Evaluation of Modified Sorghum Starches and Biodegradable Films

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The properties of bio-films prepared from sorghum starch using three different modification techniques (hydrothermal treatment (HTT), acid-alcohol treatment (AA) and acetylation (A)) were studied. The physical properties of modified starches were significantly different from unmodified starches. More specifically, acetylated starch had significantly higher swelling power and solubility, where-as HHT starch had the highest water binding capacity. Starch films prepared from hydrothermal treatment had the least solubility (26.15%) and the maximum tensile strength (TS) - (6.50MPa), whereas films prepared from acid-alcohol treated starch had the lowest water vapour transmission rate (WVTR) (720.0 g/m2/d) and acetylated starch film had the maximum elongation (19.03%). The concentration of glycerol, a plasticising agent, affected the properties. The WVTR of the films increased with an increase in glycerol concentration; while tensile force and elongation decreased.

Key words: Sorghum starch, Modification techniques, biodegradable film, Water vapour, transmission rate, tensile force, Percentage elongation

Introduction

Biodegradable packaging materials are environment friendly and are commonly made from renewable polymer sources such as starch, cellulose, proteins, polylactic acid (PLA), polyhydroxyalkanoate (PHA) and polyanhydrides. Of all polymer sources, starches have been proven to be more efficient base materials owing to its versatile sources and properties which can be enhanced by chemical and physical modifications (Bodîrlău, Teacă, Spiridon, & Tudorachi, 2012; Waliszewski, Aparicio, Bello, & Monroy, 2003). Some common chemical modification methods are acetylation and acid-alcohol modification. Hydrothermal treatment (HTT) of starch is one of the most commonly used physical methods to modify starch (Zhu, 2014). During HTT, reorganization of the molecular structure of starch increases the gelatinization temperature, leading to a reduction of swelling power (Olayinka, Adebowale, & Olu-Owolabi, 2008) and improves the defects of granules (Zhu, 2014). In contrast to HTT, acetylation (A), an etherification of starch polymers with acetyl groups to form starch acetates, decreases the gelatinization temperature, improves clarity and increases the swelling and solubility of the starch (Singh et. al, 2011; Ali and Hasnain, 2011). Acidalcohol (AA) treatment modifies starch by allowing both hydrolysis and re polymerization (Chung & Lai, 2006). AA specifically decreases starch swelling power, paste strength and viscosity while also, increasing the amylose fraction of starch without a significant effect on granular shape (Chang et al., 2004; John et al., 2002; Lin et al., 2003). Amylose

plays an important role in the film forming capability of starch (Bodîrlău *et al.*, 2012).

Unlike plastic derivatives, starch film possesses biodegradability. Moreover, starch derived films show promising barrier properties against air and water vapour, depending up on modification methods and additives(Kampeerapappun et al., 2007; Singh et al., 2004). Sorghum is regarded as an important crop in developing nations and is the fifth most significant crop in world. Sorghum starch from the grain is cross-linked with protein and is therefore less digestible, limiting its food application (Ezeogu et al., 2008; Wong et al., 2009) . Thus, modification of sorghum starch and using it as base material for biodegradable films may be a promising way to add value to sorghum crops. However, research into sorghum starch modification and the use of modified starches for the production of bio-film has been limited (Adebowalea et al., 2005; Olayinka et al., 2008). Thus in this study, sorghum starch was modified using both physical and chemical methods and the properties of biodegradable films based on these modified sorghum starches were investigated.

Materials and methods

Isolation of starch

Sorghum grains, purchased from a local market in Nepal, were hammer milled and packed in an air tight container for further use. Milled sorghum flour was steeped in 0.2%

NH4OH solution (1:5) and continuously stirred for 5 minutes to suspend the starch in the solution. The slurry was centrifuged at 2500 rpm for 5 minutes. The supernatant was discarded and the top, brown-yellow, protein layer was removed as described by Marshall (1969). The residual white starch layer was re-suspended in distilled water, centrifuged and the top brown-yellow protein layer was removed by decanting. The process of re-suspension of starch and removal of protein layer was repeated until there was no longer any visible protein present. The starch concentrate was then re-suspended in distilled water. The concentrate was passed through 75 µm mesh sieve and the starch solution obtained was filtered through Buchner's funnel under vacuum. The washed starch cake was dried at 50°C, manually ground and again passed through 75µm sieve and stored in airtight container at ambient temperature for further use.

Analysis of sorghum starch

Moisture (AOAC 925.10), and starch content (AOAC 996.11) were determined according to AOAC (2005). Starch recoveries were expressed as percentage yields based on weight.

