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Edible Paper Sheets from *Alternanthera philoxeroides* and *Hypophthalmichthys molitrix*: Smart Biomass Valorization

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Abstract: *Alternanthera philoxeroides* and *Hypophthalmichthys molitrix* offer significant nutritional benefits. This study evaluates the proximate composition, amino acid profile, GC-MS analysis, FT-IR spectroscopy, SEM and EDX, and color values of edible paper sheets (EPSs) derived from *Alternanthera philoxeroides* incorporating different levels of *Hypophthalmichthys molitrix* flesh. The protein content in the EPSs varied based on fish flesh incorporation, peaking at 52.66% in Ap100/Hm300 (Non-boil). Protein and carbohydrate contents showed an inverse correlation across EPSs, with the highest carbohydrate content of 60.89% in sample Ap400/Hm0 (Boil). Lipid content was also found to correlate with *H. molitrix* flesh content in EPSs, ranging from 1.59% to 18.41%. Amino acid analysis identified 11 types, with methionine as the most prevalent, followed by leucine, phenylalanine, and lysine. GC-MS analysis revealed 51 bioactive compounds, including carbonic acid, hentriacontane, and various fatty acids. FT-IR analysis showed characteristic bonds, while color analysis displayed L* values ranging from 24.37 to 30.97. SEM analyses depicted the microstructure, surface view, and elemental composition of the EPSs, and EDX showed an abundance of Ca, N, K, O, C, Mg, Na, P, Cl, Mn, and Fe. Therefore, EPSs prepared from *A. philoxeroides* and *H. molitrix* could offer a promising approach for effectively utilizing aquatic biomass and providing both plant and animal nutrients to consumers.

Keywords: aquatic weeds; edible paper sheets; silver carp; physico-chemical properties; nutritional composition

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1. Introduction

Aquatic resources including plants and animals represent a bountiful gift to humanity, offering essential sustenance and nutrition vital for the growth and protection of the human body. Bangladesh, despite its small size, boasts abundant rivers, beels, haors, lakes, baors, and floodplains, constituting one of the world's largest inland fisheries [1] and hosting diverse aquatic flora. These aquatic habitats serve as crucial sources of food and medicine for rural communities. Aquatic weeds, renowned for their renewable biomass, offer a rich array of nutrients such as protein, lipids, vitamins, minerals, polysaccharides, amino acids, polyphenols, and other bioactive compounds [2] while being low in fat, calories, and fiber content. In regions such as China, Japan, Korea, and Southeast Asia, various edible paper sheets (EPSs) are crafted from seaweeds, known commercially as nori in Japan and China, gim in Korea, and gamet in the Philippines. These are popular in Asian countries such as China, Japan, and Korea due to their nutritional richness and health benefits, often used plain or seasoned for soups and as wrappers for rice balls. An abundant reservoir of compounds that promote health, exhibiting functional properties such as antioxidative,

antihypertensive, antihyperlipidemic, and anticancer effects, nori is considered an excellent contender as a food ingredient to enhance overall well-being [3].

A. philoxeroides, commonly known as alligator weed, is traditionally used to treat various ailments such as night blindness. The stems and leaves of the plant are decocted and consumed on an empty stomach, believed to alleviate vomiting when ingested with salt [4,5]. It has been historically employed to address a range of health issues including night blindness, hazy vision, post-natal complaints, malaria, diarrhea, dysentery, and puerperal fever. Given the absence of commercial seaweed harvesting and cultivation in Bangladesh, efforts have been made to produce edible paper sheets (EPSs) from freshwater aquatic weeds [6] that demonstrate nutritional and technological viability. Phenolics and flavonoids in plants are associated with free radical scavenging activities [7]. However, EPSs derived solely from aquatic plants lack animal protein and essential amino acids crucial for optimal health. Animal protein typically contains all essential amino acids in proportions closer to human requirements compared to most plant proteins [8]. Consequently, this study proposes the incorporation of fish flesh into the EPS formulation sourced from aquatic weed. To the best of our knowledge, no previous studies have been found on enriching the protein content in EPSs prepared from aquatic weeds. Therefore, this research focuses on a novel approach of incorporating fish flesh with aquatic weeds for the formulation of EPSs. Fish protein contains essential amino acids in the right proportions that are easily digestible and bioavailable [9]; additionally, it is a very available and cheap source of animal protein. EPSs prepared from aquatic weeds, incorporating fish muscles, hold importance in cuisine for various dish preparations, such as rice rolls, dish toppings, and wrappings for non-traditional fillings. Additionally, they serve as health supplements and can be incorporated into various snacks as flavorants and nutritional supplements [10].

Hypophthalmichthys molitrix, commonly known as silver carp, is a widely recognized and affordable fish species in Bangladesh. It is extensively cultivated in freshwater aquaculture and often referred to as Chinese carp. Silver carp is esteemed for its richness in protein and fat content [11]. Its appealing white flesh and high nutritional value make it a preferred choice for the production of ready-to-eat and premium-quality fish products suitable for human consumption. Rich in essential polyunsaturated fatty acids, silver carp offers significant health benefits, boasting high levels of protein, essential fatty acids, and necessary amino acids, thus serving as a valuable source of nutrients for human consumption [12]. Despite its nutritional value, some individuals, particularly young consumers, may exhibit reluctance to consume silver carp due to taste preferences, concerns about bones, and texture. By adopting preservation, processing, and product development techniques for affordable fish varieties such as silver carp, a consistent year-round supply to consumers can be ensured, while also generating employment opportunities and enhancing income within the fisheries sector [13]. In this context, the production of edible aquatic weed paper sheets incorporating fish flesh emerges as an economical, straightforward, and safe method for fish flesh consumption and storage. Therefore, incorporation of *H. molitrix* flesh for the preparation of EPSs could provide balanced nutritional supplements to consumers. Therefore, the present study aimed to assess the feasibility of preparing EPSs of *A. philoxeroides* incorporating *H. molitrix* flesh, evaluating their physico-chemical and nutritional properties such as proximate composition, GC-MS, amino acids, SEM, EDX, FT-IR, and color analysis to reveal the potential of EPSs as a sustainable and nutritious food candidate.

2. Materials and Methods

2.1. Sample Collection and Preparation of EPSs

A. philoxeroides was collected from Jashore district, while *H. molitrix* was purchased from Churamankathi bazar, Jashore, in Bangladesh. Two types of paper sheets were prepared: one boiled and the other non-boiled. Initially, the *A. philoxeroides* was thoroughly cleaned with water. The *H. molitrix* was descaled, cleaned, and filleted with a knife. The flesh was then washed with water and boiled for 20 min. After boiling, the fish flesh was cooled and small bones were manually separated. The *A. philoxeroides* was ground properly

using an electric blender (model: WBL-13EC25N, Walton, Dhaka, Bangladesh) after adding water at a ratio of 2:1 (*w/v*) (*A. philoxeroides*:water). The *H. molitrix* paste was added to the *A. philoxeroides* in different proportions and treated with both boiling and non-boiling methods as per the experimental design outlined in Table 1. The mixture was blended again and settled in a cheesecloth-covered tray, then placed in a hot air oven (model: BOV-T270C, Biobase, Jinan, China) at 65 °C for 12 h. After drying, the thin EPS was carefully separated from the cloth. All dried paper sheets were ground separately using an electrical crusher, and the resulting powders were stored in different airtight plastic bags within a plastic container at room temperature for further analysis.

