

Original Research

**VALORIZATION OF BREWERY SPENT GRAIN INTO BIOPLASTIC FILMS:
INFLUENCE OF BIOMASS LOADING ON BARRIER AND ANTIOXIDANT
PROPERTIES**

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**Despina Kostadinova Jankovski^{1,a*}, Milica Arizanova^{1,b}, Bozidar Ristovski^{1,c},
Elena Velickova^{1,d}, Donka Doneva-Shapcheska^{1,e}**

Faculty for Technology and Metallurgy, University of Ss. „Cyril and Methodius“, Ruđer Bošković 16,
1000 Skopje, NORTH MACEDONIA

^a <https://orcid.org/0000-0001-9999-9798>

^b <https://orcid.org/0000-0003-0245-6901>

^c <https://orcid.org/0000-0003-4493-2144>

^d <https://orcid.org/0000-0003-3259-5726>

^e <https://orcid.org/0000-0003-3618-9872>

Abstract

Brewery spent grain (BSG), the major by-product of the brewing industry, represents a promising feedstock for the development of sustainable bioplastic materials. In this study, chitosan-based bioplastic films containing different BSG loadings were prepared using glycerol as a plasticizer to evaluate the influence of biomass incorporation on film properties. Prior to film preparation, BSG was subjected to mild acetic-acid hydrolysis to improve the release of bioactive compounds. The films were characterized in terms of thickness, water vapor permeability, and antioxidant activity using the DPPH radical scavenging assay. The incorporation of BSG influenced the structural and barrier properties of the films while enhancing their antioxidant potential due to the presence of phenolic compounds naturally associated with lignocellulosic biomass. Increased BSG loading resulted in higher radical scavenging activity, indicating the potential of these materials for active packaging applications. Overall, the results demonstrate the feasibility of valorizing brewery spent grain into functional bioplastic films, contributing to sustainable packaging development and circular bioeconomy strategies.

Keywords: brewery spent grains, chitosan, bioplastic films, antioxidant activity, active packaging.

INTRODUCTION

The global packaging industry is currently facing a significant challenge due to the environmental contamination caused by the widespread use of fossil-fuel-derived synthetic plastics [1,2]. Transitioning toward sustainable alternatives and a circular bioeconomy is now imperative to mitigate the environmental impact of plastic pollution [3,4]. In this context,

* despina@tmf.ukim.edu.mk

agricultural side streams and lignocellulosic residues have emerged as strategic renewable feedstocks to replace petroleum-based industrial components [2,5].

Brewer's spent grain (BSG) is one attractive agri-food wastes for the fabrication of bio-based materials [6]. It is the primary solid by-product of the brewing process, accounting for approximately 85% of the total brewery waste output. Annually, the brewing industry generates between 38 to 39 million tons of BSG worldwide [7]. Despite its potential, BSG is currently underutilized, often limited to low-value applications such as animal feed or disposed of in landfills [7,8]. Valorizing this waste stream into bioplastic films represents a promising strategy for reintegrating brewery residues into the production cycle [5].

The suitability of BSG for film formation stems from its rich chemical composition [6]. It is a lignocellulosic material composed of dietary fibers, including cellulose (12%–33%), hemicellulose (20%–42%), and lignin (10%–28%), as well as a significant protein fraction (19%–30%) [7,8]. Furthermore, BSG contains valuable phytonutrients, notably phenolic compounds such as ferulic and p-coumaric acids, which are concentrated in the barley grain's outer layers [7]. These compounds impart important functional characteristics, such as radical scavenging and antioxidant capacity, making BSG-based materials ideal for active food packaging designed to extend the shelf life of oxidative-prone products [2,9,10].

However, biopolymers derived from BSG often yield films that are inherently brittle [10]. To overcome this, glycerol is frequently employed as a plasticizer; it decreases the attractive forces between polymer chains, thereby enhancing film flexibility, handling, and bulk mechanical properties [11]. While several studies have explored films made from isolated BSG proteins or specific fractions like arabinoxylans [9,11], research into the direct use of whole BSG waste in varying loads for film fabrication remains a growing area of interest.

