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MICROBIOLOGICAL STABILIZATION AND SHELF-LIFE MODELLING OF BREWERS SPENT GRAINS THROUGH ACIDIFICATION AND ALGINATE ENCAPSULATION

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ABSTRACT

Brewer's Spent Grain (BSG), the primary by-product of the brewing industry, is highly susceptible to rapid spoilage due to its elevated moisture content and nutrient-rich composition. This perishable nature necessitates the application of effective stabilization strategies to extend shelf life and enable its safe and sustainable utilization in food, feed, and bioenergy sectors.

The present study aimed to evaluate the safety and shelf life of BSG intended for both human and animal consumption by investigating the influence of key physicochemical parameters, namely temperature, pH, and water activity (a_w), on microbial spoilage. Additionally, innovative stabilization approaches, including acidification and alginate-based encapsulation, were explored to establish optimal microbiological stability conditions.

Fresh BSG samples were obtained from the Stefani & Co Brewery (Tirana, Albania) and subjected to microbiological analysis using Plate Count Agar (pour plate method) and Wort Agar (spread plate method). Predictive modeling of microbial degradation was performed using a bioengineering-based approach, integrating degradation kinetics with an exponential decay function to estimate shelf life under varying environmental conditions.

Acidification with food-grade acids effectively reduced the pH to 3.2–4.5, significantly inhibiting microbial growth while inducing moderate changes in soluble protein and sugar content. Furthermore, a novel stabilization technique employing sodium alginate (1.5–2%) cross-linked with $CaCl_2$ reduced water activity from approximately 0.96 to 0.90–0.92, resulting in a shelf-life

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extension of up to two weeks. The combined application of acidification and alginate treatment exhibited a synergistic effect, further enhancing microbiological stability.

The results demonstrate that simultaneous control of multiple intrinsic factors temperature, pH, and water activity is significantly more effective than the modification of a single parameter. The proposed stabilization strategies are not only technically feasible but also cost-effective, contributing to improved storage stability and reduced risks of microbial contamination and aflatoxin formation.

Overall, these findings support the sustainable valorization of BSG by enabling its safe reuse in food and feed applications.

Keywords: Brewery Spent Grain, mathematical modeling, pretreatment, microbiological stabilization, shelf life, alginate treatment.

1. INTRODUCTION

Brewers' Spent Grains (BSG) are nutrient-rich by-products with diverse applications in the food industry, agro-food sector, and environmental management. However, their high moisture content and nutrient density make them highly susceptible to microbial contamination, especially at elevated storage temperatures, leading to rapid degradation. Acid treatment is a simple, low-cost, and effective method to enhance the shelf life of BSG. By lowering the pH, this treatment inhibits microbial growth and prevents aflatoxin formation without compromising nutritional quality. Acidification can extend BSG's microbiological stability for over 20 days at 20°C, and at 25°C, mycotoxin levels remain low or undetectable for at least one week. The efficacy of acidification increases as pH levels drop, as they significantly delay spoilage. Conversely, storage temperatures above 18°C substantially raise the risk of contamination. When properly acidified and stored, BSG can maintain quality and safety for up to one month. Brewers' spent grain (BSG) is inherently microbiologically stable immediately after lautering, exhibiting low total aerobic mesophilic bacterial counts (10^2–10^3 CFU/g), as stated by Robertson et al., (2010a), but its high moisture content and accessible nutrients render it highly perishable under ambient conditions, as published by Lynch, et al. (2016). Storage at 20 °C leads to rapid microbial proliferation, total aerobic mesophilic rise from ~10^2–10^3 CFU/g to ~10^6 CFU/g within 5 days and to ~10^8 CFU/g by day 16, whereas refrigeration at 4 °C can limit these counts to <10^6 CFU/g for at least 16 days, underscoring the critical role of temperature control in BSG preservation as published by Robertson et al., (2010b). Microbial growth rates are temperature sensitive, typically following a Q_{10}≈2 relationship (i.e., rates double per 10 °C increase). For many bacteria (e.g., *Escherichia coli*), Q_{10} values near 2 have been measured, indicating that a 2 °C drop (from 20 °C to 18 °C) reduces growth rates to ~87% of those at 20 °C Milo et al. (2014). Consequently, at 18°C the onset of exponential microbial growth is modestly delayed compared to 20 °C, but the overall

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progression through lag, exponential, and stationary phases remains similar Milo et al. (2014). The microbiota of fresh BSGs remains largely unknown, but human pathogenic microorganisms do not survive the beer production process. BSG has low microbial contamination and is considered stable, within acceptable limits for food consumption. However, failure to meet storage and preservation conditions can cause BSG spoilage and make it unsuitable for human consumption Terefe (2022). In principle, the environment for microbial growth requires light, temperature, pH, moisture, and suitable chemical composition Rhezqy Furwati Jufri (2020b). BSGs have shown potential for use as a carbon source. Additionally, their high content of minerals and bioactive compounds, along with the absence of toxins, make BSGs a suitable medium for the growth of bacteria that produce biosurfactants Moshtagh et al., (2019). The high content of proteins, carbohydrates, and free amino acids creates favourable conditions for the laboratory growth of microorganisms. There is still a need for further studies regarding the formulation of media that recreate natural conditions, as well as identifying new roles and functions of certain microorganisms Pham V.H.T. & Kim J. (2012). Different stages of the process from barley harvest to storage revealed various species of Penicillium spp. Hill & Lacey (1984). The bacteria isolated from the grain were *Rhizospheric* (*Kocuria rhizophila, Microbacterium aerolatum* and *Bacillus pumilus*), as well as *Staphylococcus spp*. Among the identified yeasts the most common were *Cryptococcus spp.* and *Rhodotorula spp.,* less common species was *Saccharomyces cerevisiae* Bianco et al. (2019). Wet BSG may contain yeasts, moulds, and *Clostridia*, and it can support the growth of mycotoxigenic moulds such as *Aspergillus spp., Penicillium spp.,* and *Fusarium spp.* since these can contaminate raw materials Terefe (2022), as well as filamentous moulds like *Alternaria* and *Rhizopus* Bianco et al., (2019). The Isolation of microorganisms is the process of growing microbes from various contamination sources in a sterile artificial medium for various phytopathological and microbiological research purposes Golam Dastogeer (2022). Cultivation techniques play an essential role in identifying new species, though they have low efficiency as concluded by Pham V.H.T. & Kim J., (2012). Studies have shown that simple methods are more helpful in cultivating microorganisms, even when dealing with samples obtained from extreme environments Pulschen et al. (2017). Different preservation methods, including freezing, sealing, and food additives, have been used to address transportation and storage issues as published by Terefe (2022).

