ISSN: 2230-9926

Availableonlineathttp://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 10, pp. 23501-23506, October, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

DEVELOPMENT OF STARCH BASED EDIBLE FILMS

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

[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

ARTICLEINFO

Article History:
Received 19th July, 2018
Received in revised form
20th August, 2018
Accepted 06th September, 2018
Published online 30th October, 2018

Key Words:
Starch Films, Essential oils,
Antimicrobial activity,
Escherichia coli, Bacillus cereus.

ABSTRACT

The present study was conducted with an objective to standardize the formulation and process protocol for the development of starch based biodegradable antimicrobial films for the extension of shelf-life of food and food products. The processing technology of starch edible films was optimized using response surface methodology with different levels of starch (4%, 5%, 6% w/v) incorporated with 0.3% level of Sodium alginate and 5% of glycerol at drying temperature 35^{0}C. The starch level was standardized as 6% on the basis of biochemical characteristics viz. thickness, penetrability, moisture, WVTR, solubility, L*, a* and b* values. Different essential oils were selected on the basis of their antimicrobial activity. The standardized formulation of starch was incorporated with 0.5% of essential oils viz. cinnamaldehyde, peppermint oil, clove oil and lemongrass oil. The antimicrobial efficacy was then assessed on the nutrient agar medium against pathogenic micro-organisms viz. E. coli and B. cereus. This was experimented by well diffusion method on the basis of zone of inhibition.

Citation: Prerna Singh, Anshu Sibbal Chatli and Harneet Kaur Mehndiratta, K. K. 2018. "Development of starch based edible films", *International Journal of Development Research*, 8, (10), 23501-23506.

INTRODUCTION

Increased use of synthetic polythene bags has led to serious ecological problems due to their total non-biodegradability. To overcome these problems, there is an urgent need to develop packaging films, which are safe and eco-friendly. One possible solution may be utilization of naturally derived materials for the development of biodegradable films. Biodegradable films are generally used for food packaging due to its edibility and safety. Biodegradable films degrade naturally. Biodegradability varies with the conditions of sunlight, moisture, oxygen and composting (Tharanathan, 2003). Biodegradation is enhanced by reducing the hydrophobic properties and increasing hydrophilic properties. Biodegradable plastics or bio-plastics are made from renewable raw materials such as starch, protein, polysaccharides, lipids, fibers etc. Among all the natural polymers, starch has been considered as one of the most promising candidate for future material, because of its attractive combination of price, abundance and renewable in addition to biodegradability. All the plant seeds and tubers contain starch, which is predominantly present as amylose and amylopectin. Plants use starch as way to store excess glucose. Foods that are high in starch include bread, grains, cereals, rice, potato, peas, corn etc. Starch exhibits thermoplastic behaviour (Choi *et al*, 2007). The essential oils like cinnamaldehyde, lemongrass oil, clove oil, peppermint oil etc are traditionally being used in food and medicines due to their antimicrobial effects. The biodegradable packaging films with antimicrobial properties can be developed with the addition of these essential oils. The developed antimicrobial films can be used to enhance the storage stability of different perishable foods.

In view of the above discussion, the present study was conducted with the following objectives:

- To develop starch based biodegradable films and their quality evaluation.
- To develop antimicrobial biodegradable films with the incorporation of selected levels of different essential oils.
- To elucidate the antimicrobial efficacy of the developed biodegradable films.

MATERIALS AND METHODS

Standardized Procedure for the Preparation of Potato Starch Edible Films (sef): The formulation and process protocol for the development of starch based edible films were

Corresponding author: **Prerna Singh**
Department of Biotechnology, Guru Nanak Girls College, Model Town, Ludhiana 141002, Punjab, India

standardized in the laboratory by conducting various preliminary trials with the modification of procedure documented by (Talja, 2007). Potato starch (6%) powder and (0.3%) sodium alginate were mixed in 100ml of distilled water with regular stirring. The mixture was heated to temperature 70□-72□ C for 10 minutes on the magnetic stirrer hot plate. The glycerol (5% V/V) was added slowly in the mixture. The hot mixture was tempered for some time at room temperature and thereafter poured in rectangular plastic tray size (10×15 cm), 80ml and 30 ml in petriplates (150 mm diameter). These plastic tray and plates were gently moved on the plane surface so that the mixture can be uniformly distributed. The films were dried at 35±2□C for 7 hours in hot air oven. Thereafter these films were gently peeled off with the help of forceps.
The dried films were stored in a humidity chamber with 50% relative humidity at 25±2□C temperature until evaluation. The films were analyzed for extensibility, penetrability, color profile (*L*, a*, b**), water vapour transmission rate (WVTR).

