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Research Article

Development of Biodegradable Packaging Film using Potato Starch

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Abstract

This research work deals with the possibility of extraction of starch from the potato and incorporating this starch to make biodegradable packaging film using glycerol as a plasticizer. This work report deals with the methodoly of preparation of film by casting method and the results of tests which were performed to the mechanical property of the film. The test included tensile strength of the film, puncture strength of the film, moisture permeability of film, water absorbed by the film and elongation at break. The films were formed by making a filmogenic solution of potato starch (7.5gm,11.25gm),glycerol(1.5ml,2ml) and citric acid(1 gm each).Two samples of films were made i.e. sample 1 (7.5 gm starch,1.5ml glycerol and 1gm citric acid),sample 2 (11.25 gm starch,2ml glycerol and 1gm citric acid).The result of this study demonstrated that the film made by sample 1 were having god tensile and puncture strength compared to sample 2. While considering other parameters in combination with tensile and puncture strength such as moisture permeability, water absorbed by film and elongation at break the results showed that the film made by sample 1 showed better results than sample 2.The sample 1 was found more appropriate in enhancing the shelf life of the food as it showed lesser amount of moisture permeability and water absorption. Both the samples were used to test the performance evaluation of film by packing chikki in it. Moisture Content, Ash Content and Total Plate Count Method were used to perform microbial and physio- chemical analysis of chikki. The results of moisture content, ash content and total plate count of sample 1 were more suitable and acceptable in comparison to sample2.The films was found appropriate and suitable for packaging of chikki and similar bakery products,

Keywords: *Biodegradable, Starch, Fimogenic Solution Glycerol, Food Packaging, Chikki, Performance Evaluation*

1. Introduction

Petrochemical based plastics films such as polyolefin, polyesters and polyamides have been increasingly used as packaging materials because of their availability in large quantities at low cost and favorable functionality characteristics such as good tensile and tear strength, good barrier properties to oxygen and aroma compounds and heat seal ability. However, these plastics are made of petroleum based materials that are not readily biodegradable and therefore lead to environmental pollution, the most obvious form of pollution associated with plastic packaging is waste plastic dump to the landfills.

In order to overcome these problems, several studies are concentrated on the development of new biodegradable packing film materials that can be manufactured with the utilization of environmentally friendly raw materials.

Biodegradable plastic films can form the basis for environmentally preferable, sustainable alternative to current material exclusively based on petroleum feed stocks. Biopolymers are generally capable of being utilized by living matter (biodegraded), and so can be disposed in safe The use of biopolymers within this field appears as an excellent alternative for reducing current environmental problems (Pablo *et al.* 2007).These bio-based materials offer value in the sustainability of life-cycle equation by being a part of the biological carbon cycle. hough somewhat expensive, biopackaging is tomorrow's need for packaging especially for a few value added food products (Tharanathan 2002). Among the natural polymers, starch has been considered as one of the most promising candidates for future materials because of its attractive combination of price, abundance and renewable in addition to biodegradability.

Starch a renewable source, appears to be the best raw material of biodegradable polymer with low cost. Starch from different sources has been studied as a potential film-forming agent, including that from potato and barley, wheat, tapioca, and rice. Films developed from starch are described as isotropic, odorless, colorless, non-toxic and biologically degradable (Flores *et al.* 2007).

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Potato (*Solanum tuberosum L.*) is one of the world's major agricultural crops and is member of the Solanaceae family. It has been in cultivation since its introduction in the early part of the 17th century and is considered of significant importance as food crop. India is the second largest producer of potato with a production of about 34.4 million MT after China which produces 69.06 million MT .India contributes about 11.26% of the total potato production in the world. Potato starch based biodegradable packaging film is prepared by the method of casting.

The performance evaluation of film was done by Chikki. Chikki is a traditional Indian ready-to-eat Indian sweet generally made from groundnuts and jaggery . Jaggery is obtained by concentrating sugar cane juice to solid or semi solid state. It is a natural sweetener having a sweet winy flavor (Shahi 1999) and contains protein, minerals and vitamins and is a potent source of iron and copper (Manay and Swamy 2001).

