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Evaluation of the Effect of Hydrothermal Pretreatment on Kitchen and Fruit Wastes for Bacterial Cellulose Fermentation Medium

Muhammad Irhamni Haziqi Nasharudin[1] · Chua Gek Kee[2] · Mohd Hairul Ab Rahim[1] · Nazira Mahmud[1]

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Abstract

Purpose Hydrothermal pretreatment stands out for its efficiency and simplicity yet offers economic and environmental benefits. Its application on non-fibrous organic waste such as kitchen and fruit waste are deemed efficient and does not require extreme working parameters. This study evaluated the effect of predetermined parameters on the hydrothermal pretreatment of various food waste. We aimed to establish a set of parameters for the hydrothermal pretreatment, to promote the utilisation of waste for fermentation into value-added products.

Methods The kitchen and fruit wastes were homogenized and treated at varying temperatures, incubation times, and water ratios. Reducing sugar, protein, and lipid content was analysed pre- and post-treatment. The treated waste was used as a medium for bacterial cellulose fermentation and the yield was compared to chemical medium.

Results The models identified the optimum parameters for maximizing glucose production were at incubation temperature of 115 °C for 75 min and 1:2 waste-to-water ratio, achieving 449.031 mg/mL (kitchen waste), 382.033 mg/mL (guava waste), and 358.840 mg/mL (watermelon waste), with satisfactory desirability and model fit for kitchen waste and guava waste. Attempts to produce bacterial cellulose (BC) from the treated waste recorded promising results. While kitchen waste had higher glucose content post-hydrothermal pretreatment, guava waste and watermelon waste produced more BC due to lower oil content, which did not interfere with the oxygen transfer.

Conclusion The conducted hydrothermal pretreatment at the pre-optimized conditions increased the potential of the food waste tested as a media substitute for BC production.

Keywords Hydrothermal pretreatment · Bioconversion · Kitchen waste · Fruit waste · Bacterial cellulose

Introduction

Food waste (FW) generation is a global problem that has been of concern to various stakeholders, including researchers, policymakers, and environmental agencies, due to its interrelated impacts on the environment, society, and economy. Approximately 1.05 billion tonnes of FW are generated globally in 2022, equivalent to 19% of the total food production in the same year [1], and it was estimated that 38% of global FW was fruit and vegetable waste [2]. FW creates problems in managing landfills and uses up valuable space. FW includes food that is thrown away at different stages of the food supply chain, such as during farming, processing, selling, food services, and in homes [3–5]. It can be grouped into three types: avoidable, possibly avoidable, and unavoidable. Avoidable FW is food that is still safe to eat but is thrown away. Possibly avoidable FW refers to food that some people may eat or cook properly, depending on habits

✉ Nazira Mahmud
nazira@umpsa.edu.my

Muhammad Irhamni Haziqi Nasharudin
irhamnihaziqi@gmail.com

Chua Gek Kee
chua@umpsa.edu.my

Mohd Hairul Ab Rahim
mhairul@umpsa.edu.my

1 Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuh Persiaran Tun Khalil Yaakob, Kuantan, Pahang 26300, Malaysia

2 Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al- Sultan Abdullah, Lebuh Persiaran Tun Khalil Yaakob, Kuantan, Pahang 26300, Malaysia

or knowledge. Unavoidable FW includes parts that are not usually eaten, such as bones or eggshells [6]. Although it is possible to reduce FW by cutting down on avoidable and possibly avoidable waste, this depends on people's awareness and habits, which makes it difficult to achieve. FW also harms the environment because it breaks down and releases methane. This adds to the emissions already produced during farming, such as from using energy, pesticides, and raising animals for meat [7–10]. It was recorded that FW generated 3.3 billion tonnes of CO_2, making it the major contributor to greenhouse gas emissions [11]. Despite the many initiatives in reducing FW, it is beneficial if a low-cost and reliable solution could be figured out as a solution for the already high amount of FW. The idea of utilising FW as a primary substrate for the production of value-added compounds and materials seems promising in this context. Various microorganisms could metabolise FW through their specific metabolic pathway to produce important chemicals [12].

Currently, FW is most often reused in the agro-industry as a source of nutrients for diverse living organisms in the form of fertiliser and feed [13]. FW contains various nutrients such as carbohydrates, proteins, lipids, and other micronutrients. These properties encouraged studies that focus on the utilisation of FW as the growth nutrients for bacterial fermentation, such as for *Methanosarcina thermophila*, *Clostridium stercorarium*, *Thermoanaerobacterium*, and *Escherichia cloacae* [14–17]. Various types of FW were explored, for instances, Rambabu et al. [18] used rice mill wastewater as the energy source for dark fermentation by *Clostridium beijerinckii*, with the biohydrogen yield of 214.9 mL H_2/L. Other example includes the use of FW mixed with straw in an anaerobic digester, which recorded biogas production of 0.392 m^3 CH_4/kg at the ratio of 5:1 [19]. Lee et al. [20] also reported that about 440 mL/g of methane was produced by converting the FW to methane in a continuous digester. The key to a high reaction yield is having a significant amount of nutrients released, particularly fermentable sugar, through a pretreatment process. Nutrients from various carbohydrate waste sources are commonly trapped within the cell wall of the molecules, often the time from the original plant sources, which will be released upon a pretreatment process [21]. A pretreatment will also reduce the microorganisms present in the FW, ensuring successful fermentation of the assigned microorganism [22]. A high-temperature-based pretreatment process often combines with high-pressure steam, which has been proven to increase the efficiency of the process [23]. The method demands a special high-pressure reactor that costs more but possesses limitations on the amount of sample that can be run at one time [24, 25]. Elimination of high-pressure elements is expected to benefit to the whole process through simplicity of the working conditions, unit operation required, and handling. Such a method would be sufficient for common FW materials that lack fibrous structure such as rotten fruits and dining residue. Our previous study proved that nutrients released from treated FW are significantly higher than those of untreated [26]. A similar work was conducted earlier by Chua et al. [27], which attempted several pretreatments on kitchen waste. They found that the increase in the macronutrients after hydrothermal pretreatment was comparable to that of enzymatic pretreatment. Another study highlights other type of hydrothermal pretreatment, namely microwave assisted hydrothermal pretreatment, that was used on mango peel to achieve high extraction of pectin [28]. Similarly, microwave hydrothermal pretreatment was conducted on watermelon peel, which recorded a remarkable yield of hyroxymethylfurfural [29]. Ding et al. [28] reported that higher temperature hydrothermal pretreatment is much better at releasing nutrient compared to low temperature hydrothermal pretreatment up until a temperature limit of 180 °C. Similar findings reported that the rate of sugar released tends to increase followed by a decrease within the temperature range of 100 °C to 200 °C [28]. Our previous study suggested that variation in temperature of 100 °C and 140 °C during hydrothermal pretreatment did not give any significant difference in glucose released [26]. Harsh reaction temperatures will promote decomposition of water-soluble products (i.e., sugar and organic acids), leading to an increase of pH, rendering it unsuitable for fermentation application [30]. It is therefore important to find the right temperature for hydrothermal pretreatment of FW to ensure cost efficiency at large scale processing.

This study used a central composite design (CCD) to evaluate the effect of pre-determined parameters on the hydrothermal pretreatment of FW, including kitchen waste (KW) and several fruit waste. We aimed to determine the optimum parameters for the hydrothermal pretreatment of FW to give high reducing sugar content for application in bacterial fermentation processes. We tested the treated FW for bacterial cellulose production. The output can maximise the utilisation of an easy pretreatment method, such as hydrothermal pretreatment, to promote the utilisation of waste for value-added products.

Methodology

Sample Collection and Preparation

Two types of FW involved in this study are kitchen and fruit wastes. The kitchen waste (KW) was collected from the local restaurant that serve various western and Malaysian cuisine located in Kuantan, Pahang. While rotten guava

(GW) and watermelon (WW) were obtained from fruit vendors in Kuantan, Pahang. Each FW was analysed according to the sample characterization procedure to determine the exact amount of water required to achieve the desired waste-to-water ratio. The preparation of the FW involved homogenising each type of FW separately using a kitchen blender. While homogenising, the waste-to-deionised water ratio was adjusted to 1:2. Following that, the homogenised wastes were kept in a sealed plastic bag and stored in a chiller at 4 °C separately.

