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Solid–Liquid Extraction of Carbohydrates from Defatted Rice Bran Using Green Techniques: An Optimization Study

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Abstract: Defatted rice bran (DRB), a by-product of rice processing primarily used as animal feed, holds potential for food and pharmaceutical production applications due to its carbohydrate content. This study aimed to optimize and compare two solid–liquid carbohydrate extraction methods: ultrasound-assisted extraction (UAE) and hydrothermal treatment. A progressive experimental design strategy was employed for both methods. Carbohydrates were quantified using the Anthrone method, and FTIR analysis was conducted to identify functional groups. Optimal UAE conditions included a DRB-to-water ratio of 65 $g \cdot L^{-1}$, ultrasonic power of 350 W, and an extraction time of 20 min, yielding 60 $g_{CHO} \cdot 100\ g_{DRB}{}^{-1}$ with an extraction efficiency of 98.30%. For the hydrothermal method, the optimal conditions were a DRB-to-water ratio of 100 $g \cdot L^{-1}$, a pH of 6.0, and an extraction time of 35 min, achieving a yield of 48 $g_{CHO} \cdot 100\ g_{DRB}{}^{-1}$ with a 78.30% efficiency. The UAE method demonstrated higher efficiency, faster processing time, and lower operating temperatures compared to the hydrothermal treatment. FTIR analysis confirmed the successful identification of carbohydrate functional groups with spectral similarities to standard sugars. This study highlights the potential of DRB as a carbohydrate source and demonstrates the value of optimizing extraction methods to enhance yield and efficiency.

Keywords: ultrasound-assisted; hydrothermal; surface response; experimental design

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Academic Editor: Francesca Blasi

Received: 2 December 2024
Revised: 20 December 2024
Accepted: 24 December 2024
Published: 26 December 2024

Citation: Ferreira, A.L.; Peron-Schlosser, B.; Magro, D.R.; Spessato, A.; Baraldi, I.J.; Drunkler, D.A.; Colla, E. Solid–Liquid Extraction of Carbohydrates from Defatted Rice Bran Using Green Techniques: An Optimization Study. *Processes* **2025**, *13*, 30. https://doi.org/10.3390/pr13010030

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1. Introduction

Rice (*Oryza sativa*) is the third most produced cereal globally, with estimates indicating a worldwide production of approximately 522 million metric tons during the 2023/2024 season. Outside of Asia, Brazil ranks as the largest rice producer, with an estimated 7.2 million metric ton yield for the same period [1].

According to Dhankhar [2], rice kernels comprise approximately 69% starchy endosperm, 20% rice husk, and 11% rice bran. The primary by-products of rice processing include husk, broken rice, and the bran layer [3]. These by-products contain bioactive compounds and nutrients that can be extracted for food applications, generating additional income streams and promoting food sustainability [4].

Rice bran, the outer layer of the rice grain, accounts for approximately 8–12% of the total grain weight. The growing global demand for rice bran oil has concurrently driven the production of defatted rice bran (DRB), which represents about 78–85% (*w*/*w*) of the original rice bran [5]. DRB is commonly used as a low-cost ingredient in animal feed or

discarded [6]. Its composition typically includes 66% total carbohydrates, 15% proteins, 12% lipids, 11% moisture, and 7% ash [7]. These values, however, depend on the rice variety and the efficiency of the milling system [3]. Recent studies have explored the potential of DRB for diverse applications, including bioethanol production [8–11], functional cookies [6], vinegar production [12], and protein extraction [13].

Rich in complex carbohydrates, DRB offers significant functional and nutritional value, including prebiotic properties that benefit human health [14,15]. Carbohydrates extracted from rice bran have shown potential as safe and effective antitumor agents or functional food components [16]. These findings underscore the importance of developing and optimizing carbohydrate extraction methods.

Traditional techniques such as solid–liquid extraction (SLE) and liquid–liquid extraction (LLE) have long been used for carbohydrate recovery. However, conventional SLE methods often present drawbacks, including high energy consumption, lengthy extraction times, and the degradation of thermosensitive compounds. To address these challenges, environmentally friendly techniques have been developed, including ultrasound-assisted extraction (UAE) [17–19] and hydrothermal treatment [14,20,21].

UAE relies on cavitation, a process in which particle collisions and shock waves disrupt cells, reduce particle size, and enhance mass transfer, thereby improving extraction efficiency [17]. In contrast, hydrothermal treatment utilizes high temperatures and pressures to hydrolyze polysaccharides, with hydronium ions acting as catalysts [21].

This study aimed to optimize and compare two green SLE methods—hydrothermal treatment and UAE—for carbohydrate recovery from DRB. The comparison of green methods for carbohydrate extraction from DRB remains underexplored in the literature, which gives this study particular relevance. Furthermore, the application of optimization techniques to identify the ideal extraction conditions and maximize carbohydrate recovery stands out as a significant contribution to advancing the valorization of rice by-products.

2. Materials and Methods

2.1. Raw Material

The DRB in pellet form was supplied by IRGOVEL—Indústria Rio Grandense de Óleos Vegetais (Pelotas, Rio Grande do Sul, Brazil). For the experiments, the DRB was ground using a knife mill (Solab, SL31, Brazil) and standardized to a particle size of 70 mesh. Reducing the particle size will result in solids with larger surface areas, thereby increasing the extraction rate [22]. The processed DRB was then stored at −12 °C until further analysis.

