Tensile Strength and Solubility Studies of Edible Biodegradable Films Developed from Pseudo-cereal Starches: An Inclusive Comparison with Commercial Corn Starch

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ABSTRACT

Edible biodegradable films were prepared from amaranth, buckwheat and commercial important corn starches by casting method. Starch, glycerol and carboxymethyl cellulose (CMC) were used to prepare filmogenic starch solutions in potable luke warm water, followed by ultra-sonication for homogenous mixing of the mixed ingredients. After this, heating was applied to starch based slurried solutions until it turned into gel solutions. These filmogenic gel solutions were then dried and films were peeled off and stored in desiccator. Stored films were analyzed for functional properties *viz.*, tensile strength, solubility and water vapor permeation. Amaranth starch based edible biodegradable films presented considerable clarity values however buckwheat starch and corn starch based films exhibited good tensile strength and better solubility values. Developed edible biodegradable films from amaranth starch were analysed for surface structure examination by SEM. Evaluation of surface revealed uniformity, homogeneity with no surface crack on the surface of developed edible biodegradable films.

Key words: Edible films, Properties, SEM, Starches, Ultra-sonication.

INTRODUCTION

Pseudocereals (amaranth, buckwheat and chenopodium) are unexplored new century crops comprise innumerable treasure of life supporting essential constituents like quality starch, proteins, fats and soluble fibres. Psuedocereals are gaining popularity due to their enormous functional properties their use in developing gluten free food products for celiac patient (Caselato-Sousa and Amaya-Farfan, 2012).

Among psuedocereals family of grains, amaranth grain is widely used, to feed the starving population of across the globe as per the United Nations Millennium Development Plans (MDP) (Chaudhari et al., 2009) Amaranth grain belongs to family Amaranthaceae and genus Amaranthus. Amaranthus hypochondriacus (L.), is the main amaranth grain species which is most explored till date across the world and in India (Rana et al., 2007). Some of the amaranth varieties viz. red amaranth (Amaranthus tricolor L.) and green amaranth (Amaranthus dubius L.) are tried after quality evaluation to develop feed and food products (Hoang, 2019). Amaranth (Amaranthus hypochondriacus) varieties namely; Annapurna and Durga are grown in many parts of Uttaranchal and Himachal Pradesh by National Bureau of Plant Genetics Resources, (NBPGR), New Delhi, to propagate the amaranth grain cultivation and their utilization in developing value added food products (Chandla et al., 2017a; Rana et al., 2007; Arya and Singh, 2004). Effect of blending of amaranth and bengal gram on the organoleptic quality of wheat based weaning mixes was also investigated (Anand and Malhotra, 2010).

Grain of amaranth seed majority contains starch as a chief fraction (40-50%). Amaranth starch extracted

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possesses small granule size, an intermediate range of viscosities and most significantly, high paste clarity which make possible this starch to be usable for product preparation (Caselato Sousa *et al.*, 2012; Rana *et al.*, 2007).

Another psuedocereals is buckwheat (Fagopyrum esculentum) which belongs to the family Polygonaceae. Buckwheat is economically important pseudocereal grown chiefly for carbohydrates and protein content (Dogra, 2019). Buckwheat, a pseudocereal is a nutritionally rich and gluten-free grain and it has a great prospective as raw, germinated and roasted grain flour to develop the

functional food (Tanwar, 2019). Buckwheat contains good amount of starch and quality protein of high nutritional value, dietary fiber, vitamins and minerals. Starch is a major component of the buckwheat seed (Charista *et al.*, 2008). In comparison to amaranth and buckwheat starch, the corn starch is a commercial available food starch and commonly utilized by food processing industries (FPIs) to develop new food textures. Mostly, corn starch in food processing is generally used in thickening of sauces/soups and in making corn syrup (Singh and Singh, 2003; Singh *et al.*, 2009). Since amaranth, buckwheat and corn starches all are odorless, tasteless and non-toxic therefore biodegradable edible films of desired attributes could be developed.

Edible coatings are mainly derived from food ingredients that are generally recognized as safe (GRAS). Edible films are fundamentally edible and also possess the newest trend in the processing of films in biodegradable industry (Sukhija *et al.*, 2016: Thakur *et al.*, 2016). These edible biodegradable films could be utilized in food processing for wrapping and coating food products as primary packaging material (Chandla *et al.*, 2017a).