2. MATERIALS AND METHODS

The microbiological stability assessment was conducted via two parallel methodologies.

i. Empirical Analysis

Identification of the Contaminating Population. To assess microbial contamination, BSG samples from various sources (e.g., post-mashing) were analyzed for the presence of bacteria, yeasts, and

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molds. Identification methods included cultivation on selective media, microscopic examination, and biochemical assays.

Investigating conditions that inhibit microbial growth under varying parameters such as pH, water activity (aw) and temperature. Empirical data were used to determine critical thresholds, such as $pH < 4.5$ and $aw < 0.80$, under which the activity of indigenous microflora was significantly reduced.

ii. Experimental Analysis

The study by (Drazho et al., 2025) laid the foundation for understanding the microbial dynamics BSG, establishing baseline data on microbial growth, safety thresholds, and the impact of environmental factors such as temperature, pH, and water activity on shelf life. That work demonstrated that untreated BSG stored under mesophilic conditions has a microbiological shelf life of approximately five days, and that integrated optimization of temperature, pH, and water activity could, in theory, extend stability to more than two months.

The present paper is a continuation of that research, shifting the focus from predictive modelling to experimental stabilization strategies. Specifically, we investigate the effectiveness of acidification with food-grade acids and alginate encapsulation, applied individually or combined, as practical methods to extend the shelf life of BSG and ensure its safety for food and feed applications.

3. RESULTS

The first paragraph under each heading or subheading should be flush left, and subsequent paragraphs should have a five-space indentation. A colon is inserted before an equation is presented, but there is no punctuation following the equation. All equations are numbered and referred to in the text solely by a number enclosed in a round bracket (i.e., (3) reads as "equation 3"). Ensure that any miscellaneous numbering system you use in your paper cannot be confused with a reference [4] or an equation (3) designation.

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Table 1: Safety analysis of malt (raw material) and BSG stored for 20 days at 20°C.

Parameter	MALT	Limit	Typical level fresh BSG	Typical level after 20 days at 20 °C	Allowable limit value (EU feed)
Total Aerobic Count cfu/g	<10	10^5	10^2–10^3	10^8-10^9	≤10^6 cfu/g (EC, 2011)
***Enterobacteriacea* cfu/g**	<10	10^4	10-10^2	10^5-10^6	≤3×10^2 cfu/g (EC 2011)
***Yeasts* cfu/g**	<10	10^4	2-4	10^8-10^9	≤10^4 cfu/g (EC, 2011)
***Moulds* cfu/g**	<10	10^4	2-4	10^6-10^7	Not regulated
Total aflatoxins (µg/kg)	< 2	4	< 2	300	≤20 µg/kg (European Parliament & Council, 2002)
Aflatoxin B1 (µg/kg)	< 0.5	2	<0.5	190	≤8000 µg/kg (EC, 2006)
Deoxynivalenole (µg/kg)	< 50	750	<50	6000	≤250 µg/kg (European Commission, 2006)
Ochratoxin A (µg/kg)	< 2	3,0	<2	450	≤2000 µg/kg (European Commission, 2006)
Zearalenone (µg/kg)	< 3	75	< 3	2100	≤10^6 cfu/g (European Commission, 2011)

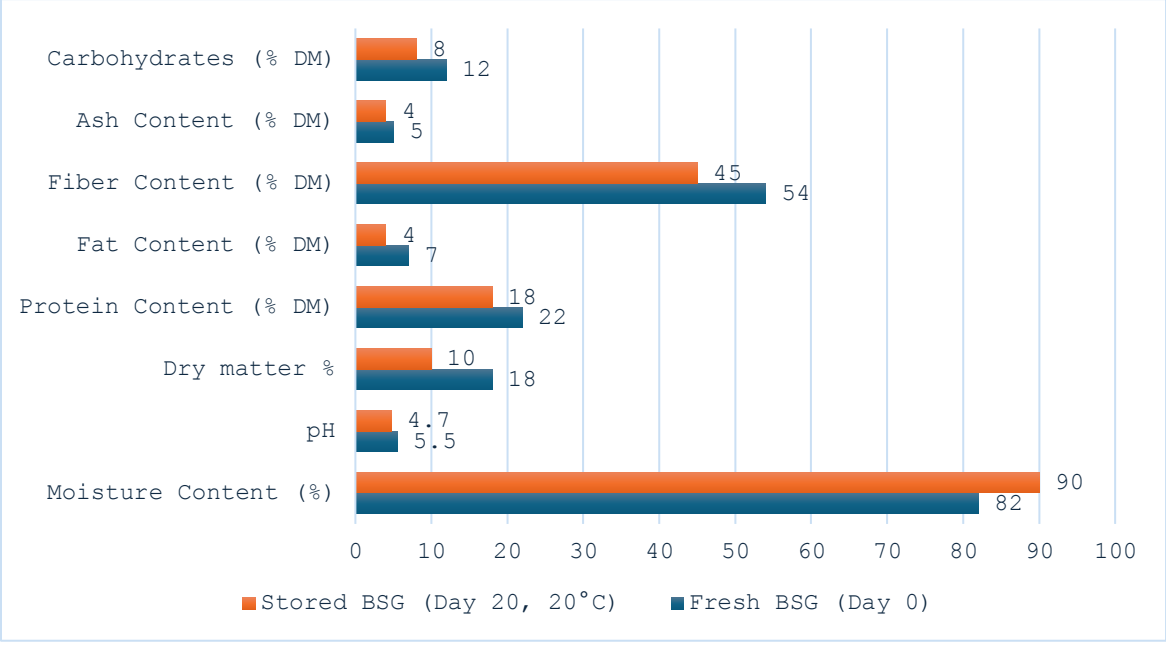

Figure 1: Chemical composition of fresh Brewers Spent Grains straight from the source and after 20 days of being stored at 20 °C.

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Table 2: Comparison of content in Fresh BSG (0 days) and stored BSG (20 days, 25°C)

Parameter	Fresh BSG (Day 0)	Stored BSG (20 days at 20 °C)
Moisture content (%)	82	90
pH	5.5	4.7
Dry matter (%)	18	10
Organic content (% in dm)	92	75
Organic content (% in wet sample)	18	8
Aflatoxins (ppb)	Not detected	High levels detected
Total microbial count (cfu/g)	$<10^3$ - 10^4	$<10^8$
Mold & yeast (cfu/g)	$<10^2$	$<10^8$

During storage, we noticed an increase in moisture content, due to microbial activity and degradation. The pH dropped, indicating fermentation and the production of organic acids. Protein and carbohydrate content slightly decreased, because of microbial consumption. Aflatoxin levels rose, suggesting potential fungal contamination, while microbial counts significantly increased, leading to spoilage.

