

#### Research Article

# High Strength Bio-Foams of Cassava Starch/Wheat Gluten Blends by Microwave Processing

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#### **Abstract**

Environmental issues have a high impact on the selection of materials for packaging. Advanced research in biodegradable materials has shown that starch-based foams could be an effective replacement for petroleumbased polystyrene foam. However, pure starch (ST) foam has some disadvantages, including low water resistance and poor mechanical properties. In this study, wheat gluten (WG) protein was added to starch-based foams, and the foam structure was formed using microwave processing to improve those properties. This was the first report on a novel bio-foam made from ST/WG blend utilizing a rapid and energy-efficient microwave foaming process that resulted in improved foam structure and high strength. The effect of foam blending ratios (ST100, ST95/WG5, ST90/WG10, ST80/WG20, and ST70/WG30 by weight) were studied. It was found that due to WG's nucleating effect, the cellular microstructure of the ST/WG blend foams was denser with smaller cell size and thicker cell walls than the pure ST foam. Mechanical properties of the foams compared by the flexural strength and modulus have shown that increasing the amount of WG significantly enhanced the foam's properties. Based on the findings of this study, ST90/WG10 exhibited a notably high flexural strength and modulus of 9.5 MPa and 412.8 MPa, respectively, which were more than 9 times stronger than expanded polystyrene foam. Furthermore, the addition of WG protein improved the water resistance of the blend foams. This study demonstrates that the new bio-foam based on ST and WG (as a blending component), which can be quickly produced by microwave heating, is a very promising alternative for high strength, good water resistance, and eco-friendly foam packaging applications.

Keywords: Bio-Foam, Blend, Cassava starch, Microwave processing, Wheat gluten

#### 1 Introduction

The most common foaming materials used nowadays are expanded polystyrene (PS) because of its economical price, rigidity, lightweight, water resistance, chemical stability, shock resistance, thermal insulation, and flexibility in molding. However, PS is not compostable or degradable, disposing of PS foam causes serious environmental problems [1], [2]. With increasing awareness of environmental protection and sustainability, many researchers have been focusing on the selective

biodegradable materials to produce bio-foam packaging. Starch (ST) is an important economic crop and a preferred raw material for bio-foam packaging development since it has excellent biodegradability and can be decomposed by microorganisms in the natural environment [3], [4]. Compared to traditional PS foams, however, pure starch-based foams have limitations in uses mainly due to their poor mechanical properties and hydrophilicity [4]–[7]. Various approaches have been investigated to overcome these drawbacks [8].



In recent decades, many researchers have attempted to improve ST foam's performance by adding or blending with several other components such as natural fibers, industrial waste materials, and natural protein polymers [9]–[11]. Natural fiber (e.g., malt bagasse, sugarcane bagasse, asparagus peel fiber and sesame cake) and industrial waste materials (e.g., fish scale, shells of egg, wastepaper pulp and shrimp) have caught more attention to incorporate in baked starch foams due to differences in nature of these materials were improved or affected foam's properties differently [12], [13]. Natural proteins are also an interesting economic choice for blends with ST foam. As previously reported [14], adding plant proteins such as sunflower, zein, gluten, and soy proteins can help in reducing the water sensitivity of the ST foam. Wheat gluten (WG) is a cheap byproduct natural protein from starch and bioethanol industries with large-scale availability and has the benefits of environmental friendliness. viscoelastic good properties, low water solubility, and good strength [15], [16]. WG has also been explored for various nonfood applications, including use in films, adhesives, binders, biomedical products, biodegradable plastics, and composite materials [17]-[20].

Bio-foams based on starch manufactured by various methods, including extrusion, oven baking, compression molding, solvent exchange, supercritical fluid, and microwave heating [8]. Microwave-assisted processing has emerged as an innovative technique to enhance the production of biobio-composites, offering advantages, including ensuring uniform heating, rapid volumetric heating, minimizing thermal degradation, enabling selective heating at the molecular level, and providing better structural control during foaming, as well as reducing processing time and highly energy efficient when compared to conventional conduction or convection heating [21]–[23]. Furthermore, prior studies have demonstrated that microwave processing can greatly improve the mechanical strength and thermal stability of starch-based foams [24]-[26]. To the best of our knowledge, no research has been conducted into the structure and properties of microwave-heated foam made from ST/WG blends.