Table 1. Experimental design used in the present study.

Sample Tags	*A. philoxeroides* (g)	*H. molitrix* (g)	Normal Salt (g)	Water (mL)	Technique
Ap400/Hm0 (Boil)	400	0	2	200	Boil
Ap300/Hm100 (Boil)	300	100	2	200	Boil
Ap200/Hm200 (Boil)	200	200	2	200	Boil
Ap100/Hm300 (Boil)	100	300	2	200	Boil
Ap400/Hm0 (Non-boil)	400	0	2	200	Non-boil
Ap300/Hm100 (Non-boil)	300	100	2	200	Non-boil
Ap200/Hm200 (Non-boil)	200	200	2	200	Non-boil
Ap100/Hm300 (Non-boil)	100	300	2	200	Non-boil

2.2. Determination of Proximate Composition

The proximate composition of moisture, protein, lipid, and ash content was analyzed following the methods of AOAC [14]. The carbohydrate content was calculated by subtracting the sum of the percentages of protein, moisture, ash, and lipid contents from 100%.

2.3. Determination of Amino Acid Composition

The amino acid content of the paper sheets was determined using an amino acid analyzer (LA 8080, Hitachi, Japan) equipped with a Hitachi high-performance cation-exchange column, with the column temperature maintained at 57 °C with some alternations as previously described [15]. Each sample, weighing one gram, was added to 25 mL of 6 M HCl in individual plastic tubes. These tubes were then placed in a sand bath and heated to 110 °C for 24 h. Following the HCl evaporation process, the solid samples were dried and mixed with 6 mL of distilled water. Subsequently, the mixture was filtered using a 0.45 µm syringe filter.

2.4. Determination of Major Bioactive Compounds by GC-MS Analysis

To prepare the samples, 5 g of each sample was placed in a beaker. Subsequently, 50 mL of ethanol was added to each sample, and they were covered with aluminum foil paper before being placed on a magnetic stirrer at 400 rpm for 6 h. Following this, the samples were filtered using double filter paper and then filtered again using a syringe filter before being stored in tubes. These tubes were kept at room temperature and covered with foil paper. The analysis was conducted using a Clarus 690 gas chromatograph equipped with an Elite-35 column (30 m × 0.25 mm, 0.25 µm film thickness; PerkinElmer, Waltham, MA, USA). Initially, a 1-microliter sample was injected, and pure helium (99.99%) was utilized as a carrier gas at a constant flow rate of 1 mL/min for a 40-min run time. The material was evaluated using high-energy (70 eV) electron ionization mode. The inlet temperature remained constant at 280 °C for 4 min. The substances present in the samples were identified by referencing the database of the National Institute of Standards and Technology (NIST).

2.5. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

To prepare the samples, 1 g of each sample was measured and placed in a beaker. Potassium bromide (99%) was then added to each sample to create a mixture. Hydraulic pressure was applied to shape this mixture into pellets. These resulting sample pellets were positioned in the path of the IR source. A detector captured the analog signal and converted it into a spectrum. Subsequently, the signals were analyzed and peaks were identified using OMNIC software (version 9.2).

2.6. Scanning Electron Microscope (SEM) and Energy-Dispersive X-ray (EDX) Analysis

The surface morphology and microstructure of the EPSs were examined using a field emission scanning electron microscope (FE-SEM) equipped with an energy-dispersive X-ray (EDX) microanalyzer (Zeiss Sigma300, Berlin, Germany). Initially, carbon tape was attached to a metallic stub, onto which a small quantity of EPS samples was dispersed. Each stub was then subjected to gold sputtering for 2 min. Subsequently, each stub was affixed to a sample holder and inserted into the FE-SEM machine, initiating the pumping process. After completion of pumping, images of the samples were captured using the FE-SEM at a magnification of 100 and an electron accelerating voltage of 5.00 kV. For EDX analysis, measurements were performed at an electron accelerating voltage of 20.0 kV and a magnification of 3000.

2.7. Color Value Measurement of EPSs

For color analysis, 2 g of each sample was placed on separate slides. Each slide was positioned at the bottom of the color reader device (model: CS-826, Konica Minolta, Hangzhou, China). The power button of the device was then activated, and the camera was passed over the sample.

2.8. Statistical Analyses of Experimental Data

Microsoft Excel was utilized to generate the graph and table. All experiments were conducted three times, and the results are presented as the mean ± standard deviation (SD). Data analysis was performed using a one-way analysis of variance (ANOVA) on IBM SPSS software version 29 (SPSS Inc., Chicago, IL, USA). Significant differences between the means were determined using Duncan's Multiple Range Tests, and $p \leq 0.05$ was regarded as significant.

3. Results and Discussion

3.1. Proximate Composition of EPSs

3.1.1. Protein Content

The protein content in the edible paper sheets produced from *A. philoxeroides* incorporating *H. molitrix* fish flesh exhibited significant variation ($p \leq 0.05$) based on the concentrations of fish flesh; however, there were no significant differences observed regarding the boiling process (Figure 1). The inclusion of *H. molitrix* flesh led to an increase in protein content in the produced EPSs. In the boiled samples, the highest protein content was recorded as 51.12% in Ap100/Hm300, followed by 40.12%, 29.43%, and 17.25% in samples Ap200/Hm200, Ap300/Hm100, and Ap400/Hm0, respectively. A similar increasing trend of protein content in the EPSs was observed with the non-boiling treatment due to the augmentation of *H. molitrix* flesh. In a previous study, the protein contents of edible paper sheets produced from *A. philoxeroides*, *E. fluctuans*, and *I. aquatica* were reported to be 17.12%, 16.23%, and 15.25%, respectively [6]. They also noted a reduction in protein content in EPSs compared to raw aquatic weeds due to processing. Aquatic weeds are considered to have low-protein-content biomass, with the highest reported protein content in *A. philoxeroides*, *I. aquatica*, and *L. adscendens* being 2.18%, 1.52%, and 2.0%, respectively, on a wet weight basis [16]. The study also documented that the protein content in aquatic weeds was found to be lower than that of terrestrial plants. They are expected to have a high moisture level, resulting in a reduction in protein content. Therefore, the incorporation

of *H. molitrix* in EPSs resulted in an increase in protein content. *H. molitrix* is recognized for its high protein content, ranging from 64.5% to 68.7% on a dry weight basis [17]. The primary objective of the present study was to enhance the fish protein content in EPSs, making them suitable for consumption by individuals who are not interested in consuming fish directly.