Development of Potato starch based edible films incorporated with essential oils: The antimicrobial potato starch based edible films were developed with the incorporation of different essential oils viz. Lemongrass oil, clove oil, peppermint oil, cinnamaldehyde at concentration level of 0.5% each. All the essential oils were added in the formulation mixture at a temperature 50±2□C and mixed for 15 min. at magnetic stirrer. It is assumed that the essential oil should be uniformly mixed into the formulation mixture. The films were developed by casting method as described in 2.1. The developed films were subjected to physico-chemical analysis to evaluate various parameters viz. thickness, penetrability, WVTR, GTR etc.

Analytical technique

pH: The pH of mixture was determined as per the method given by Trout *et al* (1992) using digital pH meter equipped with a combined glass electrode in the homogenized mixture. The formulation mixture tempered at room temperature before measuring pH.

Microbiological analysis:

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

Optimization of Inoculation Dose: The dose rate of the inoculum 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□-10□ cfu/ml.

Well Diffusion Method: Formulation solutions of the starch edible films incorporated with the known levels of cinnamonaldehyde, lemongrass oil, peppermint oil and clove oil. The well of size 10mm of diameter cut by using sterile cork borer on solid media (Nutrient agar media) which was inoculated with 0.1ml of inoculums of containing *E. coli and B. cereus* in the range of 10□-10□ cfu/ml. The plates were then incubated at 37□C for 24 hours. The diameters of inhibitory zone surrounding the SEF wells were measured with the help of digital vernier calipers.

Processing Quality Characteristics of Starch based edible films

Colour Profile Analysis: Colour profile was measured using LovibondTintometer (Lovibond RT-300, Reflectance Tintometer, United Kingdom) set at 2□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 SEF films. The instrument was caliberated using light trap (back hole) and white tile provided with the instrument. The instrument was directly put on the surface of 3 individual SEF 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□C for these measurements. The whole assembly was kept at 25□C and weight loss of the test cell was measured after storage for 24 hour. WVTR of thefilm 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 20 mm 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 were calculated automatically by the preloaded software in the texture analyzer (TMS-Pro, Food Technology Corporation, USA) from the force-time.

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 the present study was to develop antimicrobial biodegradable films using potato starch as base material incorporated with selected levels of essential oils viz.

Table 1. Physicochemical Parameters of starch based edible films

Physicochemical parameters	Value*
Film thickness (µm)	151.2±0.79
Penetrability(N)	0.90±0.07
Water vapor transmission rate (WVTR) (g/m²/t)	0.00254±0.0001
Extensibility(N)	10.67±0.16
*L**(lightness)	21.64±0.38
*a**(redness)	5.78±0.18
*b**(yellowness)	11.21±0.51

n=6, *Mean ± S.E. (Standard error)

Table 2. Physicochemical characteristics of edible films added with different essential oils

Parameter	Control	Starch+ Cinnam-aldehyde	Starch+ peppermint oil	Starch + Clove oil	Starch+ Lemon-grass oil
Essential oil conc. (%v/v)	0	0.5	0.5	0.5	0.5
Thickness (mm)	0.151±0.04^{a}	0.174±0.008^{b}	0.159±0.009^{a}	0.180±0.012^{bc}	0.188±0.011^{c}
Moisture content	21.95±1.69^{c}	18.80±1.10^{b}	19.85±1.10^{b}	16.90±1.15^{a}	16.54±0.59^{a}
Solubility in water (%)	27.88±2.01^{c}	25.25±1.78^{c}	26.92±0.95^{c}	24.55±0.75^{b}	22.86±1.49^{a}
Penetrability (N)	0.90±0.07	0.45±0.05	1.09±0.08	0.67±0.09	0.79±0.04
Extensibility (N)	10.67±0.16	4.53±0.20	8.04±0.22	2.05±0.18	4.80±0.20

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

Table 3. Colour profile of starch edible films incorporated with different essential oils

Parameter	Control	Starch+ cinnam-aldehyde	Starch+ peppermint oil	Starch+ clove oil	Starch+ Lemon grass oil
Essential oil conc. (v/v %)	0	0.5	0.5	0.5	0.5
L *	78.09±1.32	76.67±2.06	77.02±1.97	77.45±1.79	76.97±1.90
a *	1.67±0.11^{b}	1.67±0.002^{b}	1.68±0.01^{c}	1.66±0.08^{a}	1.66±0.05^{a}
b *	14.70±1.11	14.14±2.83	14.23±1.98	14.68±0.50	14.29±1.76

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

Table 4. Antimicrobial effect of developed films with the different essential oil against test organisms