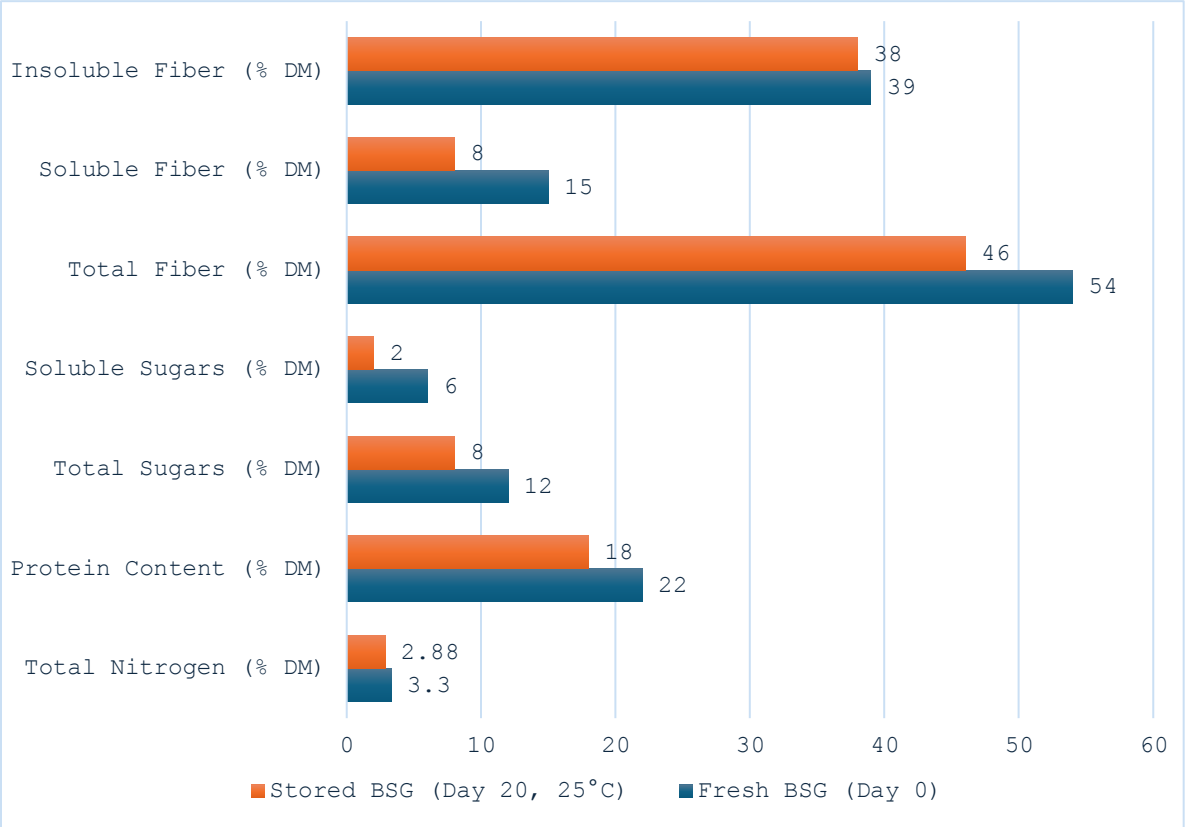

Figure 2: Chemical Composition of Fresh vs. Stored BSG (20°C, 20 Days).

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Total nitrogen and protein levels decrease due to microbial activity. Both total and soluble sugars decline as microbes consume them. Total fiber remains relatively stable, while soluble fiber slightly decreases, likely due to enzymatic degradation. The presence of aflatoxins in BSG stored at high temperatures indicates contamination by *Aspergillus* (aflatoxin), *Fusarium* (deoxynivalenole and zearalenone) and *Aspergillus* (ochratoxin). The high moisture content, along with the abundance of proteins and sugars, makes BSG a highly unstable product, susceptible to microbial growth and contamination.

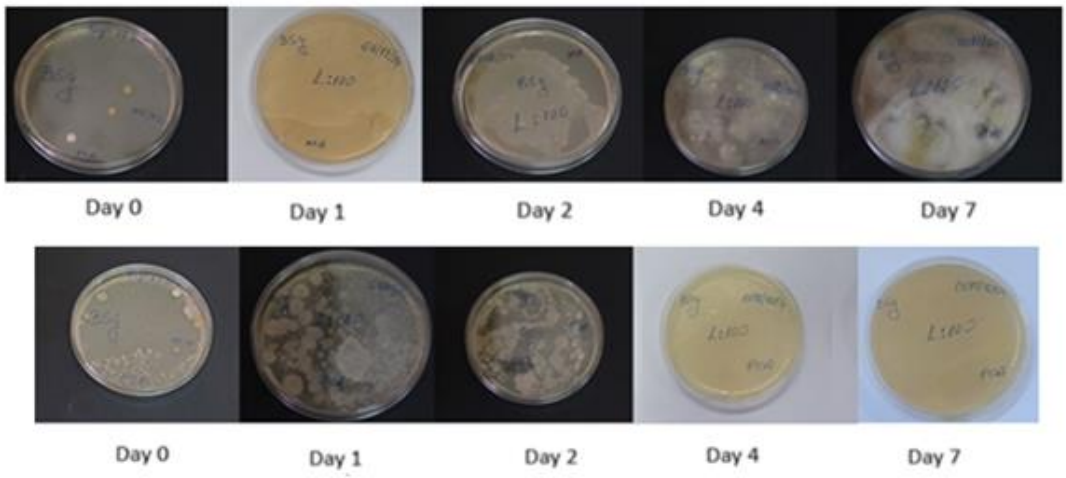

Figure 3: Growth dynamics of microorganisms on Wort Agar (1:100 dilution) and Plate Count Agar (1:100 dilution). Source: (Drazho et al., 2025)

These observations confirm the microbial succession described previously (Drazho et al., 2025) and serve as a baseline for evaluating the stabilization treatments tested here. The images illustrate the gradual transition from fresh BSG (day 0) to older samples (day 8), highlighting increasing colony density and morphological diversity. Colonies evolved from scattered yeast-like growth in the early days to dense bacterial and fungal populations at later stages. These visual observations were used to support the quantitative data collected in microbial counts.

4. DISCUSSIONS

The microbiological succession observed during storage of fresh BSG (0–8 days) is consistent with our previous kinetic study (Drazho et al., 2025). Initially, yeast populations (*Pichia*, *Rhodotorula*, *Saccharomyces*) predominated, followed by the emergence of bacterial species (*Micrococcus*, *Diplococcus*), and subsequently filamentous fungi (*Penicillium*, *Aspergillus*, *Mucor*) at later stages of storage. This sequential development reflects the progressive adaptation of microbial communities to changing substrate conditions and nutrient availability.