Edible biodegradable films are generally made up of starch, protein and fibres and sometimes a biocomposite of two or more than two macromolecules. However, starch is the most important bio-macromolecule and basically a polysaccharide that is used to develop edible biodegradable films because of its capability of forming a continuous starch gel matrix. Film components such as water, plasticizers and additives have significant effects on granule swelling, the disintegration of amylose/ amylopectin chains, glass transition temperature that shows the integrated influence of these components on edible film properties (Saberi *et al.*, 2017; Thakur *et al.*, 2019)

Starch based edible films are generally transparent, colorless, flavorless, tasteless and odorless, hence considered ideal for presenting real appearance of food product contained with edible film (Chandla et al., 2017a). Many researchers tried to produce edible and biodegradable starch based films by casting techniques and then evaluated the functional properties of developed films (Sukhija et al., 2016; Lopez et al., 2015; Almasi et al., 2010; Bergo et al., 2008; Mali et al., 2005). Depending upon the characteristics of developed film and its formulation, it could be sought for its application either in edible or biodegradable, sector (Santhoskumar et al., 2019). Many researchers utilized the developed edible film in coating purposes to extend the shelf life of fresh produce and processed food products (Sharma et al., 2018). In addition to shelf life extension and to ensure quality intact of the food product, edible biodegradable films could lead to solve many environmental issues of plastic handling, reprocessing and landfilling. On the other side marine life and stray animals that sometimes eat polymeric films due to the food adhered to film is becoming an important issue which is leading to plastic deposition in rumen of the animals.

Therefore, in view of the above demand and future perspectives of edible biodegradable films, amaranth starch,

buckwheat starch and corn starch are studied to explore the possibility to utilize them to develop biodegradable edible films. Developed edible biodegradable films from amaranth starch, buckwheat starch and corn starch, are finally evaluated for properties *viz.* tensile strength, solubility and water vapor permeability.

MATERIALS AND METHODS

Amaranthus hypochondriacus Durga and buckwheat (Fagopyrum spp) grains samples were obtained from National Bureau of Plant Genetic Resources (NBPGR), Phagli, Shimla, H.P., India. Corn starch (CS) sample was procured from Starch Company namely; M/S. Sukhjeet Starch and Chemicals Ltd., Phagwara, Punjab, India. Chemicals used in the entire research were of AR grade.

The experiments were conducted at two different places namely at Department of Food Engineering and Tech., SLIET, Longowal, Sangrur, Punjab, India and Department of Dairy Engineering, College of Dairy Science and Tech. (CoDST), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India. in session 2018-19.

Amaranth starch was extracted from defatted flours of amaranth grain by modified method of Choi et al., (2004). Defatted amaranth grain flours were then soaked for 20 h in NaOH solution of 0.25%, (w/v), at 4°C. In case of buckwheat starch, defatted buckwheat flour was soaked overnight in NaOH solutions of 0.2 N at ambient temperature in the month of March (23-30°C). The treated amaranth and buckwheat flours slurries were screened through British Standard Size sieve (250 mesh) for proper removal of fibrous portion of grain. Thus starch milk was collected as filterate and centrifuged (Eltek 4100 F) at 3000 rpm for 15 min. After centrifugation, protein layer was scraped off and starch was dried at 40°C and packed in polyethylene (PE) bags for further study. However, corn starch was directly purchased and packed in PE bags for its analysis and till its usage.

Starch Properties

Color

Hunter colorimeter (Model i5 Green Macbeth, USA) was used to estimate the color of amaranth starch, buckwheat starch and corn starch samples. Data obtained was noted down for color determination of starch samples.

Amylose content

Amaranth and buckwheat starch samples (70 mg) were mixed with 10 ml of urea and in DMSO (Di-methylsulphoxide) solution in 1:9 ratio. The mixed solution incubated and is added (0.5 ml) to 25 ml of dis. water, along with 1 ml of lodine (I) and potassium lodide (KI) solutions. This 1 ml solution was prepared by the addition of 2 mg lodine and 20 mg potassium lodide and then the volume was make-up to 1 ml by distilled water. (Morrison and Laignelet, 1983).