Finally, the typical petroleum based plastics takes a long time to degrade because of the molecular bonds that make the plastics so durable and equally resistant to natural processes of biodegradation.

The main objectives of this research were to produce biodegradable plastic films which are obtained from Potato starch and to study its suitability for the food packaging. These plastic films are to be tested to ensure that these are appropriate for the food packaging.

2. Material and Methods

2.1 Raw materials

Potaro starch was used for preparation of biodegradable plastic film from starch. Glycerol was used as a plasticizer in the filmogenic solution to increase the flexibility and plasticity of the film. Citric Acid was also added to the solution. In packaging films, citric acid is added to increase the antimicrobial, plasticizing and dispersing effect in biodegradable/edible films and to improve the mechanical properties and water vapor permeability (Cagri and Ustonol 2004 and Wang *et al.* 2007). Distilled water was added to solution as it acts as the plasticizer and it decreases the brittleness of plastic films. So water is used to make the solution of starch.

2.2 Extraction of Starch

Starch was isolated from potato as per the method described. Potatoes were washed, peeled and shreds were put into pestle and mortar and distilled water was added to it. Potato shreds were crushed hard in pestle and mortar and then straining of potatoes was done by tea strainer and left over potatoes were kept in mortar. Then distilled water was added and strained twice. The mixture was left in beaker undisturbed until entire starch was settled down at the bottom. White starch was settled at the bottom. Again add distilled water to the beaker and stir it. Water was decanted from the beaker and pure starch was obtained which can be used for film formation.

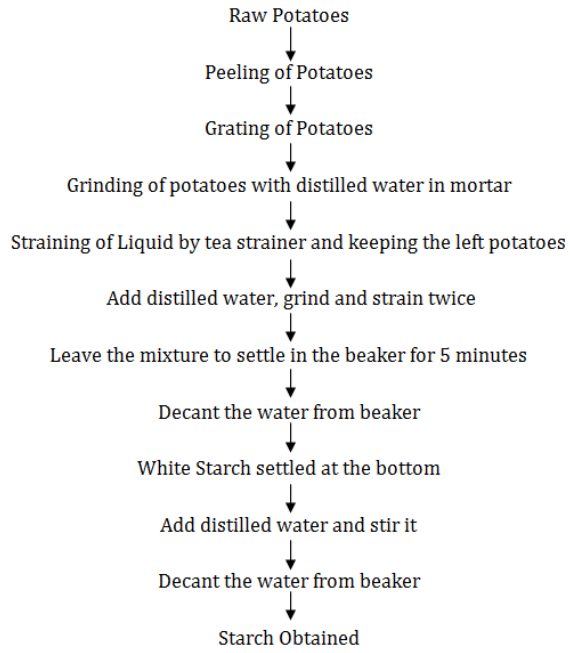


Fig.1 Flowchart for extraction of Starch

2.3 Preparation of film

The films were prepared by casting technique using a film-forming solution containing potato starch. Glycerol was used as plasticizer. The mixture of dry starch, water and glycerol was taken in a beaker. Distilled water was added to it. Then required amount of citric acid was added to the solution. The entire mixture was filtered with the help of muslin cloth. The mixture was mixed with the help of glass rod on hot plate at 40˚C for 5 mins. Now the mixture was kept in water bath at 70°C temperature for 10 minutes. Now a cast was prepared and the entire solution was poured on the cast and was left for drying at room temp for 48 hrs. After drying the films were peeled off and were kept in poly bags away from moisture.