Therefore, this work aimed to prepare the novel bio-foams based on ST/WG (cassava starch/wheat gluten) blends by microwave processing approach at different blending ratios of ST100, ST95/WG5, ST90/WG10ST80/WG20, and ST70/WG30 by weight. Then, the effect of WG blending content on the cellular microstructural, physicochemical, and

mechanical properties, as well as water resistance of the resulting blend foams, was investigated.

#### 2 Materials and Methods

#### 2.1 Materials

Cassava starch (food-grade quality) manufactured by Bangkok Inter Food Co., Ltd. (Baiyok, Jade Leaf<sup>TM</sup>, Thailand), was locally obtained and utilized as received. Guar gum (1 wt.% based on the dried weight of starch), used as a thickening agent, was purchased from Sigma-Aldrich Co. (USA). Controlled relative humidity (RH) environments were established using magnesium nitrate (52.9% RH) were sourced from Ajax Finechem Pty Ltd. (Australia) and sodium chloride (75.3% RH) from RCI Labscan Co. (Thailand). Wheat gluten (WG) was supplied by Zhang Jia Gang Heng Feng Starch Products Co., Ltd. (China).

# 2.2 Preparation of ST/WG foam using microwave heating

The procedure illustrated in Figure 1 was followed to prepare the microwave-heated foam samples. A kitchen mixer (OTTO, 250 W) was used to mix the ST/WG blends in various weight ratios (ST100, ST95/WG5. ST90/WG10, ST80/WG20, ST70/WG30) at a medium speed (400 rpm) for 5 minutes. Following that, each batter formulation was loaded into the ceramic mold's lower cavity, which had dimensions of 130 mm × 130 mm × 3 mm. The mold's upper plate was then closed and fastened with a cotton string. Thin polytetrafluoroethylene (PTFE) films were used as release films to make it easier to remove the foam sample later. To produce a foam sample, the mold was placed in the centre rotating plate of a microwave (Toshiba ER-B7TC), and heated for 5-8 minutes at a maximum power of 600 watts. After allowing the mold to cool for about 10 minutes, the foam sample was gently taken out of the mold and placed inside a zip-lock plastic bag made of polyethylene.

#### 2.3 Foam characterization

#### 2.3.1 Color parameter

Color analysis of the foam samples was conducted using a CIE range spectrophotometer (UltraScan® VIS, HunterLab, Virginia, USA). Three foam



specimens from each formulation were evaluated under daylight conditions based on the CIE Lab color space, reporting  $L^*$ ,  $a^*$ , and  $b^*$  values. The  $L^*$  parameter represents lightness (ranging from 0 for black to 100 for white), while  $a^*$  denotes the red-green axis (+60 indicating red and -60 indicating green), and  $b^*$  corresponds to the yellow-blue axis (+60 for yellow

and -60 for blue). The overall color difference ( $\Delta E$ ) between samples was determined using Equation (1).

$$\Delta E = \int (L - L^*)^2 + (a - a^*)^2 + (b - b^*)^{1/2}$$
 (1)

where  $L^*$ ,  $a^*$ , and  $b^*$  values were the averaged color values of the pure starch foam.

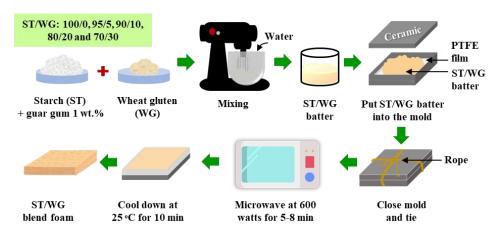


Figure 1: A schematic diagram shows the steps in preparation of starch (ST)/wheat gluten (WG) blend foams using microwave processing.

#### 2.3.2 Scanning electron microscopy (SEM)

The microstructural properties of foams from each formulation were examined using a scanning electron microscope (Leo 1450 VP, Germany) set to an accelerating voltage of 10 kV. To view their cross-sections, each foam sample was meticulously cut and affixed to aluminum stubs with carbon double-sided adhesive tape. Before observation, all specimen surfaces were coated with a thin layer of gold through sputtering. The average cell size and wall thickness of the foam samples were directly measured from the SEM images using ImageJ 1.6.0 software (Image Processing & Analysis in Java).

