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Full Length Research Article

Development of Eco-Friendly Edible Packaging Films Using Rice Starch

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Abstract

The edible packaging is a new trendsetter in the era of modern packaging. The researchers and food scientist recognize edible packaging as a useful alternative or addition to conventional packaging to reduce waste and to create novel applications for improving product stability. The rice based edible films were developed as an alternative for conventional packages providing the nutritional benefit as when consumed along with the food. The development of rice based edible films, by the extraction of rice starch at lab scale level. The films were developed by the employment of plasticiser at different concentrations of 5ml and 10ml. the films developed using glycerol as plasticiser in filmogenic solution to increase the flexibility and plasticity of film. The films developed were tested for its functional properties such as thickness, tensile strength, elongation at break, moisture permeability, moisture content, puncture strength. The overall results showed that potato starch based edible films absorbed less moisture and they also contributed to the low moisture permeability and they exhibited good tensile strength. The rice starch films were firm and transparent white in colour. There was a growth of bacteria and yeasts, but within the permissible limits and standards i.e., 8 cfu/gm in combination of both different glycerol concentration of edible films as 3.5 cfu/gm in 5ml concentration and 5.2cfu/gm in 10 ml concentration of glycerol in rice-based starch films. The rice films developed out of rice when observed highlighted that the moisture content of rice films was medium and they tend to exhibit good functional properties.

***Key words*:** Edible films, Glycerol, Plasticiser, Rice starch, Microbial load, Functional properties

Foods are generally protected using packages as they are susceptible to microbiological, physical and chemical deterioration during storage and distribution, both as a function of food components and the environmental conditions. Selection of appropriate packaging materials can prevent food from deterioration by providing barrier, more over choosing materials which are biodegradable, non-toxic and biocompatible is also essential. Bio-based materials offer value in the sustainability of life-cycle equation by being a part of the biological carbon cycle. Bio packaging is tomorrow's need for packaging especially for a few value-added food products Edible film packaging is a relatively new and environmental friendly food preservation technique compared to the use of plastic packaging that tends to damage the environment. Edible film that is used to coat food, or placed between components that functions as a barrier to mass transfer such as water, oxygen, and fat. Edible films are environmental-friendly and have the ability to protect perishable food from deterioration by reducing microbial growth. An edible and biodegradable film is one which is typically produced from food derived ingredients using wet or dry manufacturing process. The resulting edible film should be a free-standing sheet that can be placed on or between food components. Starch from different sources has been studied as a potential film-forming agent, including that from potato, barley, wheat, tapioca and rice. Films developed from starch are described as isotropic, odourless, colourless, non-toxic and biologically degradable, thus prevent a change of taste, flavour and appearance of food products Rice starch with high amylose is an attractive raw material for use as barriers in packaging materials. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of the low cost and renewability, as well as possessing good mechanical properties. The main advantage of edible films over synthetics is that they can be consumed with the packaged products. There is no package to dispose even if the films are not consumed, they could still contribute to the reduction of environmental pollution. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials.

MATERIALS AND METHODS

Raw materials

The raw materials required for the study was choosen based on natural plant sources. These plant sources are easily available and cultivable crops. They are of local origin and raw materials identified for the development of edible films are of low-cost sources and they are easily accessible. The purpose of the study is to develop edible films out of food substances as they are harmless and they reduce the environment load. food sources of natural origin were selected based on their high starch content, especially their amylose and amylopectin. The selected source was a cereal based source rice, as per the previous literature studies. The extraction of starch from rice was emphasized on a large scale as a cereal based alternative plastic packages.

