Characterization of Barnyard Millet Starch Films Containing Borage Seed Oil

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Abstract: In this study, barnyard millet starch (BMS) was used to prepare edible films. Antioxidant activity was conferred to the BMS film by incorporating borage seed oil (BO). The physical, optical, and thermal properties as well as antioxidant activities of the films were evaluated. The incorporation of BO into the BMS films decreased the tensile strength from 9.46 to 4.69 MPa and increased the elongation at break of the films from 82.49% to 103.87%. Water vapor permeability, water solubility, and moisture content of the BMS films decreased with increasing BO concentration, whereas Hunter L and a values of the films decreased. The BMS films containing BO exhibited antioxidant activity that increased proportionally with increased BO concentration. In particular, the BMS film with 1.0% BO exhibited the highest antioxidant activity and light barrier properties among the BMS films. Therefore, the BMS films with added BO can be used as an antioxidant packaging material.

Keywords: antioxidant activity; edible films; essential oils; starch

1. Introduction

Biodegradable films have been applied in food packaging to resolve problems related to environmental impact [1]. Among bio-materials used for films, starch is popular in edible film production because of its widespread distribution in nature, ease of isolation, and low cost [2]. Recent studies have been conducted to improve the functional properties of starch films, such as antimicrobial, antioxidant activities, and light barrier properties [3]. In addition, economic and environmental benefits from utilizing agricultural byproducts or underutilized natural materials for film preparation have attracted attention for edible film production [4,5].

Barnyard millet is a cereal cultivated in Asian countries for human food or animal feed [6]. Owing to its extreme hardiness, this cereal is appreciated as a dominant crop on harsh lands [7]. Barnyard millet contains starch (66%), protein (15%), lipid (7%), and various micronutrients [8,9]. However, this cereal has a hard pericarp and contains high amylose content, which reduces its culinary and organoleptic properties [10]. In addition, this grain possesses low digestibility [11]. These disadvantages have limited the popular consumption of this grain and confined it as a coarse food [10], but starch containing high amylose content is considered a raw material for edible films with good oxygen barrier properties [12].

Fatty acids have been incorporated into starch films as a chemical modifier [13,14]. In particular, the incorporation of fatty acids decreases the hydrophilicity of starch films. Borage (Borago officinalis L.) seed consists of various bioactive compounds including fatty acids and phenolic substances. Mhamdi et al. [15] investigated fatty acid contents in ripe borage seed and found that unsaturated fatty acids accounted for more than 80% of total fatty acids, while rosmarinic and p-coumaric acids were dominant phenolic substances. In addition, Khan and Shahidi [16,17] reported the presence of...
δ-tocopherols and γ-tocopherols—compounds with strong antioxidant activities—in borage seed oil. Borage seed oil (BO) is odorless, and it can be used as a chemical modifier to improve the functional properties of films prepared from the underutilized barnyard millet starch (BMS). Moreover, BMS films containing BO have not been studied. Therefore, the objective of this study was to develop BMS films and characterize the physical, optical, and thermal properties as well as antioxidant activity of the BMS films incorporated with BO.

2. Materials and Methods

2.1. Materials

Barnyard millet (*Echinochloa utilis*) was purchased from Grainlife Co., Goesan, Korea. Commercial BO (purity 100%) was provided by The Witch Company (Seoul, Korea). Sorbitol and Tween 80 were obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

2.2. Starch Extraction

Barnyard millet grains were pulverized in a Waring blender (Osaka Chemical Co., Osaka, Japan), then soaked in 0.3% NaOH solution (flour: NaOH solution, 1:6, w/v), homogenized at 12,000 rpm for 5 min, incubated at 4 °C for 24 h with constant stirring at 300 rpm, sieved through a 200-mesh screen, and centrifuged at 10,000 × g for 15 min. The precipitate was obtained after removing the dark yellow layer from the surface. Afterwards, the precipitate was washed with distilled water, neutralized to a pH of 7.0, and centrifuged at 10,000 × g for 15 min. The extracted starch was dried on a clean bench at 25 °C for 18 h. Finally, dried starch was ground using a mortar and pestle, then sieved through a 200-mesh screen.