Sample Characterisation

The raw sample were characterised for their total solids (TS), moisture content (MC), volatile solids (VS), and fixed solids (FS) [31]. Firstly, the weight of empty crucible was measured ($W_{crucible}$) by using a digital analytical balance (Mettler Toledo JS1203C, Switzerland). Then, 4 g of raw sample was put in a crucible (W_{raw}) and dried in an oven for 24 h at 105 °C. After that, the dried sample was cooled inside a desiccator for 15 min before the measurement of the weight (W_{dry}). The TS and MC were calculated based on Eq. 1 and Eq. 2, respectively.

$$TS\,(\%) = \frac{W_{dry}}{W_{raw}} \times 100\% \tag{1}$$

$$MC\,(\%) = \frac{W_{raw} - W_{dry}}{W_{dry}} \times 100\% \tag{2}$$

The dried sample was then used for VS determination. It was started by igniting the dried sample in a muffle furnace for 5 h at 550 °C. Following that, the sample was cooled in a desiccator and weighted ($W_{volatile}$). The $W_{volatile}$ was used to determine the VS content according to Eq. 3.

$$VS\,(\%) = \frac{W_{volatile}}{W_{dry}} \times 100\% \tag{3}$$

FS was obtained by repeating the ignition process every 30 min at 550 °C, cooled, and weighted until the changes recorded were less than 4% and recorded as W_{fixed}. FS was calculated according to Eq. 4.

$$FS\,(\%) = \frac{W_{fixed} - W_{crucible}}{W_{fixed}} \times 100\% \tag{4}$$

Hydrothermal Pretreatment

Two different waste-to-water ratios of 1:2 and 1:4 were used. The initially prepared FW with waste-to-water ratio of 1:2 was further added with water to adjust the waste-to-water ratio to 1:4. The prepared FW (100 g, wet basis) was then placed into a 250 mL conical flask and subjected to hydrothermal pretreatment at different temperatures (100, 105, 110, 115, and 120 °C) and incubation times (30, 45, 60, 75, and 90 min). The hydrothermal pretreatment parameters were summarized in Table 1, and the experiments were run following a CCD experimental design. After treatment, the samples were centrifuged at 5,000 rpm for 10 min, resulting in separation into solid and liquid phases. The liquid phase (supernatant) was stored at 4 °C for reducing sugar and protein analysis, while the solid phase was used for lipid analysis.

Analysis

Reducing Sugar Analysis

Phenol-sulphuric acid method was used to determine the reducing sugar content of the FW [32]. The 5% (w/w) phenol solution was prepared by homogenising 5 g phenol pellet with 95 mL distilled water and was kept in a dark condition. For the working solution, 2.5 mL concentrated sulphuric acid was mixed with 1 mL FW supernatant, and 0.5 mL of 5% (w/w) phenol solution. The mixture was then vortexed and incubated in room temperature for 30 min. The absorbance reading of the mixture was determined at 490 nm by using UV-Vis spectrophotometry.

Protein Analysis

Bradford assay was used to determine the protein concentration in the treated waste [33]. The Bradford reagent was prepared by adding 20 mg Coomassie Brilliant Blue G-250 into 20 mL methanol. Then, 40 mL of 85% (w/v) orthophosphoric acid was mixed into the mixture until the colour changes from blue to reddish. Subsequently, distilled water was added until the final volume of 400 mL. The working

Table 1 The numeric and categoric factor values for independent variables

Independent variable	Range and level					
Numeric factors						
Temperature (°C)	100	105	110		115	120
Incubation period (mins)	30	45	60		75	90
Categoric factor						
Waste-to-water ratio	1:2			1:4		

solution was prepared by mixing 0.25 mL FW supernatant with 2.5 mL Bradford reagent in a test tube. Then, it was vortexed and incubated at room temperature for 2 min. The protein concentration was analysed by obtaining the absorbance reading of mixture at 595 nm. The bovine serum albumin (BSA) standard curve was used as a reference.

Lipid Analysis

The raw and treated KW were subjected to Bligh and Dyer method for lipid determination while the lipid content in the GW and WW were assumed to be negligible [34]. The KW of 4 g was mixed with 20 mL chloroform, 20 mL methanol and 10 mL distilled water. The mixture was centrifuged at 5,000 rpm for 10 min to separate the methanol and chloroform layer. The chloroform layer (5 mL) was placed onto a pre-weighted aluminium dish (W_0) and dried in an oven at 105 °C for 15 min. The weight of dried dish was denoted as W_1 and the lipid content was calculated according to Eq. 5, where V_a is total aliquot volume and V_c is chloroform amount used in the analysis.

$$\text{Lipid content (g)} = \frac{(W_1 - W_0) \times V_c}{V_a} \tag{5}$$

Table 2 The designated CCD experiments in sequence

Std. order	Temperature (°C)	Incubation period (min)	Waste-to-water ratio
1	105	45	1:2
2	115	45	1:2
3	105	75	1:2
4	115	75	1:2
5	100	60	1:2
6	120	60	1:2
7	110	30	1:2
8	110	90	1:2
9	110	60	1:2
10	110	60	1:2
11	110	60	1:2
12	105	45	1:4
13	115	45	1:4
14	105	75	1:4
15	115	75	1:4
16	100	60	1:4
17	120	60	1:4
18	110	30	1:4
19	110	90	1:4
20	110	60	1:4
21	110	60	1:4
22	110	60	1:4

Experimental Design Based on Response Surface Methodology (RSM)

Design Expert (DE) software (Version 13.0.5, State-Ease) was used to determine the best experimental parameters for FW pretreatment process. A CCD was designed to get the best amount of carbon source (glucose), protein, and lipid, contributing to 22 experimental runs with triplicates at centre points. CCD with 5 levels and 3 independent variables (2 numeric and 1 categoric variable) were created. The numeric variables consisted of temperature (°C), incubation period (minutes), and waste-to-water ratio for categoric. The running order of experiments that the software has set, as shown in Table 2 was strictly followed without changing the position to avoid any bias in the analysis [35]. Table 1 shows the list of variable ranges, in which low and high ranges are according to the proposed range. The data was later validated statistically using variance analysis (ANOVA). The second-order polynomial equation was created (Eq. 6) based on the variables present to analyse the effect of independent variables towards the response.

$$\begin{aligned} Y =& \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 \\ &+ \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \end{aligned} \tag{6}$$

Where Y represents the response value, which is glucose content, β_0 is the constant coefficient, β_1, β_2, and β_3 are the linear parameters, while β_{12}, β_{13}, β_{23} cover the cross-product coefficients. Quadratic terms will only appear in numeric factors β_{11} and β_{22}. The temperature, incubation periods, and waste-to-water ratio contribute to X_1, X_2, X_3 terms, respectively. The RSM analysis in this study focuses exclusively on glucose content as the response variable (y-value), due to glucose is a key macronutrient influencing bacterial cellulose (BC) production [36]. Although protein and lipid analyses were also conducted on FW, they were not included in the RSM model, as the primary objective was to optimize glucose availability for bacterial cellulose synthesis.

Utilization of Treated Food Waste for Bacterial Cellulose (BC) Production

A 10% concentration of *Komagataeibacter nataicola* inoculum was used for bacterial cellulose (BC) production through fermentation in yeast-glucose-calcium carbonate (YGC) media and treated food waste (FW) media for 16 days under slow agitation mode. The YGC media was prepared by dissolving 50 g/L D-glucose, 5 g/L yeast extract, and 12.5 g/L calcium carbonate in distilled water, followed by pH adjustment to 5 using 2 M acetic acid. The treated FW media was prepared by centrifuging at 10,000 rpm for 5 min, and the supernatant was collected. Both the YGC media and FW supernatant were sterilized via autoclaving at 121 °C for 15 min. Post-sterilization, 150 mL of both types of growth media were inoculated with 15 mL of *K. nataicola* and incubated at 30 °C under slow agitation (150 rpm) for 16 days. BC pellicle formation was observed on the surface of the media. The wet BC pellicle was harvested and purified by immersion in 1 M NaOH at 80 °C for 2 h, then rinsed with tap water until a neutral pH was achieved [37].