#### 2.3.3 Density and thickness

Three 20 mm × 20 mm rectangular specimens were cut from the starch foam sheets using a rotating saw. The specimens were dried for two hours at 105 °C in the hot-air oven. Following that, all specimens were allowed to cool for an additional hour at room temperature in a desiccator. The mass of each sample was measured using a four-digit analytical balance. Subsequently, a digital vernier caliper was employed to accurately measure the thickness and width of each specimen, allowing for the calculation of foam volume

based on its dimensions. The density of each foam was then determined by calculating the ratio of its weight (g) to its volume (cm<sup>3</sup>).

#### 2.3.4 Mechanical properties

The mechanical characteristics of the foams were assessed through a three-point bending test conducted on a universal testing machine (UTM, Instron 4201, US) fitted with a 1 kN load cell. This flexural test was carried out at a crosshead speed of 2 mm/min, following the guidelines of ASTM D790. Foam strips, measuring 100 mm in length and 20 mm in width, were cut and then conditioned in a controlled environment with 52.9% relative humidity at 25 °C for four days prior to testing. The findings were presented as the average of five measurements for each foam type.

# 2.3.5 X-ray diffraction (XRD)

The samples were analyzed with an X'pertPro MPD diffractometer (X'Pert Pro MPD, UK) using copper  $K\alpha$  radiation at 40 kV and 30 mA. The X-ray diffraction analysis was performed in reflection mode, scanning over a 2 $\theta$  angular range of 5° to 45°, with a step size between 0.01° and 0.03°, and a dwell time of



5 to 10 seconds per step. Before the test, the foam samples were dried at 105 °C for 2 h in a hot air oven, then ground with a mortar and pestle to powder, and stored in a chamber with the controlled RH of 52.9% (using the saturated solution of magnesium nitrate (Mg (NO<sub>3</sub>)<sub>2</sub>; ASTM E104) for 4 days.

## 2.3.6 Fourier transform-infrared spectroscopy

FT-IR analysis was conducted using a Perkin Elmer spectrophotometer (Spectrum GX, USA). Before analysis, the foam samples were ground, dried, mixed with potassium bromide (KBr), and pressed into pellets. The measurements were performed in absorption mode over the spectral range of 4000–500 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>.

# 2.3.7 Moisture absorption

To observe the moisture absorption of the foam samples at 75% relative humidity (RH), square specimens (approximately  $20 \times 20$  mm) were first dried in an oven at 105 °C for 2 h. After drying, they were cooled to 25 °C in a desiccator before recording the initial weight (Weight i). The specimens were then placed in a conditioning chamber containing a saturated sodium chloride solution, maintaining a controlled RH of  $75.3 \pm 0.4\%$  at 25 °C in accordance with ASTM E104. Over a period of 3 days, the samples were weighed periodically (Weight t), with measurement intervals ranging from 15 minutes to 12 hours. All measurements were conducted in triplicate. Moisture absorption (%) was calculated using Equation (2).

% Moisture absorption = 
$$\frac{Weight_t - Weight_i}{Weight_i} \times 100$$
 (2)

#### 2.3.8 Water absorption capacity

Foam square samples (approximately 20 × 20 mm) were measured using a four-digit analytical balance and then fully immersed in distilled water maintained at 25 °C for 1, 15, and 30 min. After immersion, samples were removed, blotted with tissue paper to eliminate surface water, and reweighed. Water absorption was determined by calculating the weight gain and expressed as grams of water absorbed per 100 grams of the initial dry sample (g water/100 g solid). Each reported value represents the average of five independently tested specimens per formulation.