Blue Value (%) =	
Absorbance	~ 100
$2 \times \text{gram}$ of solution \times weight of starch sample)
	(1)
Amylose content (%) = 28.414 Blue Value	(2)

Water/Oil Absorption Capacity (W/OAC)

Amaranth starch, buckwheat starch and corn starch samples were determined for water binding and oil binding, capacities by the method described by Medcalf and Gills, (1965). Starch sample of 5 g was taken and dissolved in 75 ml of dis. water and groundnut oil. The starch samples were then agitated for 1h and centrifuged at 3000 rpm for a period of 10 min. Excess water and oil recovered from every starch sample was removed however the weight of the sendiment was observed. The water and oil binding, capacity was calculated as follows;

W/OAC (g/g) =
$$\frac{\text{Sendiment weight}}{\text{Weight of starch sample}} \times 100$$
 (3)

Swelling power

Homogeneous mixture of amaranth starch, buckwheat starch and corn starch (1.0 g, dry basis) in luke warm water (35mL) were heated at 95°C for 30 min. Samples were then cooled for 1 h in cold water and then centrifuged (Made: Remi Laboratory Ltd., Mumbai, India, Model; C-24, BL) at 12,500 rpm for 30 min. The supernatant layer was poured through double folded muslin cloth and gel is collected on filter muslin cloth (Kong *et al.*, 2010). The weight of sediment was recorded for swelling power.

Swelling power
$$(g/g) =$$

$$\frac{\text{Sendiment weight (wet mass)}}{\text{Weight of starch sample}} \times 100$$
(4)

Edible films preparation process

The film formation process was standardized for obtaining polymeric edible film of desired quality attributes according to Chandla et al., (2017a) with little modification as per variation in starch source. After carrying out the primary trials, separately for amaranth starch, buckwheat starch and corn starch, the edible biodegradable film formation process was standardized. During amaranth starch films development, 8.0 gm of starch, 2.0 gm of the plasticizer was added in 100 ml of luke warm water and solution prepared was heated to 90°C till gelation occur. During the buckwheat and corn starch film formations, 5.0 gm of starch, 2.0 of the plasticizer was added in 100 ml of water and thus the solution prepared was mixed well and heated to 90°C till gelation appears in the starch solutions. The petri dish is used for casting the slurries. Before pouring the cooked starch solutions, groundnut oil is applied on the surface of petri dishes, to ease the peeling of edible films after it gets dried. After ensuring the gelation point of cooked starch solutions, pouring and casting was done in petri dishes. Petri dishes which contain the cooked starch solutions were dried in cabinet dryer without disturbing the casting starch solutions at 45°C for 20 hours as shown in Fig 1. Films were then

peeled off after ensuring their thorough matrix formation as shown in Fig 2. Developed edible biodegradable films were then placed in desiccators and stored in desiccators till evaluation.

Functional properties of films

Film thickness

The thickness of the developed edible films from amaranth starch, buckwheat starch and corn starch was determined with a manual micrometer (Mitutoyo Corporation, Kanagawa, Japan: Mitutoyo 2046F) with an accuracy of $\pm 1\mu$ m. The average value of five thickness measurements at different locations of the film was recorded.

Moisture content

Moisture content (MC) of the amaranth starch, buckwheat starch and corn starch based edible film was determined using standard analysis methods of the AOAC (1995). Edible films were cut into small pieces of each (~1.0 g) and dried in cabinet oven at 120°C, for approximate 5 h to 6 h and till the weight of sample becomes constant. The reported results represent the average of five samples in case of films



Fig 1: Casted films from amaranth (A), buckwheat (B) and corn (C), starches after drying in oven.



Fig 2: Transparent edible films produced from amaranth starch.

prepared from amaranth starch, buckwheat starch and corn starch. The percentage of moisture content in the edible films was calculated as follow.

MC (%) =
$$\frac{M_{W} - M_{D}}{M_{D}} \times 100$$
 (6)

 $\rm M_{_w}$ is the mass of the wet sample and $\rm M_{_D}$ is the mass of the dried sample.