Results and Discussion

Raw Material Characteristics

The characteristics of homogenised FW including the TS, MC, VS, FS, and their macronutrients are summarised in Table 3.

Based on Table 3, the MC recorded for KW was relatively low (64.90%) compared to GW and WW, which were 92.45% and 96.82%, respectively. MC refers to the amount of water content in the waste material, which is greatly influenced by the type of waste and storage method. Most vegetables and fruits have high MC, between 70.00 and 95.00%, which causes the life expectancy of the food to be short if it is not stored correctly. The MC for cooked food, such as chicken, fish, or meat, is relatively low (50.00–75.00%), suggesting its longer shelf life [38]. In addition, the MC significantly affects the handling requirements of each raw material. Materials with MC that are too high will likely employ more complex processing steps as such require lower storage temperature, moisture reduction and larger unit operation for sterilisation. TS and VS for KW were relatively high, which are 35.11% and 34.10%, respectively. Followed by GW containing around 7.55% (TS) and 7.03% (VS). WW recorded the least amount of TS and VS, which are 3.19% and 2.73%, respectively. While VS represents the fraction of the total solids in a sample that can be volatilised or burnt off when the sample is exposed to high

Table 3 The characteristics of untreated FW

Properties	Food Waste (%) *		
	Kitchen waste (KW)	Guava waste (GW)	Watermelon waste (WW)
MC	64.90 ± 1.077	92.45 ± 0.137	96.82 ± 0.027
TS	35.11 ± 1.077	7.55 ± 0.137	3.19 ± 0.027
VS	34.10 ± 0.615	7.03 ± 0.174	2.73 ± 0.014
FS	0.10 ± 0.045	0.24 ± 0.022	0.58 ± 0.041
Glucose**	216.357 ± 1.190	123.397 ± 1.745	91.691 ± 0.885
Protein**	0.071 ± 0.001	0.024 ± 0.002	0.012 ± 0.001
Lipid***	0.0088 ± 0.001	-	-

*The values are means ± standard deviation of three replications

** The unit sets as mg/mL

***The unit sets as g

temperatures (600 °C), TS refers to the part of a substance or sample that remains as solid material after all the moisture or water content has been eliminated [39]. It shows that the solid material present in the KW was relatively higher than GW and WW. The TS content of GW and WW can vary depending on factors such as ripeness, variety, and growing conditions [31]. Usually, GW contains around 9.00% − 14.00% TS, while WW has around 5.00% − 7.00% of TS. The TS consists of sugars (like fructose and glucose), dietary fibre, pectin, vitamins (particularly vitamin C), and minerals. Meanwhile, VS primarily consist of organic materials that can be vaporized when subjected to heat, in which it represents the organic compound contains in the TS. The final product of VS commonly biogas, specifically methane [40]. FS contribute to the total solids content of food waste, which typically comprises a mix of fibers, residual proteins, and undigested material [41]. The compositions of KW collected will differ according to different types of food and diet practised by a place or region. The information is important to determine the appropriate processing step for the processing of certain waste material. One with very high solid content will likely require more operational cost from the extensive solid separation step and waste treatment process.

KW showed higher levels of glucose (216.357 mg/mL), protein (0.071 mg/mL), and lipid (0.009 g) than GW and WW. This is due to the higher content of nutrients and organic matter in KW. In addition, fruits contain higher levels of fructose than glucose. The rate of glucose production for fruits is also affected by the level of ripeness and growing conditions of the fruits. The riper the fruit, the higher the sugar content. The data are in line with previous study that recorded the amount of glucose in fresh and fully ripe conditions of guava of 3.00% and 5.42%, respectively [42]. A study on different fruits found that fructose content in watermelon juice was the highest (0.6 mg/mL), followed by glucose (0.5 mg/mL) [43]. Similarly, Sabeetha et al. [44] also recorded lower glucose than fructose, which was 29.510 mg/g and 49.590 mg/g respectively. KW recorded a higher glucose content due to the variation of food component and food consumed by people. Organic material produced in the kitchen and often thrown away is called KW, sometimes known as kitchen scraps or FW. Food trimmings, food scraps, and other organic waste that is no longer fit for human consumption are all included in the KW [8]. As much as 69.00% and 62.70% sugar were recorded in KW, which shows that more than 50.00% of the nutritional composition in KW is made up of sugar [45, 46]. High sugar content in KW is due to the presence of various food residues, including leftover fruits, vegetables, and processed foods. These carbohydrates, upon decomposition, yield simple sugars through enzymatic breakdown or fermentation processes

Table 4 The result of CCD for hydrothermal treated KW

Std. order	Temp (°C)	Time (min)	Kitchen waste: water ratio	Glucose (mg/mL)	Protein (mg/mL)	Lipid (g)
1	105	45	1:2	200.43	0.073	0.011
2	115	45	1:2	238.926	0.074	0.007
3	105	75	1:2	320.064	0.072	0.011
4	115	75	1:2	379.308	0.087	0.012
5	100	60	1:2	182.513	0.083	0.007
6	120	60	1:2	443.202	0.090	0.014
7	110	30	1:2	201.430	0.084	0.007
8	110	90	1:2	341.667	0.067	0.012
9	110	60	1:2	317.586	0.084	0.012
10	110	60	1:2	317.152	0.086	0.009
11	110	60	1:2	317.587	0.078	0.009
12	105	45	1:4	111.485	0.052	0.004
13	115	45	1:4	142.362	0.054	0.004
14	105	75	1:4	174.212	0.068	0.005
15	115	75	1:4	283.661	0.059	0.005
16	100	60	1:4	123.734	0.056	0.003
17	120	60	1:4	292.299	0.075	0.008
18	110	30	1:4	110.608	0.050	0.002
19	110	90	1:4	270.825	0.041	0.006
20	110	60	1:4	179.086	0.044	0.005
21	110	60	1:4	171.172	0.047	0.005
22	110	60	1:4	192.212	0.047	0.006

Table 5 The result of CCD for hydrothermal treated GW

Std. order	Temp (°C)	Time (min)	Guava waste: water ratio	Glucose (mg/mL)	Protein (mg/mL)
1	105	45	1:2	201.076	0.039
2	115	45	1:2	348.262	0.038
3	105	75	1:2	259.790	0.061
4	115	75	1:2	374.000	0.035
5	100	60	1:2	201.880	0.056
6	120	60	1:2	421.453	0.019
7	110	30	1:2	259.790	0.061
8	110	90	1:2	317.699	0.040
9	110	60	1:2	302.420	0.044
10	110	60	1:2	308.852	0.042
11	110	60	1:2	300.809	0.042
12	105	45	1:4	113.407	0.020
13	115	45	1:4	230.835	0.025
14	105	75	1:4	156.035	0.051
15	115	75	1:4	298.396	0.031
16	100	60	1:4	101.343	0.045
17	120	60	1:4	319.308	0.013
18	110	30	1:4	182.577	0.022
19	110	90	1:4	284.723	0.029
20	110	60	1:4	280.701	0.029
21	110	60	1:4	265.420	0.033
22	110	60	1:4	248.529	0.033

[47]. It aligns with the results of this study, which show that glucose levels are higher than proteins and lipids. High sugar content is an important criterion for growth medium substitute in bacterial fermentation as it serves as carbon source that is essential for cell growth [48].