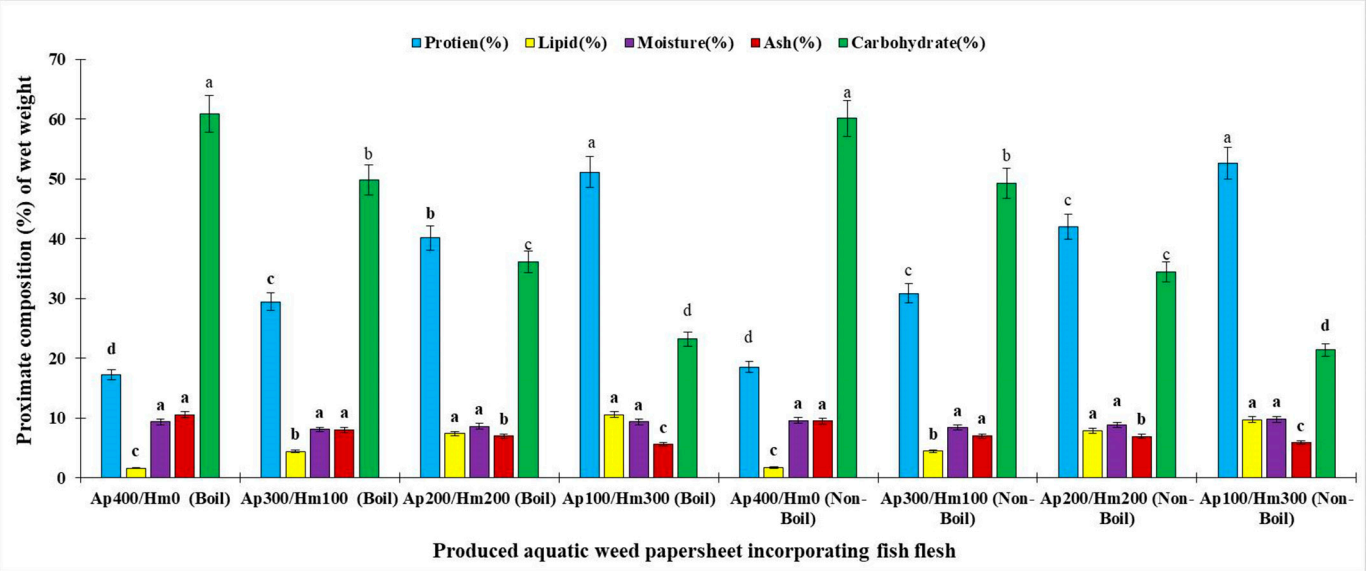

Figure 1. Proximate composition of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*. Values are the results of mean ± SD (n = 3). Different letters on each column bar for the same composition indicate significant differences ($p < 0.05$).

3.1.2. Lipid Content

The changes in lipid content in EPSs produced with *A. philoxeroides* incorporating *H. molitrix* flesh are depicted in Figure 1. The lipid content in the EPSs was found to increase with higher *H. molitrix* flesh contents; however, there were no significant changes due to boiling treatment. The maximum lipid content was recorded as 10.59% in Ap100/Hm300 (Boil), followed by 9.72% in Ap100/Hm300 (Non-boil). The lowest lipid content was observed as 1.59% in Ap400/Hm0 (Boil). Suraiya et al. [6] prepared edible paper sheets using *A. philoxeroides*, *E. fluctuans*, and *I. aquatica* without incorporating fish flesh and reported lipid contents of 1.59%, 1.95%, and 2.11%, respectively. It is noteworthy that aquatic weeds typically have a low lipid content. A study examining four edible aquatic weeds found that *E. fluctuans* and *A. philoxeroides* leaves contained lipid contents ranging from 1.12% to 2.96%, while stems ranged from 1.11% to 2.02% [18]. The lipid content observed in the present study is consistent with previous reports. The lipids found in aquatic weeds play a significant role in promoting health as they offer essential fatty acids renowned for their beneficial impacts on cardiovascular health, brain function, inflammation regulation, and more [18,19]. Lipids derived from aquatic organisms are considered healthy due to their contents of long-chain and essential fatty acids [15].

3.1.3. Moisture Content

The moisture content in the EPSs prepared using *A. philoxeroides* incorporating *H. molitrix* flesh did not vary significantly depending on the concentration of fish flesh and boiling treatment. The moisture content was approximately 8.09–9.82% (Figure 1). In a previous study conducted by Suraiya et al. [6], the moisture content in edible paper sheets produced from aquatic weeds such as *A. philoxeroides*, *E. fluctuans*, and *I. aquatica* was 9.36%, 10.62%, and 8.61%, respectively. During processing, the EPSs were dried in a hot air oven at a certain temperature for a certain period, which reduced the mois-

ture content. The moisture content in raw fish and aquatic weeds depends on environmental factors, nutritional parameters, seasonal variations, etc. [11]. Pulipati et al. [20] reported that the moisture content in *A. philoxeroides* was 10%. These findings closely correspond with the aggregated results from previous studies as well as the outcomes of the current investigation.

3.1.4. Ash Content

The ash content of the EPS samples exhibited significant variation depending on the incorporation levels of *A. philoxeroides* and *H. molitrix*. The ash content in the EPS samples ranged from 5.58% to 10.58%. The highest ash content, 10.58%, was observed in Ap400/Hm0 (Boil), which decreased significantly with decreasing concentrations of *A. philoxeroides* in the EPSs. The lowest ash content, 5.58%, was found in Ap100/Hm300 (Boil). However, there were no significant changes in ash content due to boiling treatment. The ash content in edible paper sheets produced from aquatic weeds such as *A. philoxeroides*, *E. fluctuans*, and *I. aquatica* was reported as 10.58%, 10.16%, and 9.30%, respectively [6]. The findings of the present study align with this previous report. The ash content in food is determined by inorganic minerals such as sodium, potassium, calcium, phosphorus, magnesium, and trace elements [21].

3.1.5. Carbohydrate Content

In this study, carbohydrate emerged as the most abundant compound in the EPSs, with its content varying from 21.38% to 60.89% depending on the proportion of *A. philoxeroides* and *H. molitrix* in the EPS. The highest carbohydrate content, 60.89%, was observed in Ap400/Hm0 (Boil), gradually decreasing with increasing *H. molitrix* flesh content. Conversely, in the EPS sample Ap100/Hm300 (Non-boil), the carbohydrate content was the lowest at 21.38%. There was no significant difference in carbohydrate content between boiled and non-boiled EPS samples. The carbohydrate content in edible paper sheets prepared from *I. aquatica*, *A. philoxeroides*, and *E. fluctuans* was reported as 64.63%, 60.89%, and 60.04%, respectively [6]. In a study examining wild edible plants, the carbohydrate content in dhekishak, helencha, kalmishak, patshak, and shapla stems was reported as 57.69%, 61.61%, 52.78%, 60.21%, and 76.34%, respectively [22]. Umar et al. [23] investigated the nutritional composition of water spinach leaves and recorded a carbohydrate content of 54.20%. Previous studies have revealed similarities in carbohydrate content to the results of the current study. Environmental factors, such as variances in soil composition, harvesting techniques, plant varieties, and temperature, have been identified as potential factors contributing to the nutritional variations observed among various aquatic weeds.