#### 3 Results and Discussion

#### 3.1 Color Parameter

The color characteristics for the foams prepared in this study are shown in Table 1 as luminosity  $(L^*)$ , redness  $(a^*)$ , yellowness  $(b^*)$ , and overall color difference  $(\Delta E)$ . It was found that all starch-based foams showed a high level of lightness  $(L^*)$ , a low level of redness  $(a^*)$ , and an increasing level of yellowness  $(b^*)$ , as well as total color difference ( $\Delta E$ ) with the addition of WG. It was evident that the integration of the darkyellow WG powder into the white ST foam matrix was the cause of the rising yellowness and overall color difference of the ST/WG blend foams. A significant alteration in color became apparent when the weight ratio of WG exceeded 5 percent. However, it should be noted that  $\Delta E$  of the blend foams after the ratio of ST90/WG10 began to decline, indicating a more color uniform surface in these foam samples.

**Table 1:** Colors parameter of starch (ST) based foam blended with different amounts of wheat gluten (WG).

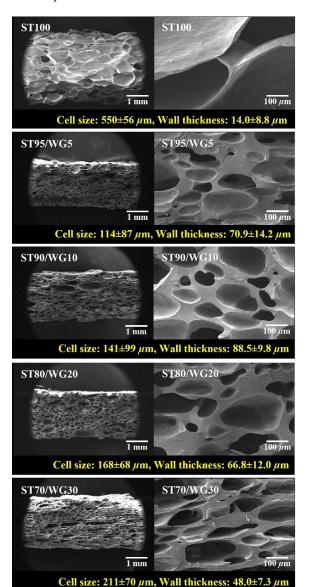
Sample	L*	a*	<i>b</i> *	<u>ΔΕ</u>
ST100	69.32	-0.08	2.62	0.00
	(2.20)	(0.04)	(0.58)	(0.00)
ST95/WG	74.41	0.21	7.03	6.80
5	(1.15)	(0.18)	(0.62)	(0.87)
ST90/WG	79.07	0.55	12.51	13.90
10	(1.94)	(0.16)	(0.95)	(1.84)
ST80/WG	74.41	0.49	13.94	12.45
20	(1.00)	(0.17)	(0.87)	(0.91)
ST70/WG	69.94	0.21	13.80	11.47
30	(2.61)	(0.28)	(1.44)	(1.12)

# 3.2 Morphology characterization

Figure 2 displays representative SEM micrographs that reveal the cellular structure morphologies of the pure ST and ST/WG blend foams. Pure ST (ST100) foam (Figure 2) showed large cells with thin cell walls after the microwave expansion. For blending conditions, the ST/WG foams (ST95/WG5, ST90/WG10, ST80/WG20 and ST70/WG30) clearly showed pores with smaller sizes and thicker cell walls than the pure ST foam sample. These observations can be explained by an increase in the batter's viscosity of the ST/WG blends, leading to less expandable gelatinized starch dough during microwave heating. Besides, a nucleating effect of WG powders was indeed expected to create denser foams with more cells in smaller sizes inside their structures [15], [27]. However, at the highest WG ratio of ST70/WG30, it was noticed that the shape of foam pore cells became



more elongated in the horizontal direction with smaller sizes and thinner cell walls, hence, being more anisotropic and less uniform cellular structure.

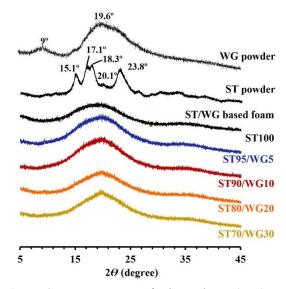


**Figure 2:** Cross-sections observed by SEM of pure starch foam and starch/wheat gluten blend foams at different ratios.

#### 3.3 XRD Analysis

Figure 3 presents the XRD patterns of wheat gluten (WG) powder, starch (ST) powder, pure ST foam (ST100), and ST/WG blend foams with compositions of ST95/WG5, ST90/WG10, ST80/WG20, and

ST70/WG30. The WG sample exhibits broad diffraction peaks near 9° and 19.6°, which are characteristic of the  $\alpha$ -helix and  $\beta$ -sheet structures commonly found in wheat gluten proteins, respectively [28]. The XRD pattern of ST shows the peaks at 15°, 17°, 18°, 20°, and 23°, which are in agreement with the literature on the semi-crystalline structure of common cassava starch granules [29]. After the foaming process, the XRD pattern of ST foam indicated that the crystal structure of starch granules was mostly destroyed, turning them into an amorphous foam material. It was implied that the starch granules were completely gelatinized and ruptured during the microwave heating and foamforming process [26]. For the conditions of ST/WG blend foams (ST95/WG5, ST90/WG10, ST80/WG20 and ST70/WG30), the increasing amount of WG ratio from 5–30 wt.% led to a slightly sharper peak intensity at 19.6°. This confirmed that a β-sheet structure of WG was increased in these blend foams.



