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Development of Native and Hydrothermally Modified Amaranth Starch Films

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Abstract: Amaranth (*Amaranthus spp*.) is a dicotyledonous plant belongs to the family *Amaranthacea*. It is a pseudocereal, well known for good nutritional starch is the major component of its grains. Starch was isolation from amaranth seeds and hydrothermally modified at 120°C for 6hr keeping the moisture content (30%) constant. Film were prepared from native and modified starches of amaranth and evaluated for various parameters. All film samples were continuous, transparent and crack free. Heat-moisture treated starch film had higher tensile strength and water vapour permeability than native amaranth starch film. Yellowness was higher and lightness was lower in modified starch film.

Keywords: Amaranth, heat-moisture treatment, edible films, starch.

1. INTRODUCTION

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 main component of amaranth seed varying from 48 to 69% depending upon the species and cropping conditions. Very small size of starch granules of amaranth has amplified 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). The applications of starch in food industries are mainly governed by its characteristics including functional, pasting, gelatinization, and structural properties. Starch from each source is unique in nature having different characteristics determining its suitability for particular application. Irrespective of sources, native starches are undesirable for many applications due to their inability to withstand processing conditions. In order to meet the new technological requirements of specific industrial processes, starches are modified by physical, chemical, enzymatic treatments or genetic transformation. Modification of starches is done with a purpose of improving the functional characteristics of starches and tailoring them to specific applications. Heat moisture treatment of starch is a physical method of starch modification involving heat treatment (80 to 120°C) of starch at restricted moisture level (10 to 30%) for a time period ranging from 15 min to 16 hr. Starch as biopolymer suits all the major aspects like edibility, large availability, nutritional value, biodegradability, biocompatibility, diverse functional properties which make it as a potential material for preparation of edible coatings or films (Dang and Yoksan, 2015; Reis et al., 2015). Starch films 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). Therefore, keeping the above in view, present investigation was carried out with the objective of isolation and hydrothermal modification of amaranth starch; and preparation of edible films using native and modified starches of amaranth and their evaluation.

2. MATERIALS AND METHODS

Grains of amaranth (*Amaranthus hypochondriacus*) of cultivar named Durga 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. Starch was isolated from buckwheat grains according to the alkaline steeping method (Choi et al., 2000). Grains were steeped in 0.25% aqueous NaOH solution for 18 hr 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 25000g 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 to 10 hr and stored at room temperature in sealed container.

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The heat moisture treatment of amaranth 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. Sample was sealed in polyethylene pouches and equilibrated at 10°C overnight. After the incubation, starch was filled in air tight glass containers and heated for 6 hr 120°C. The sample was shaken occasionally for uniform distribution of heat. The starch was cooled to room temperature and dried at 40°C for 6 to 8 hr and equilibrated at room temperature for 4hr. The dried powder was sealed in polyethylene bags, labelled and stored at room temperature for further analysis.

Starch films using native and heat moisture treated starches of amaranth 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.

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. 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. 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. Color of native and modified starch films was measured using CR-300 Chroma meter (Minolta, Japan). 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-

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

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). Tensile strength of films was determined 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-

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

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). Analytical determinations were done in triplicate.

3. RESULTS AND DISCUSSION

Films prepared from native and heat moisture treated starches of amaranth were continuous and easy to peel from the polypropylene surface; all film samples were visually transparent. The results of moisture content, thickness, solubility, water vapour permeability and tensile strength of films are presented in Table 1. Native amaranth starch film had 13.21% moisture which 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. Various factors like drying rate, relative humidity of drying chamber, starch properties and film thickness affects the moisture content of starch films. 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. Film thickness made from native and modified starches of amaranth was 0.158 and 0.161mm respectively. Zavareze et al. (2012) reported the film thickness of oxidised and heat moisture treated potato starches varying from 0.10 to 0.16mm. Chandla et al. (2017) observed comparatively higher range of thickness of films of amaranth starches from different cultivars.

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Water solubility of edible film plays an important role in deciding its applicability. High solubility of film could result in fast disintegration of film whereas low solubility could slow down the rate of degradation of film. Heat moisture treated amaranth starch film samples were intact while native starch film samples disintegrated after immersion in water for 24hr at room temperature. The water solubility of amaranth starch films was higher than that reported by Chandla et al. (2017) ranging from 33.64 to 37.56% solubility of film of amaranth starches from various cultivars. Zavareze et al. (2012) observed decreased solubility of starch film formed of hydrothermally modified potato starches than that of native potato starch films. 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 hydrothermally modified starch than 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. (2017) reported tensile strength ranged from 2.30 to 2.61MPa for films of amaranth starches of different cultivars. The variation in mechanical strength of films prepared from amaranth starch in present study and previous reports could be due 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 increased tensile strength of films. 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. Additional interaction among amylose and amylopectin molecules caused by hydrothermal treatments could be the cause for increased tensile strength of films.

Table 10.1 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	0.158±0.00	48.11 ±0.34	6.88±0.03	0.734±0.00
HMT	15.31±0.10	0.161±0.00	34.00±0.10	8.10±0.10	0.816±0.00

All values are mean of triplicate determinations ± standard deviation mean. Values within same column with different letters are significantly different ($p \leq 0.05$). NS: native starch; HMTS: heat moisture treated starch; mm: millimetres; kPa: kilopascal; MPa: megapascal.

Water vapour permeability is the measure of easiness with which moisture can permeate through the film. Water vapour permeability of native amaranth starch film was 6.88g.mm/m^2.day.kPa and heat moisture treated starch film showed higher value of water vapour permeability. Zavareze et al. (2012) observed similar trend for films of potato starches showing increased water vapour permeability of hydrothermally modified starches. 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. Higher 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 as corresponding increasing water vapour permeability with increasing thickness and hydrophilicity of starch films was recorded for different starches in earlier studies (Cuq et al., 1996;Mali et al., 2004; Zavareze et al., 2012).

Table 2 Color properties of films of native and modified starches of amaranth

Treatments	L*	a*	b*
NS	83.48±0.02	-0.25±0.01	3.26±0.04
HMT	82.91±0.17	-0.32±0.02	3.49±0.11

All values are mean of triplicate determinations ± standard deviation mean. Values within same column with different letters are significantly different ($p \leq 0.05$). NS: native starch; HMTS: heat moisture treated starch L*: black to white; a*: green to red; b*: blue to yellow

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 hydrothermally modified starch of amaranth are presented in Table 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 and heat moisture treated starch film

showed lower value than native starch film. Negative values of a* indicated slight greenish shade in amaranth starch films and higher value was recorded for modified starch samples as compared to native starch. Yellowness in starch films represented by positive b* values was noticed lower in native starch film. Change in color of heat moisture treated starch films could be attributed to the occurrence of Millard reaction during modification treatment of starch.

4. CONCLUSION

It can be concluded from the present investigation that starch can be successfully isolated and modified by heat-moisture treatment. Amaranth starch was found applicable for edible film development. Films prepared from native and modified starch were continuous and transparent. Heat moisture treatment of amaranth starch increased the tensile strength of film. Water solubility, moisture barrier capacity and lightness of film was decreased following hydrothermal modification of amaranth starch.

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