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Development of Edible Films from Heat Moisture Treated and Acetylated Starches of Amaranth

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Abstract: Starch was isolated from amaranth grains and subjected to heat moisture treatment (30% moisture content at 100°C for 6hr) and acetylation. Starch films were formed using native and modified starches of amaranth and analysed for thickness, mechanical, barrier and color properties. Amaranth starches produced continuous and transparent films with different characteristics. Tensile strength, L value and water vapour permeability of films increased following the acetylation and heat moisture treatment of amaranth starch. Water solubility of films of acetylated starch was higher while lower water solubility was exhibited by films of heat moisture treated starch as compared to native starch films of amaranth.

Keywords: Amaranth, acetylation, heat moisture treatment, modification, films.

1. INTRODUCTION

Rising environmental awareness and health consciousness of consumers has rehabilitated the interest in edible and biodegradable films. The food packaging industries have promoted to make use of edible films and coatings due to some challenges such as non-sustainable production, less suitability for recycling and inadequate mechanical and barrier properties of packaging materials. Various types of biopolymers including protein, starch, lipids and polysaccharides are used to form edible films and coatings due to their environment-friendly nature, good keeping quality and safety records for foods (Pierro et al., 2007; Tanese et al., 2008; Mihindukulasuriya and Lim, 2014). Starch is the important biopolymer used as thickening agent, emulsifiers, and stabilisers and for productions of bio-plastics in industries. Among the renewable sources with film-forming properties, starch suits all the major aspects like edibility, large availability, good isolation yield with low expenditure, nutritional value, biodegradability, biocompatibility, diverse functional properties which make it as a potential material for preparation of edible coatings or films. Edible films prepared from starch are odourless, neutral in taste, colourless, free from toxic, and semi-permeable to moisture, carbon dioxide, oxygen, and lipid as well as flavour components (Shah et al., 2016). However, the native starches have some shortcomings such as hydrophilic nature, poor solubility, weak mechanical characteristics, varying paste consistency, and low freeze-thaw stability during film formation (Xie et al., 2013; Dang and Yoksan, 2015; Sabetzadeh et al., 2015). Various modification techniques such as physical, chemical, enzymatic, and genetic, or a combination of treatments have been developed to change the starch characteristics. The starch films prepared from modified starches show altered properties. Zavareze et al. (2012) developed oxidised and heat-moisture treated potato starch films and noticed significant changes in mechanical properties of films. Effect of acid and oxidative modification of sorghum starch on properties of film was analysed by Biduski et al. (2017) and observed improved tensile strength.

Amaranth (Amaranthus spp.) is a dicotyledonous plant belongs to the family *Amaranthacea* consisting of around 60 species. Three species of amaranth namely *Amaranthus hypocondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus* are grain species. Grains of amaranth are well known for its high nutritional value containing high level of carbohydrates. Starch is the major component of amaranth grain accounting for 48-69% of its weight depending upon the species and cropping conditions. Extremely small size of starch granules of amaranth has increased its suitability for various food applications like as stabilizers, thickeners and non-food applications such as dusting powders, cosmetics and biodegradable plastics (Sindhu and Khatkar, 2016, a). Amaranth starch is characterized as 'waxy' type starch due to low amylose and high amylopectin content, which in turn provide excellent freeze/thaw stability. High clarity and good gel strength of starch are important properties increasing utilization of starch in processed food products (Sindhu and Khatkar, 2016, b). Starches from various traditional sources utilized in food and non-food applications and has been reported in previous studies. Scanty of literature is available on utilization of starch extracted from amaranth and its application in development of biodegradable films. Therefore, objective of present investigation was to exploit the amaranth starch in development of edible film; and to assess the effects of heat moisture treatment of starch on the properties of film.

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2. MATERIALS AND METHODS

2.1 Materials

Grains of amaranth (*Amaranthus hypocondriacus*) used in this study were procured from National Bureau of Plant Genetic Resources Regional Station, Shimla, India. The grains were screened to remove foreign matter and stored in sealed container at room temperature. The flour was prepared by grinding seeds on laboratory mill and stored in polyethylene bags at 10º C.

2.2 Starch Isolation

Starch was isolated from amaranth grains according to the alkaline steeping method (Choi et al., 2000). Grains were steeped in 0.25% aqueous NaOH solution for 18 h at room temperature and stirred three times during this period. After steeping, the grains were washed with distilled water and ground in a blender at full speed for 2 min, and slurry was filtered step wise through 100 mash (150µm), 270 mesh (53µm) and 400 mesh (38 µm) sieves. The starch was isolated from the filtrate by centrifugation at 25,000g for 20 min. The supernatant was discarded, and the top yellowish layer of protein was removed. This step was repeated to obtain a white starch layer. The starch layer was re-suspended in distilled water, shaken and centrifuged as described above. Thereafter, the isolated starch was dried in hot air oven at below 40ºC for 8- 10 hr and stored at room temperature in sealed container.