The objective of this study was to valorize brewery spent grain (BSG) by incorporating it into bioplastic films at different loading concentrations (5, 15, 25, and 50%) using glycerol as a plasticizer. The effect of BSG addition on the physical and barrier properties of the films, including water vapor permeability (WVP), was evaluated. Antioxidant activity was also determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay to assess the potential of the films for packaging applications.

MATERIALS AND METHODS

Preparation of BSG and bioplastic films

Brewery spent grain (BSG) was obtained from a local brewery in Skopje, North Macedonia. The material was dried at 50 °C for 24 h, milled, and sieved to obtain a particle size below 150 µm. Mild hydrolysis was applied to partially disrupt the lignocellulosic structure of BSG and improve the extractability of bound antioxidant compounds. Briefly, BSG (5 wt%) was dispersed in 1 M acetic acid and continuously stirred at 40 °C for 24 h, according to the methodology described by Arizanova et al. (2026) [12]. Medium molecular weight chitosan (190,000–310,000 Da; degree of deacetylation >75%) was purchased from Merck (Italy). Glycerol, used as a plasticizer, was obtained from Fisher Scientific (USA). All chemicals and reagents used in the study were of analytical grade. Four film formulations

were prepared by mixing the chitosan and BSG solutions in different ratio, as presented in Table 1. Films' appearance is shown in Figure 1.

Table 1 Formulations of the prepared bioplastic films

Formulation	Chitosan:BSG ratio	Chitosan (g)	BSG (g)	Total Glycerol (g)
Ch	100:0	1	0	0.3
Ch-BSG-1	85:15	0.85	0.15	0.3
Ch-BSG-2	75:25	0.75	0.25	0.3
Ch-BSG-3	50:50	0.50	0.50	0.3
Ch-BSG-4	25:75	0.25	0.75	0.3

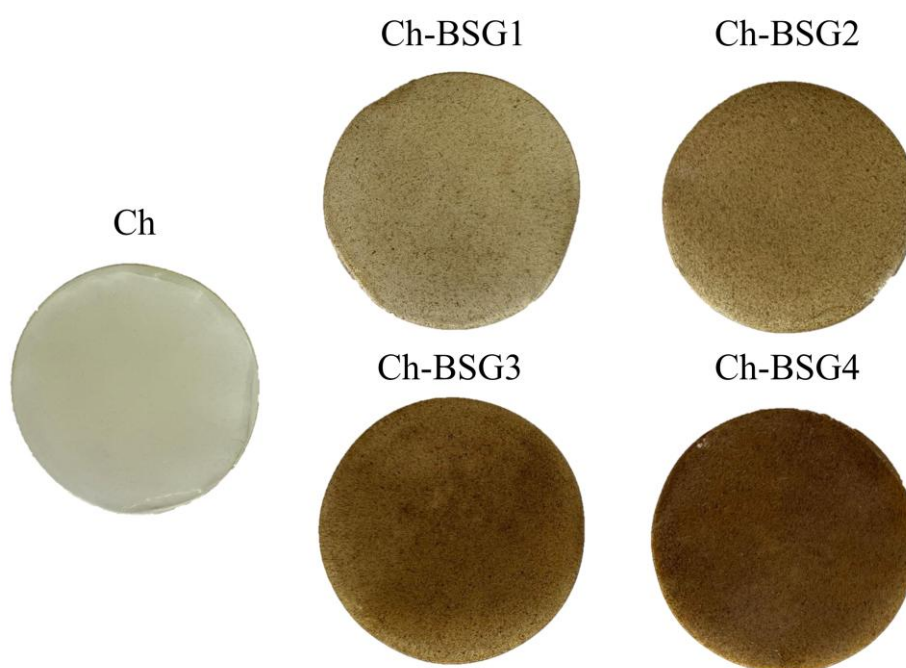


Figure 1 Appearance of prepared bioplastic films

Film thickness (δ)

The thickness of the prepared films was determined using a digital caliper (Extol, China) with a precision of ± 0.02 mm. Measurements were taken at ten randomly selected locations across each film sample to account for possible heterogeneity. The final thickness values are expressed as the average $\pm$ standard deviation (SD).