Film Formation

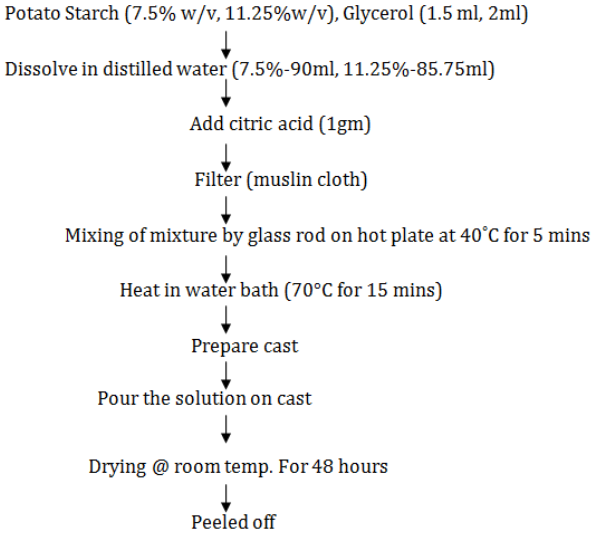


Fig.2 Flowchart for Film Formation

2.4 Thickness of the Film

The thickness of the film was measured using micrometer made by Singhla scientific industries, model: MM156.The micrometer used in research work was manual one

2.5 Mechanical Properties of the Film

The mechanical properties of the film were calculated by using texture profile analyzer made by Stable Micro Systems, model: TA TXT plus. It was used to calculate the tensile strength, puncture strength and elongation at break of the film. Moisture permeability test was conducted to check amount of moisture absorb by salt packed in the film. Water absorption test was conducted to test the hygroscopic nature of film by dipping film in water and then measuring it weight at regular intervals.

2.6 Performance Evaluation of the Film

Performance of the film was checked by packaging chikki (a bakery product) in the film. The microbial analysis was conducted by total plate count method and physio-chemical analysis was done by calculating moisture content and ash content of the film.

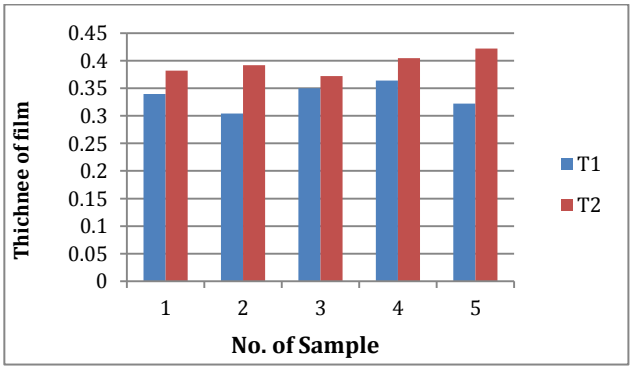
2.6 Statistical Analysis

The experiment was conducted by adopting completely randomized design the data recorded during the course of investigation were statistically analyzed by the 'Analysis of variance- One way classification or single factor 'ANOVA'(Fisher 2000). It gives an appropriate method capable of analyzing the variation of population variance. The significant effect of treatment was judged with the help of 'F' (variance ratio). Calculated F value was compared with the table value of F at 5% level of significance. If calculated value exceeded the table value the affect was considered to the significant. The significance of the study was tested at 5% level.

3 Results and Discussion

3.1. Thickness of Film

The film thickness was approximately constant.



Graph 1 Thickness of film

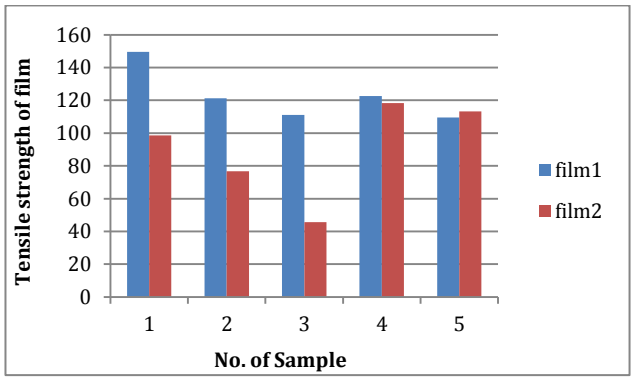
All the samples were measured and the mean thickness were then calculated with the use of micro meter and showed that the mean thickness were in the range of 0.322 mm to 0.425 mm similar results for the film thickness were reported by Wang *et al*. 2007..