2.3 Heat Moisture Treatment of Starch

The heat moisture treatment of amaranth and buckwheat starch was carried out according to the method of Franco et al. (1995) with minor modifications. The moisture level of starch was adjusted to 30% by adding appropriate volume of distilled water (the moisture level of native starch was predetermined).The addition of distilled water was done slowly and simultaneously mixed for uniform distribution of water in starch powder. Samples were sealed in polyethylene pouches and equilibrated at 10°C overnight. After the incubation, starches were filled in air tight glass containers and heated for 6 hr 100°C. The sample was shaken occasionally for uniform distribution of heat. The samples were cooled to room temperature and dried at 40° C for 6 to 8 hr and equilibrated at room temperature for 4hr. The dried samples were sealed in polyethylene bags, labelled and stored at room temperature for further analysis.

2.4 Acetylation of Starch

The acetylation of starch was done by following to the method described by Wurzburg (1964). The native starch (100g, db) was placed in a 500ml beaker and 137ml distilled water was added at 25°C to prepare a 42.4% w/w (db) starch suspension. The mixture was stirred with a magnet stirrer to obtain homogeneous slurry. The pH of slurry was adjusted to 8.0 by adding drop-wise 3% aqueous NaOH solution. The calculated amount of 8ml vinyl acetate was added (8% of starch, db) drop-wise with simultaneously maintaining the pH of mixture at 8.0 to 8.4 by adding drop-wise 3% NaOH with constant stirring. On completion of vinyl acetate addition, the reaction was terminated by adjusting the pH at 4.5 using 0.5N HCl and slurry was centrifuged at 4,000rpm for 10 min. The starch cake obtained was washed 4 to 5 times with distilled water and dried at 40°C in hot air oven. The acetylated starch was ground and passed through 75µm sieve, packed in polyethylene bags, labelled and stored at room temperature till further analysis.

2.4.1 Acetyl content

The acetyl group (%, db) and the degree of substitution (DS) of starch were calculated using the method of Smith (1967). The starch sample of 5g was taken in a 250ml conical flask and added 50ml distilled water. The starch was dispersed thoroughly in distilled by mixing manually. Few drops of phenolphthalein indicator were added and titrated against 0.1N NaOH to permanent pink color. Subsequently, 25ml of 0.45N NaOH was added to it and mixed vigorously for 30min. The stopper and neck of flask was flushed with little distilled water and the excess alkali was neutralized by titrating with 0.2N HCl to disappearance of pink color. A total of 25ml of 0.45N NaOH was titrated as blank. Acetyl group and degree of substitution were calculated as follows-

$$\text{Acetyl group (\%)} = \frac{[(\text{Vb} - \text{Vs}) \times \text{N} \times 0.043] \times 100}{\text{Wt. (db)}}$$

Where Vb is the volume of 0.2N HCl used to titrate blank (ml), Vs is the volume of 0.2N HCl used to titrate sample (ml), N is the normality of 0.2N HCl, and Wt. is the weight of sample (g).

$$\text{Degree of substitution (ds)} = \frac{162 \times \text{A}}{4300 - 42\text{A}}$$

Where A is the acetyl group (%) of starch on dry basis

The degree of substitution and acetyl content of acetylated starch was 0.022 and 0.58%, respectively which were in the range recommended by FDA for food application of acetylated starches. Therefore, acetylated starch of amaranth in present investigation was utilised in the starch film formation.

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2.5 Preparation of Starch Films

Starch films using native and modified starches of amaranth and buckwheat were prepared by following the method described by Chandla et al. (2017) with minor modifications. Filmogenic solutions were prepared by dispersion of 5g starch in 100 ml distilled water with continuous stirring at magnetic stirrer for 15min. Glycerol at rate of 3g/100g starch was added as plasticizer and mixed thoroughly. The solution was magnetically stirred for 15min at 85°C. The resulting solution was cooled at room temperature to avoid air bubbles during pouring. Casting technique was used to prepare films. The prepared solution was poured onto the polypropylene round trays of diameter 12.5cm and dried at 40°C for 16hr in hot air oven with circulating air in chamber.