Water vapor permeability

The water vapour permeability of the prepared films was evaluated using the gravimetric cup method based on the ASTM E96 (2015) standard, with slight modifications [13]. Permeability cells containing 3 mL of distilled water were sealed with the film samples and subsequently transferred to a desiccator maintained at low relative humidity using silica gel. The experiment was conducted at 25 °C, and the mass loss of the cups was monitored periodically over a 24 h period. The reduction in mass corresponded to the migration of water vapour through the film matrix from the high-humidity side (100 % RH inside the cup)

toward the lower-humidity environment (30 % RH outside the cup). The WVP values were determined according to the following equation:

$$WVP = \frac{WVTR * \delta}{P_{H_2O} * \Delta RH}$$

where δ represents the average thickness of the film, WVTR is the water vapor transmission rate, P_{H_2O} denotes the saturation vapor pressure of water at 25 °C, and ΔRH corresponds to the relative humidity gradient across the film.

Antioxidant properties

The antioxidant potential of the bioplastic films was assessed using the DPPH radical assay, based on a modified procedure described by Merino et al. (2023) [14]. Film samples weighing ~0.05 g were immersed in 4 mL of a 0.1 mM DPPH solution prepared in ethanol. The interaction between the films and the radical solution was monitored by measuring the reduction in absorbance at 517 nm over a period of 24 hours, using a Cary 6000i UV–Vis–NIR spectrophotometer (USA). The radical scavenging activity (RSA, %) was calculated according to the following equation:

$$RSA (\%) = \frac{A_0 - A_{1,t}}{A_0} \times 100$$

where A_0 represents the initial absorbance of the DPPH solution at 517 nm, and A_t corresponds to the absorbance of the solution in the presence of the film sample at a given time.

RESULTS AND DISCUSSION

Film thickness (δ)

The thickness of the prepared films is presented in Figure 2. The pure chitosan film (Ch) exhibited the lowest thickness (0.26 ± 0.03 mm), while the incorporation of brewer's spent grain (BSG) increased the thickness up to 0.37 ± 0.01 mm in Ch-BSG2. This increase is attributed to the lignocellulosic and predominantly insoluble nature of BSG particles, which are dispersed within the biopolymer matrix as a reinforcing biofiller and contribute to a bulkier structural organization [5,7]. Similar thickness ranges (0.25–0.38 mm) have been reported for thermoplastic starch composites reinforced with BSG fibers, confirming that the incorporation of lignocellulosic biomass typically increases the total volume and structural complexity of biopolymer films [7]. The initial rise in thickness is associated with the presence of bulky insoluble particles that partially disrupt the compact polymer network and restrict biopolymer chain mobility. Interestingly, further increases in BSG loading (Ch-BSG3 and Ch-BSG4) did not result in continuous thickening, with both films stabilizing at approximately 0.35 mm. This plateau likely reflects particle aggregation and more efficient packing at higher biomass concentrations, combined with a reduced proportion of continuous hydrated matrix surrounding the particles as the chitosan fraction decreases. Similar non-linear thickness trends have been observed in other BSG-derived composite systems, as well as for poly(vinyl alcohol) composite films [10,15].