2.3. Preparation of the BMS Films

BMS (3.5%, w/v) suspension was prepared by dispersing starch in distilled water. Sorbitol (50% of starch weight, w/w) was added to the starch suspension as a plasticizer, and the suspension was heated at 95 °C for 20 min with constant stirring at 260 rpm. The film-forming solution was prepared by adding BO at different concentrations (0.2%, 0.4%, 0.6%, 0.8%, and 1% of film-forming solution, w/v) and Tween 80 (10% of BO, w/w), followed by homogenization at 12,000 rpm for 5 min. A film-forming solution without BO and Tween 80 was used as the control film. The film-forming solutions were casted on plates and dried at 25 °C, relative humidity 50% for 15 h.

In this study, Tween 80 was used as a surfactant in the BMS film containing BO. The effect of Tween 80 on the physical and optical properties of the films was evaluated, and the obtained results showed that the amount of Tween 80 used did not affect the physical and optical properties of the films.

2.4. Determination of Physical Properties of the BMS Films

An Instron machine (M250-2.5 CT, The Testometric Company Ltd., Lancashire, UK) was used to determine tensile strength (TS) and elongation at break (E). Film sheets (2.54 cm × 8 cm) were prepared and fitted into the grips with an initial clearance of 5 cm. The stretching was performed at a speed of 50 cm/min. At least five sheets of each sample were measured to evaluate TS and E. These two parameters were calculated based on the equations described by Yang et al. [18].

Five pieces of each film (2 cm × 2 cm) were dried at 105 °C for 24 h to determine the moisture content (MC) of the films. MC was calculated using Equation (1).

\[
MC(\%) = \left(\frac{W_0 - W}{W_0}\right) \times 100
\]

where \(W_0\) is the initial weight of the film and \(W\) is the weight of the film after drying for 24 h.

To determine water solubility (WS), dry films were gently shaken in 50-mL tubes containing 20 mL deionized water at 25 °C for 24 h. The films were then placed on aluminum dishes and dried.
at 105 °C for 24 h. The WS was expressed as the percentage of the decrease in film weight. Five film squares (4 cm²) were analyzed for each type of film.

The water vapor permeability (WVP) of the films was evaluated at 25 °C and relative humidity of 50%, according to the method described by Song et al. [19]. The experiment was carried out in triplicate.

2.5. Measurement of Optical Properties

Color and opacity of the films were determined based on the method described by Kim et al. [20]. The L, a, and b values of the white standard plate were 96.79, −0.15, and 2.03, respectively.

2.6. Determination of Antioxidant Activities

Ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays were carried out to determine the antioxidant activity of the films. The film extract solution for the assays was obtained by shaking tubes containing 19.5 mL deionized water and 0.5 g of the films (0.3 cm × 0.3 cm) at 37 °C, 150 rpm for 30 min, followed by centrifugation at 10,000 × g for 5 min to remove solid materials. FRAP reagent was prepared according to methods described by Da Silva and Jorge [21]. A mixture of FRAP reagent (5 mL) and film sample (2 mL) was placed in the dark for 30 min and centrifuged at 3000 × g for 3 min to eliminate precipitate. The absorbance of the supernatant was measured at 593 nm. The ABTS test was carried out according to the method described by Lee et al. [22]. A mixture containing 4 mL of ABTS reagent and 1.8 mL of film sample was prepared to measure the absorbance at 734 nm.

2.7. Scanning Electron Microscopy (SEM)

Microscopic images of the films were obtained using a focused beam of high-energy electrons generated by a scanning electron microscope (LYRA3 XMU; Tescan, Warrendale, PA, USA). An accelerating voltage of 5.0 kV was used, and magnifications of 3000× and 2000× were utilized to examine surface and cross-section microstructures, respectively.

2.8. Differential Scanning Calorimetry (DSC)

DSC was carried out with a Mettler Toledo DSC 1 (Mettler Toledo, Schwerzenbach, Switzerland) with 10 °C/min as the heating rate from 50 to 200 °C to determine the melting temperature (Tm) and fusion enthalpy (ΔH) of the films (10 ± 0.35 mg).

2.9. Statistical Analysis

Significant differences among experimental data were determined by analysis of variance and Duncan’s multiple range tests (p < 0.05) using the SAS program (SAS Institute, Cary, NC, USA). The results were expressed as the mean ± standard deviation.