2.6 Analysis of Films

2.6.1 Thickness

The thickness of starch films was determined using Digital micrometer with an accuracy of ±0.001mm. The average value of 10 thickness measurement at different locations on each film was used in all calculations.

2.6.2 Moisture Content

Moisture content of starch films was determined by drying the pre-weighed pieces of films at 110°C for 6 to 8hr or till the weight comes constant. Moisture content was measured as loss in weight of film sample during heating.

2.6.3 Water Solubility

Water solubility of starch films was determined by following the method of Gontard et al. (1994). Pre-weight piece of starch film was immersed in water at room temperature for 24hr. The immersed film piece was removed from water and dried in oven at 110°C for 4 to 5 hr, cooled and weighed. The water solubility of starch film was measured as the difference in weight of dried piece of film before and after immersion in water.

2.6.4 Color Parameters

Color of native and modified starch films was measured using CR-300 Chroma meter (Minolta, Japan). The system determines the L, a^* and b^* values, where L represents lightness and darkness; a^* represents the opposition between green and red color ranging from positive (red) to negative (green) values; and b^* is the yellow/blue opposition ranging from positive (yellow) to negative (blue) values. The average value of three measurements were calculated and used.

2.6.5 Water vapour permeability

Water vapour permeability of starch films was determined by following the E96-95 ASTM standard method (ASTM, 1995). Each film sample was sealed over the circular opening of a permeation cell containing anhydrous $CaCl_2$ (0% RH) and weighed. These cells were placed on desiccators with a saturated NaCl solution (75% RH) at 25°C. The weight of each permeation cell was recorded after 24hr and water vapour permeability of films was calculated using following formula-

$$\mathrm{WVP} = \frac{\Delta W \times X}{t \times A \times \Delta P}$$

Where WAP is the water vapour permeability (g.mm/m^2.day.kPa);ΔW is the weight gain by descant (g); X is the film thickness (mm); t is the incubation period (days); A is the area of the exposed film surface (m^2); and ΔP is the difference of partial pressure (kPa).

2.6.6 Tensile Strength

Tensile strength of films was determined by a tensile test based on ASTM D-882-91 method (1996) using texture analyser (TA-XT 2i Stable Micro Systems, UK). The films were cut in strips (20mm × 50mm) and thickness of strips was measured at eight points. The strip was gripped from both the edges of width on 'tensile grip' probe and initial grip separation was set at 30mm. The force and distance were recorded during extension of strips at 0.8mm/s up to break. The tensile strength of films was calculated using following formula-

$$\mathrm{TS} = \frac{F}{A}$$

Where TS is the tensile strength (MPa); F is the maximum force (N); A is the area of film cross-section (thickness × width; m^2).

2.7 Statistical Analysis

Analytical determinations were done in triplicate, and Duncan test was conducted to examine significant differenced among experimental mean values. The statistical significance was observed at $p < 0.05$. Data were analyzed using Statistical Analysis System SAS, version 8.2 and SPSS software version 16.0 (SPSS Inc).

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3. RESULTS AND DICUSSION

3.1 Thickness, Tensile Strength, Solubility and Water Vapour Permeability of Films

The native and heat modified starches of amaranth produced continuous and easy to peel films which were visually transparent as shown in Figure 3.1. The results of moisture content, thickness, solubility, water vapour permeability and tensile strength of films are presented in Table 3.1. Moisture content of films varied from 13.21 to 15.90% with the minimum value of native starch film. The values of moisture content of acetylated and heat moisture treated starch films were statistically similar. The range of moisture content of films was comparable with the findings of Chandla et al. (2017) observed 16 to 20.50% moisture content in starch films of amaranth of different cultivars. Moisture content of starch films depends on various factors like drying temperature, relative humidity of drying chamber, starch properties and film thickness. Moisture content of modifies amaranth starch films showed higher values as compared with native amaranth starch films. Colussi et al. (2017) observed higher moisture content in the acetylated rice starch film than that of native starch film. Introduction of bulky acetyl groups promoted large space between the starch chains, decreased retrogradation and syneresis, consequently retained more water in film. Increased moisture content of hydrothermally modified starch films could be attributed to the increased hydrophilicity (lower retrogradation in waxy starch) and more thickness of modified starch films than native starch film. The thickness of film is an important parameter as it influences the film properties like transparency and water vapour permeation rate; and uniformity in film thickness is requisite for attaining good and consistent mechanical strength. Thickness of films made from native and modified starches of amaranth varied from 0.15 to 0.16mm. These thickness results were similar to the findings of Zavareze et al. (2012) reported that the film thickness of native and heat moisture treated potato starches ranged from 0.10 to 0.16mm and 0.10 to 0.14mm respectively. Chandla et al. (2017) observed comparatively higher range of thickness for films of amaranth starches from different cultivars.