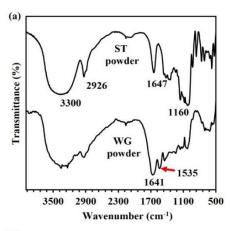
**Figure 3**: XRD patterns of wheat gluten (WG) and starch (ST) raw materials and starch-based foam blended with different amounts of wheat gluten.

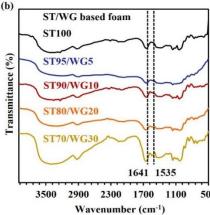
#### 3.4 FTIR analysis

The FTIR spectra of ST and WG powders are shown in Figure 4(a). The characteristic peaks at 3300 cm<sup>-1</sup>, 2926 cm<sup>-1</sup>, 1647 cm<sup>-1</sup> and 1160 cm<sup>-1</sup> attributed to the O-H stretching, the CH<sub>2</sub> deformation, O-H bending and C-O stretching [6] of ST molecules, respectively. For WG spectra, the C=O (amide I) and N-H (amide II) presented at the peak positions of 1655 cm<sup>-1</sup> and



1536 cm<sup>-1</sup>, respectively [30]. Following the microwave processing, the FTIR spectra of the blend foams exhibited the distinctive peaks of both the ST and WG components. It was noticeable that when the WG ratio in the foams increased, so did the peak intensity of amide at 1655 and 1536 cm<sup>-1</sup>. The high peak intensity of C=O (amide I) and N-H (amide II) in the blend foam at ST70/WG30 ratio was attributed to an increase in the content of the  $\beta$ -sheet structure of WG protein aggregation or crosslinking in this foam sample [31].





**Figure 4**: FTIR spectra of (a) starch (ST) and wheat gluten (WG) raw materials and (b) starch-based foam blended with different amounts of wheat gluten.

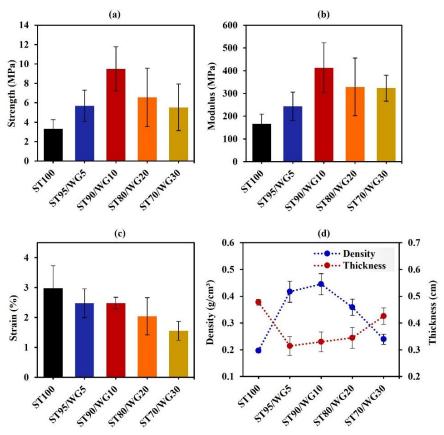
#### 3.5 Mechanical properties

The flexural strength, flexural strain and modulus of pure ST foam (ST100) were averaged at 3.3 MPa, 2.9 % and 166 MPa, respectively (Figure 5(a)–(c)). When

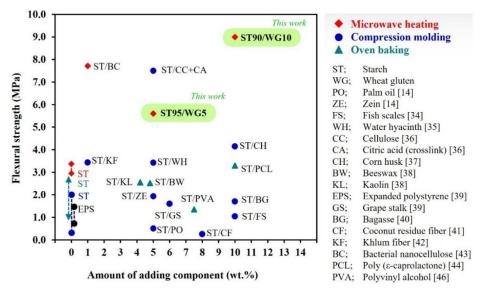
WG was blended in at contents of 5 to 30 wt.%, the flexural strength and modulus of all blend foams were noticeably improved, while their flexural strain slightly decreased as compared to those of pure ST foam. The ST/WG foam at WG blending content of 10 wt.% (ST90/WG10) exhibited the highest flexural strength and modulus of 9.5 MPa and 413 MPa, respectively. This ST90/WG10 foam also had the highest density (Figure 5(d)) with more cells and the thickest cell wall (Figure 5(e)) as confirmed by SEM results, which led to an almost 3-fold increase in its flexural strength. Undoubtedly, an increase in foam density usually leads to stiffer and higher load-bearing foam materials [32]. In addition, between the two macromolecules, WG can create intermolecular interactions with ST molecules as well as a crosslinked network of WG disulfide bonds itself, both of which can strengthen the blend foam's structure at the molecular level [28].