3. Results and Discussion

3.1. Physical Properties of the BMS Films

Prior to the measurement of physical properties of the BMS films containing BO, the effect of Tween 80 on the physical and optical properties of the BMS films was examined. The BMS films with sorbitol and Tween 80 only at 10% (w/w) of 1.0% BO as a maximum amount used in this study had the TS, E, WVP, and opacity of 9.24 ± 0.41 MPa, 83.56% ± 2.85%, (4.48 ± 0.19) × 10⁻⁹ g/m·s·Pa, and 0.55 ± 0.06 A/mm, respectively, and these values were similar with those for the BMS film containing sorbitol only as a control (Tables 1–3), suggesting that Tween 80 used as a surfactant in this study did not affect the physical and optical properties of the BMS films.
Table 1. Physical properties of the BMS films containing BO.

| Borage Oil (%) | Tensile Strength (MPa) | Elongation at Break (%) |
|----------------|------------------------|-------------------------|
| 0              | 9.46 ± 0.38 \(^a\)     | 82.49 ± 2.95 \(^c\)     |
| 0.2            | 6.99 ± 0.24 \(^b\)     | 85.74 ± 4.34 \(^d, e\)  |
| 0.4            | 6.54 ± 0.40 \(^c\)     | 86.38 ± 1.46 \(^d\)     |
| 0.6            | 5.61 ± 0.13 \(^d\)     | 90.65 ± 2.83 \(^c\)     |
| 0.8            | 4.87 ± 0.22 \(^e\)     | 94.48 ± 2.71 \(^b\)     |
| 1.0            | 4.69 ± 0.14 \(^e\)     | 103.87 ± 2.16 \(^a\)    |

Means ± S.D. \(^{a-f}\): any means in the same column followed by different letters are significantly \((p < 0.05)\) different by Duncan’s multiple range test.

TS decreased gradually from 9.46 to 4.69 MPa as BO concentration increased up to 1.0%, whereas E increased proportionally from 82.49% to 103.87%. In particular, the addition of 1.0% BO decreased TS by 50% and increased E by 26%, compared to the film without BO. It appears that distribution of BO in the film matrix induces an obstruction between hydrophilic starch polymers and decreases the cohesiveness of the film network [23]. Moreover, fatty acid molecules in BO are embedded inside helical amyloses, which can reduce the interactions between starch chains and weaken the film network [13,14]. In addition, unsaturated fatty acids (USFAs), the major component of BO, might behave as a grease for the polymers in the films, increasing E [24,25]. Similar results were reported regarding changes in TS and E of the films containing USFAs for sweet potato and corn starch films [13,25].

The changes in MC, WS, and WVP of the BMS films are shown in Table 2. The MC of the films decreased as BO was added from 0.2% to 1.0%. This decrease in MC could be explained by the increase in hydrophobicity from BO. The incorporation of BO into starch films rearranged the amylose and amylopectin molecules, resulting in decreased affinity of amylose for water molecules by producing amylose-fatty acid complexes [26].

Table 2. Moisture content, water solubility, and water vapor permeability of the BMS films containing BO.

| Borage Oil (%) | Moisture Content (%) | Water Solubility (%) | Water Vapour Permeability \(10^{-9} \text{ g/m-s-Pa}\) |
|----------------|----------------------|----------------------|-----------------------------------------------|
| 0              | 7.41 ± 0.26 \(^a\)  | 38.70 ± 0.28 \(^a\)  | 4.44 ± 0.09 \(^a\)                           |
| 0.2            | 7.00 ± 0.29 \(^b\)  | 35.42 ± 0.29 \(^b\)  | 4.30 ± 0.08 \(^b\)                           |
| 0.4            | 6.73 ± 0.24 \(^b,c\) | 34.58 ± 0.38 \(^c\)  | 4.11 ± 0.12 \(^c\)                           |
| 0.6            | 6.57 ± 0.29 \(^c,d\) | 33.68 ± 0.25 \(^d\)  | 3.92 ± 0.11 \(^d\)                           |
| 0.8            | 6.40 ± 0.12 \(^d\)  | 31.41 ± 0.24 \(^e\)  | 3.83 ± 0.14 \(^d\)                           |
| 1.0            | 5.88 ± 0.19 \(^e\)  | 31.11 ± 0.08 \(^e\)  | 3.65 ± 0.11 \(^e\)                           |

Means ± S.D. \(^{a-f}\): any means in the same column followed by different letters are significantly \((p < 0.05)\) different by Duncan’s multiple range test.