Plasticiers

The plasticizers should be generally compatible to the structure of the polymer that they plasticize and the permeability be present within the solvent-polymer system and under the conditions used and also it must be compatible with the polymer, which results in the inter-molecular reactions. It is important to note that the formulation of the whole film system (polymer, solvent, plasticizer, and other additives) has a direct effect on the nature and characteristics of the film produced. Glycerol is used as plasticiser to modify the certain properties of starch molecules and to incorporate firmness to the films. Glycerol is one of the most popular plasticizers used in film-making techniques, due to its stability and compatibility with hydrophilic bio-polymers of edible polymers such as starch (Chandran, 2008)

Methods

Evaluation of properties of film

The edible films are evaluated for the properties to test for the optimum frameability of the edible films and for the co-relation of characteristics of the edible film.

Thickness of films

Thickness is an important parameter that affects the use of film in the formation of the product to be packaged. Thickness of the films was measured using screw gauge. Thickness also affects the mechanical properties such as tensile strength and elongation of the films, which might increase or decrease according to the end use (Anandito *et al*., 2013). A screw gauge was employed to measure the film thickness to the nearest 0.001 mm. Thickness of each film was measured at room temperature (230C and 45% RH) and expressed as an average of three random measurements.

$$t = m/Ad$$

Where;

m - Mass of the film; A - Area of the film; D - Density of the film

Tensile strength

Tensile strength is the maximum tensile force that could be held by a film. It is one of the most important mechanical properties that reflects the maximum stress a film sustains before it eventually breaks. Tensile strength shows the film strength in resisting the mechanical damage. Edible films with high tensile strength are needed for food packaging industry that aims to protect foodstuffs during handling, transporting, and marketing (Pitak and Rakshit 2011). Tensile strength was measured using Universal Testing Machine where the value of tensile strength was determined by dividing the maximum force (F) employed with the surface area (A) of edible films. Tensile strength could be calculated using the following equation (Fransisca *et al*. 2013).

Principle of universal testing machine

The principle of operation of the machine is by hydraulic transmission of load from the test specimen to a separately housed load indicator. The system is ideal since it replaces transmission of load: through levers and knife edges. Load is applied by a hydrostatically lubricated ram. Main cylinder pressure is transmitted to the cylinder of the pendulum dynamometer system housed in the control panel. The cylinder of the dynamometer is also of self - lubricating design. The load transmitted to the cylinder of the dynamometer is transferred through leverage to the pendulum.

Displacement of the pendulum actuates the rack and pinion mechanism which operates the load indicator pointer and the autographic recorder. The deflection of the pendulum represents the absolute load applied on the test specimen (Mechatronics guide, 2013).

$$t = \frac{Fmaks}{A}$$

Where;

t - tensile strength (MPa), Fmaks - force of tensile strength (N), A - sample surface area (cm^2)

Elongation at break measurement

Elongation is the maximum extension percentage that an edible film can achieve before it is finally broken / torn. The measurement of elongations was measured out using Universal Testing Machine where the value of elongation at break was determined by dividing the strain at which the film is broken to the initial length. The extension percentage of the film could be calculated by equation (Clause *et al*. 2011).

$$\text{Elongation at break (\%)} = \frac{\text{Strain when broken (mm)}}{\text{Initial length}} \times 100$$

Puncture test

Puncture strength test is used to determine the puncture or rupture characteristics of a material. It is measured using puncture tester. Puncture test is generally a compressive test where a material is compressed by a probe or other device until it ruptures. Puncture test was commonly used to determine the strength of a material such as film, rubber or membrane (Oliver *et al*. 2012).

$$\text{Puncture test} = \frac{Fmax}{Acs}$$

Fmax is the maximum applied force, **Acs** is the cross-sectional area of the edge of the film located in the path of the gap.