3.2 Mechanical properties

Following mechanical tests were performed to check the efficiency of the film. The results of the tests are discussed below:-

3.2.1 Tensile strength of Film

The plasticized films were prepared and tested for its tensile strength using a texture profile analyzer. By this analyzer the exact tensile strength can be measured according to ASTM D882. The change in mechanical properties is characterized by the effect of plasticizers which weakens the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of mechanical resistant (Sorbal *et al*., 2001). Thus the increase in the plasticizer concentration causes a reduction of the TS due to the decrease in the intermolecular interactions. Therefore the films prepared with lesser glycerol content i.e. 1.5 ml glycerol was having higher tensile strength than those having higher glycerol content i.e. 2 ml glycerol.



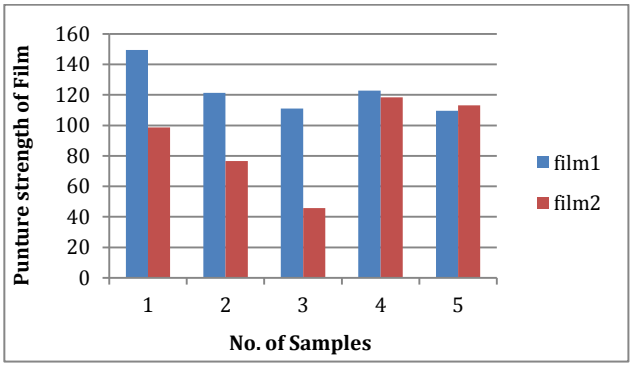
Graph 2 Tensile strength of film

3.2.2 Puncture strength of Film

The plasticized films were prepared and tested for its tensile strength using a puncture profile analyzer. By this analyzer the exact tensile strength can be measured according to ASTM D882.

In general, it was observed that the films became more extendible when the concentration of plasticizer was increased which results in the reduction of puncture and tensile strength of the film. The reduction of the puncture force was consequences of the incorporation of plasticizers, and to water molecules absorbed by the samples, a common phenomenon of edible films, as has been revealed in other studies (Sobral *et al*., 2001)

Thus when glycerol concentration increased from 1.5 ml to 2ml, the greater incorporation of glycerol into starch resulted in reduction of puncture strength of the film.

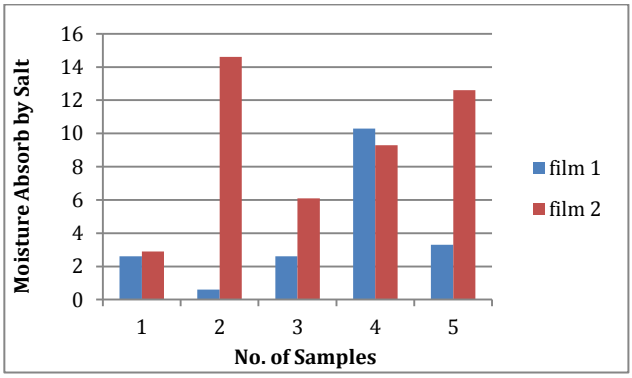


Graph 3 Puncture strength of Film

3.2.3 Test for Moisture Permeability

Moisture permeability is a major property of films that is related to the structural properties of film and the presence of components in the films, since potential applications may require water insolubility to enhance product integrity and water resistance. Addition of glycerol at a level of 20% w/w reduced the water solubility value. Thus, might interact with water and interrupting the network by hydrogen bonds, reducing the cohesiveness of the natural gum matrix and increasing its solubility in water (Altiok *et al*., 2010). Tunc and Osman obtained similar results for methylcellulose films (Tunc *et al*., 2007).

Moisture gained by 1.5 ml glycerol concentration film was lower than that gained by 2ml glycerol concentration film.