Tensile strength

Where

Tensile strength (TS) of edible films was determined using a texture analyzer, Model; TA.XT2i, SMS, Surrey, England. Edible films were cut into strips of size 20 mm wide and 50 mm long and these film strips were fixed between the grips of the texture analyzer. The initial grip separation was set at 30 mm and speed at 1.0 mm/s.

Solubility

Solubility of prepared edible films in water was determined by the method of C. A. Romero-Bastida *et al.*, (2005). Dried film samples comprising size of 20 mm × 20 mm dried were weighted. Edible films were dried at 105°C for 24 h in an oven (Macro Scientific Works (MAC) Pvt. Ltd., New Delhi, India. Dry weight of the edible films samples was obtained after drying. Edible films samples were then submerged in a flask containing 80 mL of distilled water, at 20°C for 1h. The edible films samples were then collected gently and dried again at 105°C for 24h. Dry weights of the edible films were recorded and subsequently, reduction in weight of edible films was noted carefully. Percentage loss in weight in 1 h is considered as, per cent solubility.

Light transmittance

Transparency values of edible films were observed by UV spectrophotometer (Model; ID 5000 HACH, USA). Edible films were cut into long strips of sizes 15 mm wide and 50 mm and edible were mounted between the cuvette of spectrophotometer and transparency value of edible films from amaranth starch, buckwheat starch and corn starch were recorded at 600 nm.

Scanning electron microscopy

Optimized edible film was dried at a moisture content of 5-6% before its surface analysis. Edible film was mounted on stub and coated in auto fine coater, Model No. JEOL-JFC-1600 with gold palladium (60:40, w/w). Edible film sample was analyzed by scanning electron microscope, JEOL, Tokyo, Japan, Model No. JSM 6610-L.

Statistical evaluation

Mean value and standard deviation value was reported for the every statistical analysis. All the analysis was determined in the triplicates and then one and two way analysis of variance (ANOVA) was done, followed by Duncan's by Mini Tab Statistic 7. (Statesoft Inc., OK, USA).

RESULTS AND DISCUSSION

Functional Characteristics of Starch

Nutritional composition

It was observed that there was significant different (p<0.05) in the nutritional composition of amaranth, buckwheat and corn starches. The moisture content of all the starch samples was varied from 10-12% on dry basis and the residual protein contents in amaranth, buckwheat and corn starch was observed 0.24%, 0.29% and 0.30%, respectively. As all the starch samples were de-fatted during sample preparation therefore, there was no fat residue in starch samples. The fiber contents of amaranth, buckwheat and corn starch samples were observed in the range of 0.02% - 0.04%.

Color

Amaranth, buckwheat and corn starches exhibited very high instrumental L* value i.e. 97.03, 96.23 and 92.13, respectively that indicated high purity of starch samples. Boudries *et al.*, (2009) also acknowledged that L* value greater than 90 indicates high purity of starches. Individually, the amaranth starch sample exhibited highest L* value as compared to other samples.

Amylose content

Amylose content of amaranth, buckwheat and corn starch samples was found 3.47%, 18.2% and 16.03%, respectively and buckwheat starch sample showed highest amylose content whereas amaranth starch sample had significantly (p<0.05) lesser fraction of amylose content as compared to other samples (Table 1). Hoover, (2010) has also shown positive impact of amylose content in development of edible films and also suggested that better starch matrix formation occur during gelation of starch slurries as of amylose content present in the starch solutions.

Water/oil absorption capacity (W/OAC)

The water binding capacity (WBC) of all samples was observed significantly different ($p \le 0.05$) and individually, the buckwheat starch sample showed highest followed by corn

Table 1: Functional properties of starches from amaranth, buckwheat and corn.

	Color	Amvlose	Water binding	Oil binding	Swelling power
Starches	(L Value)	content(%)	capacity(%)	capacity(%)	(g/g)/(90°C)
Amaranth starch	97.03 ± 0.06ª	3.47±0.03°	199.60±0.92°	190.33±0.76°	10.10±0.05°
Buckwheat starch	96.23 ± 0.10^{b}	18.20±0.82 ª	99.20±0.35 ^b	100.02±0.45 ^b	16.11±0.03 ^b
Corn starch	92.13 ± 0.01°	16.03±0.02 ^b	299.21±0.76ª	285.67±3.40ª	19.29±0.08ª

Results are expressed as mean value± standard deviation of three determinations.