2.2. Chemical Analysis of DRB

Chemical analyses were conducted to determine the composition of DRB. Moisture content was measured using the gravimetric method in an oven at 105 °C [23]. Protein content was determined using the Micro–Kjeldahl method 960.52 [24], applying a nitrogen-to-protein conversion factor of 5.7. Ash content was quantified by incineration in a muffle furnace at 550 °C, following initial carbonization at 200 °C [23]. Lipid content was measured according to method 945.16 [23]. Carbohydrate content was calculated by subtracting the sum of the other constituents, as shown in Equation (1).

$$\text{Carbohydrates (\%)} = 100 - (\text{moisture\%} + \text{protein\%} + \text{lipid\%} + \text{ash\%}) \quad (1)$$

2.3. Sequential Strategy of Experimental Design for Extracting Carbohydrates from DRB

Two methods were employed to extract carbohydrates from DRB: UAE and hydrothermal treatment.

2.3.1. Ultrasound-Assisted Extraction (UAE)

A 2^{4-1} Fractional Factorial Design (FFD), comprising 11 runs, was conducted to analyze the effects of four independent variables on the UAE: DBR·water^{-1} ratio (g·L^{-1}), ranging from 100 to 200; temperature (°C), ranging from 50 to 90; sonication time (min), ranging from 10 to 20; sonication power (W), ranging from 100 to 300. Table 1 presents the experimental design, including the coded and real values of the studied variables.

Table 1. FDD 2^{4-1} matrix with coded and real values for the variables and response to carbohydrate (%) (g_{CHO}·100 $g_{DRB}{}^{-1}$) UAE of DRB.

Run	x_1 [a]	x_2 [b]	x_3 [c]	x_4 [d]	y [e]
1	−1 (100)	−1 (100)	−1 (10)	−1 (50)	27.14 ± 0.94
2	+1 (200)	−1 (100)	−1 (10)	+1 (90)	18.00 ± 0.61
3	−1 (100)	+1 (300)	−1 (10)	+1 (90)	43.29 ± 0.81
4	+1 (200)	+1 (300)	−1 (10)	−1 (50)	32.54 ± 0.02
5	−1 (100)	−1 (100)	+1 (30)	+1 (90)	45.35 ± 3.51
6	+1 (200)	−1 (100)	+1 (30)	−1 (50)	19.89 ± 0.56
7	−1 (100)	+1 (300)	+1 (30)	−1 (50)	48.37 ± 1.57
8	+1 (200)	+1 (300)	+1 (30)	+1 (90)	38.30 ± 0.78
9	0 (150)	0 (200)	0 (20)	0 (70)	34.97 ± 1.34
10	0 (150)	0 (200)	0 (20)	0 (70)	41.31 ± 1.09
11	0 (150)	0 (200)	0 (20)	0 (70)	35.36 ± 1.88

[a] Ratio of DRB·water^{-1} (g·L^{-1}); [b] power (w); [c] time (min); [d] temperature (°C); [e] carbohydrates (%) (g_{cho}·100 $g_{drb}{}^{-1}$) ± standard deviation.

In the UAE process, DBR was suspended in ultrapure water (produced using a reverse osmose water purification system, GEHAKA, OS10 LX, São Paulo, Brazil) according to the design parameters (Table 1). The suspension was transferred to a jacketed beaker connected to a temperature-controlled bath. The extraction was performed using an ultrasonic probe (VCX 500 and VCX 750, brand: SONICS, model: Vibra cell, United States) operating at a frequency of 20 kHz. The power and the sonication time were set according to the FFD matrix. Following sonication, the suspension was centrifuged at 5000× *g* at 25 °C for 5 min (Rotina 420 R, Hettich, Germany).

This initial FFD identified the statistically significant variables ($p < 0.10$) contributing to carbohydrate extraction and their effects. Based on these findings, a subsequent Central Composite Rotational Design (CCRD) was performed to develop a second-order model for carbohydrate extraction (dependent variable) as a function of the studied variables (independent variables). In the CCRD, the ranges of the significant variables were adjusted to wider or narrower intervals, depending on the positive or negative effects observed during the FFD analysis [25–27]. Table 2 presents the CCRD 2^3 design matrix with the coded and actual values of the variables.

Table 2. CCRD 2^3 matrix with coded and real values for the variables and response to carbohydrate (g_{CHO}·100 $g_{DRB}{}^{-1}$) UAE of DRB.