Graph 4 Test for Moisture Permeability

3.2.4 Water absorb by the Film

Water is a polar compound having a small size that tends to favor both sorption and diffusivity in dense materials. This ability makes these materials more permeable to moisture than to non condensable gases. Water sorption test are useful for determination of film stability under various conditions, as many film constituents, especially hydrocolloids, they are sensitive to relative humidity and temperature. Water absorption test of sample T1 and T2 were done.

The weights of the sample were monitored at the end of the first 30min, 60min, and 180min. The percentage increase in weight was tabulated and that was taken as a measure of the water absorption of film. The water absorption percentage of films is given in Table 4.3.

Fig 1 Film cut into pieces

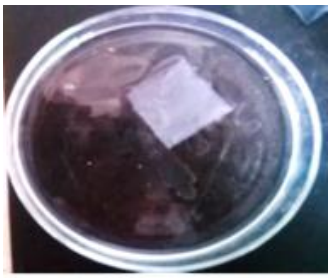
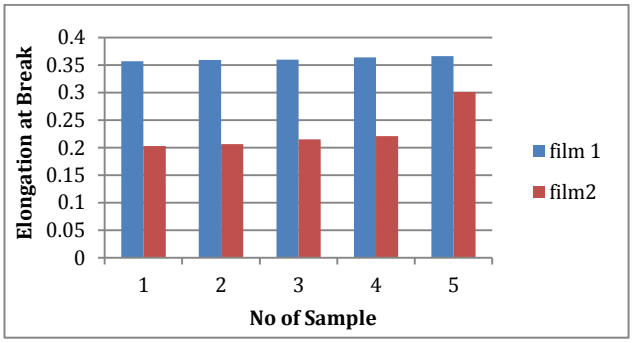


Fig 2 Water Absorption Test

3.2.5 Elongation at Break

With the increase of glycerol loading, a monotonous increase of Elongation at break values was observed. Effect of Glycerol addition of 10% glycerol in starch/chitosan-based films, the flexibility increased by 118%. (Wafa Tonny, Mohammad Oliuddin Tuhin, Rafiqul Islam and Ruhul Amin Khan 2014). (Bourtoom and Chinnan *et al* 2009) reported that strength of rice starch-chitosan composite film decreased with the addition of lipids, whereas plasticity increased.

Thus it can be observed that an increase in glycerol concentration results in decrease the percent elongation at break of the films.



Graph 5 Elongation at Break

3.3 Performance Evaluation of Film

The performance evaluation of the film was done by packing chikki with the sample. Three plates of samples were kept for twenty days to study the physio-chemical parameters and to perform microbiological analysis. The results obtained are shown under the following headings.

3.3.1 Microbial Analysis

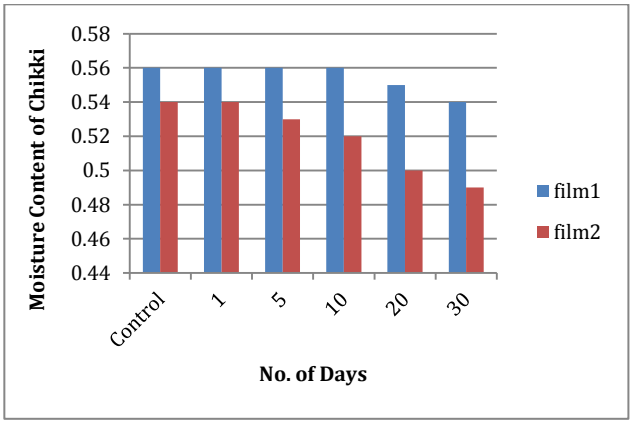
Total Plate Count Method was used to perform the microbiological analysis. The colonies were counted on a regular interval of 10 days as per the prescribed method in Ranganna 2002. The results obtained are shown in the Table 4.7. It was observed that there was a constant growth of yeasts and molds, but within the permissible limits and standards i.e. 2 x 10^3 cfu/ ml. similar results were obtained in the research conducted by Ferrer *et al.* 2002.