Table 3. Optical properties of the BMS films containing BO.

| Borage Oil (%) | L         | a   | b   | Opacity (A/mm) |
|----------------|-----------|-----|-----|----------------|
| 0              | 96.09 ± 0.20 \(^a\) | −0.23 ± 0.02 \(^a\) | 3.20 ± 0.03 \(^a\) | 0.55 ± 0.04 \(^e\) |
| 0.2            | 95.65 ± 0.07 \(^b\) | −0.35 ± 0.01 \(^b\) | 3.78 ± 0.03 \(^d\) | 2.84 ± 0.02 \(^d\) |
| 0.4            | 95.19 ± 0.08 \(^c\) | −0.46 ± 0.03 \(^c\) | 4.54 ± 0.06 \(^c\) | 3.57 ± 0.08 \(^c\) |
| 0.6            | 94.83 ± 0.09 \(^d\) | −0.50 ± 0.02 \(^d\) | 5.00 ± 0.05 \(^b\) | 3.64 ± 0.08 \(^c\) |
| 0.8            | 94.53 ± 0.10 \(^e\) | −0.56 ± 0.03 \(^e\) | 5.52 ± 0.04 \(^a\) | 4.62 ± 0.22 \(^b\) |
| 1.0            | 94.32 ± 0.06 \(^f\) | −0.59 ± 0.03 \(^e\) | 5.56 ± 0.06 \(^e\) | 6.26 ± 0.19 \(^a\) |

Means ± S.D. \(^{a-f}\): any means in the same column followed by different letters are significantly \((p < 0.05)\) different by Duncan’s multiple range test.

Table 1 shows the effect of BO incorporation into the BMS films on the TS and E values. TS decreased gradually from 9.46 to 4.69 MPa as BO concentration increased up to 1.0%, whereas E increased proportionally from 82.49% to 103.87%. In particular, the addition of 1.0% BO decreased TS by 50% and increased E by 26%, compared to the film without BO. It appears that distribution of BO in the film matrix induces an obstruction between hydrophilic starch polymers and decreases the cohesiveness of the film network [23]. Moreover, fatty acid molecules in BO are embedded inside helical amyloses, which can reduce the interactions between starch chains and weaken the film network [13,14]. In addition, unsaturated fatty acids (USFAs), the major component of BO, might behave as a grease for the polymers in the films, increasing E [24,25]. Similar results were reported regarding changes in TS and E of the films containing USFAs for sweet potato and corn starch films [13,25].

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The WS of the films ranged from 38.70% for the film without BO to 31.11% for the film containing 1.0% BO. The addition of BO reduced the diffusion of soluble solid substances into water due to the formation of an insoluble outer layer of starch granules, which decreased the WS of the films [26]. According to Amini et al. [27], the addition of fatty acids showed similar results on the WS of basil seed gum films.

The incorporation of BO into BMS films decreased the WVP. The improved moisture barrier property indicates the formation of hydrophobic layers around the starch granules, which prevents water vapor permeation through the films [26,28]. In addition, interactions between amylose and fatty acids minimize the affinity of amylose for water molecules and reduce the hydrophilicity of the films [26]. BO contains various fatty acids including saturated fatty acids with different hydrocarbon chain lengths (palmitic, stearic, and arachidic acids) and USFAs (palmitoleic, oleic, linoleic, α-linolenic, γ-linolenic, and eicosanoic acids) [15,29], which create a barrier against moisture permeation. Several studies have similarly reported that increasing the fatty acid concentration decreased the WVP [14,23].

3.2. Optical Properties

The changes in colors of the films are presented in Table 3. While $L$ and $a$ values tended to decrease, the $b$ value slightly increased with increased BO content. This change can be explained by the dispersion of light yellow BO in the colorless starch film. With the increasing concentration of fatty acids or essential oils into chitosan films, similar results were reported by Silva et al., where $L$ decreased and $b$ increased [30]. Decreased $L$ value was also observed when stearic acid was added into Bambara groundnut starch films [14].

Opaque packages with light barrier properties have been applied in food packaging to protect foods from oxidation, especially photodegradation of lipids. Opacity of the BMS films proportionally increased with the addition of BO. The opacity of the BMS film with 1.0% BO was more than 11-fold higher than that of the BMS film without BO. A similar result was obtained when stearic acid was incorporated into the Bambara groundnut starch film [14]. The addition of corn oil also increased the opacity of gelatin films [23].