However, the loading of WG over 10 wt.% did not further improve the flexural properties of the ST/WG blend foams. At high WG contents (ST80/WG20 and ST70/WG30), the density of foam as well as its uniformity were decreased. With more protein (WG) in the system, phase separation between WG and ST may occur, followed by inhomogeneous foam expansion, resulting in a less dense and weaker foam structure [33]. Moreover, the higher crosslinking of WG molecules might lead to the formation of more protein aggregates [34], separated from the ST molecular network, and thus lessen the reinforcing effect on the blend foams. Evidently, starch-based foams can be made using a variety of processing techniques, including microwave heating. compression molding, and oven baking. mechanical properties of these foams are clearly influenced by processing techniques and parameters. The mechanical properties of these foams are clearly influenced by processing techniques and parameters [32]. According to Figure 6, the flexural strength of pure ST foams produced by compression molding and oven baking is comparable to or better than that of expanded polystyrene foam (EPS). Compression molding or baking techniques may be especially useful for applications that require well-defined shape foam products, such as disposable foam bowls, dishes, and trays. These foaming techniques allow foam to expand within a tightly sealed mold, resulting in the desired product shape [14].





**Figure 5**: Flexural strength (a), flexural modulus (b), flexural strain (c) and foam's density and thickness (d) of ST and ST/WG foam samples.



**Figure 6**: Flexural strength of ST/WG foams in this work compared with prior starch-based foams in the literature and conventional expanded polystyrene (EPS) foam.



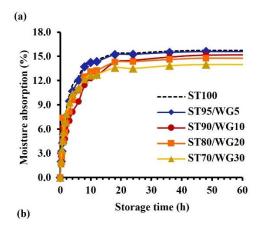
Typically, the disposal foam containers require moderate flexural strength, ranging from 0.1 to 5 MPa. However, compression molding often involves preheating and long pressing cycles at elevated temperatures (120-150 °C for 5-10 min), which results in high energy consumption and longer processing times. Similarly, oven baking, while effective for basic foam expansion, suffers from nonuniform heating, limited structural control, and extended processing durations (typically over 30 minutes). These limitations may hinder large-scale, energy-efficient production. In contrast, microwave processing enables rapid volumetric heating by directly interacting with polar molecules in the foam matrix, allowing uniform heat distribution and expansion within just 2–5 min [34]–[46]. In this study, pure ST foams prepared with microwave heating, on the other hand, have a higher flexural strength than foams prepared using other foaming techniques. The results from this study confirmed that starch-based foams produced by microwave processing can have a dense and more uniform cellular structure, as well as a thicker cell wall, making them suitable for applications requiring high strength and rigidity.

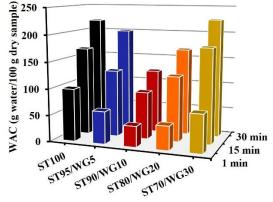
Moreover, microwave processing is very energy efficient, offers lower operational costs, and supports industrial scalability. Although the initial cost of industrial microwave systems may be higher, the benefits of shorter cycle times and improved energy efficiency indicate a strong promise for sustainable, large-scale manufacturing.

Figure 6 compares the flexural strength of starchbased foams blended or composited with various components from prior studies and the current ST/WG foams. When other components are combined or added to starch foams, their strength appears to increase to some extent. Only the composite foam of citric acid-crosslinked starch/cellulose (ST/CC+CA) [36] and the starch foam reinforced with bacterial nanocellulose (ST/BC) [43] have superior strength compared to other foams. This suggested that both macromolecule's crosslinking network and nanosized reinforcing agent are particularly effective at increasing the strength of starch-based foams. Still, our ST90/WG10 blend foam had the highest flexural strength among all previously reported starch-based foams. These remarkable improvements can be attributed to the superior cellular structure achieved with the microwave foaming approach, as well as WG's nucleating effect and increased intermolecular interactions between WG and ST macromolecules.