3.2. Amino Acid Composition of EPSs

The amino acid chromatogram and composition are depicted in Figure 2 and Table 2, respectively. A total of 11 different amino acids were detected in the EPSs produced from *A. philoxeroides* and *H. molitrix*. Methionine emerged as the predominant amino acid in the EPSs, with a content of 12,161.79 mg/100 g in the Ap100/Hm300 (Non-boil) EPS, followed by leucine, phenylalanine, and lysine. The amino acid contents in the prepared EPSs increased with the increment in *H. molitrix* flesh. *A. philoxeroides* exhibited a poor amino acid content, with no serine, cysteine, and threonine detected in EPSs produced solely with *A. philoxeroides*. There was no significant variation in amino acid content due to boiling treatment. Other amino acids found in the EPSs included valine, tyrosine, histidine, and aspartic acid. Both *A. philoxeroides* and *H. molitrix* contributed to the amino acid content in the EPSs. Fish is recognized as a high-protein and amino-acid-rich food with high digestibility properties. Additionally, some essential and rare amino acids are also available in fish, such as methionine and lysine. Dewanji et al. [24] found the amino acids lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, leucine, tyrosine, and phenylalanine in *A. philoxeroides*. Sabetian et al. [25] found that the amount of essential amino acids in rainbow trout was

(35.74 ± 0.1)%. Among the amino acids, glutamic acid, aspartic acid, and lysine were predominant, with values of (11.1 ± 0.4)%, (9.53 ± 0.1)%, and (7.72 ± 0.1)%, respectively, in rainbow trout (*Oncorhynchus mykiss*).

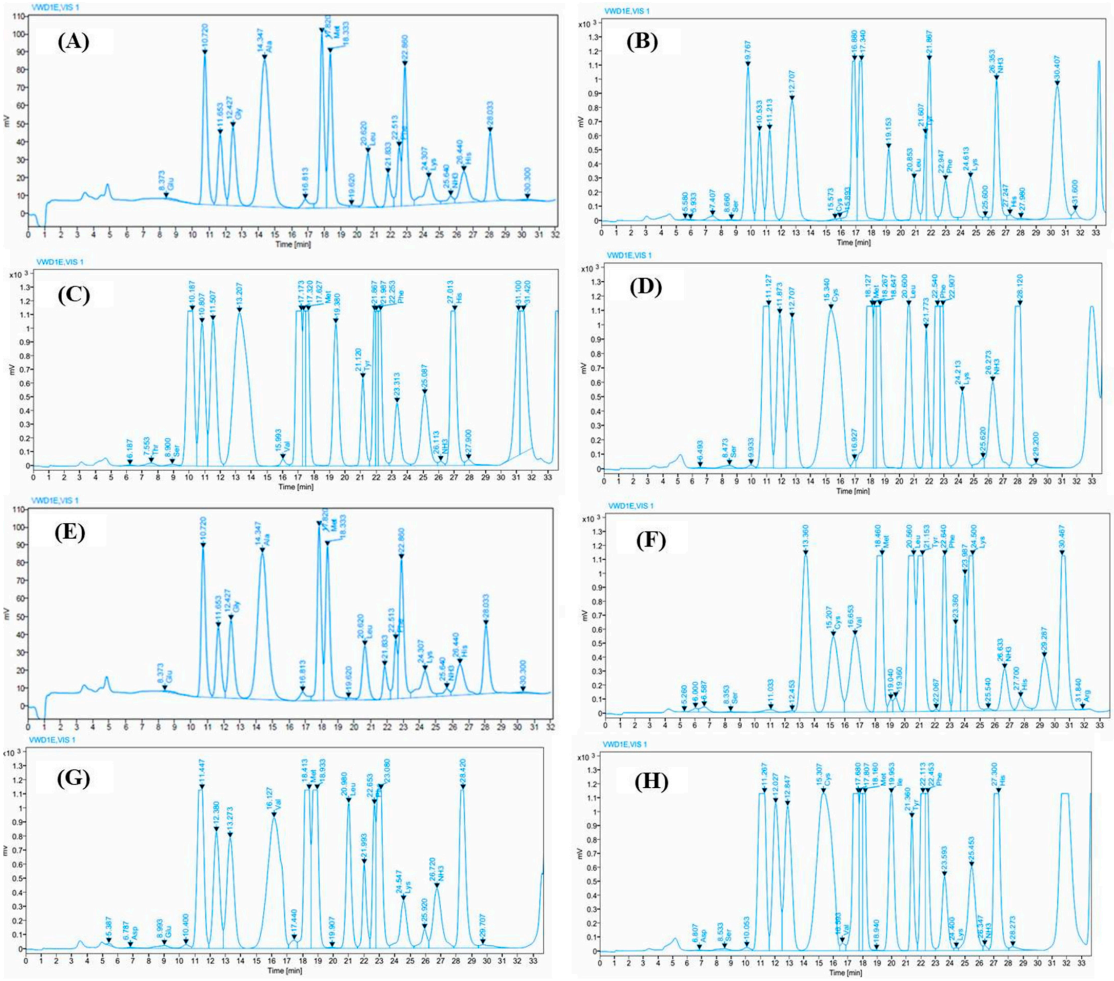

Figure 2. Amino acid chromatogram of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*. (**A**) Ap300/Hm100 (Boil); (**B**) Ap300/Hm100 (Boil); (**C**) Ap200/Hm200 (Boil); (**D**) Ap100/Hm300 (Boil); (**E**) Ap400/Hm0 (Non-Boil); (**F**) Ap300/Hm100 (Non-Boil); (**G**) Ap200/Hm200 (Non-Boil); and (**H**) Ap100/Hm300 (Non-Boil).

Table 2. Amino acid composition and contents (mg/100 g) of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*.

Amino Acids	Ap400/Hm0 (Boil)	Ap300/Hm100 (Boil)	Ap200/Hm200 (Boil)	Ap100/Hm300 (Boil)	Ap400/Hm0 (Non-Boil)	Ap300/Hm100 (Non-Boil)	Ap200/Hm200 (Non-Boil)	Ap100/Hm300 (Non-Boil)
Ser	n.d.	88.31 ± 2.24 ^{c}	179.02 ± 5.85 ^{b}	298.33 ± 4.65 ^{a}	n.d.	97.11 ± 2.54 ^{c}	191.87 ± 6.25 ^{b}	286.68 ± 5.50 ^{a}
Cys	n.d.	126.80 ± 5.58 ^{c}	728.14 ± 12.25 ^{b}	1544.88 ± 18.25 ^{a}	n.d.	113.65 ± 5.25 ^{c}	750.52 ± 10.25 ^{b}	1532.69 ± 20.25 ^{a}
Met	804.77 ± 14.25 ^{c}	465.24 ± 5.25 ^{d}	6666.62 ± 75.25 ^{b}	11,990.94 ± 780.25 ^{a}	901.55 ± 18.54 ^{c}	458.33 ± 5.57 ^{d}	6680.19 ± 60.25 ^{b}	12,161.79 ± 87.25 ^{a}
Leu	2072.24 ± 45.52 ^{d}	2705.27 ± 50.25 ^{c}	4348.96 ± 98.22 ^{b}	4938.94 ± 140.25 ^{a}	2194.50 ± 85.22 ^{d}	2648.83 ± 75.25 ^{c}	4397.27 ± 83.25 ^{b}	4905.93 ± 90.25 ^{a}
Phe	3394.24 ± 45.67 ^{c}	3067.87 ± 48.25 ^{d}	4138.08 ± 73.25 ^{b}	4677.47 ± 82.25 ^{a}	3693.12 ± 76.35 ^{c}	2904.41 ± 49.25 ^{d}	3975.66 ± 80.25 ^{b}	4762.60 ± 92.25 ^{a}
Lys	4270.07 ± 140.25 ^{d}	4768.48 ± 87.01 ^{c}	5476.14 ± 58.47 ^{b}	4640.63 ± 76.35 ^{a}	4294.84 ± 82.25 ^{d}	4676.02 ± 47.58 ^{c}	5543.00 ± 90.25 ^{b}	4604.12 ± 45.36 ^{a}
Thr	n.d.	203.60 ± 12.21 ^{c}	314.19 ± 14.20 ^{b}	376.79 ± 5.25 ^{a}	n.d.	192.70 ± 4.58 ^{c}	304.07 ± 8.52 ^{b}	350.90 ± 9.35 ^{a}