Modification of starch

The native (N) sorghum starch was modified by three modification methods namely hydrothermal treatment (HTT), acetylation (A) and acid-alcohol treatment (AA). HTT, A and AA modifications were conducted as described by Collado *et al.* (2001), Bello-Perez *et al.* (2000) and Chang *et al.* (2006) respectively.

Physico-chemical characteristics of native and modified starch

Starch swelling power and solubility were determined by the method described by Subramanian, Hoseney, & Bramel-Cox (1994). Water binding capacity (WBC) of the sample starch was determined using the method described by Medcalf and Gilles (1965). The acetyl group (AG, expressed as percentage dry basis) and the degree of substitution (DS) of sorghum starch were determined according to Smith (1967).

Preparation of starch films

The starch films were prepared according to the method described by Müller *et al.* (2008) with minor modifications. The films were prepared by a casting technique using a film-forming solution containing 5% of native or modified sorghum starch. Glycerol at various concentrations (0.25, 0.35 and 0.45 g/g dry starch) was used as plasticizer. The mixture was heated to boiling temperature with constant stirring for 10 minutes by magnetic stirrer on a hot plate. The mixture was then cooled until the bubbles vanished, and 45ml of mixture was poured homogenously into the non-sticky plastic trays of diameter 13cm. The trays containing the film forming solution were then dried in a cabinet drier at 50°C for 5h. The dried films were peeled from the trays and kept in air tight polyethylene bags until further analysis.

Analysis of starch based films

Film thickness was determined using a micrometer screw gauge. The film thickness was measured at 5 different points for each sample. The percent solubility was measured as a percentage of dry matter remaining in the film after being immersed in water for 24h (Irissin-Mangata *et al.*, 2001). The tensile force and elongation of sample films were measured according to the standard method D882-02 ASTM (2006) at room temperature using a texture analyser (TA.XTplus model, Stable Micro Systems). Samples of each film, measuring 100 x 35 mm (length x breadth), were analysed. Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by initial specimen cross-sectional area. Percent elongation at break was calculated as follows:

E = 100 X (Da - Db) / Db

Where, Da and Db were the distance between grips holding the specimen before elongation and after the break of the specimen respectively.

2.6.1 Water Vapour Transmission Rate (WVTR)

WVTR (g water/m2/d) of the plasticized sorghum starch film was measured as described by Robertson (2006). A film of 2.5 cm diameter was used to seal a testing cup containing anhydrous calcium chloride (75% by volume of cup). The cup was then placed in a controlled chamber with relative humidity (RH) of 75% and temperature ranging from 27 to 30°C. The weight of the cup was measured intermittently at intervals of 24h up to 6 days. The weight of the arrangement was plotted against time, and WVTR was calculated from the slope of curve.

2.7 Statistical analysis

The data was analyzed by statistical software, GenStat discovery edition 3 (2008). Differences between group means were investigated by analysis of variance (ANOVA). Statistical significance was set at a 0.05 probability level and experiments were performed in triplicate.

Results and discussion

Starch extracted from sorghum was analysed to determine moisture and starch content, which were found to be 10.65 % (wet basis) and 95.63% (dry basis) respectively. The starch content in the extracted sample was similar to the results obtained by Marshall (1969) and Sira and Amaiz (2004) in pigmented sorghum. The yield of starch was 47% which is slightly higher than that obtained by Sira and Amaiz (2004).

Physicochemical properties of native and modified sorghum starches

Swelling power and solubility

Swelling power is a measure of a swollen starch granules' ability to occlude water. This reflects the degree of damage inflicted upon granular structure and the formation of new binding sites on the granular surface. The swelling power of native starch (N) was found 25.23% whereas, for modified starch, the mean swelling power was 24.67%, 26.09% and 29.22% for hydrothermal treated (HTT), acid-alcohol treated (AA) and acetylated (A) starches respectively.

Similarly, the solubility of native starch was found to be 13.63% whereas, for modified starches, the values were found to be 12.85%, 14.75% and 24.52% for HTT, AA and A starches respectively.

The results obtained showed no significant difference (P>0.05) between percentage solubility of HTT and native starches, however, AA and A starches varied significantly (P<0.05) when compared to native starches. The percentage solubility of the modified starches (HTT, AA and A) varied significantly (P<0.05) when compared to each other.

The acetylated sorghum starch sample showed highest solubility and swelling power, 24.52% and 29.01% respectively. This is in agreement with Lawal (2004) who demonstrated that acetylation increases swelling power and solubility of maize starch. Acetylation causes rapid hydration of starch granules and eventually increases solubility and swelling power.

