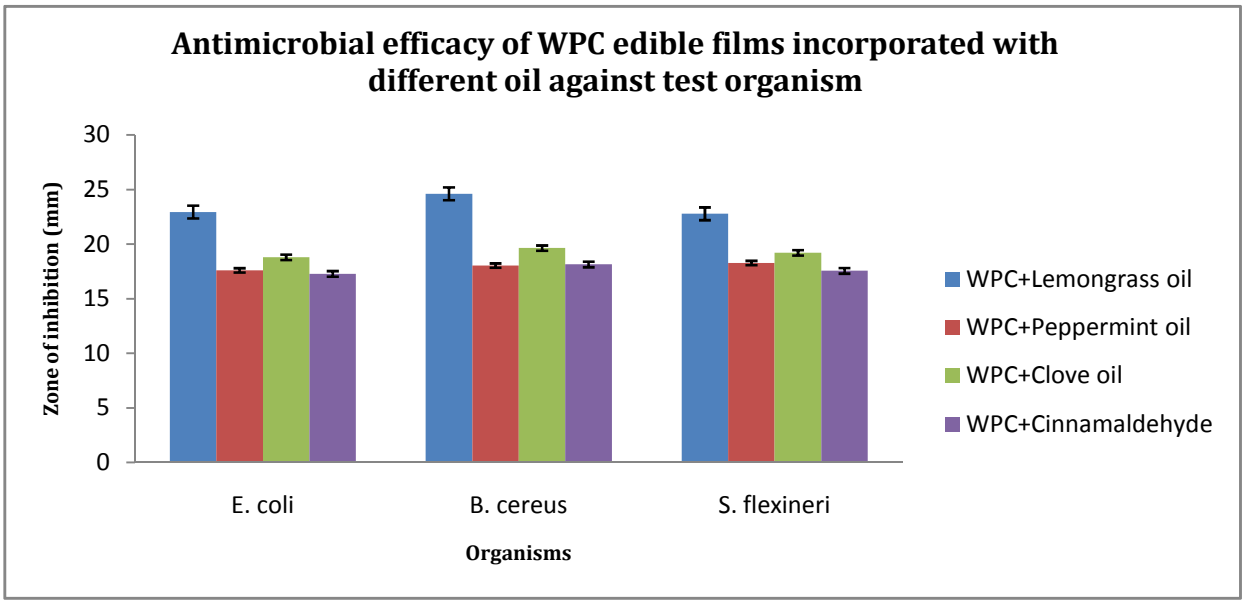


Table 4. Antimicrobial efficacy (Zones of inhibition) of WPC edible films incorporated with different oils against test organisms

Zone of Inhibition (mm)	WPC+Lemongrass oil	WPC+Peppermint oil	WPC+ Clove oil	WPC+ Cinnamaldehyde
E.coli	22.94±0.78[b]	17.60±0.54[a]	18.80±0.44[a]	17.28±0.51[a]
B.cereus	24.61±0.63[b]	18.04±0.18[a]	19.64±0.72[a]	18.14±0.63[a]
S.flexineri	22.78±0.92[c]	18.28±0.16[b]	19.21±0.48[b]	17.56±0.23[a]

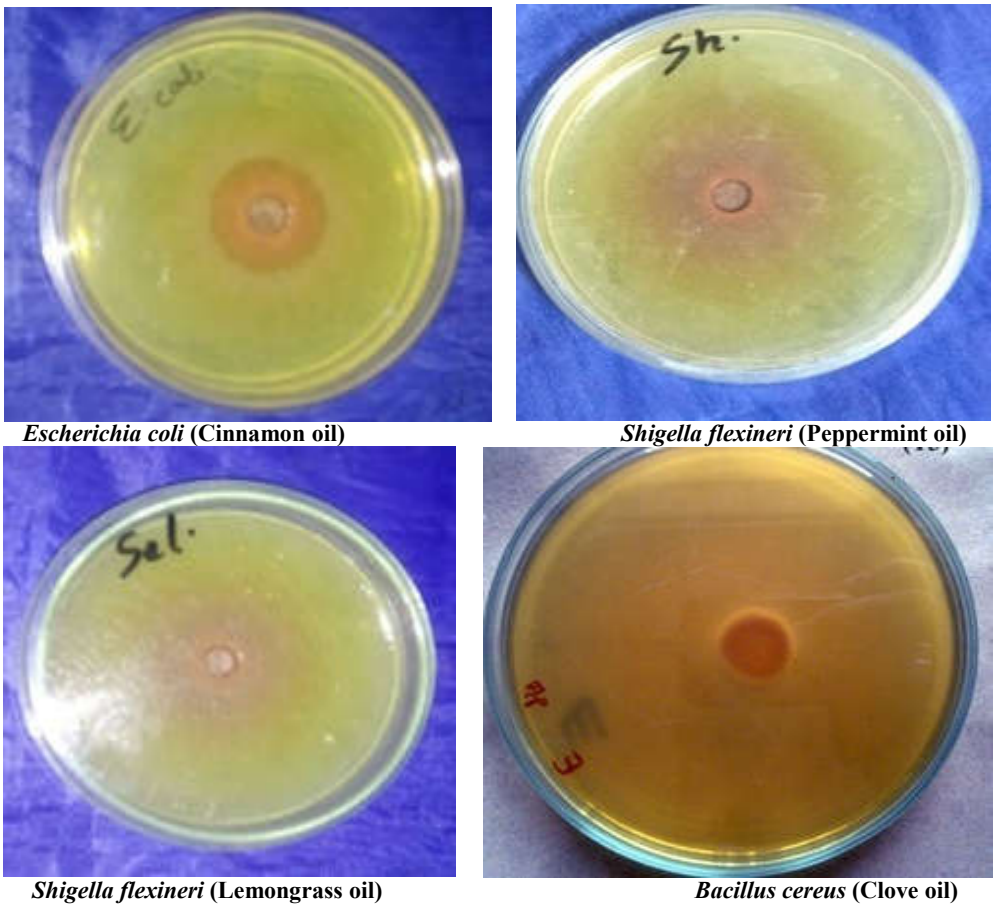
n = 6 Mean ± S.E with different superscript differ each other significantly (P<0.05)