Central Composite Design (CCD) Experimental Result

Three reaction parameters (temperature, incubation period, and waste-to-water ratio) were studied for their effects on reducing sugar, protein, and lipid content following hydrothermal pretreatment. The condition settings employed in the current investigation (100 °C < temp < 120 °C; 30 min < time < 90 min; 1:2 < waste to water ratio < 1:4) were chosen based on predetermined parameters from previous study [27]. The results of CCD experiments for KW, GW, and WW are presented in Tables 4, 5, and 6, respectively. The data showed that the amount of glucose, protein, and lipid in treated FW was significantly improved compared to the untreated FW. The highest amount of glucose recorded for KW, GW, and WW were 443.202 mg/mL, 421.453 mg/mL, and 295.484 mg/mL, respectively, given by set of parameters including the temperature of 120 °C, 60 min incubation time and the waste-to-water ratio of 1:2. The water used during pretreatment also helps in solubilising the FW, which later increase the degradation rate and substrate surface area towards complex cellulose, hemicellulose and lignin [27]. The structure of complex compounds in the FW is quite substantial due to long-chain glucose units that bond with hydrogen bonds that turn into fibres [49]. Thus,

Table 6 The result of CCD for hydrothermal treated WW

Std. order	Temp (°C)	Time (min)	Watermelon waste: water ratio	Glucose (mg/mL)	Protein (mg/mL)
1	105	45	1:2	205.580	0.014
2	115	45	1:2	159.301	0.014
3	105	75	1:2	215.811	0.019
4	115	75	1:2	252.921	0.013
5	100	60	1:2	121.708	0.018
6	120	60	1:2	295.484	0.010
7	110	30	1:2	210.280	0.011
8	110	90	1:2	227.778	0.014
9	110	60	1:2	211.709	0.011
10	110	60	1:2	211.467	0.015
11	110	60	1:2	213.349	0.014
12	105	45	1:4	100.426	0.010
13	115	45	1:4	94.925	0.011
14	105	75	1:4	116.158	0.016
15	115	75	1:4	189.124	0.012
16	100	60	1:4	82.474	0.015
17	10	60	1:4	194.867	0.008
18	110	30	1:4	100.550	0.010
19	110	90	1:4	180.534	0.012
20	110	60	1:4	128.174	0.012
21	110	60	1:4	119.391	0.013
22	110	60	1:4	114.083	0.012

to obtain more nutrients from FW, pretreatment is required to break the complex structure. Effective solubilisation of organic material was reported when the ratio of solids-to-water between 1:1 and 1:2 [50]. In this study, the solids-to-water ratio of 1:2 was recorded higher yield than those of 1:4 ratio. Chua et al. [27] reported that the hydrothermal pretreatment at 120 °C, 75 min, and 1:2 solids-to-water ratio gave comparably high glucose yield than those of enzymatic pretreatment.

The protein content showed a slight increase after the pretreatment, the KW recorded more protein content (0.090 mg/mL), followed by GW (0.061 mg/mL) and WW (0.019 mg/mL) at 120 °C; 60 min; 1:2 waste-to-water ratio for KW and 105 °C; 75 min; 1:2 waste-to-water ratio for GW and WW. The KW has higher protein concentration due to various components of the food source, like chicken, meat, egg, and many more. The three-dimensional shape of proteins given its specific function. High temperatures can damage these structures by causing the protein to unfold or change shape, which might result in a loss of function. At high temperatures, the weak connections that support the protein's structure, such as hydrogen bonds and van der Waals forces, are broken, which results in the protein losing its natural shape [51]. The availability and use of proteins and carbohydrates as macronutrients for microbial development can affect microorganisms' metabolism, growth, and survival. For bacteria, carbohydrates are their primary source of energy. It converts carbohydrates into adenosine triphosphate (ATP), the cell's energy currency, through glycolysis and oxidative phosphorylation. ATP fuels various cellular functions [52]. On the other hand, microbes use proteins for structural functions. Flagella, essential in motility of bacteria, are made up of proteins such as flagellin. Structural proteins are present in fungi's cytoskeleton and cell wall [53]. It also acts as a nitrogen source for growing bacteria [54]. The results obtained suggests that the parameters used for the pretreatment able to retain and slightly increase the protein content of the waste. However, the functionality and denaturation of the protein can only be confirmed with detail analysis of the sample such as differential scanning calorimetry (DSC), Raman spectroscopy, and the use of denaturants like urea and guanidinium chloride, which help elucidate the conformational stability and potential unfolding mechanisms of proteins [55–57].

Lipid analysis was only conducted on the treated KW using the Bligh and Dyer method. A low lipid concentration was recorded at a temperature of 110 °C, 30 min incubation time, and 1:4 waste-to-water ratio (0.002 g). Several reactions occur when lipids (fats and oils) are exposed to high temperatures with oxidation as the undesired one. It causes the production of free radicals and several chemical compounds, such as aldehydes and ketones, which can develop off-flavours and odours in food items [58]. Lipid concentrations that are too high can stop microbial development. This inhibition may be brought on by the build-up of lipids on cell surfaces, which may obstruct waste disposal and nutrient absorption [59]. In the production of bacterial cellulose (BC), Wu et al. [60] have found that oil and fat were interfering with the BC yield due to the obstruction of oxygen transfer into the media. All the tested KW generally had significantly low lipid content.

Reducing Sugar Released from Treated KW, GW and WW

Analysis of Variance (ANOVA)

The ANOVA analysis was conducted to assess the variables and potential interactions between them. The independent variables tested were temperature, incubation period, and waste-to-water ratio. Transformation of the data was conducted on the KW and WW response data set to minimize the effect of variance and improve the fit of the model [61, 62]. Nevertheless, the lack-of fit of WW is still showing a significant value, suggesting that a substantial discrepancy exists. In this scenario, the variability among replicate measurements around their mean values is markedly smaller compared to the variability of the design points around the predicted values. In simpler terms, this means that there are influential factors or variables not accounted for in the

Table 7 ANOVA for best parameters of KW pretreatment in glucose production

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	8.233e-05	8	1.037e-05	48.126	< 0.0001	Significant
A-Temp	1.722e-05	1	1.722e-05	80.547	< 0.0001	
B-Time	2.649e-05	1	2.649e-05	123.885	< 0.0001	
C-Food waste-to-water ratio	3.099e-05	1	3.099e-05	144.929	< 0.0001	
AB	2.709e-10	1	2.709e-10	0.0013	0.9721	
AC	1.502e-06	1	1.502e-06	7.023	0.0200	
BC	3.997e-06	1	3.997e-06	18.691	0.0008	
A^2	6.609e-07	1	6.609e-07	3.091	0.1022	
B^2	1.961e-06	1	1.961e-06	9.105	0.0099	
Residual	2.780e-06	13	2.138e-07			
Lack of Fit	2.573e-06	9	2.859e-07	5.524	0.0575	Not significant
Pure Error	2.070e-07	4	5.175e-08			
Cor Total	8.570e-05	21				
R-squared	0.9673					

Table 8 ANOVA for best parameters of GW pretreatment in glucose production

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	126905.398	8	15863.175	32.544	< 0.0001	Significant
A-Temp	81218.897	1	81218.897	166.622	< 0.0001	
B-Time	11077.192	1	11077.192	22.725	0.0004	
C-Guava waste: water ratio	30174.04	1	30174.04	61.903	< 0.0001	
AB	8.086	1	8.086	0.017	0.8995	
AC	0.941	1	0.941	0.002	0.9656	
BC	487.279	1	487.279	0.999	0.3356	
A^2	2565.786	1	2565.786	5.264	0.0391	
B^2	2531.642	1	2531.642	5.194	0.0402	
Residual	6336.772	13	487.444			
Lack of Fit	5782.602	9	642.510	4.637	0.0769	Not significant
Pure Error	554.169	4	138.542			
Cor Total	133237.193	21				
R-squared	0.9524					