Table 2. *Cont.*

Amino Acids	Ap400/Hm0 (Boil)	Ap300/Hm100 (Boil)	Ap200/Hm200 (Boil)	Ap100/Hm300 (Boil)	Ap400/Hm0 (Non-Boil)	Ap300/Hm100 (Non-Boil)	Ap200/Hm200 (Non-Boil)	Ap100/Hm300 (Non-Boil)
Val	408.17 ± 10.25 ^{d}	3278.15 ± 98.25 ^{c}	4054.30 ± 80.35 ^{b}	4455.23 ± 48.25 ^{a}	410.14 ± 10.24 ^{d}	3114.36 ± 57.36 ^{c}	4111.46 ± 83.25 ^{b}	4204.43 ± 86.25 ^{a}
Tyr	2399.43 ± 45.25 ^{d}	2704.87 ± 75.28 ^{c}	4274.41 ± 46.45 ^{b}	4746.36 ± 46.25 ^{a}	2555.32 ± 74.18 ^{c}	2630.74 ± 80.29 ^{c}	4278.56 ± 46.80 ^{b}	4498.97 ± 47.58 ^{a}
His	408.17 ± 12.25 ^{d}	442.47 ± 10.27 ^{c}	1105.99 ± 8.25 ^{b}	1511.63 ± 17.25 ^{a}	455.50 ± 18.34 ^{d}	3819.45 ± 20.27 ^{c}	1192.77 ± 42.25 ^{b}	1680.86 ± 41.73 ^{a}
Asp	242.92 ± 10.27 ^{d}	5619.94 ± 44.25 ^{c}	762.22 ± 8.25 ^{b}	975.81 ± 6.34 ^{a}	240.03 ± 4.35 ^{d}	4447.39 ± 4.58 ^{c}	763.72 ± 8.54 ^{b}	1169.06 ± 8.25 ^{a}

Values are presented as mean ± standard deviation of triplicates. Different superscript small letters on each row indicate significant differences ($p < 0.05$). n.d.: not detected.

3.3. Major Bioactive Compounds Available in EPS Detected by GC-MS Analysis

The major bioactive compounds, along with their molecular weight, molecular formula, and quantity, are presented in Table 3. A total of 51 bioactive compounds were identified in the EPSs produced from *A. philoxeroides* and *H. molitrix*. Key compounds included N-hexadecanoic acid, 1,2,3,4-tetrahydro-3-(phenylacetamido)quinolone, tetradecanoic acid, N-hexadecanoic acid, pentadecanoic acid, 14-bromo-, 6-octadecenoic acid, trans-2-methyl-4-n-butylthiane, s,s-dioxide, and 9-octadecenoic acid. Other common compounds included carbonic acid, 2-dimethylaminoethyl ethyl ester, hentriacontane, pentadecanoic acid, N-hexadecanoic acid, tetradecanoic acid, 10,13-dimethyl-, methyl ester, and 1,2,3,4-tetrahydro-3-(phenylacetamido)quinolone. Most of the phenolic and flavonoid compounds in the EPSs originated from the aquatic weed sample, while the fatty acid compounds were sourced from fish oil. Increased antioxidant activity was associated with rises in the levels of phenolic and flavonoid contents, which in turn scavenge free radicals [26]. In a previous study, 33 compounds were identified in an edible aquatic weed paper sheet produced from *A. philoxeroides* [6]. Akbar et al. [27] conducted a gas chromatography–mass spectrometry (GC-MS) analysis on *A. philoxeroides* and discovered the presence of acetic acid 2-(2-methoxycarbonylamino-5-nitrophenylthio)-, methyl ester, at the highest concentration (31.19%), followed by 1,4-benzenediol, 1,2,5-bis(1,1-dimethylethyl)- (15.06%). Suraiya et al. [6] conducted a similar analysis on *A. philoxeroides* and found the presence of dodecanoic acid (8.71%) and phytol (14.02%).

Table 3. GC-MS analysis of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*.

Sl No	Identifying Compound	Molecular Weight	Molecular Formula	Peak Area (%)							
				Ap400/Hm0 (Boil)	Ap300/Hm100 (Boil)	Ap200/Hm200 (Boil)	Ap100/Hm300 (Boil)	Ap400/Hm0 (Non-Boil)	Ap300/Hm100 (Non-Boil)	Ap200/Hm200 (Non-Boil)	Ap100/Hm300 (Non-Boil)
1	Carbonic acid, 2-dimethylaminoethyl ethyl ester	161	$C_7H_{15}NO_3$	1.54	1.42	2.04	0.02	0.56	0.53	1.77	0.25
2	Acetamide, 2-amino-n-ethyl-2-thioxo-	132	$C_4H_8N_2OS$	-	-	0.03	-	-	0.09	-	-
3	Propane, 2-chloro-2-nitro-	123	C3H6ClNO$_2$	-	-	0.15	-	-	-	0.49	-
4	Propamocarb	188	$C_9H_{20}N_2O_2$	-	-	0.45	-	0.28	0.21	0.49	-
5	Oxime-, methoxy-phenyl-_	151	$C_8H_9NO_2$	-	0.53	1.24	2.99	-	0.20	1.33	2.14
6	Oxime-, methoxy-phenyl-_	151	$C_8H_9NO_2$	-	0.53	-	2.99	-	0.20	0.33	-
7	D-Methionine	149	$C_5H_{11}NO_2S$	3.22	1.25	0.21	-	-	3.25	2.40	1.19