Table 3.1 Moisture content, thickness, solubility, water vapour permeability and tensile strength of films of native and modified starches of amaranth

Treatments	Moisture content (%)	Thickness (mm)	Solubility (%)	WVPR ($g.mm/m^2.day.kPa$)	Tensile strength (MPa)
NS	13.21 ± 0.10^{a}	0.158 ± 0.00^{a}	48.11 ± 0.34^{a}	6.88 ± 0.03^{a}	0.734 ± 0.00^{a}
Ace-S	15.85 ± 0.05^{b}	0.152 ± 0.00^{a}	52.39 ± 0.19^{e}	7.35 ± 0.04^{e}	1.06 ± 0.03^{e}
HMTS	15.90 ± 0.10^{b}	0.162 ± 0.00^{b}	30.35 ± 0.35^{c}	6.91 ± 0.02^{c}	2.24 ± 0.00^{c}

All values are mean of triplicate determinations ± standard deviation mean. Values within same column with different letters are significantly different ($p\leq0.05$). NS: native starch; HMTS: heat moisture treated starch; Ace-S: acetylated starch; WVPR: water vapour permeability rate; mm: millimetres; kPa: kilopascal; MPa: megapascal

Solubility of edible film plays an important role in deciding its applicability. Higher solubility of film could result in disintegration of film with in short time while lower solubility could decrease the rate of degradation of film. After immersion in water for 24hr, amaranth starch films prepared from hydrothermally modified starch were intact while native and acetylated starch film samples got disintegrate. The solubility of amaranth starch films prepared from native starch was significantly higher than that of heat moisture treated starch films while lower than solubility of acetylated starch films. The water solubility of films made from native starch of amaranth was higher than that reported by Chandla et al. (2017) ranging from 33.64 to 37.56% solubility of films of amaranth starches from various cultivars. Similar results were observed by Zavareze et al. (2012) recorded decreased solubility of starch film made up of hydrothermally modified starches of potato relative to solubility of native potato starch films. Higher water solubility of films following acetylation of rice starch was reported by Colussi et al. (2017), and suggested that increased water solubility of acetylated starch films could be attributed to easily penetration of water inside the acetylated starch film as a result of lower retrogradation than native starch. The reduced water solubility of the hydrothermally modified amaranth starch films might be attributed to the increased interactions between amylose-amylose, amylose-amylopectin chains and strengthened intermolecular bonds promoted during modification treatments.

The tensile strength is the measure of the maximum force used during a stress-strain testing or the force applied at the break point of film sample. Considerably increased values of tensile strength were noticed for films of modified starches as compared to films of native starch. The value of tensile strength of films made from native starch was 0.734MPa indicating lower strength of films as compared with findings of Chandla et al. (2016) reported tensile strength ranged from 2.30 to 2.61MPa for films of amaranth starches of different cultivars. The variation in mechanical

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strength of films prepared from amaranth starch in present study and previous reports could be attributed to the differences in level of starch used for film formation, thickness, film formation conditions like drying temperature and rate. Heat moisture treated starches showed the highest values of tensile strength among native and acetylated starch films in present study. It has been reported by Zavareze et al. (2012) that heat moisture treatment of potato starch increased the tensile strength of the films from 3.53 to 6.07MPa. However, Majzoobi et al. (2015) revealed decreased tensile strength of films from 2.22 to 1.84MPa after heat moisture treatment of rice starch. The mechanical properties of the starch films depend on various factors such as polymeric chain arrangement, molecular chain interactions, film thickness, quantity and type of the plasticizer, and relative humidity of the environment. Additional interaction among amylose and amylopectin molecules caused by hydrothermal treatments could be the cause for increased tensile strength of films.