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In our earlier work, we developed a predictive modeling framework grounded in classical bioengineering principles, integrating logistic and exponential growth kinetics, Arrhenius-type temperature dependence, and correction factors accounting for pH and water activity effects on microbial growth rates. The model demonstrated that untreated BSG stored under mesophilic conditions (20–28 °C, pH ≈ 6.0, a_w ≈ 0.98) exhibits a predicted shelf life of approximately 5 days. In contrast, optimized storage conditions combining low temperature (4 °C), acidification (pH ≈ 4.5), and reduced water activity (a_w ≈ 0.85) were estimated to extend shelf life to over two months.

Building upon this modeling framework, the present study experimentally evaluates novel stabilization strategies, specifically acidification using food-grade acids and alginate-based encapsulation, both individually and in combination. The objective is to validate model predictions and assess their effectiveness in enhancing microbiological stability and prolonging BSG shelf life.

Table X presents representative values of the optimal growth rate constant (k_opt) and the effective rate constant (k_eff) for the dominant microorganisms under mesophilic conditions (20 °C), expressed in d^{-1}.

Furthermore, the graphical analysis illustrates the predicted spoilage time of BSG (days) as a function of temperature (4, 18, 28, and 38 °C) and pH (3.0–7.5) for *Saccharomyces carlsbergensis*, a representative brewing yeast. The results indicate that spoilage rates are strongly temperature- and pH-dependent, with shorter shelf life (darker regions) observed near optimal growth conditions, and significantly prolonged stability at lower temperatures and at pH values deviating from the microbial optimum.

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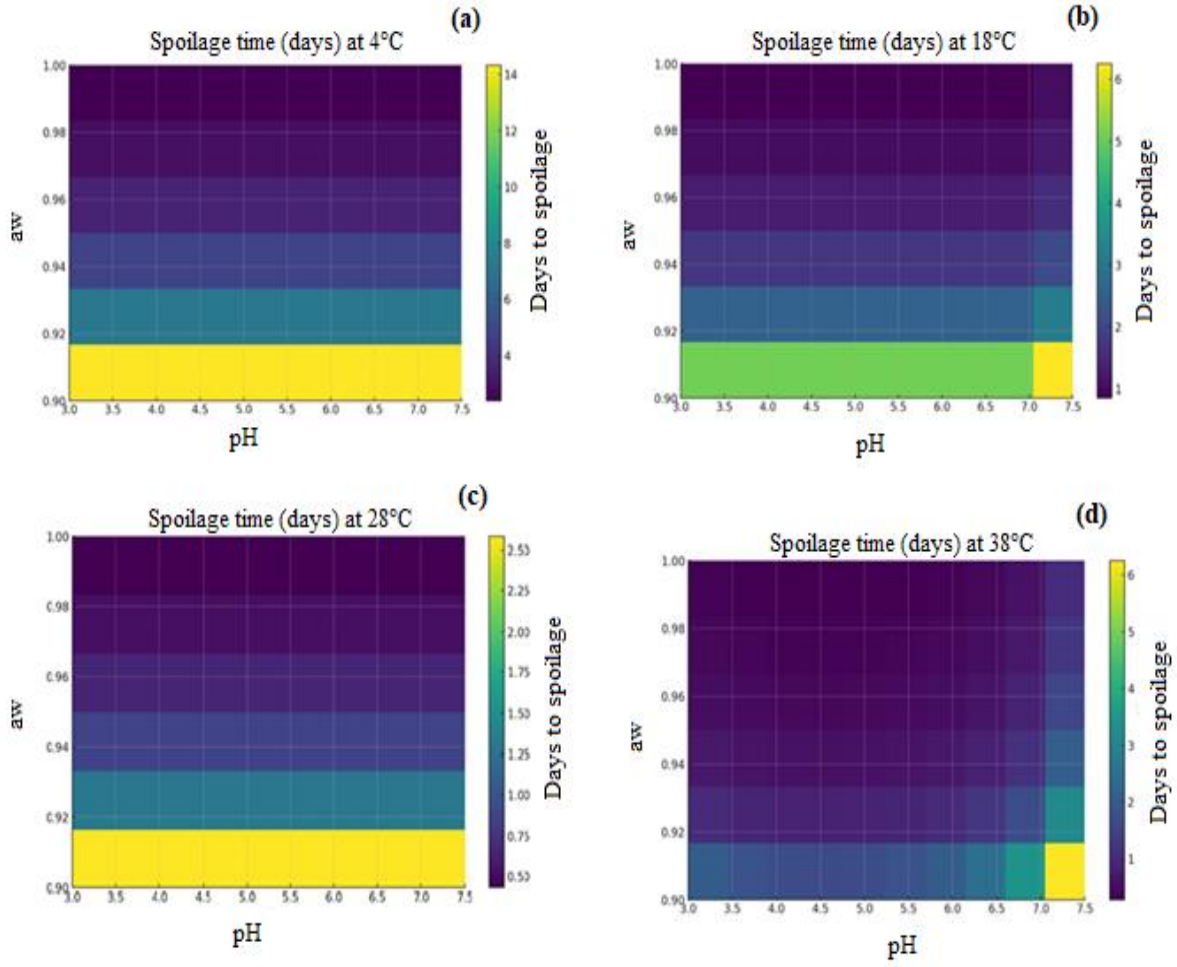

Figure 4: Spoilage time (days) of BSG in different temperatures, depending on aw and pH.