Table 3. *Cont.*

Sl No	Identifying Compound	Molecular Weight	Molecular Formula	Peak Area (%)							
				Ap400/ Hm0 (Boil)	Ap300/ Hm100 (Boil)	Ap200/ Hm200 (Boil)	Ap100/ Hm300 (Boil)	Ap400/ Hm0 (Non-Boil)	Ap300/ Hm100 (Non-Boil)	Ap200/ Hm200 (Non-Boil)	Ap100/ Hm300 (Non-Boil)
8	Phthalan	120	C_8H_8O	-	-	1.07	1.12	-	2.82	-	-
9	Dimethy-lisopropylsi-lyloxycy-clobutane	172	$C_9H_{20}OSi$	1.45	0.25	-	-	-	1.25	-	-
10	Tetrazolo[5,1-a]phthalazin-6-amine, n-methyl-	200	$C_9H_8N_6$	-	2.25	-	--	-	-	0.37	-
11	Diethyl 1-methyl-3-hydroxy-5-phenylpyrrole-2,4-dicarboxylate	317	$C_{17}H_{19}NO_5$	-	-	-	3.45	0.31	0.37	0.59	2.21
12	Hentriacontane	436	$C_{31}H_{64}$	-	0.51	-	-	0.13	0.20	-	-
13	Hexadecane	226	$C_{16}H_{34}$	-	0.46	0.50	-	0.18	0.26	0.31	-
14	Hentriacontane	436	$C_{31}H_{64}$	-	0.51	0.44	0.98	0.13	0.20	0.25	-
15	Hentriacontane	436	$C_{31}H_{64}$	-	0.51	0.44	0.98	0.13	0.20	0.25	-
16	S-butyl n-hexyl disulfide	206	$C_{10}H_{22}S_2$	-	1.87	0.50	-	0.72	0.54	-	-
17	Phosphoric acid, bis(tri-methylsi-lyl)monomethyl ester	256	$C_7H_{21}O_4PSi_2$	39.54	35.02	29.88	20.40	37.64	31.38	28.33	19.25
18	Triacontane	422	$C_{30}H_{62}$	-	-	-	0.71	0.32	-	0.36	-
19	Hexadecane	226	$C_{16}H_{34}$	-	0.46	0.50	-	0.18	0.26	0.31	-
20	Pipradrol	267	$C_{18}H_{21}NO$	0.88	-	-	-	-	-	-	1.46
21	2,4-difluorobenzoic acid, 2-formyl-4,6-dichlorophenyl ester	330	$C_{14}H_6Cl_2F_2O_3$	-	-	0.5	1.24	0.17	0.16	-	1.05
22	Dodecanoic acid	200	$C_9H_8N_6$	2.50	2.25	1.58	--	2.47	2.14	1.37	-
23	Tritetracontane	604	$C_{43}H_{88}$	-	-	-	-	-	0.26	-	-
24	Triacontane	422	$C_{30}H_{62}$	-	-	-	-	0.32	-	0.36	-
25	Quinoline, 2-Ethyl-	157	$C_{11}H_{11}N$	0.27	-	-	-	-	-	-	0.19
26	Dl-Alanyl-Dl-Alanine	482	$C_{17}H_{28}N_2O_5$	-	-	-	-	-		-	0.16
27	Oleic acid	282	$C_{18}H_{34}O_2$	21.01	20.41	15.00	10.42	21.83	12.20	10.25	5.34
28	N-decanoic acid	172	$C_{10}H_{20}O_2$	-	0.98	-	-	5.10	1.34	0.31	-
29	Isoheptadecanol	256	$C_{17}H_{36}O$	28.24	24.24	21.88	11.83	37.64	39.38	38.33	24.02
30	Methyl 11-Methyl-Dodecanoate	228	$C_{14}H_{28}O_2$	4.46	3.25	2.14	1.24	3.54	2.47	1.43	2.54
31	Undecanoic Acid,10-Methyl-,Methyl Ester	214	$C_{13}H_{26}O_2$	0.05	-	-	-	-	-	-	8.05
32	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	2.14	2.36	3.50	4.80	1.31	1.30	1.91	2.14
33	N-hexadecanoic acid	256	$C_{16}H_{32}O_2$	2.14	5.24	9.88	0.40	7.64	9.38	8.33	10.25
34	Phytol	296	$C_{20}H_{40}O$	2.89	-	-	-	-	-	-	14.02
35	13-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	-	-	-	0.50	2.15	-	-	-
36	Heptadecanoic acid, 16-methyl-, methyl ester	298	$C_{19}H_{38}O_2$	-	-	-	0.03	0.08	-	-	-
37	Methyl 8,11,14-heptadeca-trienoate	278	$C_{18}H_{30}O_2$	0.56	1.43	1.33	1.14	-	1.00	1.43	1.51

Table 3. *Cont.*

Sl No	Identifying Compound	Molecular Weight	Molecular Formula	Peak Area (%)							
				Ap400/ Hm0 (Boil)	Ap300/ Hm100 (Boil)	Ap200/ Hm200 (Boil)	Ap100/ Hm300 (Boil)	Ap400/ Hm0 (Non-Boil)	Ap300/ Hm100 (Non-Boil)	Ap200/ Hm200 (Non-Boil)	Ap100/ Hm300 (Non-Boil)
38	Ethyl 9,12,15-octadeca-trienoate	306	$C_{20}H_{34}O_2$	-	-	-	-	0.56	0.74	-	-
39	11,14,17-Eicosatrienoic Acid, Methyl Ester	320	$C_{21}H_{36}O_2$	6.33	12.52	13.54	15.27	5.25	11.24	13.25	13.81
40	N-propyl 11-octadecenoate	324	$C_{21}H_{40}O_2$	0.25	3.79	5.25	6.96	0.12	8.25	10.81	12.47
41	Pentadecanoic acid, 14-bromo-	320	$C_{15}H_{29}BrO_2$	2.14	5.25	11.24	15.58	1.24	4.25	10.70	14.27
42	N-propyl 11-octadecenoate	324	$C_{21}H_{40}O_2$	-	3.79	11.47	15.24	-	2.25	5.81	7.5
43	3-cyclopentyl-propionic acid, 2-dimethy-laminoethyl ester	213	$C_{12}H_{23}NO_2$	2.74	8.12	4.22	5.86	-	4.04	-	-
44	Cis-2-methyl-4-n-butylthiane, s,s-dioxide	204	$C_{10}H_{20}O_2S$	-	-	-	1.65	-	1.95	-	-
45	Glycidyl palmitate	312	$C_{19}H_{36}O_3$	-	1.33	1.42	2.84	1.94	2.13	2.85	3.25
46	Glycidyl palmitate	312	$C_{19}H_{36}O_3$	-	1.33	-	6.84	1.94	-	2.85	-
47	2-amino-4-methyl-6-(2-thienyl)pyri-midine	191	$C_9H_9N_3S$	-	-	-	5.47	-	-	0.73	-
48	Phenol, 2,5-bis(1,1-dimethyl-ethyl)-	206	$C_{10}H_{22}S_2$	-	1.87	0.50	-	0.72	0.54	-	-
49	1,2,3,4-tetrahydro-3-(phenylacet-amido)quinoline	266	$C_{17}H_{18}N_2O$	9.28	8.95	7.22	5.25	10.56	8.32	6.28	4.25
50	1,2,3,4-tetrahydro-3-(phenylacet-amido)quinoline	266	$C_{17}H_{18}N_2O$	-	2.14	1.85	1.64	2.63	2.32	1.28	0.76
51	Acetic acid, (dodecahydro-7-hydroxy-1,4b,8,8-tetramethyl-10-oxo-2(1h)	405	$C_{24}H_{39}NO_4$	-	1.46	1.74	-	-	-	-	-

3.4. FT-IR Analysis of EPSs

The FT-IR spectra of the EPSs produced from *A. philoxeroides* and *H. molitrix* are depicted in Figure 3. FT-IR spectra are utilized to determine the molecular composition and structure by measuring the absorption of infrared light by materials in solid, liquid, and gas states. Their most common application is in identifying unknown materials and confirming product composition, as well as determining the chemical bonds of compounds. The figure illustrates bonds ranging from 440 cm^{-1} to 3500 cm^{-1}. Specifically, the Si-O-Si bond appears at around 440 cm^{-1}, the C-H bond at 667 cm^{-1}, the C-O bond at 1080 cm^{-1}, the C = O bond at 1660 cm^{-1}, the C-C bond at 2930 cm^{-1}, and the O-H bond at 3500 cm^{-1}. The absorption peak observed at 2985 cm^{-1} is attributed to the asymmetric stretching vibration of methylene groups within the CH_2-OH present in cellulose components. Additionally, the band located approximately at 1160 cm^{-1} is attributed to the asymmetrical stretching vibration of C-O-C, associated with cellulose and hemicellulose [28]. The bands

at 1633 and 1536 are responsible for amide-I and amide-II, respectively [29]. In this work, the evident bands higher in Ap100/Hm300 (Boil) are indicative of the high protein content among these EPSs. Sanden et al. [30] found that the connective tissue of Atlantic cod contains spectral regions ranging from 1800 to 800 cm^{-1}. Careche et al. [31] observed that frozen hake (*Merluccius merluccius*) muscle exhibits C-H vibration (3100–2750 cm^{-1}), C = O vibration (1800–1670 cm^{-1}), and phospholipid vibration (1330–1127 cm^{-1}).