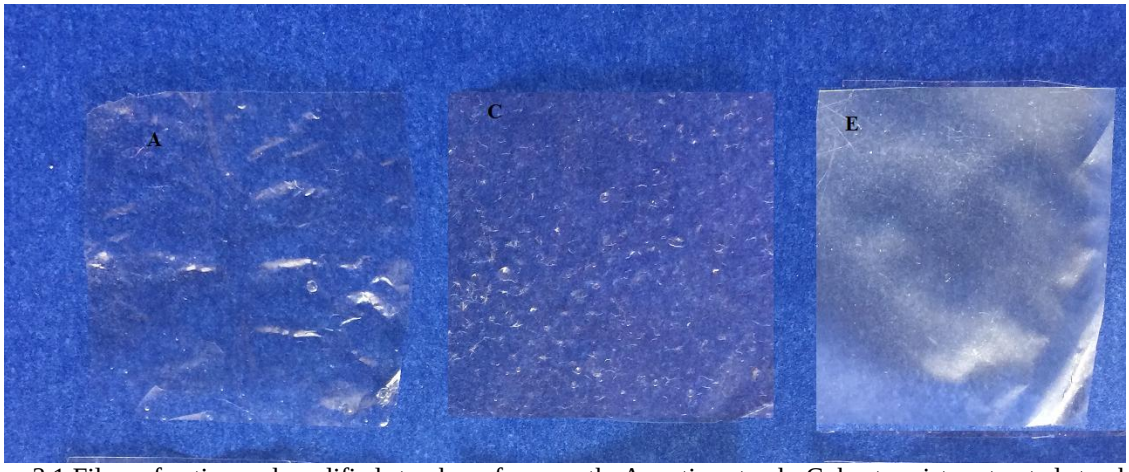

Figure 3.1 Films of native and modified starches of amaranth. A: native starch; C: heat moisture treated starch; E: acetylated starch

Water vapour permeability of film is the measure of easiness with which moisture can permeate through the film. To provide a barrier or to decrease the transfer of moisture between the food and the surrounding atmosphere, the water vapour permeability of film should be low. The values of water vapour permeability of amaranth starch films prepared from hydrothermally modified and acetylated starches were 6.96 and 7.35 g.mm/m^2.day.kPa respectively which was significantly higher than native starch films. Modified starches showed considerably increased values of water vapour permeability of films except oxidised starches. Zavareze et al. (2012) observed similar results for films of potato starches showing increased water vapour permeability of hydrothermally modified starches. Increment of water vapour permeability of acetylated starch film was attributed to the introduction of bulky acetyl groups which enlarged the space among starch molecules. Retrogradation in starch gel takes place due to interaction in amorphous region at initial stage followed by interaction in crystalline domains. As amaranth starch is waxy type, heat moisture treatment increased stiffness in the starch granules and caused lesser retrogradation (due to absence of amylose) resulted in loose packing of gelatinised granules providing space for mobility of water molecules, consequently more hydrophilic films formed with increased water vapour permeability and stiffness. Thickness of film is an another important factor affecting water vapour permeability and increased thickness of films in case of heat moisture treated starches could be the reason for higher water vapour permeability of starch films than native starch film.

3.2 Color Parameters of Films

Color of coating material is important as it influences the appearance of product in which it is applied. Color parameters of films prepared from native and modified starches of amaranth are presented in Table 3.2. Modification treatments of amaranth starch affected color of starch, consequently altered color (L, a* and b* parameter) of produced starch films. The L value indicating the lightness of films ranged from 83.48 to 84.07 with the highest value noticed for acetylated starch films.

Table 3.2 Color properties of films of native and modified starches of amaranth

Treatments	L	a*	b*
NS	83.48 ± 0.02^{a}	-0.25 ± 0.01^{a}	3.26 ± 0.04^{a}
Ace-S	84.07 ± 0.15^{d}	-0.35 ± 0.00^{b}	3.00 ± 0.01^{b}
HMT	83.54 ± 0.35^{a}	-0.27 ± 0.02^{a}	3.13 ± 0.01^{d}

All values are mean of triplicate determinations ± standard deviation mean. Values within same column with different letters are significantly different ($p\leq0.05$). NS: native starch; HMTS: heat moisture treated starch; Ace-S: acetylated starch; L: black to white; a*: green to red; b*: blue to yellow

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Negative values of a* ranged from -0.25 to -0.35 indicated slight greenish shade in amaranth starch films and higher values were recorded for modified starch samples as compared to native starch. Yellowness in starch films represented by positive b* values ranged from 3.00 to 3.26 with the lowest value observed for acetylated starch films. Modified amaranth starch films had increased brightness indicated by higher L values as compared with native amaranth starch films. However, higher values of color parameters were observed by Zavareze et al. (2012) for films prepared from native and heat moisture treated starches of potato.

4. CONCLUSION

Amaranth starch was found suitable for production of biodegradable films. Heat moisture treatment and acetylation affected differently the properties of amaranth. Both the modification treatments of amaranth starch increased tensile strength, water vapour permeation rate and L values of films. Acetylation increased while heat moisture treatment decreased the water solubility of amaranth starch films.

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