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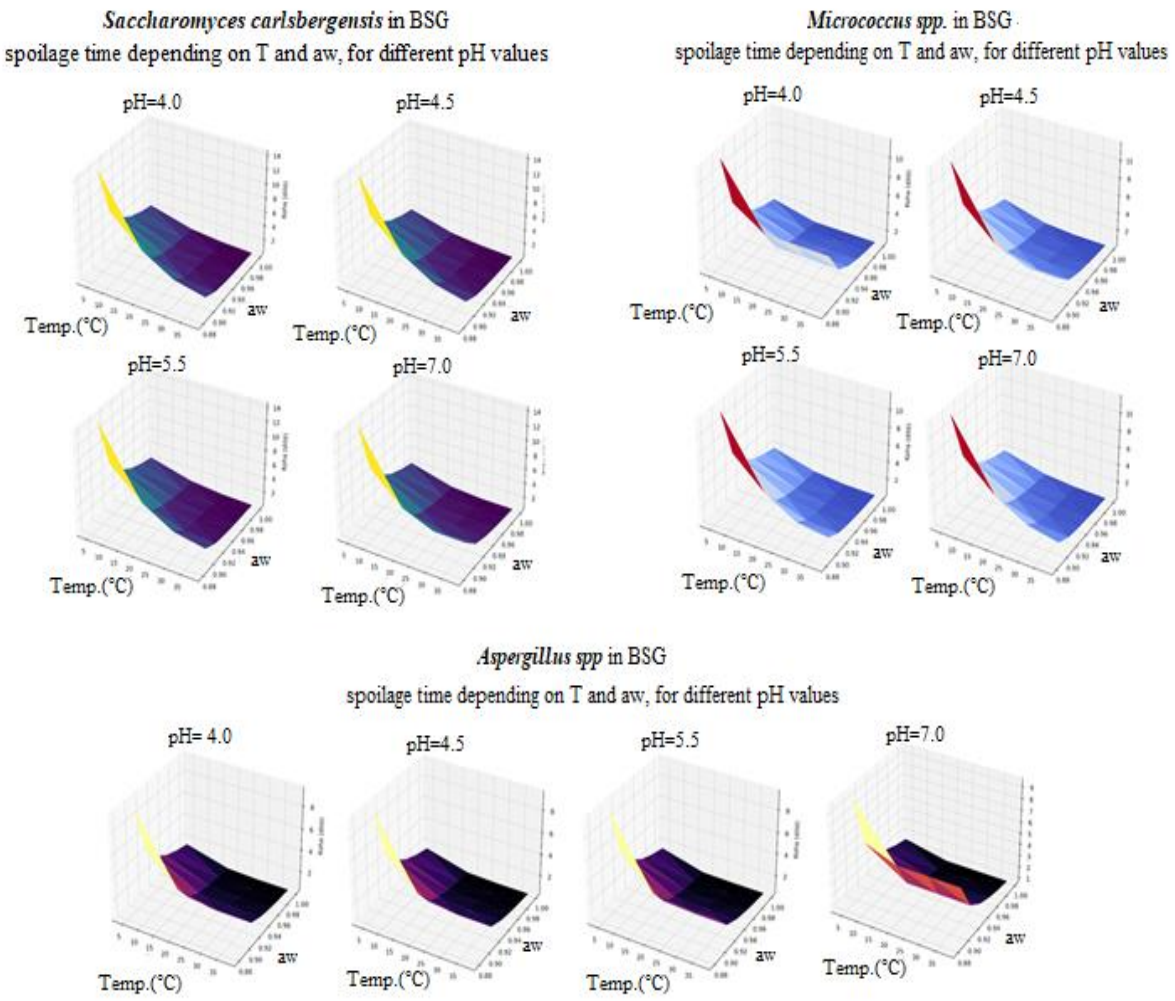

Figure 5: Spoilage time (days) depending on T and aw, for different pH values, of BSG in presence of different microorganisms, respectively *Saccharomyces carlsbergensis*, *Micrococcus spp.* and *Aspergillus spp.*

From the above mathematical analysis, it is noted that, in addition to temperature, pH and water activity have a significant impact on BSG shelf life. The effect of lowering pH on BSG shelf life was tested, as well as the effect of reducing water activity through the use of alginates.

It was tested microbiological stabilization process using different acids. The process begins with homogenization, followed by acidification to a pH of 4 or lower. Food grade organic acids are used to ensure microbiological stability and safety for human consumption. The impact of acid treatment on key parameters such as soluble protein and reducing sugars was also evaluated. Acid

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treatment, in addition to extending shelf life, increases fiber solubility, soluble protein content, and levels of reducing sugars.

Below you will find a summary of the key physicochemical parameters of BSG before treatment and after various treatments.

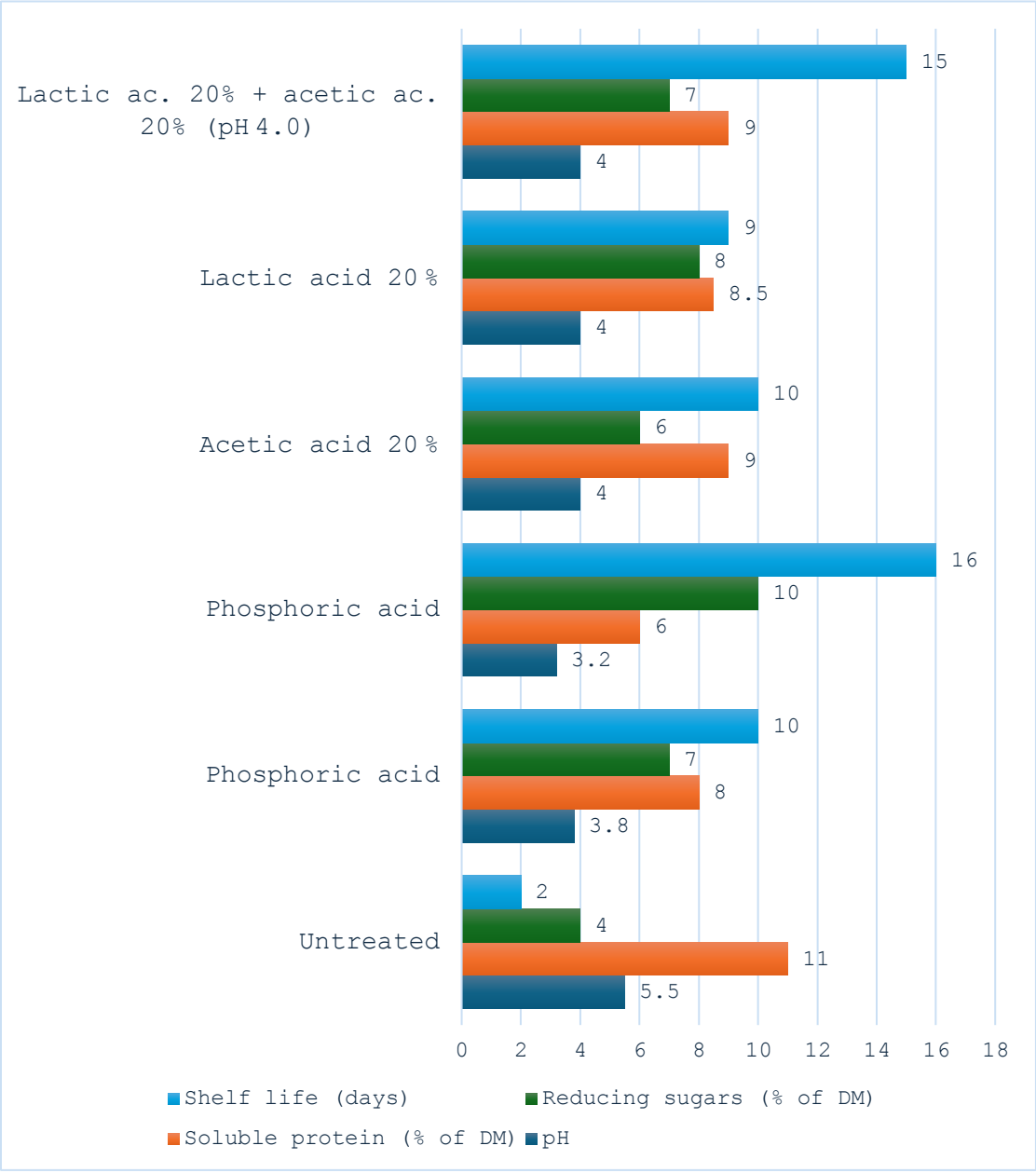

Figure 6: The impact of acid stabilization in the shelf life and chemical composition of BSG. (Storage condition: T = 18-20□C; Moisture = 80%).