Run	x_1 [a]	x_2 [b]	x_3 [c]	y [d]
1	−1 (44.17)	−1 (320.24)	−1 (12.02)	28.37 ± 1.32
2	+1 (85.83)	−1 (320.24)	−1 (12.02)	28.99 ± 2.39
3	−1 (44.17)	+1 (379.76)	−1 (12.02)	42.86 ± 2.49
4	+1 (85.83)	+1 (379.76)	−1 (12.02)	46.97 ± 2.77
5	−1 (44.17)	−1 (320.24)	1 (17.97)	10.23 ± 0.28
6	+1 (85.83)	−1 (320.24)	1 (17.97)	26.41 ± 0.55

Table 2. *Cont.*

Run	x_1 [a]	x_2 [b]	x_3 [c]	y [d]
7	−1 (44.17)	+1 (379.76)	1 (17.97)	49.56 ± 3.16
8	+1 (85.83)	+1 (379.76)	1 (17.97)	28.62 ± 0.61
9	−1.68 (30)	0 (350)	0 (15)	56.77 ± 2.94
10	+1.68 (100)	0 (350)	0 (15)	29.52 ± 2.10
11	0 (65)	−1.68(300)	0 (15)	44.50 ± 2.56
12	0 (65)	+1.68 (400)	0 (15)	51.85 ± 1.43
13	0 (65)	0 (350)	−1.68 (10)	49.88 ± 1.80
14	0 (65)	0 (350)	+1.68 (20)	60.26 ± 1.16
15	0 (65)	0 (350)	0 (15)	40.91 ± 0.70
16	0 (65)	0 (350)	0 (15)	37.49 ± 1.03

[a] Ratio of drb·water^{-1} (g·L^{-1}); [b] power (w); [c] time (min); [d] carbohydrates (%) (g_{cho}·100 $g_{drb}{}^{-1}$) ± standard deviation.

2.3.2. Hydrothermal Extraction

Hydrothermal extraction was studied using a CCRD 2^3 with a star configuration, comprising 17 runs (including three replicates at the central point and six axial points), as shown in Table 3. This experimental design was chosen because it is specifically recommended for studies with two or three independent variables [25–27]. In this case, the independent variables analyzed were the ratio of DBR·water^{-1} (g·L^{-1}), ranging from 100 to 200; pH, ranging from 3 to 7; and extraction time (min), ranging from 10 to 60. The CCRD allowed for an efficient and robust exploration of these variables and their interactions, ensuring reliable results for the hydrothermal extraction process.

Table 3. CCRD 2^3 matrix with coded and real values for the variables and response to carbohydrate (g_{CHO}·100 $g_{DRB}{}^{-1}$) hydrothermal extraction of DRB.

Run	x_1 [a]	x_2 [b]	x_3 [c]	y_1 [d]	y_1 [e]
1	−1 (100)	−1 (3.8)	−1 (20.12)	18.93 ± 0.06	19.66
2	+1 (200)	−1 (3.8)	−1 (20.12)	14.87 ± 0.40	14.97
3	−1 (100)	+1 (6.2)	−1 (20.12)	13.06 ± 0.19	16.70
4	+1 (200)	+1 (6.2)	−1 (20.12)	13.22 ± 0.82	12.01
5	−1 (100)	−1 (3.8)	+1 (49.88)	16.24 ± 0.99	21.83
6	+1 (200)	−1 (3.8)	+1 (49.88)	17.28 ± 0.30	17.14
7	−1 (100)	+1 (6.2)	+1 (49.88)	15.33 ± 0.79	18.87
8	+1 (200)	+1 (6.2)	+1 (49.88)	14.71 ± 0.81	14.18
9	−1.68 (66)	0 (5)	0 (35)	32.47 ± 2.77	25.84
10	+1.68 (234)	0 (5)	0 (35)	15.48 ± 0.71	17.96
11	0 (150)	−1.68 (3)	0 (35)	23.27 ± 0.91	20.94
12	0 (150)	+1.68 (7)	0 (35)	17.78 ± 0.22	15.96
13	0 (150)	0 (5)	−1.68 (10)	9.02 ± 0.20	8.49
14	0 (150)	0 (5)	+1.68 (60)	15.76 ± 0.35	12.14
15	0 (150)	0 (5)	0 (35)	15.18 ± 0.05	16.36
16	0 (150)	0 (5)	0 (35)	16.29 ± 0.44	16.36
17	0 (150)	0 (5)	0 (35)	16.86 ± 0.57	16.36

[a] Ratio of DRB·water^{-1} (g·L^{-1}); [b] ph; [c] time (min); [d] carbohydrates (%) (g_{cho}·100 $g_{drb}{}^{-1}$) ± standard deviation; [e] predicted values.

For the hydrothermal treatment, DRB was suspended in ultrapure water to achieve the desired DRB·water^{-1} ratio, and the pH (ranging from 3 to 7) was adjusted with HCl or NaOH (2 mol·L^{-1}), as detailed in Table 3. The samples were autoclaved at 121.2 °C and 1 atm, following the method described by Kurdi and Hansawasdi [21], with modifications. After treatment, the suspension was centrifuged at 5000× *g* at 25 °C for 5 min (Rotina 420 R, Hettich, Germany).

2.3.3. Analytical Methods

Carbohydrate Determination

The Anthrone method was employed to determine the carbohydrate percentage [28]. A sucrose solution of 0.1 $g \cdot L^{-1}$ was used as the standard, and absorbance was measured at 600 nm using a UV-VIS spectrophotometer (Lambda XLS, PerkinElmer, USA).