Means in column with different superscript differ significantly (p < 0.05).

starch and amaranth starch (Table 1) this could be due to the high amylose content and chemical composition of buckwheat and corn starch samples. On other side, corn starch sample showed highest OBC followed by amaranth starch. Amylose content and accessibility of oil to bind the specific sites of these starches has significantly affected oil binding capacity (Singh *et al.*, 2009: Leach *et al.*, 1959). In addition, smaller amaranth starch granules and starch granule's polygonal geometrical shape may also be responsible for offering more active sites to enhance the oil binding capacities.

Swelling power

Swelling power of starch is associated with granule size, granular structure and starch biochemical conformation. Swelling power values for all samples was observed significantly different ($p \le 0.05$) (Table 1). The corn starch presented highest swelling power followed by buckwheat starch however lowest swelling power values were recorded for amaranth starch which may be due to the lesser amylose and high amylopectin contents present in granules of amaranth starch. Additionally, good amount of amylose content, high hydration degree and to some extend high reaction of water molecules with starch granules is also responsible for significant increase in the swelling power starch samples (Adebooye and Singh, 2008).

Edible films properties

Moisture content and thickness

Edible biodegradable films from amaranth starch, buckwheat starch and corn starches have been developed. The moisture content, thickness, tensile strength, solubility values and surface morphology of the prepared starch based edible biodegradable films are presented in Table 2. To evaluate tensile strength and solubility properties of edible films moisture content and thickness is standardized for comparative studies of edible biodegradable films. Moisture content values of prepared edible biodegradable films from amaranth, buckwheat and corn starch samples are observed 20.5%, 20.0% and 20.1%, respectively. According to Solano and Gante (2014) moisture content of starch films should range from 16.50% to 34.39%. Thickness values of edible films for amaranth, buckwheat and corn starches observed 0.24 mm, 0.28 mm and 0.29 mm, respectively. Thickness of

buckwheat and corn starch shown increment in thickness with increase in starch concentration however less increase was observed in case of amaranth starch films. This might be due to lesser amount of amylose content in amaranth starch granules, subsequently lesser swelling occurred in the amaranth starch granules resulted in lesser expansion and gain in the thickness of amaranth starch film (Chandla *et al.* 2017b).

Tensile strength

Tensile strength (TS) values of starch based edible biodegradable films ranged from 1.9 to 4.8 MPa, to attain sufficient mechanical strength. In addition to this tensile strength subsequently affect almost all characteristics of edible biodegradable films (Tapia-Blacido et al., 2013; Garcia et al., 2008). The tensile strength values for edible films was observed significantly different ($p \le 0.05$) shown in Table 2. The tensile strength values of amaranth, buckwheat and corn based edible biodegradable films was observed 2.61 MPa, 2.92MPa and 2.97MPa, respectively (Table 2). These tensile strength values were compared with low density polyethylene films (LDPE) which had presented very high tensile strength of 7.6 MPa to 17.3 MPa (Jaramillo et al., 2016; Sukhija et al., 2016). Corn starch based films presented highest values of tensile strength (2.97MPa) followed by buckwheat starch (2.92MPa) and amaranth starch (2.61MPa) which may be due to structural and granular size differences within the these non-conventional starches and commercial corn starch. Tensile strength value observed in corn starch and buckwheat starch is considerably high which may be due to amylose fraction of starch is hydrated to swell up (Chandla et al., 2017b). Heating of swollen starch granule enhanced the viscosity, gel strength and promote visco-elastic behavior of the filmogenic gels at lower gelatinization temperatures. After drying the filmogenic gel solutions a strong network of starch, plasticizer (glycerol) and hydrocolloid; carboxymethyl cellulose (CMC) is formed. This in return increased the intermolecular binding within the starch, plasticizer and hydrocolloid (CMC) and subsequently increases the tensile strength of the edible biodegradable film developed from corn starch and buckwheat starch in comparison to amaranth starch. However, amaranth starch contain very small fraction of amylose which offers it less tensile strength in this formulation.