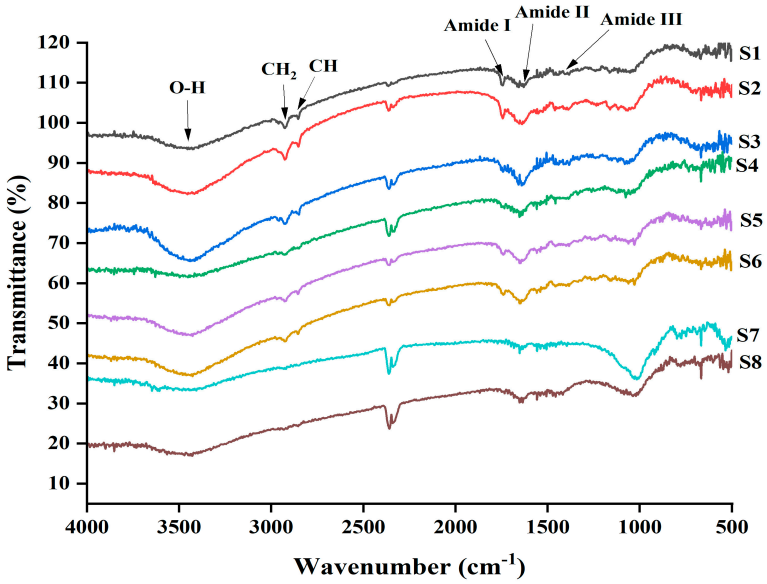

Figure 3. FT-IR analysis of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*. S1: Ap300/Hm100 (Boil); S2: Ap300/Hm100 (Boil); S3: Ap200/Hm200 (Boil); S4: Ap100/Hm300 (Boil); S5: Ap400/Hm0 (Non-Boil); S6: Ap300/Hm100 (Non-Boil); S7: Ap200/Hm200 (Non-Boil); and S8: Ap100/Hm300 (Non-Boil).

3.5. SEM View and EDX Analysis of EPS

The SEM view of EPSs produced from *A. philoxeroides* and *H. molitrix* is presented in Figure 4. The SEM offers magnified images revealing the size, shape, and physical and chemical properties of EPS samples, as well as providing insights into their surface morphology and microstructure at a very high magnification. The figure illustrates that incorporation of a high amount of H. molitrix flesh gives the sample a rough and porous appearance (Figure 4D,H). However, boiling treatment appears to improve the surface. The EPS samples prepared with a higher proportion of *A. philoxeroides* exhibited a more regular and fibrous appearance (Figure 4A,B,E,F). Ural et al. [32] demonstrated that SEM analysis of clay particles can help explain unexplained physical or mechanical behavior. SEM imaging provides valuable insights into the microstructure and surface characteristics of aquatic weed paper sheets, aiding in understanding their properties and potential applications.

The EDX technique is employed for elemental analysis of samples, operating by directing a concentrated stream of electrons onto a sample to stimulate the emission of X-rays from its atoms. These emitted X-rays are then analyzed to determine the elemental composition of the sample. The EDX chromatograms of EPSs produced from *A. philoxeroides* and *H. molitrix* are presented in Figure 5. Slight variations were observed in the different compositions in the EPS samples, possibly due to variations in the proportions of *A. philoxeroides* and *H. molitrix* in the samples. All samples contained elements such as Ca, N, K, O, C, Mg, Na, P, Cl, Mn, and Fe in their structure. From Figure 5, it is evident that minerals such as P, K, and Na showed higher peak areas in Ap200/Hm200 (Boil) and Ap100/Hm300 (Boil). Therefore, EPSs containing a high fish muscle content with boiling showed higher amounts of essential minerals. Currently, there are no available reports detailing EDX composition analyses of aquatic weed paper sheets.

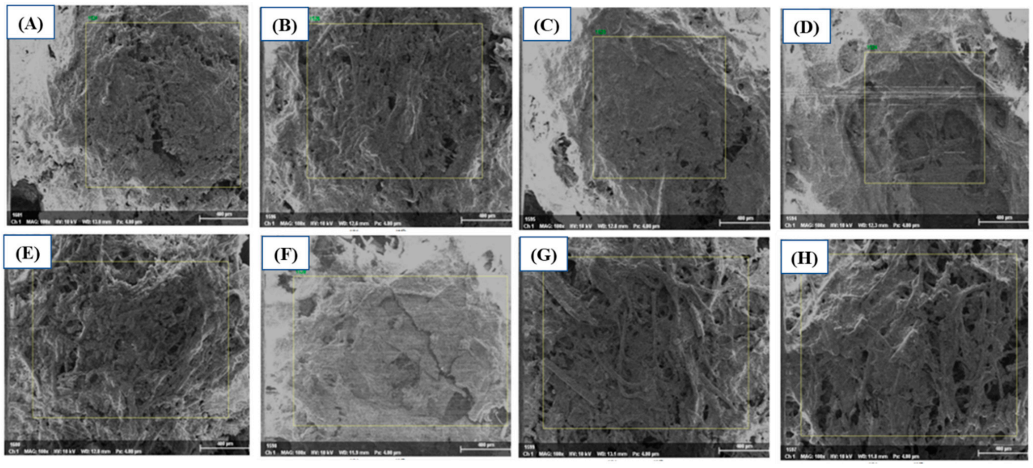

Figure 4. Scanning electron microscope pictures of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*. (**A**) Ap300/Hm100 (Boil); (**B**) Ap300/Hm100 (Boil); (**C**) Ap200/Hm200 (Boil); (**D**) Ap100/Hm300 (Boil); (**E**) Ap400/Hm0 (Non-Boil); (**F**) Ap300/Hm100 (Non-Boil); (**G**) Ap200/Hm200 (Non-Boil); and (**H**) Ap100/Hm300 (Non-Boil).