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As we can see despite moisture content and water activity remaining unchanged by the acid dose alone, the lowered pH inhibits microbial growth. Soluble protein decreases due to acidification and protein aggregation near their isoelectric points. Reducing sugars increase as acid hydrolysis partially solubilizes hemicellulose and other polysaccharides. Shelf life is extended due to suppresses spoilage organisms.

Alginate coating of BSG. Treatment of brewer's spent grain (BSG) with alginates (applying a 1.5 - 2 % sodium alginate coating and cross linking with $CaCl_2$) has a significant impact on its shelf life.

Table 3: A comparative analysis of different parameters in untreated BSG and BSG treated with an alginate coating.

Parameter	Untreated	Alginate Coating
pH	5.5	5.5
Moisture content (%)	80	80
Water activity (a_w)	0.96	0.91
Soluble protein (% DM)	10	10
Reducing sugars (% DM)	4	4
Microbiological shelf life (days)	3	12

Figure 7: Same sample treated with alginate in three different concentrations.

The alginate forms a tight film around the BSG particles, reducing water vapor and oxygen exchange with the environment. This lowers the effective water activity at the surface, slowing microbial growth and oxidative reactions. Surface water activity (a_w) drops from about 0.96 to around 0.90 – 0.92, creating a less favorable environment for bacteria, molds, and yeasts, and

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delaying spoilage. Also the alginate film protects the fibers and structure of the BSG from mechanical damage during transport and storage, preserving sample integrity and preventing internal exposure to contaminants. When combined with an acid treatment (e.g. adjusting to pH 4), the physical barrier is reinforced by antimicrobial effects, further extending shelf life.

Using pressing technology before alginate treatment reduce further aw leading to a better shelf life. Pressing (mechanical dewatering) reduces moisture from ~78 % to ~60 % and water activity (aw) from 0.98 to 0.95-0.96. The process removes some free proteins and sugars, and extending shelf life to ~5 days without changing pH. Combined Treatments (Pressing + alginate) achieves longer shelf life extension, up to ~12 days.

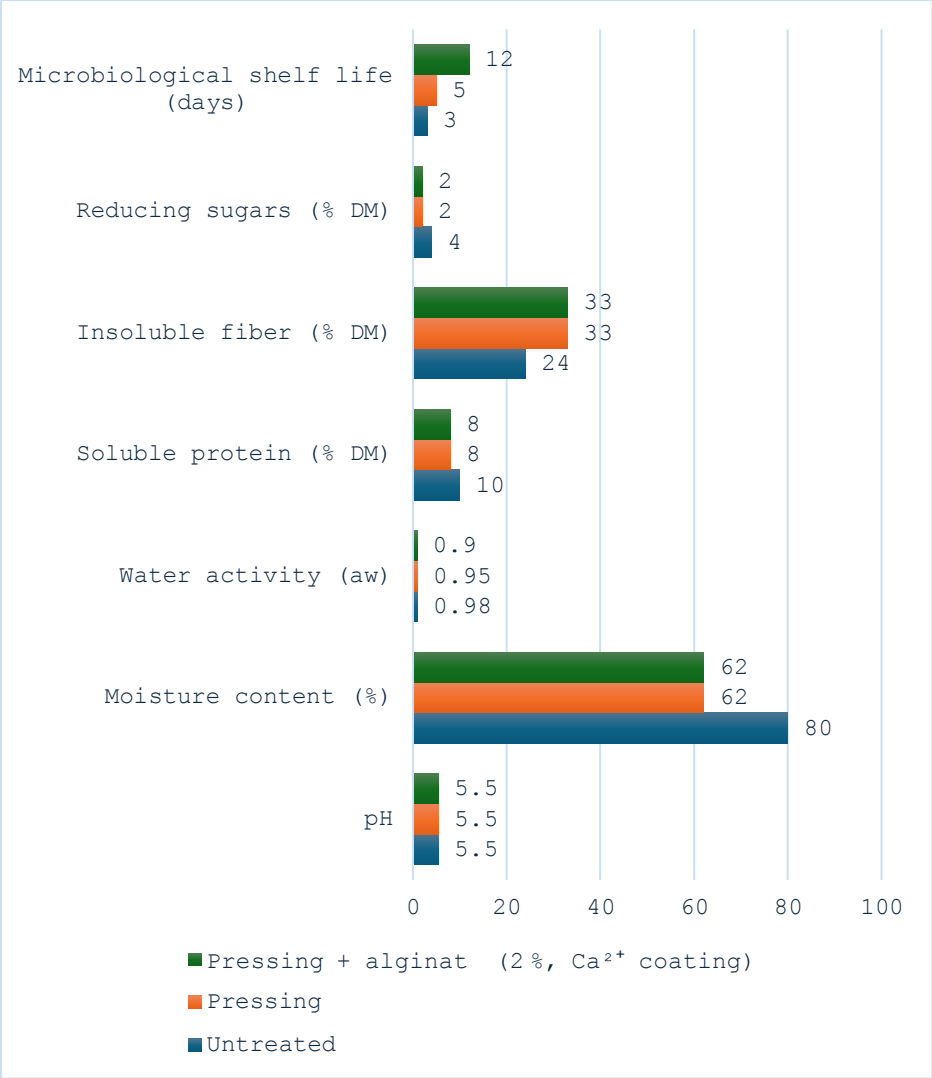

Figure 8: Dependence of shelf life to different treatment methods and combinations.