3.3.2 Pysio- Chemical Analysis

3.3.2.1 Moisture Content of Chikki

Moisture of *Chikki* is very critical as it determines the quality and stability of the product. All the products had a moisture content of 3.4 - 3.8%. Only marginal differences were observed in the moisture content Chikki samples. Similar results were reported for groundnut chikki (Chahal and Sehgal 1996) However, the moisture content increased during storage.

Thus moisture absorption of chikki packed in 1.5 ml glycerol content packaging film was less than that packed in 2 ml glycerol content of chikki due to difference in water absorption capacity of the film.

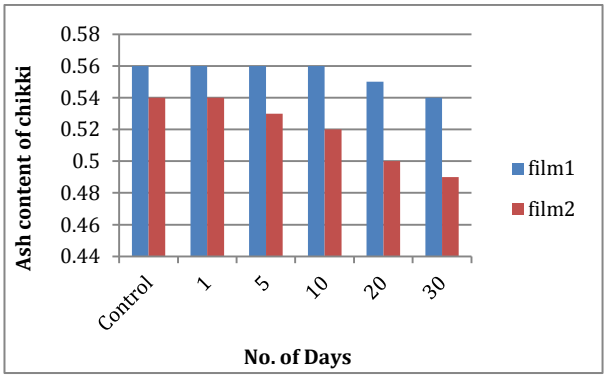


Graph 6 Moisture Content of Chikki

3.2.3.2 Ash Content of Chikki

Ash or mineral content is the portion of chikki that was left after it was burned at very high temperature. Determining the ash content is important factor for several reasons. It is part of proximate analysis for nutritional evaluation. Conversion of sample to ash is the first step in preparing a food sample for determination of specific elemental analysis.

There was no vast difference in ash content as the loss of minerals from chikki was very less due to its long shelf life.



Graph 7 Ash Content of Chikki

The table of all the tests performed is given in sequence in Appendix A.

3.4 Statistical Analysis

Complete Randomized Design was used for the statistical analysis of the results. A one way ANOVA was applied wherever possible to obtain concurrent and significant readings shown in Appendix B.

Conclusions

After the competition of the work we had ended with a cheaper alternative in comparison to other polymeric films by blending potato starch, glycerol and citric acid. The tensile and puncture test of the film helped us to find the efficiency and durability of the film which were more efficient than from films made by cassava, corn or any other starch containing product. The decrease in puncture and tensile strength with increase amount of glycerol control showed us that increased use of plasticizer would reduce the efficiency of the film. From water absorption test it was clear that pore size varies with the amount of potato starch. This data can be used to design specific food packaging film system. The water absorption test result, puncture test result, tensile strength test result , Elongation at break result are given and result are comparable those with already existing result.

The efficiency of the film was checked by packaging of chikki (a sweet bakery product) which gave appropriate result to the test done for performance analysis like total plate count, moisture content, ash content.

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Appendix A

Table 4.1.1 1.5 ml glycerol content (Thickness of Films Prepared)

Sample No.	Film thickness (mm)			
	R1	R2	R3	Mean
1	0.34	0.341	0.34	0.34
2	0.305	0.302	0.307	0.304
3	0.37	0.35	0.33	0.35
4	0.371	0.34	0.381	0.364
5	0.322	0.32	0.325	0.322

Table 4.1.2 2 ml glycerol content (Thickness of Films Prepared)

Sample No.	Film thickness (mm)			
	R1	R2	R3	Mean
1	0.381	0.38	0.385	0.382
2	0.390	0.393	0.395	0.392
3	0.372	0.371	0.373	0.372
4	0.402	0.41	0.405	0.405
5	0.422	0.421	0.425	0.422

Table 4.2.1 1.5 ml glycerol content (Tensile Strength of the Films)

Sample No.	Tensile Strength (g force)
1	366.8
2	364.2
3	366.9
4	367.5
5	366.4

Table 4.2.2 2ml glycerol content (Tensile Strength of the Films)

Sample No.	Tensile Strength(g force)
1	454.5
2	450.2
3	455.2
4	455.9
5	454.9

Table 4.3.1 1.5 ml glycerol content (Puncture Strength of the Films)