Table 9 ANOVA for best parameters of WW pretreatment in glucose production

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	1.168e-04	8	1.460e-05	12.223	< 0.0001	Significant
A-Temp	2.194e-05	1	2.194e-05	18.328	0.0009	
B-Time	1.614e-05	1	1.614e-05	13.198	0.0030	
C-Watermelon waste: water ratio	6.345e-05	1	6.345e-05	53.285	< 0.0001	
AB	4.487e-06	1	4.487e-06	3.749	0.0749	
AC	2.668e-06	1	2.668e-06	2.229	0.1593	
BC	5.949e-06	1	5.949e-06	4.970	0.0441	
A^2	1.546e-06	1	1.546e-06	1.292	0.2763	
B^2	2.835e-07	1	2.835e-07	0.237	0.6346	
Residual	1.550e-05	13	1.197e-06			
Lack of Fit	1.503e-05	9	1.677e-06	14.238	0.0106	Significant
Pure Error	4.710e-07	4	1.178e-07			
Cor Total	1.323e-04	21				
R-squared	0.8827					

model that are contributing to the observed variation. Such possible factors are omitted variable, non-linearity, measurement error, and interaction effect [63–66]. Tables 7, 8, and 9 present the differences in ANOVA data for KW, GW, and WW, respectively, in glucose yield after hydrothermal pretreatment. The data shows the second-order quadratic model with a significant F-value for KW (48.13), GW (32.54), and WW (12.22). There is only a 0.01% chance that

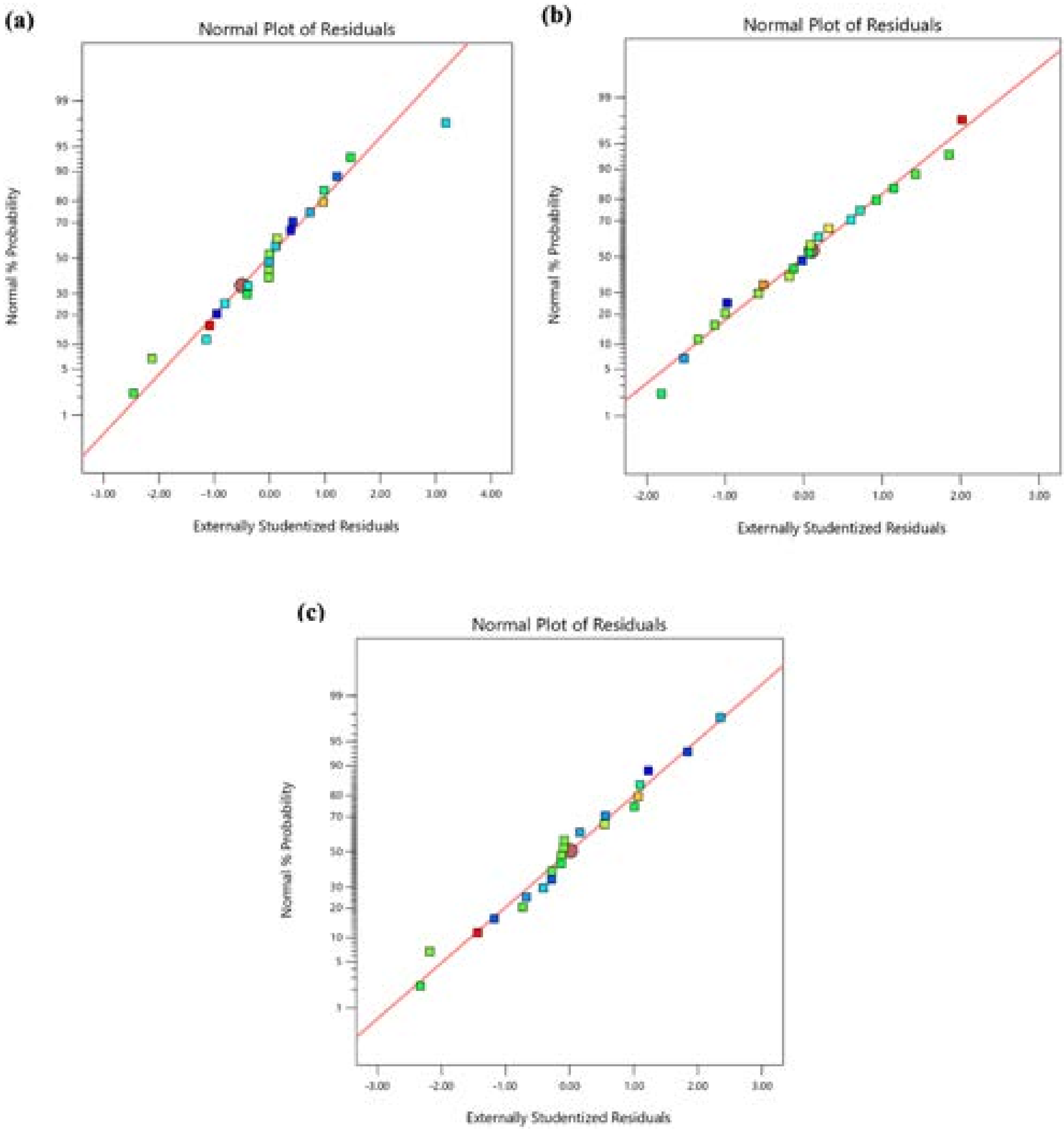

Fig. 1 The normal probability residual of (**a**) KW, (**b**) GW, and (**c**) WW

n F-value this large could occur due to noise. The lack-of-fit for the models is also not significant for KW and GW. The significant models given by *p*-value that is less than 0.05 were A, B, C, AC, BC and B^2 for KW, A, B, C, A^2 and B^2 for GW, and A, B, C and BC for WW.

The final equation in coded factor terms of KW, GW, and WW were presented in Eq. 7, Eq. 8, and Eq. 9, respectively. The intercept (0.0043, 284.46, 0.0065) represents the estimated glucose content when all factors (A, B, C) are at their zero levels. The coefficients for A, B, and C indicate the linear effects of each factor on glucose content. The coefficients for interaction terms (AB, AC, BC) suggest the combined effects of two factors on glucose content. The coefficients for squared terms (A^2, B^2) represent the curvature or non-linear effects of factors A and B on glucose content. The signs of the coefficients (positive or