The solubility and swelling power of the HTT sorghum starch sample was less than that of native sorghum starch sample. HTT starch had the lowest level of swelling power which is similar to results found by Olayinka *et al.*,(2008) and Zavareze *et al.*, (2010). This decrease in swelling power is possibly due to the separation of the double helix present in crystalline lamellae of the starch granule and interactions between amylose–amylose and amylopectin–amylopectin.

Water binding capacity

Water Binding Capacity (WBC) of modified starch corresponds to the change in its affinity towards water. WBC of native sorghum starch was 261% which was significantly different (P<0.05) from modified starches. Acid-alcohol (AA) modification decreased the water binding capacity up to 171%, whereas hydrothermal treatment (HTT) increased the water binding capacity to 310%. This result was similar to the findings reported by Olavinka et al. (2008). It has been proposed that HTT increases the hydrophilic tendency of starches increasing the water binding capacity (Abraham, 1993) where as engagement of the hydroxyl groups to form hydrogen and covalent bonds between starch chains restricts the water binding in acetylated starches (Hoover & Sosulski, 1986). Similarly, the differences in the availability of water binding sites among the starches may have also contributed to variation in water binding capacity as observed for AA starch, where a reduction of amorphous regions after AA treatment has been reported.

Degree of substitution of acetylated starch

The degree of substitution of acetyl groups has been related to extensibility and the moisture affinity of starch based bio-films. The acetyl group content on the acetylated sorghum starch was found to be 2.70%. Similarly the degree of acetylation was found 0.104 which lies in between the food application regions (0.01-0.2) recommended by Graaf *et al.*(1998). Although the degree of substitution was much lower than maximum possible limit, 3 (Xu *et al.*, 2004), these values fall within the limit

reported by Raina *et al* (2006) and (Singh *et al.*, 2004) for Indian rice starches and corn starches, respectively.

Physicochemical properties of biodegradable starch based films

Thickness

The thickness of the coating film determines the resistance a film can offer to heat and mass transfer across it. The flux reduces with an increase in thickness for same type of film. Film thickness varied from 80 µm to 116 µm. There were no significant differences (P>0.05) among thickness of films prepared from different modifications (viz. HTT, AA and A) within the same glycerol concentration. In addition, each of the films prepared from different modifications did not vary individually in terms of thickness with the film prepared from native starch. A possible reason for these results might be that there is no difference in free space within the starch network of differently treated starch films. Increased thickness may be more resistant to mass transfer across the film. However, increasing the amount of plasticizer may occupy more space causing the film to thicken. This theory was supported by our results. The increase in glycerol concentration significantly increased the film thickness. Films containing the lowest glycerol concentration (i.e. 0.25g/g) were thinner (0.0828mm) than the films containing the highest glycerol concentrations (i.e. 0.45g/g) which were the thickest (0.1057mm). These results were in agreement with results published by Laohakunjit and Noomhorm (2004). Solubility

Solubility in water is an important property of starch based films. Statistical analysis showed modification had a significant effect (P<0.05) on the solubility of films. The maximum solubility of the films were at 0.35g/g glycerol for native and acetylated starch, while AA and HTT starch films had the maximum solubility at 0.45g/g glycerol. Among the modified starch, films prepared from hydrothermal modified starches had the lowest solubility in water (26.15%) whereas films prepared from acetylated starch had the highest solubility (36.31%). HTT favours the complex formation between amylose-amylose and amylopectin-amylopectin (Zavareze et al., 2010) resulting in decreased solubility, while cumulative hydrophilicity from both acetyl groups (Yue Xu et al., 2010) and glycerol (Laohakunjit & Noomhorm, 2004) could be attributed to increased solubility. Potential applications may require both water solubility and insolubility. Insolubility is required to enhance product integrity and water resistance. However, higher water solubility of the film before product consumption may be useful for encapsulation of food or additives (Bertuzzi, Armada, & Gottifredi, 2007).

In the study, glycerol had profound impact (P<0.05) on the solubility of films. There is increase in glycerol concentration increased the solubility of the films. The results may be due to an increased hydrophilicity

attributable to added glycerol. These results are in accordance with the findings of Bertuzzi *et al.* (2007). Tensile strength