Moisture content

The moisture content of the films was determined gravimetrically by oven drying at 105°C until constant weight (dry sample weight). The results can be expressed as a percentage of the initial film weight, according to equation. At least triplicate analyses needed to be performed per variety and formulation (Bennadios *et al*. 2011).

$$\text{(\%) Humidity} = \frac{\text{(Initial Weight – 1)}}{\text{Final Weight}} \times 100$$

Moisture permeability

Moisture permeability is determined by the water uptake of the films. Initial weight (W0) of the sample film was measured. Then, the films are soaked in a flask filled with distilled water for 10 seconds. The soaked film was then lifted from the flask and weighed to obtain wet weight (W). The sample was soaked back in the flask, then it was lifted after every 10 seconds and weighed again and again. The procedure is performed continuously until a constant weight of the film is attained. The film saturated with water gives the maximum percentage of moisture permeability. The water absorbed by the film is measured using the formula, (Dios *et al*. 2012).

$$\text{Water (\%)} = \frac{W - W_0}{W_0}$$

Shelf-life analysis of films

Shelf life a product of physical, microbiological and chemical processes, triggered by any one of a multitude of contributing factors. Product characteristics, including the quality and consistency of ingredients, the moisture content and acidity levels, all play a part, as do external factors like storage, transport and packaging materials (Man, 2002). The total microbial count is the method where the edible films were analysed, and films were cut into small pieces. The edible film pieces were diluted into a series of dilution, and at a point where the colonies will be statistically significant to be counted but not so many to overgrow each other. This method that took time for the individual cells to grow into colonies.

The colonies were counted are multiplied by the dilution factor to get the number in the original sample. The results obtained here were expressed in colony forming units per milligram i.e., CFU/GM. The time taken for the cells to grow into individual colonies was called the incubation period. Counting of microbes is important as it enables us estimate the microbial population in a variety of products Breeuwer *et al.* (1994).

Microbial analysis of edible films

Microbial analysis of edible films that were carried out to evaluate the quality of edible film for maintained at constant temperature at a controlled environment for microbial load.

Total plate count method

The number of bacteria in a given sample was usually too great to be counted directly. However, if the samples were serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies, the number of colonies present could be used as a measure of the number of viable (living) cells in that known dilution. Normally, the bacterial sample was diluted by factors of 10 and plated on agar. After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies are determined.

A plate having 30-300 colonies was chosen because this range is considered statistically significant. If there was less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants would have a drastic effect on the final count. Likewise, if there were more than 300 colonies on the plate, there would be poor isolation and colonies would have grown together. Generally, one wants to determine the number of CFUs per milliliter (ml) of sample. To find these, the number of colonies (on a plate having 30-300 colonies) is multiplied by the number of times the original ml of bacteria was diluted (the dilution factor of the plate counted). Therefore, the number of CFUs per ml in the original sample was found by multiplying 150 x 1,000,000 as shown in the formula below:

$$\text{Number of CFUs per ml of sample} = \frac{\text{Number of colonies} \times \text{Dilution factor of the plate counted}}{\text{ml of sample plated}}$$

The total plate count determines for total count of viable microorganisms present in the medium. It is used to estimate the microorganisms present in the substance and it helps to analyse the microbial count present in the edible films as similar to the analysis of food stuffs. The microbial analysis of edible films is an important procedure to analyse the quality of edible films whether it would be safe when it is finally employed in packing food items. The microbes present in the edible films would be counted and determine for the types of microbes and the number of colonies present in edible films (http://www.microbialanalysisguide.co.in). The total microbial count is the method where the edible films were analysed, and films were cut into small pieces. The edible film pieces were diluted into a series of dilution, and at a point where the colonies will be statistically significant to be counted but not so many to overgrow each other. This method that took time for the individual cells to grow into colonies. The colonies were counted are multiplied by the dilution factor to get the number in the original sample. The results obtained here were expressed in colony forming units per milligram i.e., CFU/GM. The time taken for the cells to grow into individual colonies was called the incubation period. Counting of microbes is important as it enables us estimate the microbial population in a variety of products Breeuwer *et al.* (1994).