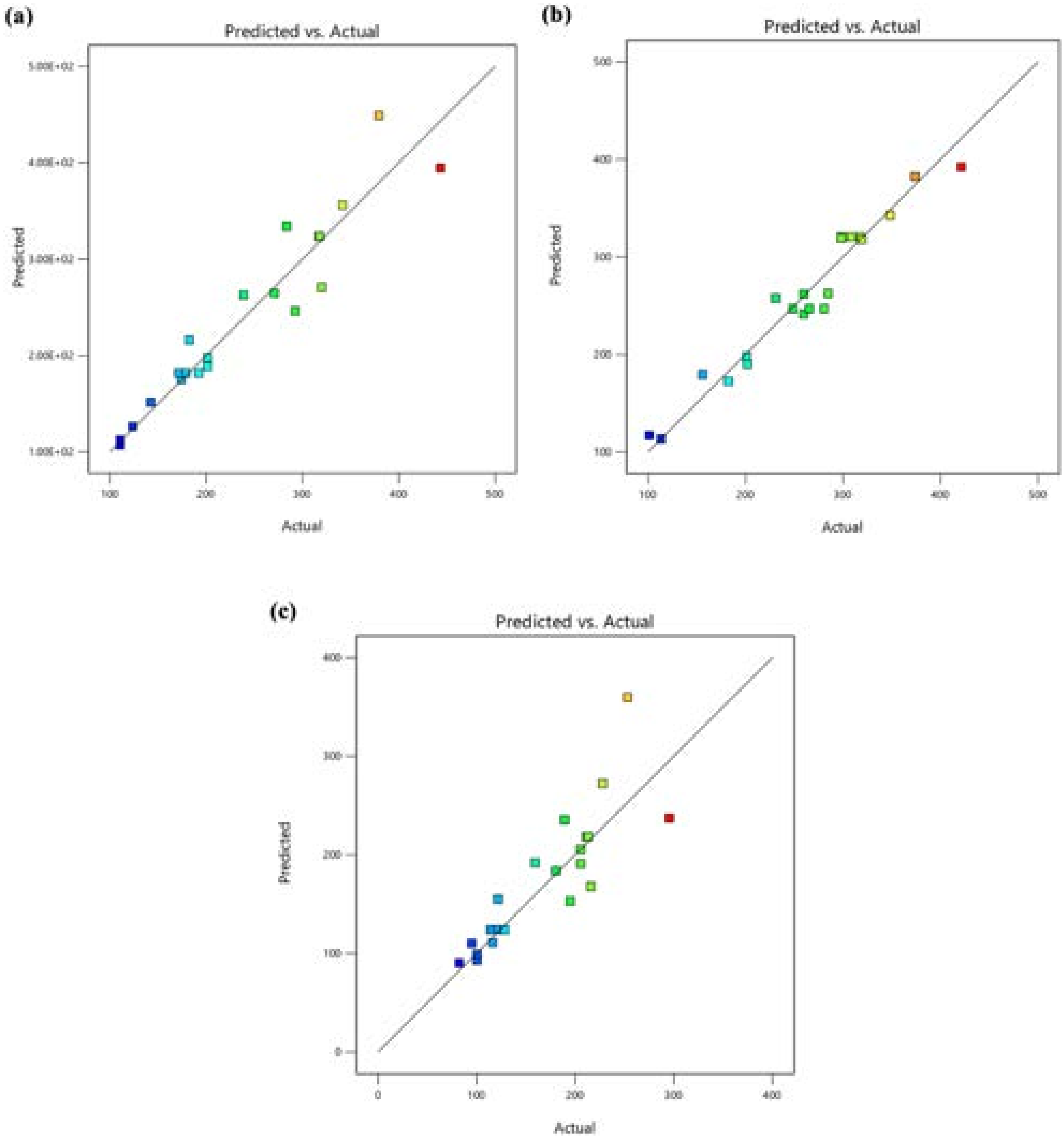

Fig. 2 The correlation between predicted and actual conversion of (**a**) KW, (**b**) GW, and (**c**) WW

negative) indicate whether the factors have a positive or negative impact on glucose content. The significant lack-of-fit for the WW model indicating it might not adequately describe the response surface, probably due to the complexity of the model or additional curvature. Besides that, the validity of regression models was assessed using the normal probability residual (NPR) graph (Fig. 1) and the predicted vs. actual plot graphs (Fig. 2). In Fig. 1, all NPR graphs for KW (a), GW (b), and WW (c) displayed straight diagonal lines, indicating that the residuals from the regression analysis followed a normal distribution. Figures 2 (a), (b), and (c) depict the relationship between predicted (y-axis) and actual (x-axis) values, showing nearly straight lines for all waste types, aligning with a normal distribution pattern.

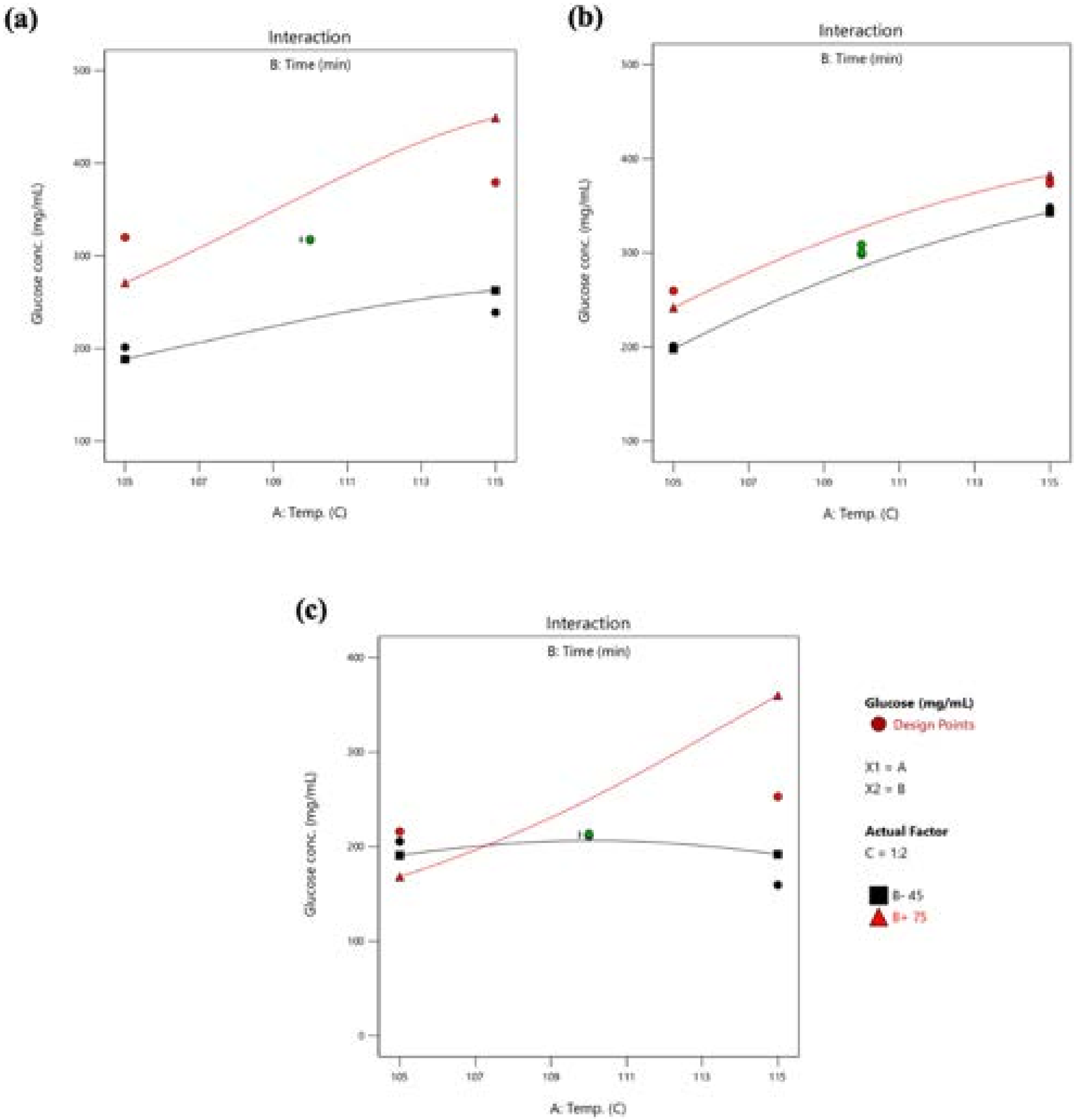

Fig. 3 The interaction between incubation temperature and time for glucose production; (**a**) KW; (**b**) GW; (**c**) WW, in which black square shape and red triangle shape indicate period of 45 min and 75 min, respectively

This alignment suggests that the observed data conforms to the expected normal distribution. However, points are scattered at high glucose concentration, suggesting that the predictions at high factor levels may be less reliable. This scattering pattern was observed exceptionally well on WW. More data at higher factor levels would probably yield a better fit in the actual versus predicted graph.

$$Y_{KW}=0.0043-0.0010A-0.0013B+0.0012C+5.819e\text{-}06AB \\ -0.0003AC-0.0005BC+0.0002A^2+0.0004B^2 \tag{7}$$