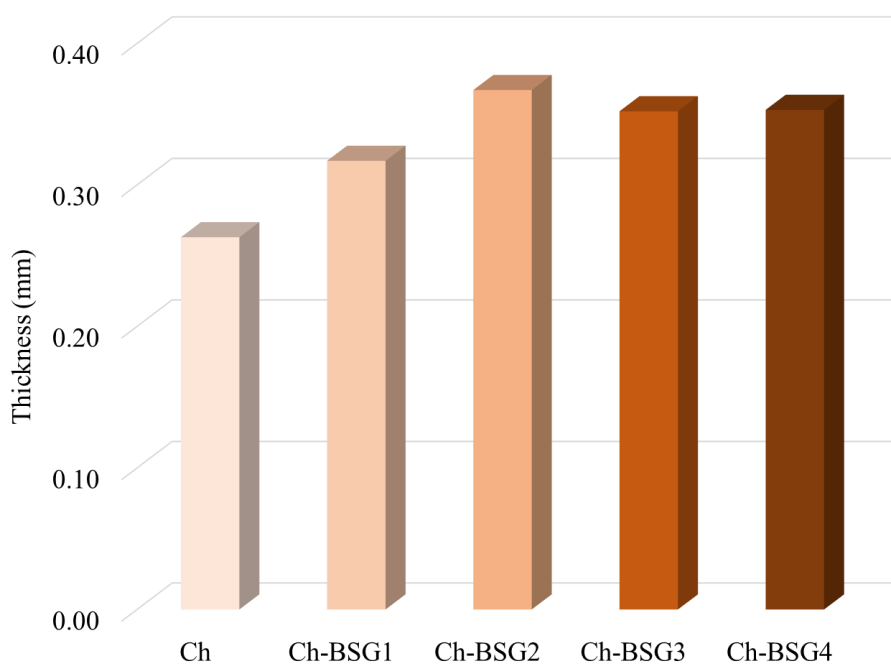


Figure 2 Films' thickness

Water vapour permeability

The water vapor permeability (WVP) values of the prepared films are presented in Figure 3. The incorporation of BSG resulted in a gradual increase in WVP, reaching approximately 4.1×10^{-10} , 4.8×10^{-10} , and 4.9×10^{-10} g/m·s·Pa for Ch-BSG1, Ch-BSG2, and Ch-BSG3, respectively, and increasing more markedly to 6.3×10^{-10} g/m·s·Pa for Ch-BSG4. This behaviour can be attributed to the hydrophilic nature of BSG constituents, including residual proteins, hemicelluloses, and cellulose, which contain polar functional groups (–OH, –NH₂, –COOH) capable of interacting with water molecules through hydrogen bonding [10]. As a result, water sorption and diffusion through the film matrix are facilitated. The obtained values are comparable to those reported for BSG protein-based films, which exhibited WVP values between 3.03 and 4.58×10^{-10} g/m·s·Pa and similarly showed increased permeability with higher biomass incorporation [11]. The increase in WVP further suggests that BSG particles partially disrupt the intermolecular interactions within the dense chitosan network, generating a less compact structure that promotes moisture transport [3]. Although lignocellulosic fillers may sometimes improve barrier performance by increasing diffusion tortuosity, the pronounced hydrophilic character of whole BSG appears to dominate in the present system, resulting in increased permeability.