The response variable for all experimental designs was the amount of extracted carbohydrates per gram of DRB, calculated according to Equation (2):

$$[CHO]\left(g_{CHO} \cdot 100\ g_{DRB}{}^{-1}\right) = \frac{[CHO]_{anthrone}\left(g_{CHO} \cdot L^{-1}\right)}{Ratio\left(g_{DRB} \cdot L^{-1}\right)} \times 100 \quad (2)$$

where $[CHO]_{anthrone}$ ($g_{CHO} \cdot L^{-1}$) is the concentration of carbohydrates determined by the Anthrone method, and Ratio ($g_{DRB} \cdot L^{-1}$) represents the amount of DRB in each suspension run.

The extraction efficiency (%) was calculated relative to the initial carbohydrate content in the DRB, as described in Equation (3):

$$Efficiency\ (\%) = \frac{CHO_{extracted}}{CHO_{DRB}} \times 100 \quad (3)$$

where $CHO_{extracted}$ ($g_{CHO} \cdot 100\ g_{DRB}{}^{-1}$) is the carbohydrate content in the supernatant determined by the Anthrone method and calculated according to Equation (2), and CHO_{DRB} ($g_{CHO} \cdot 100\ g_{DRB}{}^{-1}$) is the carbohydrate content in the DRB, calculated by difference, as described in item 2.2.

Characterization and Identification of Carbohydrate Chemical Groups

The structural elucidation of carbohydrates extracted from DRB was assessed using Fourier Transform Infrared Spectroscopy (FTIR). The analysis was performed with a spectrometer (FT-IR Spectrum 100S, PerkinElmer, Waltham, MA, USA) equipped with an Attenuated Total Reflectance (ATR) accessory. Spectral data were recorded in the 600–4000 cm^{-1} range, with a resolution of 4 cm^{-1}. For this analysis, the carbohydrates were precipitated from extracts using 80% ethanol, centrifuged, and lyophilized (Lyophilizer model 7753522, Labconco Corporation, Kansas City, MO, USA) [29].

2.3.4. Statistical Analysis

All experiments (Tables 1, 2, 3 and 6) were conducted randomly. Data analysis was performed using Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA), which was also utilized to generate contour plots and response surfaces. The adequacy of the model equations was evaluated through the determination coefficient (R^2), and their statistical significance was assessed via an F-test in the analysis of variance (ANOVA). Tukey's 95% confidence level test was applied to determine the differences between means in Table 8.

3. Results and Discussion

3.1. Chemical Composition of DRB

The chemical composition of DRB was determined as follows: 61.30 ± 0.30% carbohydrates, 15.35 ± 0.41% proteins, 12.31 ± 0.04% ash, 9.89 ± 0.14% moisture, and 1.10 ± 0.06% lipids. These results are consistent with values reported in the literature for rice bran and DRB [7,12,14,30]. As noted by Patindol et al. [31], the chemical composition of rice bran can vary depending on the milling techniques and the source material.

Polysaccharides are the primary compounds in rice bran and are classified as complex carbohydrates. They primarily consist of glucose, galactose, rhamnose, xylose, arabinose, stachyose, and other sugars [14,16,32–34]. Studies have highlighted the remarkable biological properties of DRB polysaccharides, which include prebiotic potential [14], antitumor and antioxidant activities [16,32,35], anti-hyperlipidemic effects [36], and hypoglycemic activity [32], among others.

Given their diverse bioactive properties, rice bran polysaccharides, according to Chen et al. [37], are beneficial to health and can be used in medicine, health products, beverages, food, and other fields. Thus, optimizing extraction process conditions is critical for maximizing their yield and unlocking their full potential.

3.2. Sequential Strategy of Experimental Design for the UAE Process

3.2.1. Screening of UAE Process Variables

According to Buvaneshwaran et al. [17], the efficiency of the UAE depends on factors such as temperature, time, ultrasound power and frequency, solvent concentration and type, matrix material characteristics, and pre-treatment conditions. The proper exploration and optimization of these parameters can significantly enhance the efficiency of the extraction process [38].

Carbohydrate extraction from DRB using the FFD 2^{4-1} (Table 1) yielded between 18.00% and 48.37% carbohydrates (runs 2 and 7, respectively). The FFD results identified the most significant variables as the ratio of DRB·water^{-1}, power, and time, with these variables showing significant effects ($p < 0.10$), as summarized in Table 4.

Table 4. Effect estimates from the results of FFD 2^{4-1} for the response of DRB carbohydrates extracted (%) (g_{CHO}·100 $g_{DRB}{}^{-1}$) by UAE.

Factor	Carbohydrates (%) * (g_{CHO}·100 $g_{DRB}{}^{-1}$)			
	Effect	**Standard Error**	**t(6)**	***p*-Value**
Mean	34.96	1.30	26.92	0.0000 **
x_1 [a]	−13.86	3.045	−4.55	0.0039 **
x_2 [b]	13.03	3.045	4.28	0.0052 **
x_3 [c]	7.74	3.045	2.54	0.0441 **
x_4 [d]	4.25	3.045	1.40	0.2123

[a] Ratio of DRB·water^{-1} (g·L^{-1}); [b] power (w); [c] time (min); [d] temperature (°C); * r^2: 0.89; adjusted r^2: 0.81. ** Significant factors ($p < 0.10$).