Table 2	Starch	based	biodegradable	edible	films	properties.
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Parameter	Moisture Content (%)	Thickness (mm)	Tensile Strength (MPa)	Solubility (%)	Light Transparency (%)	Reference
Amaranth starch	20.5±0.22ª	0.24±0.04°	2.61±0.12°	35.23±0.20ª	98.68±0.04ª	Current study
Buckwheat starch	20.0±0.12°	0.28±0.01 ^b	2.92±0.10 ^b	31.90±0.15 [♭]	72.25±0.02 ^b	Current study
Corn starch	20.1±0.03 ^b	0.29±0.05ª	2.97±0.10ª	30.90±0.03°	69.52±0.05°	Current study
LDPE*	-	0.20	7.6-17.3	-		Jaramillo
						et al., (2016)

Results are expressed as mean value± standard deviation of three determinations.

Means in column with different superscript differ significantly (p < 0.05).

*LDPE-low density polyethylene.

Solubility

Solubility is most critical factor analyzed for starch based edible biodegradable films. Lower solubility of edible films reduces the rate of degradation from external factors while higher solubility value would result wear and tear and sudden leakage of polymeric pack during its usage. Amaranth starch, buckwheat starch and corn starch edible biodegradable films presented solubility values 35.23%, 31.90% and 30.90% per h respectively (Table 2) which is within the optimum range for starch based edible films (Lopez-Rubio et al., 2007). Solubility values for edible films samples were observed significantly different ($p \le 0.05$) shown in Table 2. Highest solubility was observed in amaranth starch films as of more amount of soluble amylopectin content present in it and moreover solubility is also a function of temperature and ratio of amylose and amylopectin content (Rindlav-Westling et al., 2003). After heating the starch beyond 20°C, amylose swelling occurs and linear chain of amylose content and additives may diffuse through granular membrane will reduce the solubility (Vieira et al., 2011: Green et al., 1975). The study of solubility is more important and critical in case of biodegradable films to check the efficiency of developed films against external abuse by rain and their exposure at high, temperatures and humidity conditions.

Light transmittance

The transparency values of amaranth, buckwheat and corn starch sample based edible films were ranged from 69.52 -98.68% shown in Table 1. To promote the concept of real time appearance of food product, transparency is vastly desired characteristic for packing food commodities (Lopez-Rubio et al., 2008). Among all the films, highest transparency value (98.62%) was recorded for amaranth starch followed by buckwheat starch based films (72.25%) and then corn starch based films (69.52%) however the films developed from buckwheat were of reflective type appearance. Transmittance values of all edible films sample was observed significantly different (p≤ 0.05) shown in Table 2. In most of the starches, more amylose content present on the starch granule is chiefly responsible for opaque appearance as of increase in viscosity and thus reduce the transmittance through edible films however clearer viscoelastic and firm gel formation in amaranth starch gel may be due to more amylopectin content (Eliasson, 2017). On the basis of highest transparency values and intended consumer demand of clear films amaranth starch based films were chosen for surface analysis.

Scanning Electron Microscopy (SEM)

SEM graphs of optimized amaranth starch based edible films presented consistent plasticized gelification of the films with no crack appeared on the film surface. The surface structure envisioned by SEM showed that amylopectin films were very smooth whereas amylose and starch films were rougher (Rindlav-Westling *et al.*, 2003). Morphological images of edible films revealed homogenous interaction of components blended together (amaranth starch, glycerol



Fig 3: Surface morphology of optimized amaranth films.

and CMC) as shown in Fig 3. It was examined that the entire surface of amaranth starch based edible films was observed of level texture and it offered regular starch gellified matrix structure. Somewhere crumbs of incompletely gelatinized amaranth starch were also observed which may be due to some starch molecules don't melt-up on heating.

CONCLUSION

Study provided us the scientific information of starches to develop novel edible primary packaging material for dairy and food industry for enhancing the shelf life of perishables. It is concluded that amaranth starch could be better used to develop transparent edible films however buckwheat starch and corn starch could be the future for biodegradable plastic with a significant less solubility and water vapor permeation rates. The use of these non-conventional starches may be a step forward towards their utilization in fulfilling the demand of bio-plastic.

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