Modification methods and glycerol concentration affected the Tensile Strength (TS) of the film samples significantly (P<0.05). TS ranged from 0.19 MPa (A starch at 0.35g/g glycerol) to 6.50 MPa (HTT starch with 0.25g/g glycerol). A higher TS film could be suitable for tight packaging of food materials. The maximum TS for N, HTT and A starch film were found at 0.25g/g glycerol concentration. The maximum TS of AA starch film, on the other hand, was found at 0.35g/g glycerol concentration. Plasticizers extend, dilute and soften the structure, increasing chain mobility of the starch. With the exception of AA film, the results were similar to Shi et al. (2008). The low tensile force found for AA film may be due to weak bonds which have been re-structured during film formation. Glycerol concentrations had significant negative effect (P<0.05) on the mean TS of films. There was a drastic reduction on TS of film when glycerol concentration was increased from 0.25g/g to 0.35g/g compared to an increase of 0.35g/g to 0.45g/g. Although this variation is not clearly understood, almost degradation of mechanical integrity due to 0.35g/g glycerol concentration could be hypothesized. A similar trend in rice starch film has been previously reported by Laohakunjit and Noomhorm (2004). Matta et al. (2011) also found that an increase in glycerol content decreases the TS of starch based films.

Percent elongation

Percent Elongation (PE) is the extent to which film stretches before breaking when subjected to tensile force. Greater elongation reflects greater flexibility. The percent elongation varied from 2.00 (native starch at 0.45g/g glycerol) to 19.02 (acetylated starch at 0.25g/g glycerol). Acetylated film could with stand mechanical damage when used in machine and rough handling during food processing due to its higher elongation power. Among the various modifications, the maximum PE for N and HTT starch film was found at 0.35g/g glycerol, while 0.25g/g glycerol resulted in the maximum elongation for AA and A starch film. Films from both N and HTT starch showed an initial increase upto 0.35g/g glycerol concentration but decrease in elongation when glycerol concentration was further increased to 0.45g/g. The trend is in agreement with the report found by Laohakunjit and Noomhorm (2004). However, the effect of glycerol concentration analysed at P<0.05 showed a significant negative correlation with film PE when the mean values for all films were taken into considerations. Muscat et al. (2012) also reported that lower the glycerol concentration, higher the percent elongation. Therefore, it could be proposed that glycerol can add extensibility to some extent by forming new bonds by engaging hydroxyl groups. Conversely, excess glycerol

may over-soften the film, decreasing film elongation power.

Water Vapour Transmission Rate

WVTR of materials, such as starch film, reflects the sensitive of the material to moisture and thus the suitability of the material as a food packaging materials. The WVTR of the film samples films ranged from 680-890 g/m2/d. In every treatment, WVTR increased with an increase in glycerol concentration. The high affinity of water to glycerol may be the reason behind this positive relation. The addition of plasticizer increases the molecular mobility of the film, simultaneously increasing the diffusivity of the permeating molecules and thus increasing overall permeability (Arvanitoyannis, Kalichevsky, Blanshard, & Psomiadou, 1994; Biliaderis, Lazaridou, & Arvanitoyannis, 1999). ANOVA of WVTR, with respect to glycerol concentration, demonstrated that WVTR increased significantly when glycerol concentration was increased from 0.25g/g to 0.35g/g; however, further increases in glycerol concentration did not significantly affect WVTR. These findings signified that a saturation level may be reached after 0.35g/g glycerol concentration. Muscat et al (2012) also reported correlation between WVTR and glycerol concentration in their study of high and low amylose starch film formed with glycerol and xylitol. The maximum WVTR reached in present study (890 g/m2/d) much lower than the WVTR reported by was Kampeerapappun et al. (2007) and Laohakunjit and Noomhorm (2004) which were both above 1500g/m2/d.









Fig. 2 Properties of N, HTT, AA, and A films prepared at glycerol concentration of 0.25, 0.35 and 0.45 g/g dry starch (a) Thickness (mm), (b) Solubility, (c) Tensile strengths, (d) Elongation(%), (e) WVTR of films as affected by modification and glycerol concentration





Fig. 3 Properties of film affected by glycerol concentrations

(a) Thickness (b) Solubility (c) Tensile Strength (d) Percent elongation

(e) WVTR of films as. Mean is calculated combining corresponding values

from all treatments. (Bars are standard error bars. Mean separation by LSD, p<0.5)

Conclusion

Modifications improved the physicochemical properties of starch. Acetylated starch showed maximum solubility and swelling power, while hydrothermally treated starch showed highest water binding capacity. The addition of glycerol in starch film changed the film properties to varying degrees, depending upon their glycerol concentration and the method of modification Film thickness increased with glycerol concentration. An increase in plasticizer concentration increased the film solubility and WVTR; while percentage elongation and tensile force decreased. Of the three modifications, hydrothermal treatment decreased solubility and increased both tensile force and elongation. Acid-alcohol treated starch film had the lowest WVTR.

Acknowledgments

The authors express their gratitude to the Subject Instruction Committee, Central Campus of Technology, Tribhuwan University, Nepal for supporting the project.

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