The ratio of DRB·water^{-1} (100–200 g·L^{-1}) exhibited a negative effect, indicating that an increase in the ratio reduced the percentage of carbohydrates extracted. A similar trend was reported by Lupatini et al. [33] in the extraction of proteins and carbohydrates from *Spirulina platensis* biomass. These authors suggested that this behavior is possibly due to interactions between biomass and solvent. Therefore, to enhance carbohydrate extraction, the range for this variable was adjusted to 30–100 g·L^{-1} in the sequential experimental design.

The power range (100–300 W) demonstrated a positive effect. As noted by Mena-García et al. [39], high ultrasound power (range 80 and 200 W) combined with low frequencies (16 and 100 kHz) facilitates cell wall disruption, promoting the release of compounds, accelerating diffusion, enhancing mass transfer, and improving solvent penetration into the ultrasound-irradiated matrix. Considering this effect, the power range was adjusted to 200–400 W for the subsequent CCRD.

The sonication time (10–30 min) positively influenced carbohydrate extraction. Previous studies also reported that longer sonication times result in increased carbohydrate yields [38,40]. However, for the CCRD, this range was narrowed to 10–20 min to reduce energy consumption. Additionally, extended ultrasound application times tend to raise the sample temperature. It was also observed that prolonged sonication with high power increased the viscosity of the samples.

According to Soria and Vilamiel [41], the effect of ultrasound intensity on food viscosity can vary, resulting in either an increase or a decrease, which may be either temporary or permanent. In this study, UAE probably modifies the structures of macromolecules present in DRB, such as proteins and carbohydrates, enhancing intermolecular interactions and resulting in increased solution viscosity. Additionally, UAE conducted at low ultrasonic frequencies (20 kHz) favors emulsion formation [42]. The increase in viscosity following ultrasound treatment may also be attributed to a reduction in particle size [43].

The increase in the viscosity of avocado puree was reported by Bi et al. [43], who attributed this phenomenon to the homogeneous solubilization of cell wall material and polysaccharides present in the puree after ultrasound treatment. During the process, aggregated macromolecules are broken down into individual components, promoting greater interaction between them and forming a uniform network of polysaccharides. Before sonication, the viscosity was predominantly influenced by the continuous phase (water). After the ultrasonic treatment, the avocado particles are disaggregated, and the solubilized cell material creates a homogeneous structure, leading to a significant increase in viscosity.

Similarly, Krešić et al. [44] identified structural modifications in proteins following ultrasound application. These changes were associated with an increase in apparent viscosity and consistency coefficient, attributed to a greater water-binding capacity when the hydrophilic parts of amino acids are exposed to the aqueous environment.

In compound extraction, temperature plays a crucial role. Increasing the temperature to a certain point generally enhances the yield [17]. However, for DRB carbohydrate extraction, the temperature range of 50–90 °C showed no significant effect. Consequently, in subsequent stages of the study, a thermal bath with water flow was employed to maintain the temperature at a maximum of 50 °C. This precaution was taken to prevent excessive biomass heating, as high temperatures can degrade polysaccharides [45].

When combined with cavitation, these conditions typically facilitate simple, efficient, and cost-effective methods for carbohydrate extraction—particularly polysaccharides—from various by-products and food matrices. These methods offer shorter extraction times and higher yields [19].

3.2.2. Optimization of UAE of the Carbohydrates from DRB

The significant variables identified by the FFD 2^{4-1} were DRB content, power, and sonication time. Based on the findings from the FFD, a CCRD 2^3 was established to evaluate the effects of these variables on carbohydrate extraction and optimize the process. The results are presented in Table 2.

The percentage of carbohydrates extracted ranged from 10.23 to 60.26% (runs 5 and 14, respectively). The central points (runs 15–17) exhibited minimal variation, demonstrating good reproducibility of the trial data. Notably, the carbohydrate extraction observed in the FFD 2^{4-1} was lower than that achieved in the CCRD 2^3, indicating improved extraction performance in the latter.

From the regression analysis, the effects of the studied variables were calculated ($p < 0.05$) (Table 5). However, none of the variables examined in CCRD 2^3 were statistically significant. Furthermore, the coefficient of determination ($R^2 \approx 0.4$) for this model was

low, and the ANOVA results were insignificant. As a result, it was not possible to derive a second-order model equation for this design.

Table 5. Effect estimates from the results of CCRD 2^3 for the response of DRB carbohydrates extracted (%) (g_{CHO}·100 $g_{DRB}{}^{-1}$) by UAE.

Factor	Carbohydrates (%) * (gCHO·100 gDRB^{-1})			
	Effect	**Standard Error**	**t(6)**	***p*-Value**
Mean	40.41	8.83	4.58	0.0026 **
x_1 [a] (L)	−6.71	8.30	−0.81	0.4450
x_1 [a] (Q)	−4.70	9.14	−0.51	0.6226
x_2 [b] (L)	12.66	8.30	1.53	0.1709
x_2 [b] (Q)	−1.14	9.14	−0.12	0.9042
x_3 [c] (L)	−2.19	8.30	−0.26	0.7995
x_3 [c] (Q)	3.75	9.14	0.41	0.6942
x_1 [a]·x_2 [b]	−8.41	10.83	−0.78	0.4632
x_1 [a]·x_3 [c]	−2.37	10.83	−0.22	0.8329
x_2 [b]·x_3 [c]	2.27	10.83	0.21	0.8402

L: linear terms; Q: quadratic terms; [a] Ratio of DRB·water^{-1} (g·L^{-1}); [b] power (w); [c] time (min); * r^2: 0.38; adjusted R^2: 0. ** Significant factors ($p < 0.05$).