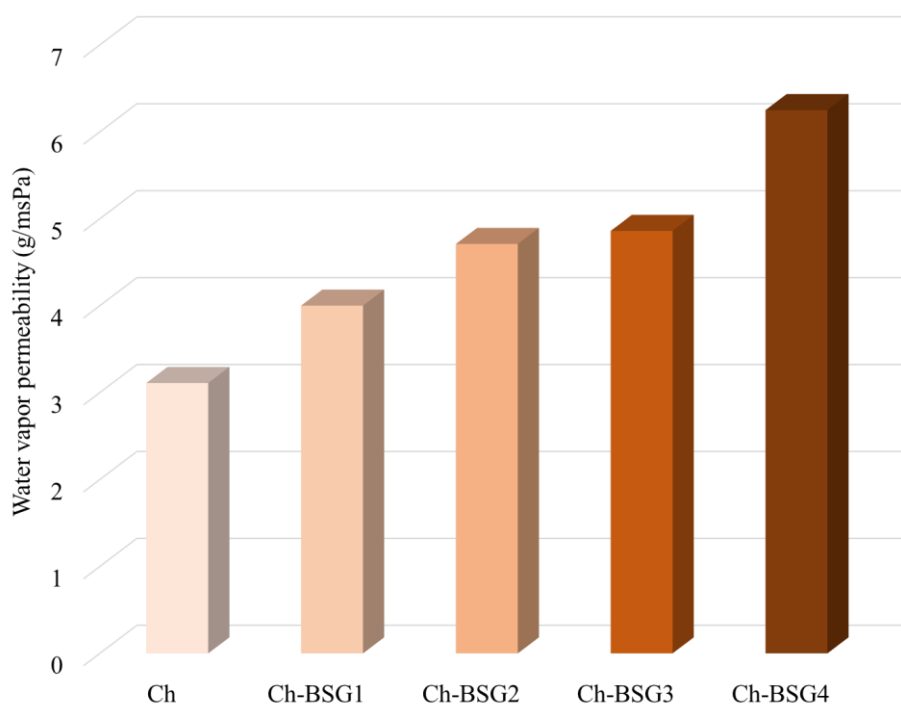


Figure 3 WVP ($\times 10^{-10}$ g/msPa) of films

Antioxidant properties

The addition of BSG enhanced the antioxidant activity of the composite films, with a marked increase observed at higher loadings: 32% for Ch-BSG2, 75% for Ch-BSG3, and nearly complete scavenging (99%) for Ch-BSG4. Notably, the antioxidant performance of Ch-BSG3 exceeded that previously reported for films based on isolated BSG proteins, which showed only 5.44% inhibition at a comparable polymer-to-BSG ratio [7]. This suggests that the utilization of whole BSG preserves a broader spectrum of bioactive constituents compared to isolated protein fractions. The observed behaviour is consistent with the presence of hydroxycinnamic acids in BSG, particularly ferulic and *p*-coumaric acids, which are recognized as the major antioxidant compounds in barley husk-derived materials [16,17]. In addition, the mild acetic-acid hydrolysis applied during film preparation likely promoted the release of bound phenolics through partial disruption of ester and ether linkages within the lignocellulosic matrix, thereby increasing their bioavailability and reactivity toward DPPH radicals [5,7]. The high scavenging activity observed at elevated BSG loadings was comparable to that reported for advanced active packaging systems, such as BSG-arabinoxylan films supplemented with ferulic acid [9]. Overall, these findings demonstrate the potential of whole BSG as a functional bioactive filler for the development of antioxidant bioplastic films.

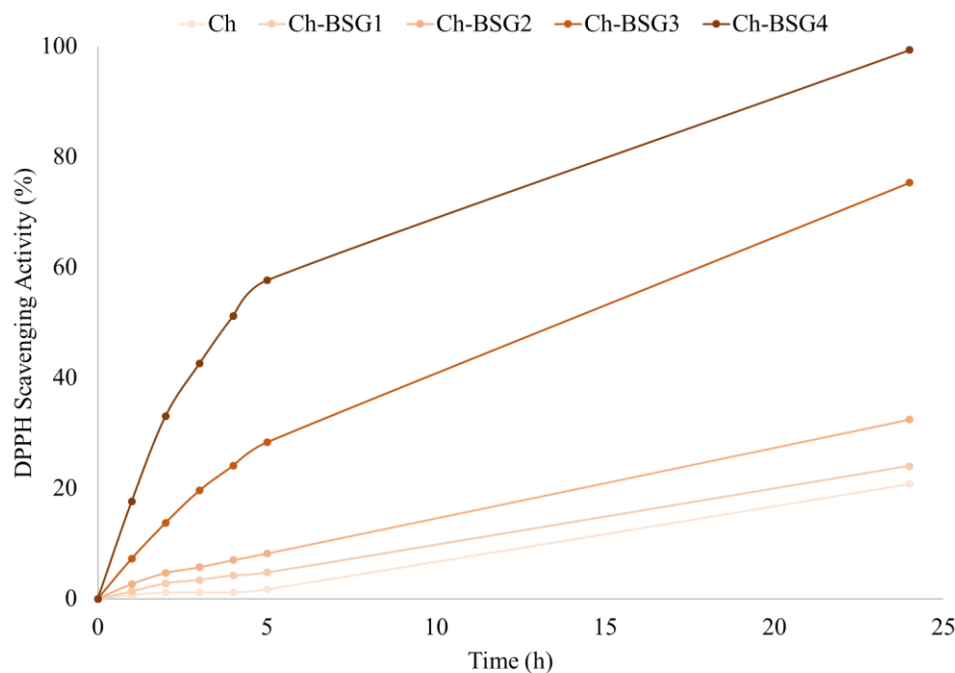


Figure 4 DPPH scavenging activity of films (%) as a function of time (h)

CONCLUSION

The present study demonstrated the successful incorporation of BSG into chitosan-based bioplastic films with improved functional properties. Increasing BSG loading enhanced the antioxidant activity of the films, while also affecting their barrier behavior through increased water vapor permeability. The highest BSG concentration resulted in nearly complete DPPH radical scavenging, confirming the contribution of phenolic compounds naturally present in BSG. These findings highlight the potential of brewery spent grain as a sustainable raw material for the development of active bioplastic packaging, contributing to waste valorization and circular bioeconomy approaches.

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