#### 3.6 Moisture and water absorption behavior

The moisture absorption of ST/WG foams is shown in Figure 7(a). At a controlled relative humidity of 75%, the moisture content in the blend foams was increased and then remained constant after approximately 24 h of storage conditioning. Initially, the foams' moisture uptake increased rapidly within the first 10 h, and then the absorption rate slowed down until reaching the plateaus.





**Figure 7:** Moisture absorption (a) and water absorption capacity (WAC) (b) of pure starch and starch-based foams with different loading amounts of wheat gluten.

The water absorption of the ST/WG blend foams was gradually decreased with increasing the portion of WG. It was undoubtedly attributable to the reduction in the ST portion, hence, fewer hydroxyl groups available to attract water molecules. WG proteins are more hydrophobic in nature and less water sensitive than polysaccharides like starch [9], [45]. For water



absorption capacity (WAC) results, it was observed that the addition of WG noticeably decreased the WAC of ST foam materials compared to pure ST foam (see Figure 7(b)). These trends are consistent with the foam's reduced moisture absorption behavior. Nonetheless, after the ratio of ST90/WG10, the WAC of the blend foams at ST80/WG20 and ST70/WG30 was increased. Perhaps it was owing to changes in the foam microstructure and the molecular interaction between WG and ST, as previously discussed. At high WG, adding contents, excess WG can form proteinrich clusters or aggregates that disrupt the continuous foam matrix, introducing structural discontinuities that facilitate moisture absorption. SEM images (Figure 2) revealed such morphological irregularities, including pore collapse and uneven cell walls. These protein aggregates have low miscibility with starch molecules, which prevents effective bonding between WG and ST chains, resulting in a less cohesive matrix [34]. As a consequence, a larger number of free hydroxyl (-OH) groups from unbound starch chains remain exposed, increasing the foam material's affinity for water and thereby contributing to the higher moisture and water absorption observed at ST80/WG20 and ST70/WG30 ratios.

#### 4 Conclusions

In this study, pure ST (ST100) foam and ST/WG blend foams (ST95/WG5, ST90/WG10, ST80/WG20, and ST70/WG30) were prepared by microwave processing. WG was added to the ST foams in an attempt to improve the properties of the bio-based foam material. The addition of dark yellow WG powder causes the ST foam color to shift slightly from white towards yellow shading. XRD confirmed that the semi-crystalline cassava starch granules were completely gelatinized during the microwave heating and foaming process, resulting in an amorphous foam upon drying. FTIR indicated characteristic peaks of ST and WG components in the blend foams. SEM images revealed that adding WG reduced cell size while increasing cell wall thickness in the foam formation. The ST90/WG10 foam had the highest flexural strength (9.5 MPa) and modulus (413 MPa), thanks to its dense and more homogenous cellular structure achieved with the microwave foaming as well as WG's nucleating effect and enhanced intermolecular interactions of WG and ST

molecules. The current blended foams demonstrated superior strength as well as reduced moisture sensitivity and water absorption, indicating great promise for use in high strength, eco-friendly foam packaging applications.

Furthermore, the microwave-assisted foaming process has advantages such as rapid, uniform, and energy-efficient heating, which results in better foam structure and strength, making it an appealing option for sustainable starch-based foam manufacture. However, achieving uniform heating in large-scale systems may be one of the industrial scalability issues. This issue could be addressed with equipment modifications, such as an advanced rotation mechanism for multiple molds within the microwave chamber. Future research on improving impact resistance, compression performance, and thermal behavior in more application-specific contexts, as well as surface coating for enhanced moisture and gas barrier properties, which are essential for high performance food packaging, should be conducted in order to advance the use of this foam material.

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### **Author Contributions**

S.K.: Methodology, Data curation, Formal analysis, Writing original manuscript. P.R.: Methodology; S.D.: Support writing original manuscript; U.I.: Validation; C.T.: Investigation, Writing-review& editing original manuscript; N.S.: Investigation, Conceptualization, Formal analysis, Writing-review & editing original manuscript, Supervision, Funding acquisition.

#### **Conflicts of Interest**

The authors declare no conflict of interest.



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