Despite this, it is worth highlighting the condition of run 14, which featured a ratio of DRB·water^{-1} of 65 g·L^{-1}, a power of 350 W, and a sonication time of 20 min. This setup yielded 60.26 g carbohydrate·100 g DRB^{-1}, with an efficiency of 98.30%. These results were achieved under relatively short extraction times and lower power than the maximum level studied, demonstrating the efficiency of these conditions.

Under similar UAE conditions, Antunes et al. [14] reported 96.72% efficiency for carbohydrate extraction from DRB. In the study of UAE of polysaccharides from purple glutinous rice bran, Surin et al. [46] identified the optimal conditions as a defatted bran-to-water ratio of 1:20 (w/v), an extraction temperature of 70 °C, and an extraction time of 20 min.

3.3. Sequential Strategy of Experimental Design for the Hydrothermal Extraction Process

Initially, a CCRD 2^3 was established to verify the effects of the ratio DBR·water^{-1} (g·L^{-1}), pH, and time (min) on carbohydrate extraction by hydrothermal treatment. The results are summarized in Table 3.

The percentage of extracted carbohydrates ranged from 9.02 to 32.47% (runs 13 and 9). Regression analysis was conducted, and the reparameterization of the mathematical model coefficients (Equation (4)) incorporated interactions between the variables studied into the residues.

$$\begin{aligned} Carbohydrates \quad &= 16.36 - 2.35x_1 + 1.96{x_1}^2 - 1.48x_2 + 0.74{x_2}^2 + 1.08x_3 \\ &\quad - 2.14{x_3}^2 \end{aligned} \tag{4}$$

where, x_1, x_2, and x_3 were the coded values for the Ratio of DRB·water^{-1}, pH, and extraction time, respectively.

The F-test (ANOVA) was used to assess the statistical significance of the model. The $F_{calculated}$ value for regression (3.29) was slightly exceeded by the critical value (3.22) (p-value = 0.0468). The model explained 66% of the variation. Based on the model, a contour plot and response surface were generated (Figure 1). The predicted carbohydrate values derived from Equation (4) are presented in Table 3.