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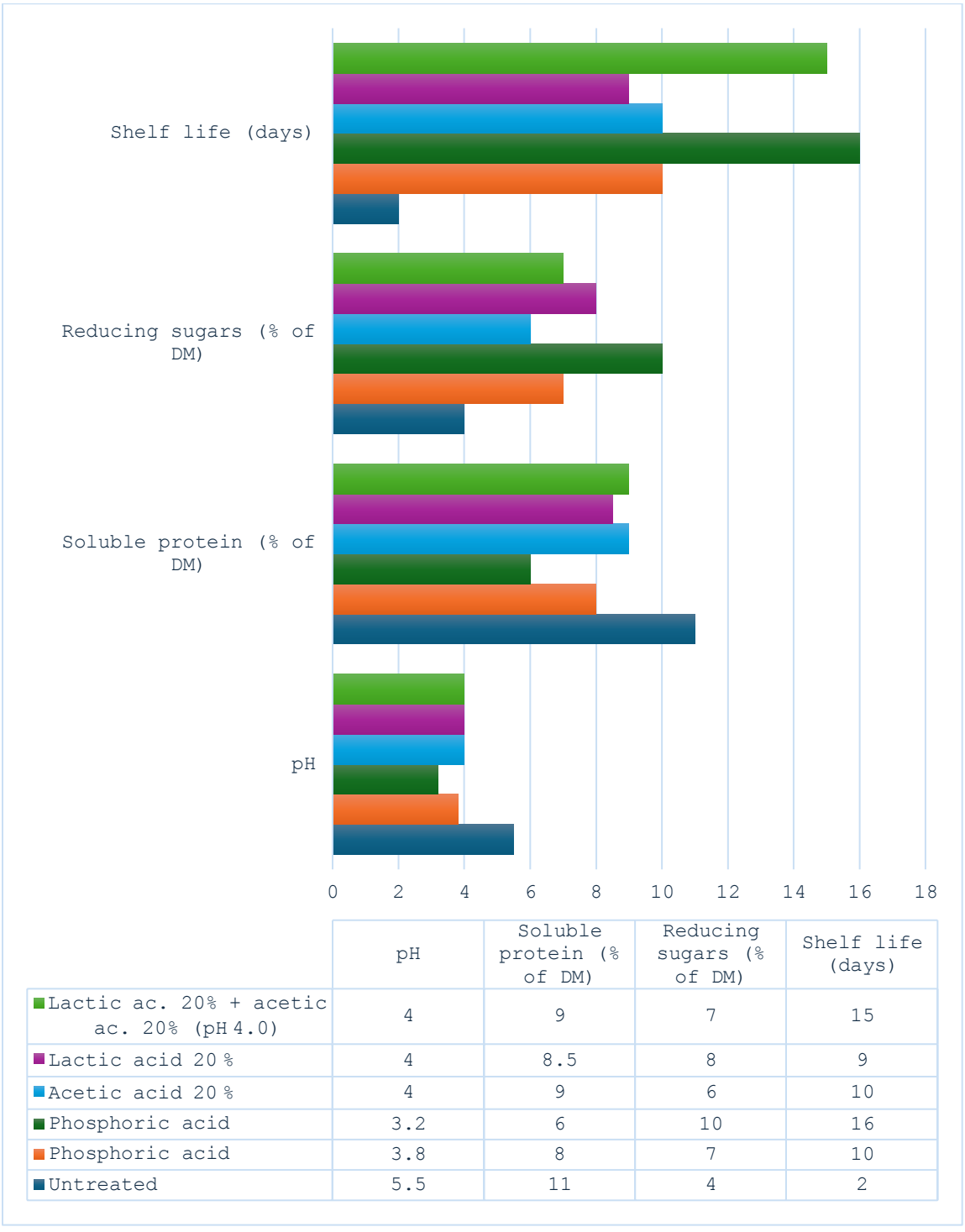

	pH	Soluble protein (% of DM)	Reducing sugars (% of DM)	Shelf life (days)
Lactic ac. 20% + acetic ac. 20% (pH 4.0)	4	9	7	15
Lactic acid 20 %	4	8.5	8	9
Acetic acid 20 %	4	9	6	10
Phosphoric acid	3.2	6	10	16
Phosphoric acid	3.8	8	7	10
Untreated	5.5	11	4	2

Figure 9: Comparative Analysis of Physicochemical Parameters in Untreated BSG, Pressed BSG, and Pressed BSG with Alginate Coating.

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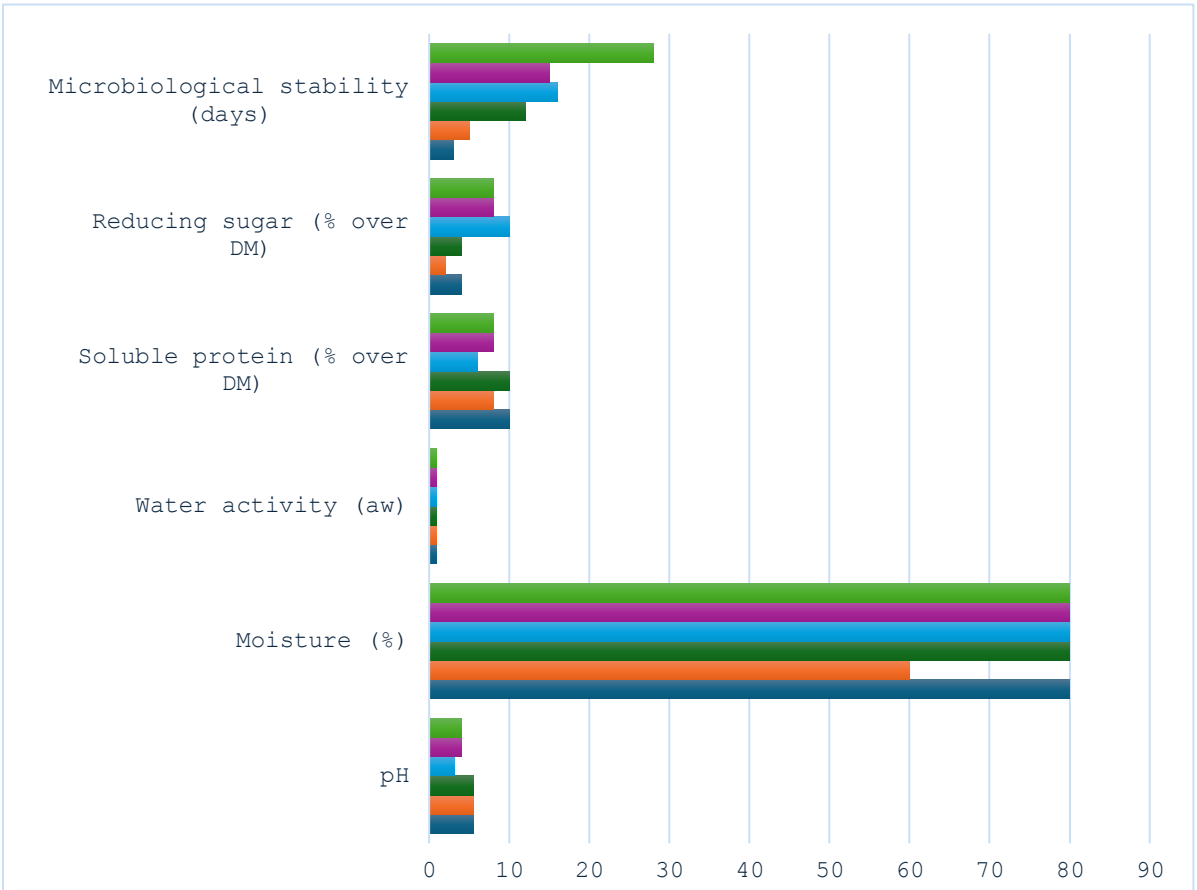
Based on the graph above, it can be observed that the use of alginate treatment alone may render pressing unnecessary. While pressing does not contribute significantly to shelf-life extension when combined with alginate, it results in a loss of proteins and soluble sugars.