Zone of inhibition (mm)	Starch+ Cinnam-aldehyde	Starch+ Peppermint oil	Starch + Clove oil	Starch+ Lemongrass oil
E. coli	16.12±0.21^{b}	15.28±0.16^{a}	17.89±0.28^{c}	19.36±0.21^{d}
B. cereus	17.06±0.34^{b}	16.12±0.11^{a}	18.24±0.16^{c}	20.18±0.26^{d}

n=3, Mean ± S.E. with different superscript differ each other significantly (P<0.05)

lemongrass oil, clove oil, cinnamaldehyde and peppermint oil. The developed films were elucidated for their antimicrobial efficacy against common spoilage organisms viz. *E. coli and B. cereus.* The above mentioned objectives were achieved by conducting 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. The inference was drawn by critically analyzing the results with a suitable support of available literature. This has been attempted systematically experiment wise in the present chapter.

Experiment No.1: To develop starch based edible films

The process and formulation of the starch based biodegradable/edible films was optimized and physicochemical characteristics viz. thickness, penetrability, WVTR, extensibility, *L*, a** and *b** value of the developed films were evaluated.

Physicochemical characteristics of Starch edible films

Thickness: The thickness of film is directed by formulation mixture material, its type, nature, interaction among the compounds and chemical nature. In the present study, the thickness of developed film was 151µm. Further, it was observed that the film thickness decreased with the increasing levels of glycerol.

Penetrability: The penetrability was recorded as 0.90 N with the area of 6.1564 mJ for the starch based films. It is directed by glycerol content which influences chain mobility during deformation leading to increase in puncture value (Sobral*et al*, 2005). Similar findings have also been reported by Singh (2015) and NurHanani*et al* (2014) in protein based edible films.

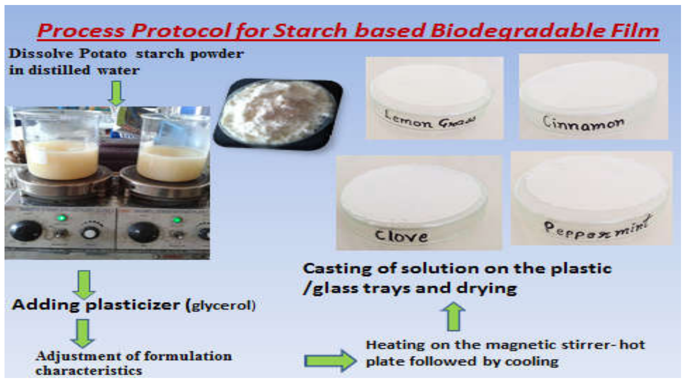


Figure 1. Process Protocol for Starch based Biodegradable Film

Water Vapour Transmission Rate (WVTR): VTR is measured as 0.00254 g/m^{2}/h for the developed films. The higher WVTR is attributed to water absorption property of starch. However, this property is reduced with the incorporation of hydrophobic plasticizers and subsequently essential oils.

Figure 2. Starch based films incorporated with different essential oils

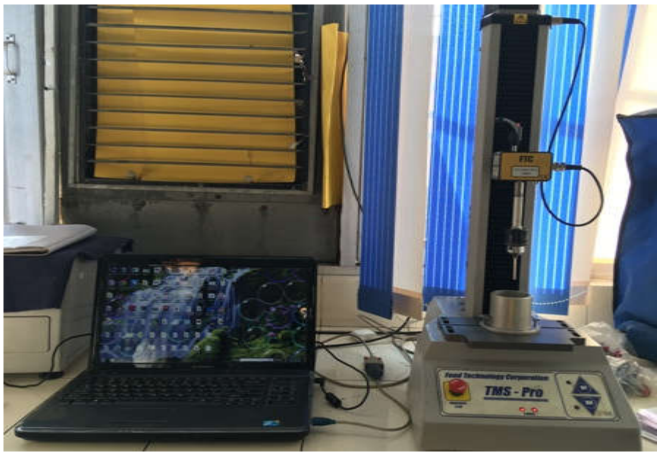
(a)

(b)

Figure 3. (a) Water Vapour Transmission Rate (WVTR), (b) Oxygen/Gas transmission Rate (GTR)

Extensibility: This percent extensibility is used as the measure of flexibility, pliability and resiliency of the film or coating. The extensibility of the developed films is measured as 10.67 N. Generally, plasticizers are added to these polymers to improve the overall flexibility and extensibility of the resulting film.