$$Y_{GW}=284.46+71.25A+26.31B-37.03C-1.01AB \\ -0.2426AC+5.52BC-15.70A^2-14.97B^2 \tag{8}$$

$$Y_{WW}=0.0065-0.0012A-0.0010B+0.0017C \\ -0.0007AB-0.0004AC-0.0006BC+0.0004A^2-0.0002B^2 \tag{9}$$

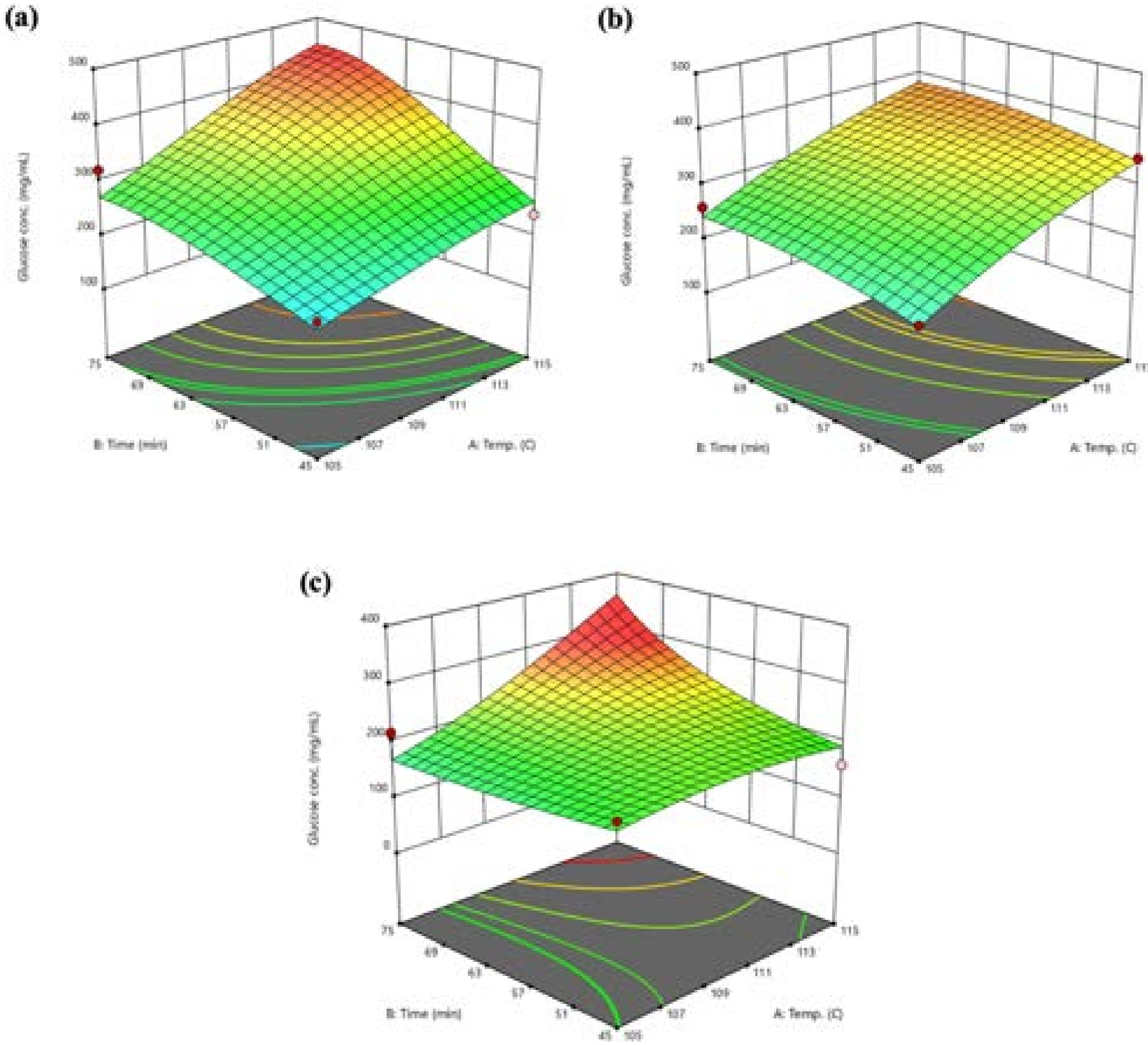

Fig. 4 The comparison of glucose produced between different waste, which are kitchen, guava and watermelon wastes shown as above (3D graph plotting); (**a**) KW, (**b**) GW and (**c**) WW

Where A is temperature, B is incubation time, and C is waste-to-water ratio. The AB, AC, BC, A^2, and B^2 shows the interaction effects between the factors. Y indicates the glucose concentration.

Main and Interaction Effects

The quadratic model was used to construct interaction plots, contour plots, and a 3D response surface curve to present the interaction between the response and the variables. These plots are valuable for visualising the behaviour of the response across different factor levels and identifying optimal conditions. Figure 3 shows the interactions between temperature (A) and time (B) of hydrothermal pretreatment on glucose content from treated FW. All interaction plots indicate an increase in glucose concentration with the increase in pretreatment time and temperature. Figure 3 (a) and (b) recorded glucose content at the same rate for short incubation periods across low to high temperatures. This suggests that KW and GW have relatively strong structures, requiring a longer incubation period compared to WW. GW comprises two types of cell wall tissues, which are stone cells and parenchyma cells. Stone cells are heavily lignified, giving them a woody texture that imparts a sandy or gritty sensation when eaten. This trait makes guava fruit resistant to enzymatic breakdown [67]. Thus, extensive pretreatment

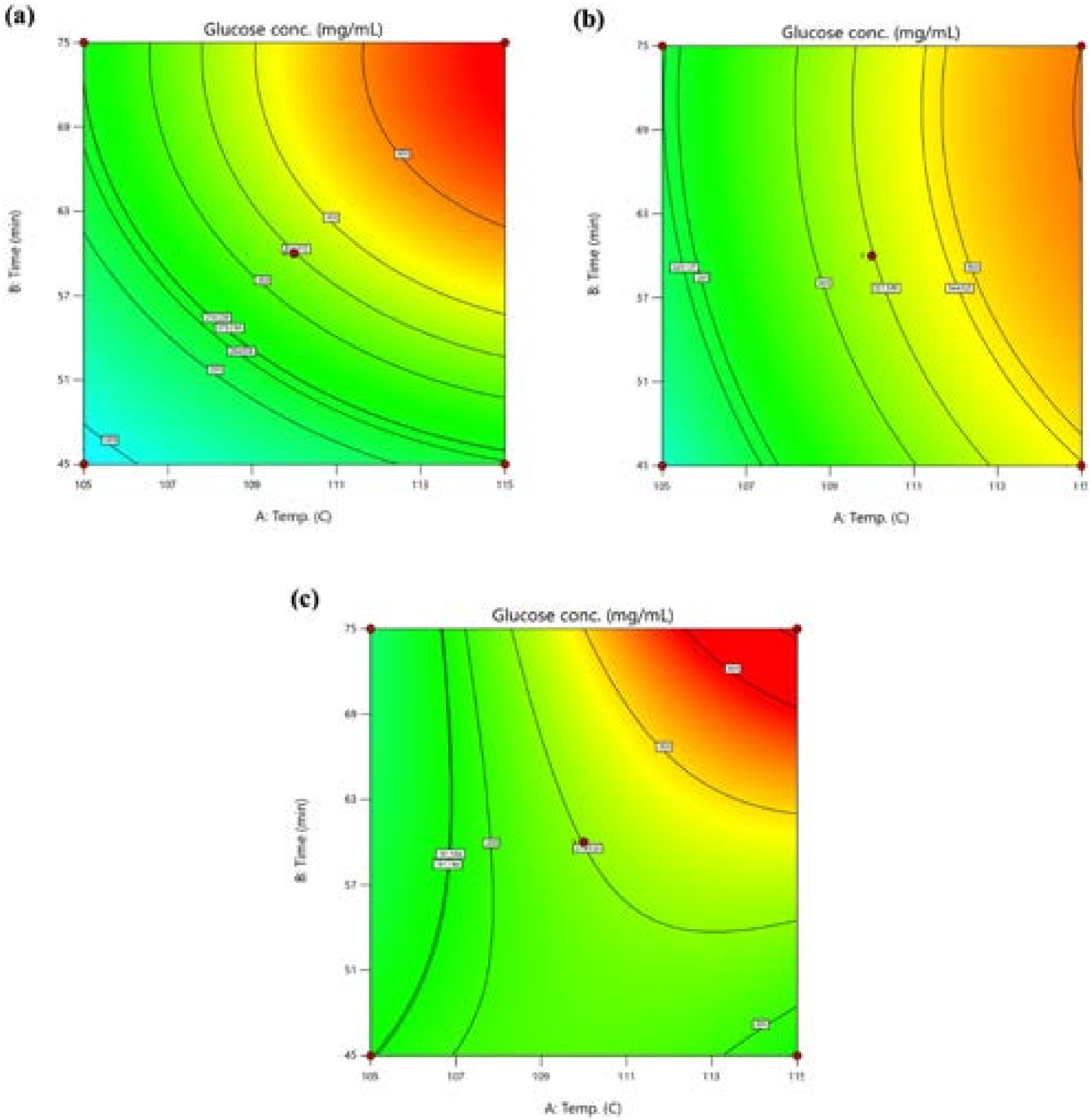

Fig. 5 The contour plot between incubation temperature and time towards glucose production of (**a**) KW, (**b**) GW, and (**c**) WW

is required to break the cell wall of GW, which might include pretreatment at prolonged time, higher temperature,