3.3. Antioxidant Activities

Plant seed oils usually contain USFAs, which are sensitive to oxidation. However, these compounds are stable because of naturally existing antioxidants [31]. Tocopherols and phenolic compounds are present in borage seed, and they protect USFAs from oxidation [32,33]. Specifically, δ-tocopherol and γ-tocopherol contents in borage seed oil are 52 and 659 mg/kg, respectively [16].

The antioxidant activities of the BMS films increased as BO concentration increased from 0.2% to 1.0%, as determined by ABTS and FRAP assays (Figure 1).

![Figure 1. Antioxidant activities of the BMS films containing BO: (a) ABTS radical scavenging activity; (b) Ferric reducing activity.](image-url)
Thus, these results suggest that the microstructure of the BMS films incorporated with BO yields a film with a distribution of oil droplets through the cross-section [25]. Similarly, Oyeyinka et al. [14] also reported that oil aggregation might form lipid aggregates, resulting in film heterogeneity [24]. Jiménez et al. observed heterogeneity in corn starch film; films incorporated with oleic acid exhibited a heterogeneous structure with the distribution of oil droplets through the cross-section [25]. Similarly, Oyeyinka et al. [14] also reported rough surfaces of Bambara groundnut starch films upon fatty acid addition. The heterogeneous structure of hydroxypropylmethyl cellulose film incorporated with fatty acids was also reported [24]. Thus, these results suggest that the microstructure of the BMS films incorporated with BO yields a film structure that is less firm and more flexible.

![Surface SEM images of the BMS films](image)

**Figure 2.** Surface SEM images of the (a) BMS film, (b) BMS-BO 0.6% film, and (c) BMS-BO 1.0% film. Cross-sectional SEM images of the (d) BMS film, (e) BMS-BO 0.6% film, and (f) BMS-BO 1.0% film. Magnification: 3000 × and 2000 × for surface and cross-section, respectively.

3.5. **Thermal Properties of Films**

The $T_m$ and $\Delta H$ of the films are shown in Figure 3 and Table 4. DSC analysis showed that BMS films possessed one endothermic peak between 110 and 122 °C, representing the melting temperatures of the films. Previously, the $T_m$ of millet amylose-fatty acid complexes was reported to be 91.7–110 °C [36].

| Borage Oil (%) | $T_0$ (°C) | $T_m$ (°C) | $T_c$ (°C) | $\Delta H$ (J/g) |
|---------------|------------|------------|------------|-----------------|
| 0             | 79.34      | 122.44     | 179.06     | 148.66          |
| 1.0           | 70.60      | 110.68     | 173.28     | 116.64          |
It is clear that BO incorporation affected the \( T_m \) and \( \Delta H \) of the film. In particular, the addition of BO decreased \( T_m \). The \( T_m \) of the BMS films with 1.0% BO was 110 °C, lower than that of the control BMS film (122 °C). The decrease in \( T_m \) of the films is mainly due to the weakened network of starch and BO. It appears that BO in the film-forming solution could interact with starch polymers and occupy space in the helical amylloses, reducing the interactions between starch polymers and weakening the film network. As a result, the \( \Delta H \) of the films decreased from 148.66 J/g for the BMS film to 116.64 J/g for the BMS films with 1.0% BO. This decrease in \( \Delta H \) indicates decreased thermal stability of the BMS films containing BO. Similarly, Jaramillo et al. reported decreases in \( T_m \) and \( \Delta H \) of cassava starch films supplemented with yerba mate extract [37]. In this study, the incorporation of BO decreased firmness and increased flexibility of the BMS films, and the energy required for melting the film was lesser than that required for the BMS film without BO. Therefore, these results are consistent with the decreased TS and increased E of the BMS film containing BO.

4. Conclusions

In this study, BMS films incorporated with BO were prepared. The increasing hydrophobicity of the BMS films containing BO was demonstrated by the decreases in MC, WS, and WVP. In addition, the BMS films containing BO exhibited antioxidant activity in accordance with increased BO concentration. In particular, incorporation with 1.0% BO into the BMS film decreased the WVP by 18%, compared to that in the film without BO. Therefore, the BMS films containing BO can be used as an antioxidant packaging material.

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Author Contributions: Thi Luyen Cao performed the experiments, analyzed the data, and wrote the first draft. So-Young Yang helped Thi Luyen Cao with discussion of experimental data. Kyung Bin Song supervised the study and prepared the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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