	pH	Moisture (%)	Water activity (aw)	Soluble protein (% over DM)	Reducing sugar (% over DM)	Microbiological stability (days)
Alginat + Lactic acid (20%) +Acetic acid pH 4.0)	4	80	0.91	8	8	28
Lactic acid (20%) +Acetic acid (pH 4.0)	4	80	0.96	8	8	15
Phosphoric acid (pH 3.2)	3.2	80	0.98	6	10	16
Alginate (2%)	5.5	80	0.91	10	4	12
Pressing	5.5	60	0.96	8	2	5
Untreated	5.5	80	0.98	10	4	3

Figure 10: Acidification and alginate treatment.

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By combining acidification (low pH), alginate coating (reduced aw), and optional pressing, BSG's microbiological shelf life can be increased from 3 days to up to 28 days, The choice of treatment depends on balancing operational costs against the added value of the final product.

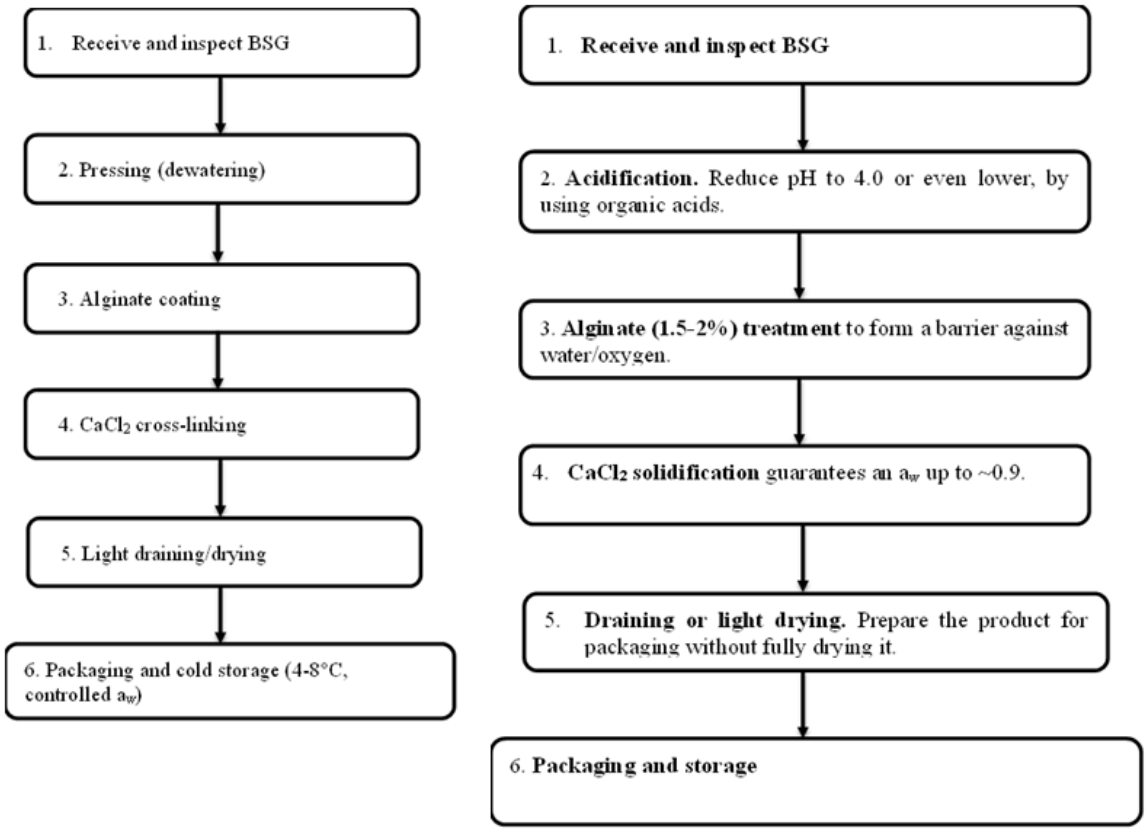

Figure 11: Proposed Process Flow chart diagram.

5. CONCLUSION

Whereas our earlier study (Drazho et al., 2025) established the microbial succession, spoilage kinetics, and predictive models of BSG shelf life, the present work demonstrates practical stabilization strategies to translate those theoretical insights into real preservation methods. The high moisture and nutrient content of BSG, combined with improper storage temperatures, significantly accelerate microbial growth and degradation. Acid treatment increases shelf life by minimizing and preventing aflatoxin formation without affecting the nutrients. Acidification provides microbiological stability for 20 days if stored at 20 °C. If stored for 1 week at 25 °C low undetectable levels of mycotoxins appear. Acid treatment at low pH, provides longer storage period. Temperatures above 18 °C significantly increase contamination. BSG acidification is an easy, low-cost, and effective method, suitable for increasing the shelf life of BSG up to 1 month,

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if properly stored. Water activity, pH and temperature act synergistically to control microbial and enzymatic degradation of BSG. By manipulating these three intrinsic factors together, the stability of BSG can be greatly extended compared to altering any single factor alone. The mathematical analysis shows that, aside from temperature, both pH and water activity critically determine the shelf life of brewer's spent grain. Two strategies were tested to extend stability. Acidifying the BSG (e.g. with phosphoric, acetic or lactic acids down to pH 3.2 – 4.0) significantly slowed microbial spoilage. Applying a calcium – crosslinked alginate film lowered surface aw from ~0.96 to ~0.91, creating a barrier to moisture and oxygen and further delaying spoilage. Together, these treatments, acidification to inhibit microbes and alginate coating to restrict water availability, synergistically prolong BSG's microbiological shelf life.

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