Selection of rice
↓
Cleaning of rice
↓
Grinding of rice
↓
Rice flour
↓
Disperse in 1500 ml Distilled water
(Adjust to pH 10 with NaOH; stand for 1 Hour with Moderate stirring)
↓
Centrifuge at 1000 rpm for 45 minutes
↓
Residue (Extracted with 1 litre Distilled water for 24 hours at 4^0C
↓
Centrifuge at 1000 rpm at 30 minutes
↓
Residue (Extracted with 300ml of 0.1 NaOH twice for 48 hours at 4^0C
↓
Resuspend in 80% ethanol
(Blended for 1 minute using stirrer)
↓
Heating on water bath at 80^0C for 1 hour
↓
Settle for 4 hours at 40^0C and discard supernatant
↓
Residue

Flow chart – 1

Extraction of Rice Starch

Method of extraction of rice starch

The rice selected for the study was soaked and was grinded into rice flour. The starch was extracted from the rice through alkaline extraction. Rice flour obtained was used for extraction of starch. 750g of rice was allowed to disperse in 1500 ml distilled water. Adjust the pH to 10 with 1 N NaOH and allowed to stand for 1 hour with moderate stirring. Then Centrifuge at 5000 rpm for 15 minutes. Residue obtained was sequentially extracted with 1 litre of distilled water and 2% NaCl (each for 24 hours at 4ºC) followed by extraction with 300 ml of 0.1 N NaOH (48 hours at 4ºC). The process was repeated once again. Subsequent to each of above the extraction, the slurry was centrifuged at 10,000 rpm for 30 minutes at 4^0C and supernatant was discarded.

The residue from the second NaOH extract was further subjected to extraction by heating using 80 % ethanol (100 ml) at 80^0C for 1hour, cool to room temperature and allowed to settle for 4 hours at 40^0C. The supernatant was discarded and the residue was dehydrated and powdered (Ericssa 2010).

Rice starch
↓
Mixed with water
↓
Added Glycerol to the rice starch
(5ml and 10ml glycerol)
↓
Mixing of glycerol with starch using a centrifuge at 2000 rpm for 25 minutes and then heated to 85^0C for 1 hour
↓
Pouring of mixture into petri plates
↓
Casting of films in petri plates
↓
Drying at 28^0C for 36 hours
↓
Removal of edible films from the petri plates

Flow chart – 2
Development of Rice Starch Based Edible Film

Development of rice starch based edible films

The formulation of starch based edible films was carried out by casting method. Casting is a process that comprises of drying of a solution or a gel. It is a simple method for developing films with desirable thickness.

Rice starch-based films were prepared by casting technique. Glycerol is used as plasticizer at different concentrations 5ml and 10ml of dry rice starch respectively. Aqueous solution containing 5% of flour were prepared and stirred for 25 min at 2000 rpm in a centrifuge. Glycerol was added to the mixture and was heated to 85^0C in thermal bath under constant stirring for 1 hour to promote the gelatinization of rice starch particles and poured homogeneously onto glass petri plates.

The contents were allowed to dry at 28^0C for 36 hours in an oven with circulating air (Amanda Bias and Carmen, 2010). The rice starch based edible films were developed with the different concentration of glycerol such as 5ml and 10ml respectively. They were subjected to analysis to study the properties of each starch-based films in depth.

RESULTS AND DISCUSSION

Extraction of starch from food sources

The starch was extracted from the Cereal based such as rice and the yield of starch extraction from the rice was 46.7% respectively out of 750g of rice. The grams of starch obtained was 350g out of 750g of rice taken for the extraction of starch. The study that concentrated on use of rice starch as a primary source of Starch for making edible based films. The results obtained in the present study are in par with the study conducted by Walstra., (2003) proving that starch was composed of amylose and amylopectin, which was primarily derived from extraction of starch from cereals-based sources like rice.