Colour Profile Analysis (*L, *a** and *b** values):** The packaging films, which are used for wrapping of food products have a direct contact with the food product and significantly contributes to the appearance and consumer acceptance of the food products. The developed films have (*L**) value lightness 21.64±0.38, (*a**) redness 5.78±0.18 and (*b**) yellowness 11.21±0.51. The lower lightness and redness value exihibited due to transparent nature of the films. The potato starch is light colour material and the developed films were transparent to transluscent. This feature provides the visibility to the packaged food.



(a)

(b)

Figure 4. (a) TMS-Pro, (b) Lovibond RT-300, Reflectance Tintometer

Physicochemical characteristics of the edible films incorporated with different essential oils: The effect of different essential oils on physicochemical characteristics of edible films can be observed in Table 2. The thickness has been increased significantly with the addition of different oils into the starch film solution than control. Due to the hydrophilic and hydrophobic interactions between water and oil, the films become more resistant to moisture and provide good barrier properties against moisture. Perusal of Table 2 shows that the solubility (%) in water is decreased significantly (P<0.05) with the incorporation of the essential oil in starch based films. It was observed lowest for lemongrass oil incorporated starch films (22.86±1.4) and highest for peppermint oil incorporated starch films (26.92±0.95) amongst the treatments. The lower values in treated films are attributed to essential oil, hydrophobic component added in the formulation mixture of starch edible films. The non-solubility of added essential oil in water and more solubility in non-polar solvents is the main reason for decreasing the percent solubility of films in water. Colour profile of starch edible films (Table 3) depicted that the basic parameters of colour i.e. Lightness (*L**), Redness (*a**) and Yellowness (*b**) did not vary significantly (P<0.05) with the incorporation of essential oils in the starch films, irrespective of the type of the essential oil added. It might be due to lower percentage (0.5%) of essential oil added in the films solution which did not influence colour characteristics. This attribute can be considered as good for food packaging as packaging material should not influence the colour of food product.

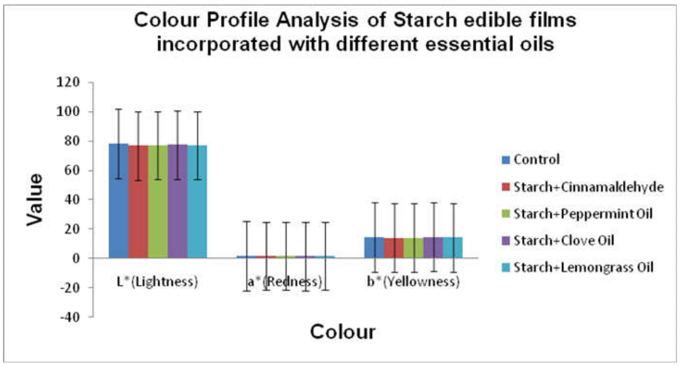


Figure 5. Colour Profile Analysis of Starch edible films incorporated with different essential oils

Starch + Clove Oil Film **Starch + Peppermint Oil Film**

Starch + Lemongrass Oil Film **Starch + Cinnamon Oil Film**

Figure 6. Starch based films incorporated with different essential oils

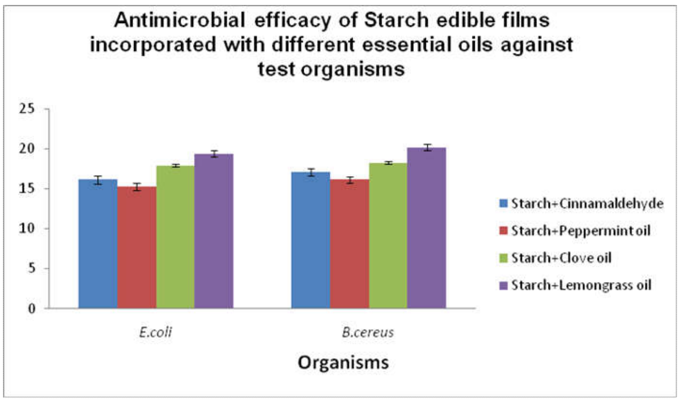
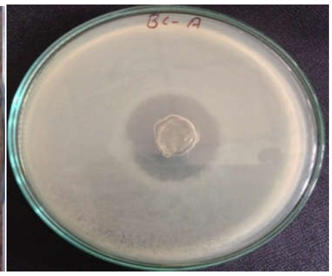


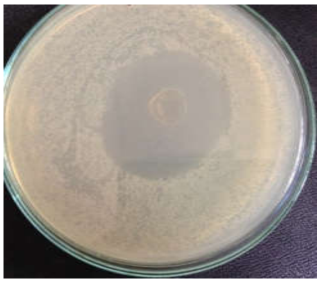
Figure 7. Antimicrobial efficacy of Starch edible films incorporated with different essential oils against test organisms