Sample No.	Puncture Strength (g force)
1	149.5
2	121.3
3	111.1
4	122.7
5	109.6

Table 4.3.2 2 ml glycerol content (Puncture Strength of the Films)

Sample No.	Puncture Strength (g force)
1	98.6
2	76.7
3	45.7
4	118.4
5	113.2

Table 4.4.1 1.5 ml glycerol content (Test for Moisture Permeability)

Sample No.	Moisture Gain by salt in 30 days (weight Increased %)			
	R1	R2	R3	Mean
1	2.8	2.7	2.5	2.6
2	0.8	0.5	0.7	0.6
3	2.3	2.9	2.6	2.6
4	10.5	9.5	11	10.3
5	3.6	3.3	3.2	3.3

Table 4.4.2 2 ml glycerol content (Test for Moisture Permeability)

Sample No.	Moisture Gain by salt in 30 days (weight Increased %)			
	R1	R2	R3	Mean
1	2.9	2.8	2.9	2.9
2	21.2	20.9	21	14.6
3	6.2	6.4	5.9	6.1
4	9.6	9.3	9.1	9.3
5	12.2	12.7	12.9	12.6

Table 4.5.1 1.5 ml glycerol content (Water absorb by the Film)

Treatments	Initial wt. (g)	Wt. at 30 min (g)	water absorption%	Wt. at 60min(g)	Water absorption(%)	Wt. at 180 min(g)	Water absorption(%)	Wt. after 24 h
1	0.48	0.98	97.09	1.41	172.07	1.79	295.18	No change
2	0.51	1.03	98.01	1.45	173.06	1.75	245.1	No change
3	0.53	1.06	98.04	1.47	173.35	1.85	246.36	No change
4	0.55	1.02	98.06	1.49	171.9	1.82	245.32	No change
5	0.56	1.04	98.08	1.43	171.8	1.88	246.9	No change

Table 4.5.2 2ml glycerol content (Water absorb by the Film)

Treatments	Initial wt. (g)	Wt. at 30 min (g)	water absorption%	Wt. at 60 min (g)	Water absorption(%)	Wt. at 180 min (g)	Water absorption(%)	Wt. after 24 h
1	0.605	1.5	125.04	1.89	196.9	2.54	272.08	No change
2	0.608	1.55	125.09	1.98	197.56	2.59	273	No change
3	0.703	1.59	126	2.09	197.68	2.62	273.01	No change
4	0.705	1.6	126.02	2.11	197.69	2.64	273.09	No change
5	0.707	1.63	126.05	2.13	197.81	2.69	273.1	No change

Table 4.6.1 1.5ml glycerol content (Elongation at break of the film)

Sample	Elongation (%)
1	0.203
2	0.206
3	0.215
4	0221
5	0.301

Table 4.6.1 2 ml glycerol content (Elongation at break of the film)

Sample	Elongation (%)
1	0.357
2	0.359
3	0.360
4	0.364
5	0.366

Table 4.7.1 1.5 ml glycerol content (Total Plate count changes observed in Chikki)

samples	Total Plate Count (cfu/g)		
	Day 1	Day 10	Day 20
Sample1	0.3×10^2	0.9×10^2	1.5×10^2
Sample2	0.5×10^2	1.1×10^2	1.9×10^2
Sample3	0.6×10^2	1.3×10^2	2.0×10^2
Sample 4	0.8×10^2	1.5×10^2	2.2×10^2

Table 4.7.2 2 ml glycerol content (Total Plate count changes observed in Chikki)

samples	Total Plate Count (cfu/g)		
	Day 1	Day 10	Day 20
Sample1	0.6×10^2	1.1×10^2	1.9×10^2
Sample2	0.9×10^2	1.3×10^2	2.0×10^2
Sample3	1.0×10^2	1.4×10^2	2.2×10^2
Sample 4	1.2×10^2	1.6×10^2	2.5×10^2

Table 4.8.1 1.5 ml glycerol content (Moisture Content of Chikki)