Development of rice starch based edible films

The starch based edible films were developed from rice was selected due to their superior film-forming properties. The films developed by casting technique, with the addition of water and then plasticiser glycerol and their properties were evaluated by testing parameters as suggested by (Hulleman *et al.*,1998, Lourdin *et al.*, 1998). Glycerol was used as a plasticiser for making the edible films as it improved the mechanical properties as per the previous literature studies. Plasticizers are added to starch mixture to increase the ductility of the material. The starch obtained from different food sources is used as the main base material for the development of starch based edible films. The rice-based films were formulated from the rice starch with different glycerol concentrations and the rice starch films developed were of using casting method as the study conducted by Razia and Florida., (2000). The films were developed from round petri plates and the films were developed with different glycerol concentrations (5ml and 10ml) and the films obtained were of round shape with good mechanical properties as in par with the study conducted by (Amanda Bias and Carmen, 2010).

Property analysis of rice starch-based films

The results of properties of rice-based starch films that has evaluated by the testing parameters are presented in the (Table 1).

Table 1 Properties of rice starch based edible films

Rice	Thickness of films (mm)	Tensile strength (g force)	Elongation at break (%)	Puncture test (g force)	Moisture content (g)	Moisture permeability (%)
			Variation-1(5ml of Glycerol)			
V1A	0.04	2.30	11.2	91.5	9.55	12.2
V1B	0.06	2.35	11.5	92.7	9.54	13.5
V1C	0.09	2.32	11.6	90.3	9.56	12.9
			Variation-2 (10ml of Glycerol)			
V2A	0.15	1.95	9.13	85.7	12.4	15.8
V2B	0.18	1.98	10.2	82.1	12.8	14.7
V2C	0.13	1.93	9.34	86.8	12.2	15.4

Thickness of films

From the (Table 2) it is evident that the results obtained for Variation - 1 (V1) of rice films in triplicates V1A, V1B, V1C showed 0.04, 0.06 and 0.09 thickness respectively. Variation - 2 (V2) of the rice films in triplicates V2A, V2B, V2C showed 0.15, 0.18 and 0.13 thickness respectively. These results obtained for rice-based films in par with the study conducted by Dia *et al.*, (2002). Dia *et al.*, (2002) stated that, higher glycerol concentrations in the plasticized rice film, the thicker the film. The thickness of the film obtained from the Variation - 2 (10ml) was thicker due to higher than the rice film obtained from 5ml of glycerol.

Tensile strength

The tensile strength of the rice based edible films obtained for the Variation - 1 (V1) of rice films in triplicates V1A, V1B, and V1C showed 2.30, 2.35 and 2.32 tensile strength respectively. Variation - 2 (V2) of rice films in triplicates V2A, V2B, V2C showed 1.95, 1.93 and 1.98 tensile strength respectively. The results obtained for rice based edible films showed that higher the concentration of the plasticizers, the less the tensile strength of rice-based films. Chris and Mulder., (2002) studied that, the plasticizer (glycerol) interfered with the arrangement of the polymer chains and the hydrogen bonding, they also decreased the polymer interaction and cohesiveness and they most likely affected the crystallinity and other physical properties of the films included the flexibility of the film. This shows that the results obtained from (Table 2) decreased tensile strength of Variation - 2 of rice starch based edible films decreased with increased plasticiser content.

Elongation at break

The elongation at break for rice starch based edible films was evaluated in (Table 2). The values obtained for Variation – 1 (V1) of rice films in triplicates V1A, V1B, V1C showed 11.2, 11.5 and 11.6 elongation at break respectively. Variation – 2 (V2) of the rice films in triplicates V2A, V2B, V2C showed 9.13, 10.2 and 9.34 elongation at break respectively. Muller *et al*., (2011) reported that an increased glycerol concentration resulted in the decreased per cent elongation at break of the rice starch films. The strength of rice starch film decreased with, whereas plasticity increased. Thus, it could be observed that an increased glycerol concentration from Variation – 2 (10ml) rice starch based edible films resulted in the decreased per cent elongation at break of the films.