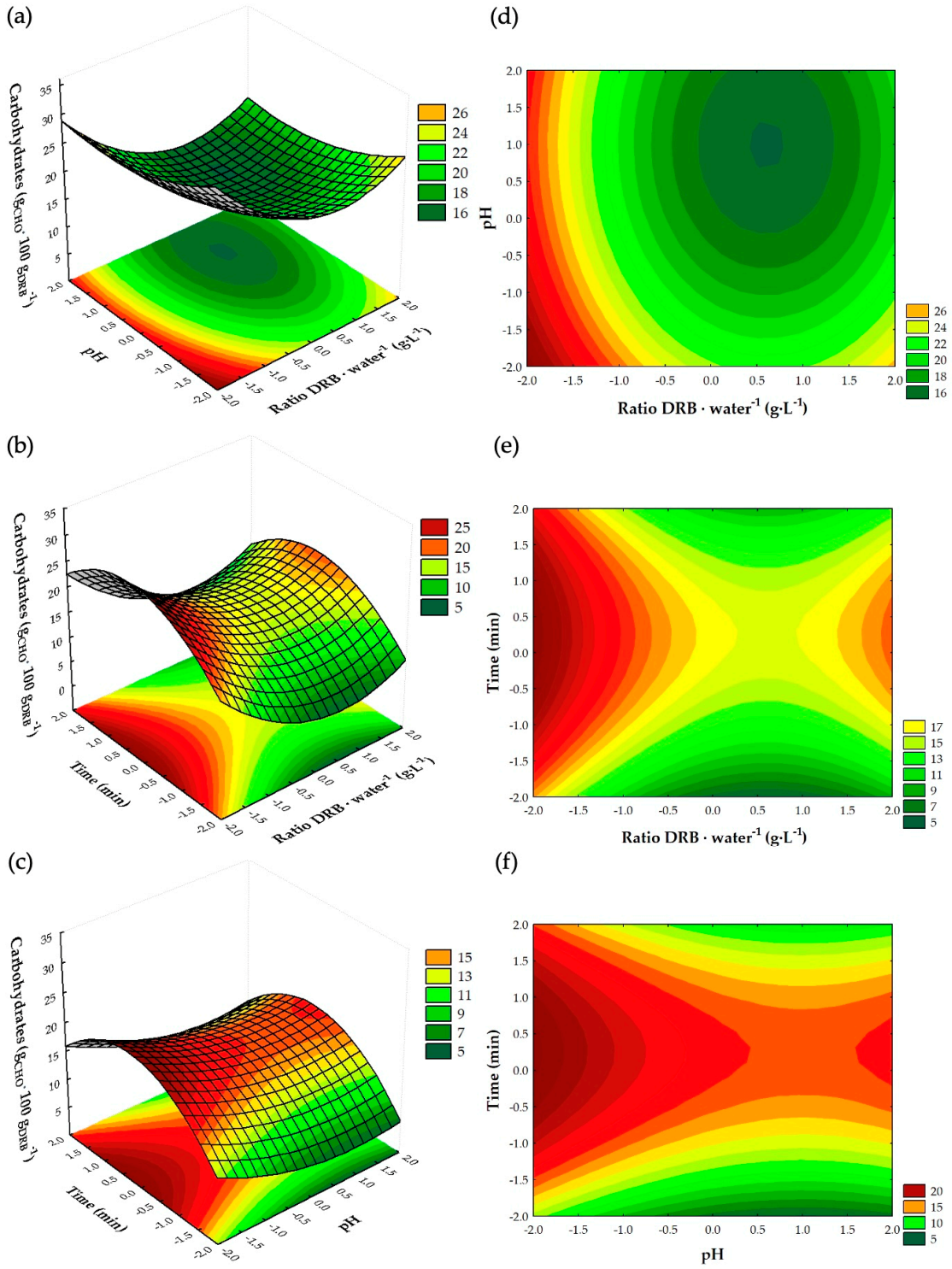


Figure 1. Response surfaces (**a**–**c**) and contour plots (**d**–**f**) for carbohydrate extraction from DRB by hydrothermal treatment.

As observed in UAE, the DBR·water^{-1} (g·L^{-1}) ratio also negatively affected the hydrothermal extraction response. Therefore, to enhance the potential for carbohydrate extraction, the range for this variable was adjusted to 40–100 g·L^{-1} in the sequential experimental design.

The pH also showed a negative effect on carbohydrate extraction. Lupatini et al. [38] reported a similar impact during carbohydrate extraction from *Spirulina platensis*. Kurdi and Hansawasdi [21] observed that rice bran oligosaccharides are efficiently released at pH

values between 4.0 and 7.0 but decrease at lower pH levels. The pH range was maintained in the sequential CCRD 2^2 since narrower pH ranges could lead to the degradation of the extracted material or the co-extraction of other compounds, such as some proteins precipitated under acidic conditions [47].

The extraction time (10–60 min) positively influenced carbohydrate extraction. According to Cui et al. [18], prolonged extraction times often enhance carbohydrate extraction. Similarly, Kurdi and Hansawasdi [21] conducted their study with an extraction time of 30 min. However, this study fixed the extraction time at 35 min to balance efficiency and minimize process costs.

As a result, the sequential CCRD 2^2 design focused on two independent variables: the ratio of DBR·water^{-1} and pH. The runs were performed for 35 min (defined in CCRD 2^3). Table 6 shows that the carbohydrate extraction ranged from 12.92% to 27.33% (runs 3 and 7, respectively).

Table 6. CCRD 2^2 matrix with coded and real values for the variables and response to carbohydrate (g_{CHO}·100 $g_{DRB}{}^{-1}$) hydrothermal extraction of DRB.

Run	x_1 [a]	x_2 [b]	y_1 [c]	y_1 [d]
1	−1 (48.73)	−1 (3.58)	25.33 ± 1.71	24.95
2	+1 (91.27)	−1 (3.58)	24.86 ± 1.60	24.95
3	−1 (48.73)	+1 (6.41)	12.92 ± 0.60	18.51
4	+1 (91.27)	+1 (6.41)	19.82 ± 1.36	18.51
5	−1.41 (40)	0 (5)	24.52 ± 4.29	19.96
6	+1.41 (100)	0 (5)	17.41 ± 0.40	19.96
7	0 (70)	−1.41 (3)	27.33 ± 1.91	27.95
8	0 (70)	+1.41 (7)	21.49 ± 1.03	18.87
9	0 (70)	0 (5)	14.32 ± 0.46	15.07
10	0 (70)	0 (5)	15.11 ± 0.90	15.07
11	0 (70)	0 (5)	15.75 ± 1.42	15.07

[a] Ratio of DRB·water^{-1} (g·L^{-1}); [b] pH [c] carbohydrates (%) (g_{cho}·100 $g_{drb}{}^{-1}$) ± standard deviation; [d] predicted values.

Table 7 presents the effects of the independent variables on the carbohydrate extraction response, with significant variables ($p < 0.05$) highlighted. The ratio of DRB·water^{-1} did not significantly contribute to carbohydrate extraction, consistent with the trend observed in the CRRD 2^3. On the other hand, the pH (3–7) significantly impacted carbohydrate extraction.

Table 7. Effect estimates from the results of CCRD 2^2 for the response of DRB carbohydrates extracted (%) (g_{CHO}·100 $g_{DRB}{}^{-1}$) by hydrothermal extraction.