Sample	Time Duration Control	Moisture Content (%)
1	0 day	0.54
2	Day 1	0.54
3	Day 5	0.54
4	Day 10	0.55
5	Day 20	0.60
6	Day 30	0.65

Table 4.8.1 2 ml glycerol content (Moisture Content of Chikki)

Sample	Time Duration Control	Moisture Content (%)
1	0 day	0.54
2	Day 1	0.57
3	Day 5	0.61
4	Day 10	0.69
5	Day 20	0.75
6	Day 30	0.80

Table 4.9.1 1.5 ml glycerol content (Ash Content of Chikki)

Sample	Time Duration Control	Ash Content (%)
1	0 day	0.56
2	Day 1	0.56
3	Day 5	0.56
4	Day 10	0.56
5	Day 20	0.52
6	Day 30	0.49

Table 4.9.2 2 ml glycerol content (Ash Content of Chikki)

Sample	Time Duration	Ash Content (%)
1	Control (0 day)	0.54
2	Day 1	0.54
3	Day 5	0.53
4	Day 10	0.49
5	Day 20	0.45
6	Day 30	0.42

Appendix (B)

4.1.1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	0.01	0.00	11.9293706	3.48	S	0.010	0.021
Error	10	0.00	0.00	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.1.2 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	0.00	0.00	267.342342	3.48	S	0.002	0.004
Error	10	0.00	0.00	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.2.1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	19.36	4.84	4.96075682	3.48	S	0.806	1.710
Error	10	9.75	0.98	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.2.2 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	61.36	15.34	5.58109784	3.48	S	1.354	2.870
Error	10	27.48	2.75	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.3.1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	3078.82	769.70	481.671907	3.48	S	1.032	2.188
Error	10	15.98	1.60	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.3.2 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	10670.36	2667.59	3475.30824	3.48	S	0.715	1.517
Error	10	7.68	0.77	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.4.1 1. 5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	166.00	41.50	303.959961	3.48	S	0.302	0.640
Error	10	1.37	0.14	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.4.1 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	581.15	145.29	2619.38101	3.48	S	0.192	0.408
Error	10	0.55	0.06	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.5.1 1.5ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	0.01	0.00	2.95343137	3.48	NS	0.023	0.049
Error	10	0.01	0.00	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.5.1 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	0.06	0.01	18.5209424	3.48	S	0.023	0.048
Error	10	0.01	0.00	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.6.1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	116973.24	29243.31	475965307	3.48	S	0.006	0.014
Error	10	0.00	0.00	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.6.2 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	4	0.00	0.00	0.2533284	3.48	NS	0.010	0.022
Error	10	0.00	0.00	-	-	-	-	-
TOTAL	14		-	-	-	-	-	-

4.7.1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	3	0.57	0.19	76	4.07	S	0.041	0.087
Error	8	0.02	0.00	-	-	-	-	-
TOTAL	11		-	-	-	-	-	-

4.7 .2 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	3	0.51	0.17	74.0606061	4.07	S	0.039	0.083
Error	8	0.02	0.00	-	-	-	-	-
TOTAL	11		-	-	-	-	-	-

4.8 .1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	5	0.78	0.16	1199.14948	3.11	S	0.009	0.020
Error	12	0.00	0.00	-	-	-	-	-
TOTAL	17		-	-	-	-	-	-

4.8 .2 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	5	0.70	0.14	961.45245	3.11	S	0.010	0.021
Error	12	0.00	0.00	-	-	-	-	-
TOTAL	17		-	-	-	-	-	-

4.9 .1 1.5 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	5	0.78	0.16	1199.14948	3.11	S	0.010	0.021
Error	12	0.00	0.00	-	-	-	-	-
TOTAL	17		-	-	-	-	-	-

4.8 .2 2 ml glycerol content

ANOVA :								
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Treatment	5	0.70	0.14	961.45245	3.11	S	0.009	0.020
Error	12	0.00	0.00	-	-	-	-	-
TOTAL	17		-	-	-	-	-	-