WPC + Clove oil film **WPC + Cinnamaldehyde oil film**

WPC + Lemongrass oil film **WPC + Peppermint oil film**

Figure 1. WPC based films incorporated with different essential oils



Escherichia coli **(Cinnamon oil)** ***Shigella flexineri*** **(Peppermint oil)**

Shigella flexineri **(Lemongrass oil)** ***Bacillus cereus*** **(Clove oil)**

Figure 2. Various zone of inhibition developed by the addition of various essential oil

The antimicrobial activity varied significantly ($P<0.05$) with respect to concentration amongst the treatments. Peppermint and clove oil were incorporated in film forming solution at level 0.5%. Perusal of Table 4 depicted that the essential oils used in the study has strong inhibitory effect against test organisms. Therefore, it is recommended that 0.5% concentration level of essential oil can be successfully incorporated in WPC films with desired antimicrobial efficacy.

Conclusion

- An attempt was made to develop an antimicrobial whey protein concentrate based edible films.
- The optimized formulation mixture for the WPC films include 6% w/v WPC, 0.3% sodium alginate, 3% glycerol and drying temperature of 45^0C for 7-8 hours have thickness of 210±4.28 µm and WVTR of 0.00187 g/m²t possessed good moisture and gas barrier properties.
- The level of incorporation of 0.5% (v/v) cinnamaldehyde, peppermint oil, clove oil and lemongrass oil in WPC based edible film was added and had effective antimicrobial activity against majority of tested food pathogen.
- Lemongrass oil possesses greater antimicrobial activity against tested pathogen whereas cinnamaldehyde possess weak antimicrobial activity against tested organisms.
- It is recommended from the results that WPC films incorporated with 0.5% lemongrass oil can be successfully used for the extension of shelf life of perishable foods.

REFERENCES

Bahram, S., Rezaei, M., Soltani, M., Kamali, A., Ojagh, S.M. and Abdollahi, M. 2014. Whey protein concentrate edible film activated with cinnamon essential oil. *Journal of Food Processing Preservation*, 38(3):1251-1258.

Kim, S.J. and Ustunol, Z. 2001. Solubility and moisture sorption isotherms of whey-protein-based edible films as influenced by lipid and plasticizer incorporation. *Journal of Agricultural Food Chemistry,* 49(9):4388-91.

Luhovyy, B.L., Akhavan, T. and Anderson, G.H. 2007. Whey proteins in the regulation of food intake and satiety. *Journal of the American College of Nutrition*, 26(6):704-712.

Nur Hanani, Z.A., Roos, Y.H. and Kerry, J.P. 2012. Use of beef, pork and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties. *Food Hydrocolloids*, 29:144-151.

Pranoto; Yudi; Rakshi; Sudip; Salokhe and Vilas, 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *Food Science and Technology*, 38:859-865.

Singh, R. 2015. Antimicrobial and antioxidant activities of *Mentha piperita L. Arabian Journal of Chemistry,* 8(3): 322-328.

Snedecor, G. W. and Cochran, W. G. 1994. Statistical Methods, 8th edition. *Journal of Educational and Behavioural Statistics*, 19(3):304-307.

Sobral, P., DeAlvarado, J.D., Zaritzky, N.E., Laurindo, J.B., Gomez-Guillen, C., Anon, M.C., Montero, P., Denavi, G., Molina Ortiz, S., Mauri, A., Pinotti, A., Garcia, M., Martino, M.N. and Carvalho, R. 2005. Films Based on Biopolymer from Conventional and Non-Conventional Sources. *Food Engineering: Integrated Approches*, *Food Engineering Series, Springer*, New York, NY, pp193-223.

Trout, E. S.; Hunt, N. C.; Johnson, D. E.; Claus, J. R.; Kashner, C. L. and Krolpf, O. H. 1992. Chemical, physical and sensory Characteristics of ground beef containing 5-30 percent fat. *Journal of Food Science,* 57(1):25-29.

Vieira, M. G. A.; Silva, M. A. D.; Santos, L. O. D. and Beppu, M. M. 2011. Natural based plasticizers and biopolymer films. *European Polymer Journal*, 47(3):254-263.

****