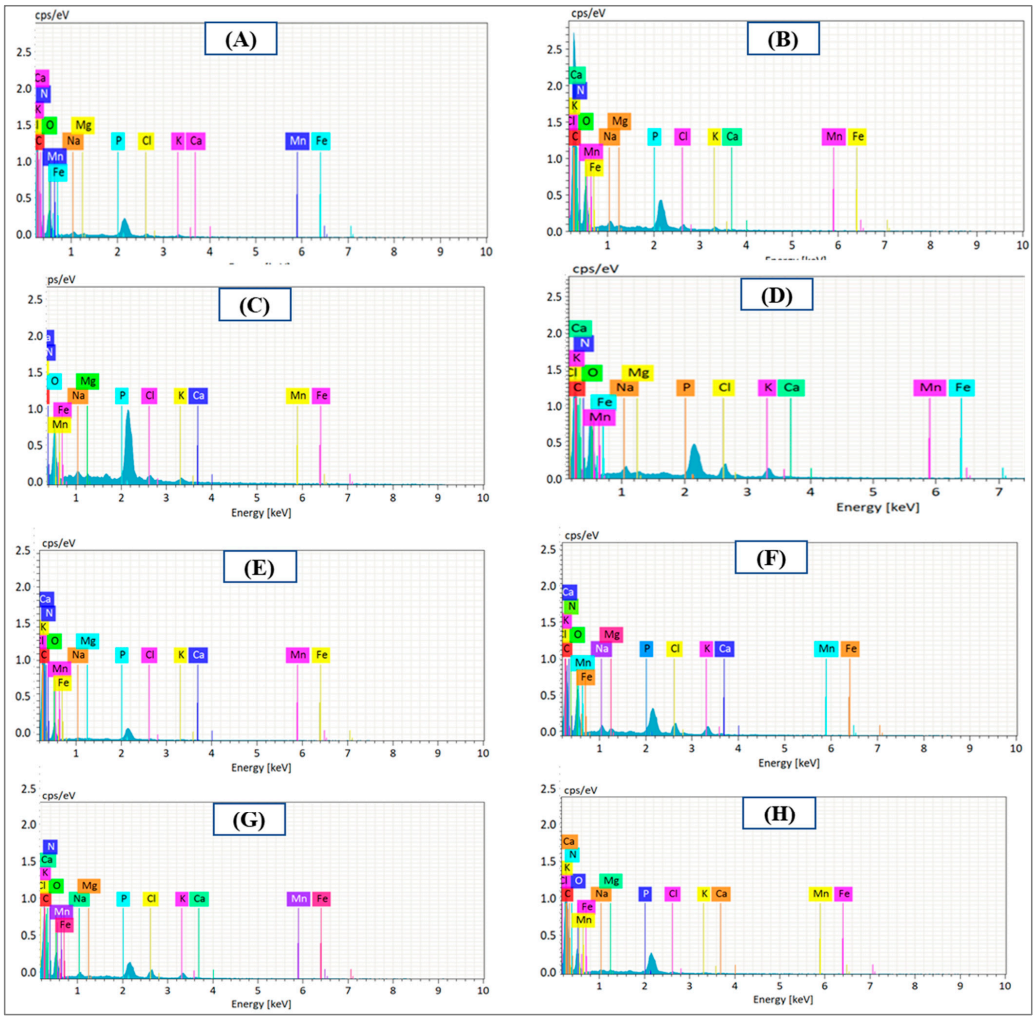

Figure 5. EDX chromatograms of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*. (**A**) Ap300/Hm100 (Boil); (**B**) Ap300/Hm100 (Boil); (**C**) Ap200/Hm200 (Boil); (**D**) Ap100/Hm300 (Boil); (**E**) Ap400/Hm0 (Non-Boil); (**F**) Ap300/Hm100 (Non-Boil); (**G**) Ap200/Hm200 (Non-Boil); and (**H**) Ap100/Hm300 (Non-Boil).

3.6. Color Values of EPSs

The color values (L*, a*, b*) of EPSs prepared from *A. philoxeroides* and *H. molitrix* are presented in Table 4. The L* value indicates the lightness of the product, while the a* and b* values denote the red to green and yellow to blue axes, respectively. The L* values ranged from 30.97 to 24.37, with the highest value (30.97) observed in Ap100/Hm300 (Boil) and the lowest value (24.37) in Ap400/Hm0 (Boil). L* increased with higher contents of *H. molitrix* flesh and decreased *A. philoxeroides* contents, as the light flesh of *H. molitrix* contributed to the overall lightness of the EPS. The a* value ranged from 5.20 to 16.70, with the highest value (16.70) observed in Ap400/Hm0 (Non-boil), which contained the highest concentration of *A. philoxeroides*. Since *A. philoxeroides* is green, its higher concentrations contributed to higher a* values. The b* value varied from 3.62 to 10.12 across the EPS samples, showing a decrease with the increasing concentration of *H. molitrix* flesh. Color is an important factor influencing food acceptance by consumers, and the lightness and green hue of the EPSs produced from aquatic weeds would likely be favorable to consumers.

Table 4. The color values of different edible paper sheets produced from *A. philoxeroides* and *H. molitrix*.

Serial	Samples	Lightness (L*)	Red to Green (a*)	Yellow to Blue (b*)
1.	Ap400/Hm0 (Boil)	24.37 ± 1.25^{d}	15.40 ± 0.58^{a}	9.24 ± 0.47^{a}
2.	Ap300/Hm100 (Boil)	25.83 ± 0.85^{c}	9.25 ± 0.10^{c}	4.77 ± 0.54^{b}
3.	Ap200/Hm200 (Boil)	28.28 ± 1.23^{b}	7.14 ± 0.76^{d}	3.62 ± 0.37^{c}
4.	Ap100/Hm300 (Boil)	30.97 ± 1.70^{a}	5.20 ± 0.47^{d}	3.63 ± 0.74^{c}
5.	Ap400/Hm0 (Non-boil)	25.95 ± 0.76^{d}	16.70 ± 0.43^{a}	10.12 ± 0.18^{a}
6.	Ap300/Hm100 (Non-boil)	26.43 ± 1.81^{c}	13.25 ± 1.07^{b}	4.52 ± 0.40^{b}
7.	Ap200/Hm200 (Non-boil)	27.38 ± 1.05^{b}	9.60 ± 1.04^{c}	3.70 ± 0.20^{c}
8.	Ap100/Hm300 (Non-boil)	29.47 ± 1.73^{a}	5.24 ± 0.74^{d}	3.47 ± 0.14^{c}

Different superscript small letters on each column indicate significant differences ($p < 0.05$).

4. Conclusions

A. philoxeroides is a nutritious aquatic weed, while *H. molitrix* contains abundant protein and nutrients. Combining both *A. philoxeroides* and *H. molitrix* to produce EPSs could yield a highly nutritious food item beneficial for various demographics. The EPSs produced in this study with the boiling treatment did not exhibit distinct variations. Therefore, decisions regarding boiling treatment could be based on organoleptic evaluation in forthcoming research. Additionally, this research focused only on physico-chemical and nutritional properties. Further research could be conducted based on biofunctional characterization through in vitro and in vivo studies in the future. These EPSs could serve as a valuable source of supplementary nutrition in the regular diets of consumers. Moreover, *A. philoxeroides* possesses medicinal qualities and can help address malnutrition issues in underdeveloped countries. *H. molitrix*, commonly known as silver carp, is rich in protein, lipids, amino acids, and other bioactive compounds, making it an excellent nutritional source for both children and adults. Despite EPSs not being widely recognized in many countries, they could introduce familiarity with paper-based food items. EPS preparation requires less time compared to cooking traditional meals, making it convenient for consumption. Utilizing edible paper sheets enables us to maximize nutritional benefits and promote healthier dietary habits.

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