Moisture content

The moisture content of the rice starch based edible films obtained for Variation - 1 (V1) of rice films in triplicates V1A, V1B, V1C showed 9.55, 9.54 and 9.56 moisture content respectively. Variation - 2 (V2) of the rice films such as V2A, V2B, V2C showed 12.4, 12.8 and 12.2 moisture content respectively. The percentage increase in weight was tabulated and that was taken as a measure of the water absorption of film. Erica *et al.,* (2007) reported that, the moisture content of the rice-based films as determined for its properties of the films was affected by the moisture content. The high amount of moisture of films that affected the stability of films due to the high glycerol content that affected the moisture content. Thus, it could be observed that, high glycerol concentration of 10ml that resulted in the high moisture content than the 5ml glycerol-based wheat films. The moisture content that affected the stability of films due to the high amount of moisture of films.

Moisture permeability

The moisture permeability of rice starch based edible films obtained for Variation - 1 (V1) of rice films in triplicates V1A, V1B, V1C showed 12.2, 13.5 and 12.9 moisture permeability respectively. Variation - 2 (V2) of the rice films in triplicates showed 15.8, 14.7 and 15.4 respectively. The values obtained showed that moisture gained from variation-1(5ml) rice films were lower than the Variation – 2 (10ml) rice films. The moisture permeability a major property of films that is related to the structural and mechanical 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 (Paras *et al.* 2006). Moisture gained by 5ml glycerol concentration of rice film was lower than that gained by 10ml glycerol concentration rice film.

Puncture strength

The puncture test of rice starch based edible films was evaluated in the Table-I. The values obtained in the study for Variation - 1 (V1) of rice films in triplicates V1A, V1B, V1C showed 85.7, 82.7 and 86.8 puncture strength respectively. Variation – 2 (V2) of the rice films in triplicates V2A, V2B, V2C showed 91.5, 92.7 and 90.3 puncture strength respectively. Chris *et al.,* (2003) reported that the films became more extendible when the concentration of plasticizer was increased which resulted in the reduction of puncture force of the film. The reduction of the puncture force was observed as the consequences of the incorporation of plasticizers, and to water molecules absorbed by the samples, a common phenomenon of edible films, The results revealed that the higher glycerol concentration of 10ml that resulted in the decreased puncture strength of rice film than the 5ml glycerol concentration rice films, the reduction of the puncture force was observed.

Microbial load analysis of rice starch-based films

The rice based edible films were developed from the rice starch in two different concentrations using glycerol as plasticiser. The resulted rice based edible films were subjected to microbial analysis to assess the shelf-life analysis of films. The results obtained were calibrated. The colonies were counted on as per the prescribed method of Total viable count. It was absorbed that there was a constant growth of yeast but within the permissible limits and standards i.e., 8cfu/gm in combination of both variation of edible films as 3.5cfu/gm in 5ml concentration of glycerol and 4.5cfu/gm in 10ml concentration of glycerol in rice starch based edible films by in rice starch based enriched films. Rover *et al.* (2006). The edible films obtained out of the starch based sources tend to extend the shelf life as it would contribute to the less water absorption capacity than other edible films as suggested by Ramos *et al* . (2000).

CONCLUSION

The global edible films and coatings market is expected to witness steady growth owing to increased use of clean label strategies by food producers. The reason for the bump in the growth of edible films and coatings in their use as an excellent solution to reduce the carbon foot print. The benefits of edible packaging are increased their acceptability by the food and packaging manufacturers and this factor is boosting revenue growth of the global edible films and coating market. Thus, the biobased edible films are strong, feasible and toxin free films which is not only favourable for human beings but also protect other living organisms resulting in reducing the environmental pollution. Thus, the present study is a small foot print towards innovation by utilizing the excess starch available which is low cost locally available with extra nutritional benefits thus reducing the effect of artificial chemicals that would affect the health by interacting with the food. The edible films thus produced can be consumed along with the food as there is no interference of any chemicals as it is purely from natural sources. The cereal based film such as starch that exhibited excellent film making properties and it contributed to the less microbial load.

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