Table 10 Constraints of factors and responds

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A: Temp	In range	105	115	1	1	3
B: Time	In range	45	75	1	1	3
C: FW: water ratio	In range	1:2	1:4	1	1	3
Glucose (KW)	Maximise	110.608	443.202	1	1	3
Glucose (GW)		101.343	421.453	1	1	3
Glucose (WW)		82.474	295.484	1	1	3

Table 11 The solution of best parameters for maximum glucose production suggested by RSM

Sample	Temp (°C)	Time (min)	FW: water ratio	Glucose (mg/mL)		Error (%)
				Predicted	Actual	
KW	115.0	75.0	1:2	449.031	379.308	15.53
GW	115.0	75.0	1:2	382.033	374.000	2.10
WW	115.0	75.0	1:2	358.890	252.921	29.53

or a combination with other treatment. Figure 3 (c) recorded a high amount of glucose for both short and long incubation times, corresponding with the increase in incubation temperature. All interaction plots show that the longer the pretreatment duration, the higher the glucose concentration recorded.

Because of the importance of the quadratic terms shown in Tables 7, 8, and 9, any two of the design parameters could interact with one another. The influence of process parameters and outcomes can also be visualised using 3D-response surface and contour plots, presented in Fig. 4 (a-c) and Fig. 5 (a-c), respectively. The shape of the distinct surface illustrated both significant and insignificant effects between the independent and dependent experimental factors. The curvature represented in Fig. 4 (a-c) shows that an interactive outcome of temperature and time used for the pretreatment process on glucose content was detected. A two-dimensional (2D) graph depicting the response in relation to different combinations of factors was created, called as contour plot (Fig. 5a-c). The surface plot for incubation temperature indicated the possibility that the optimal point lies outside the experimental range. It suggests that the true optimal conditions for the response variable have not been explored within the current experimental design. However, this study showed that pretreatment efficiency improved with increasing temperature and time, with KW showing the most notable interaction between both factors. GW and WW show weaker interaction; however, increasing temperatures and times still raise the glucose release after the pretreatment process.

Optimization and Validation of Experiment

This pretreatment procedure utilises a set of optimisation parameters suggested by CCD. Both the incubation temperature and time factors were adjusted within a specified range to maximise desirability, while the glucose yield was optimised to achieve the highest possible value. Constraints for this optimization, which were set in the software, are tabulated in Table 10.

Table 11 summarizes the optimum conditions suggested by RSM with satisfactory desirability values of 1.000 for all types of waste. The actual glucose yields for all types of FW were lower than the predicted values, with the percentage of error within acceptable range of less than 30%

Table 12 BC yield using KW, GW and WW treated by ideal parameters

Waste	Glucose concentration (mg/mL)		BC wet mass (g/L)
	Before fermentation	After fermentation	
YGC (control)	86.914 ± 1.38	73.836 ± 0.72	16.75 ± 0.32
KW	240.486 ± 7.76	152.014 ± 4.83	26.59 ± 0.39
GW	243.897 ± 2.20	122.673 ± 1.82	38.15 ± 0.22
WW	206.062 ± 1.11	116.592 ± 0.80	30.28 ± 0.02

[68]. However, it was observed that the RSM model underestimated the optimum parameters and favoured the interior point rather than the edge point (120 °C), which recorded a higher glucose yield for all types of FW with parameters combination of 60 min incubation time and 1:2 waste-to-water ratio. Further experiments with temperature parameters higher than 120 °C could potentially provide more clarification. Nevertheless, a higher temperature is impractical in bioprocessing, especially those involving fermentation as the main process, which could be seen as a challenge for large scale adoption because it contradicts with the aim to have a more environmentally sustainable process.

The Potential of Treated FW for BC Production

The FW treated using ideal parameters suggested by the CCD were then used in BC production and the yield was compared to defined media to evaluate the effectiveness of treated FW as alternative substrates. Each treated FW has different amounts of reducing sugar, especially glucose, which plays an important role in BC yield. Table 12 represents the BC wet mass with a comparison of glucose concentrations before and after 16 days of fermentation. The glucose concentration of each FW was slightly reduced due to the dilution effect from the pH adjustment and inoculum addition. The BC wet mass (g/L) recorded from YGC media was 16.75 ± 0.32 with a glucose concentration (mg/mL) of 73.836 ± 0.72 at the end of BC fermentation. GW (38.15 ± 0.22) and WW (30.28 ± 0.02) recorded higher BC wet mass (g/L) than KW (26.59 ± 0.39), even though the glucose concentration (mg/mL) before fermentation in KW was greater, at 240.486 ± 7.76. This might be due to the presence of a larger oil layer on the surface of the media, which hindered oxygen penetration into the medium. *K. nataicola* is a strictly aerobic bacterium requiring oxygen to

Table 13 BC production from various FW carbon sources with comparative research findings

Waste	Species	Additional nutrient	BC yield*	References
Watermelon and pineapple peel	*Komagataeibacter hansenii* MCM B-967	Yes	30 g/L	[71]
40% of guava extract	*Gluconacetobacter xylinus* IFO 13,693	No	37.8 g/L	[72]
Starch and grease of kitchen waste	*Acetobacter xylinum* ATCC 23,767	Yes	2.07 g/L	[60]

**Wet basis*

survive. The bacteria may experience stress due to oxygen deprivation, leading to reduced growth rates, lower yields of desired products (like bacterial cellulose), and potentially higher mortality rates [69]. The amount of oil found in guava (0.0004–0.0007%) and watermelon (0.18%) is usually minimal since these fruits are not commonly associated with oil content in their flesh [44, 70].

All data here are exhibited in the form of mean ± SD.

In this study, the BC yield obtained using watermelon waste as an alternative carbon source was 30.28 g/L (wet basis). Direct comparison with the limited published study is challenging due to the different working parameters applied. For instance, as summarized in Table 13, Kumbhar et al. [71] reported a BC yield of 30 g/L in wet weight using a combination of watermelon and pineapple peels. Slightly higher BC production in their study might be due to the use of two FW, which are watermelon and pineapple. Additional nutrients also play an important role in enhancing BC productivity. The BC yield recorded by Wu et al. [60] was smaller (2.07 g/L) than that of the present study, which is 26.59 g/L, which might be due to the additional washing and enzymatic pretreatment steps applied in their study. The slightly higher yield of BC from GW media (38.15 g/L) than Manrique et al. [72] might be due to the shorter fermentation period applied in their study.

Conclusion

This research attempt to find the optimum hydrothermal pretreatment parameters for the release of high concentration of nutrients (reducing sugar, lipid, and protein) from FW. The data showed that the incubation temperature, time, and waste-to-water ratio indeed significantly improved the reducing sugar concentration in the treated FW, while the protein and lipid content were not significantly changed in all samples. The performance of the hydrothermal pretreatment can be anticipated from the established model with the suggested optimal incubation temperature of 115 °C for 75 min and a 1:2 waste-to-water ratio. Despite the theoretical inadequacy of certain fitted model, the amount of reducing sugar at the optimum conditions was increased by 75.3%, 203.1% and 175.8% compared to untreated FW for KW, GW, and WW, respectively. Future work could explore incubation temperatures higher than 120 °C (higher observed yield). The evaluation of the performance of treated FW as an alternative substrate for BC production has recorded BC pellicle in much higher yield compared to that of defined media. KW produced much lower BC than the other waste substrates despite its highest reducing sugar content, attributed to the presence of oil which inhibited oxygen transfer. An additional stage of oil separation could also be conducted to see the full potential of KW.

Author Contributions MIHN conducted the analysis and wrote the original manuscript. NM, MHR and CGK directed the research and edited the manuscript. All authors critically revised all versions of the manuscript and approved the final version.

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Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no conflict of interest.

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