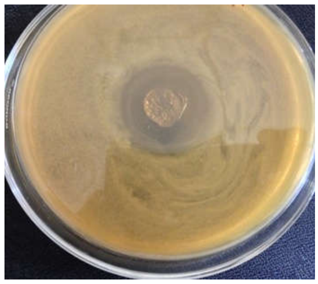
Experiment No.2 To optimize the level of incorporation of essential oils antimicrobial substances into the developed films

Incorporation of antimicrobial substances into the developed bioactive films: The starch based edible films developed in experiment 3.1 possess good moisture and gas barrier properties, thus can preserve the organoleptic properties of the product. But these lack the antimicrobial activity, which is essential to reduce microbial deteriorative changes. Thus incorporation of antimicrobial substances viz. cinnamaldehyde, lemongrass oil, peppermint oil, clove oil were carried out in the polymer matrix material, to provide the antimicrobial properties against the pathogenic and food spoilage bacteria namely *Bacillus cereus, E. coli.* The mixture formulation of the biodegradable films incorporated with 0.5% level of cinnamaldehyde, lemongrass oil, clove oil, peppermint oil. About 150µl of each solution was poured into well of 10mm of diameter made by using sterile cork borer on solid media in nutrient agar inoculated with *E. coli and B. cereus*. The media was previously inoculated with 0.1ml of inoculums of containing indicator microorganisms in the range of 10□-10□ cfu/ml.



Starch + Lemongrass oil film (*E. coli*) **Starch + Clove oil film (*B. cereus*)**





Starch + Lemongrass oil film (*B. cereus*) **Starch + Cinnamon oil film (*B. cereus*)**

Figure 8. Various zones of inhibition developed by adding different essential oils against test organisms

The plates were then incubated at 37□C for 24 hrs. The diameters of inhibitory zone surrounding the wells were measured with the help of digital vernier calipers. The antimicrobial effect of lemon grass oil incorporated starch films was highest which was followed by clove oil. The peppermint oil incorporated films exhibited lowest antimicrobial activity against both the test organisms i.e. *E. coli* (15.28±0.16) and *B. cereus* (16.12±0.11). Further it was observed that *E. coli* exhibited lower zone of inhibition diameter than *B. cereus,* irrespective of the type of oil used to develop starch based films. The inhibitory effect of clove oil and lemongrass oil was more than 80% against both the test organisms. The antimicrobial efficacy of essential oils against *E. coli* and *B. cereus* varied in a pattern peppermint oil

<cinnamaldehyde< clove oil < lemongrass oil. The lemongrass oil has highest antimicrobial efficacy whereas peppermint oil has lowest antimicrobial efficacy against test organisms.

Conclusions

- The acceptable quality starch edible films can be successfully developed with 6% potato starch and using glycerol 5% as plasticizer in the formulation mixture.
- The optimum level of incorporation of essential oils viz. peppermint oil, clove oil, lemongrass oil, cinnamaldehyde is 0.5% in starch edible films.
- The thickness of films increased with the incorporation of essential oil in the starch films.
- The moisture content and percent solubility in water of starch edible films decreased with the incorporation of essential oil.
- The colour profile characteristics remained comparable in all treated and control film.
- The starch films incorporated with lemon grass oil are able to inhibit the spoilage organisms (*E. coli* and *B. cereus*) completely whereas the starch films incorporated with clove oil inhibit test organisms 90%.
- The antimicrobial starch films incorporated lemongrass oil and clove oil can are recommended for the packaging of perishable food products.

REFERENCES

Choi, Y, Jeong, H.S. and Lee, J. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chemistry.* 103(1):130-38

NurHanani, Z.A., Roos, Y.H. and Kerry, J.P. 2014. Use and application of gelatin as potential biodegradable packaging materials for food products. *International Journal of Biological Macromolecules,* 71:94-102.

Pranoto, Y.,Rakshit, S.K. and Salokhe, V.M. 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *Food Science and Technology,* 38**:**859-865.

Singh, P.T.,Chatli, M.K. and Sahoo, J. 2015. Development of chitosen based edible films: process optimization using response surface methodology. *Journal of Food Science Technology*, 52(5):2530-2543.

Sobral, P., De Alvarado, 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.

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

Talja, R. A. 2007. Preparation and Characterization of potato starch films plasticized with polyols. *University of Helsinki, Department of Food Technology*, 1-65.

Tharanathan, R.N. 2003. Biodegradable films and composite coating: Past, present and future. *Trends in Food Science and Technology*, 14(3):71-78.

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.

****