Factor	Carbohydrates (%) * (gCHO·100 gDRB^{-1})			
	Effect	Standard Error	t(6)	*p*-Value
Mean	15.067	1.89	7.98	0.0005
x_1 [a] (L)	−0.90	2.32	−0.40	0.7130
x_1 [a] (Q)	4.93	2.76	1.78	0.1347
x_2 [b] (L)	−6.4	2.32	−2.78	0.0388 **
x_2 [b] (Q)	8.39	2.76	3.04	0.0288 **
x_1 [a]·x_2 [b]	3.69	3.27	1.13	0.3109

L: linear terms; Q: quadratic terms; [a] Ratio of DRB·water^{-1} (g·L^{-1}); [b] pH; * R^2: 0.79; adjusted R^2: 0.59. ** Significant factors ($p < 0.05$).

Regression analysis (Table 7) was performed to develop a second-order model (Equation (5)) for the carbohydrate extraction from DRB by hydrothermal treatment as a function of the ratio of DRB·water^{-1} and pH. The linear term of the ratio of DRB·water^{-1}

and the interaction between the ratio of DRB·water^{-1} and the pH were incorporated into the residues, and the mathematical model was re-parameterized.

$$Carbohydrates\ (\%) = 15.07 + 2.46{x_1}^2 - 3.22x_2 + 4.19{x_2}^2 \quad (5)$$

where x_1 and x_2 were the coded values for the ratio of DRB·water^{-1} and the pH, respectively.

The statistical adequacy of the model was evaluated using the F-test (ANOVA). The $F_{calculated}$ value (6.5) for regression exceeded the critical value (4.35) (p-value = 0.019), indicating the significance of the model. The determination coefficient (R^2 = 0.74) fits the carbohydrate extraction model well. Equation (4) was subsequently used to generate the response surface and contour plot (Figure 2). The fit between the model and the experimental data was confirmed through the predicted values (Table 6).

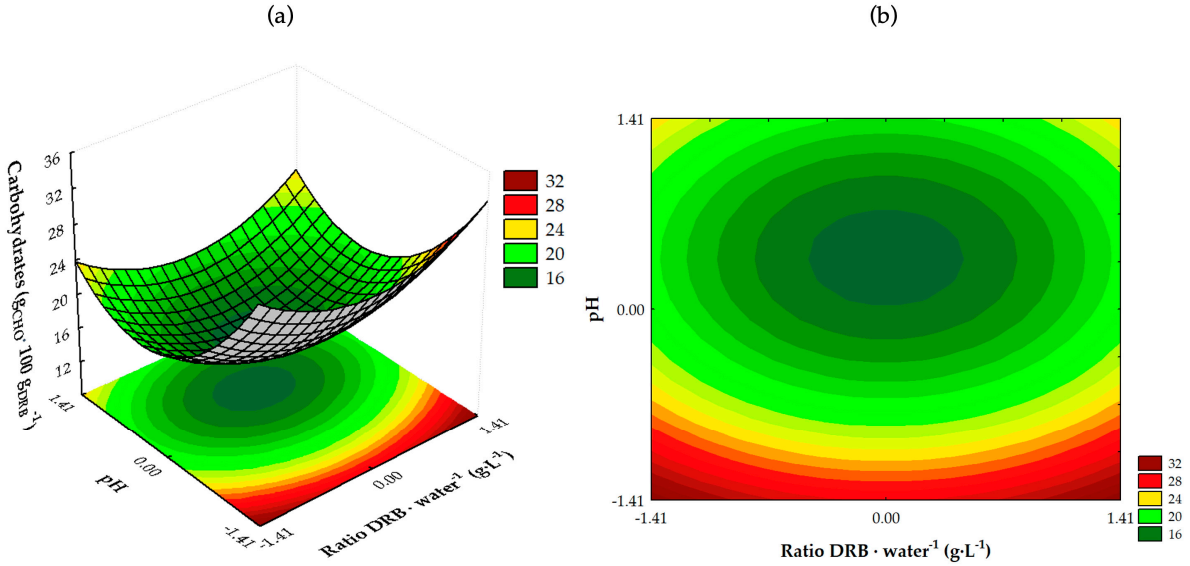


Figure 2. Response surface (**a**) and contour plot (**b**) for carbohydrate extraction from DRB by hydrothermal treatment.

The response surface and contour analysis revealed a region of minimal carbohydrate extraction. It was also observed that various combinations of studied variables yielded similar carbohydrate extraction results. According to Rodrigues and Iemma [25], identifying a range of optimal variable combinations is more advantageous than pinpointing a single condition. This approach allows variables to fluctuate near optimal values while maintaining favorable process outcomes. However, the authors also emphasize that the conditions identified from the response surface should be validated through at least three replicate runs.

To confirm the results suggested by the response surface, three runs were performed under the following conditions: 100 g·L^{-1} DRB ratio, chosen to maximize the amount of extracted carbohydrates; a pH of 6.0, close to the natural pH of DBR suspended in water; minimizing the addition of HCl or NaOH; and a time of 35 min, previously fixed to reduce process costs. Table 8 shows the carbohydrate extraction results under these defined conditions.

The validation results can be compared to those reported by Sunphorka et al. [48], who extracted carbohydrates and proteins from DRB. Under the conditions of 200 °C, 79 min, and 7 MPa of pressure, the authors achieved approximately 40 g_{CHO}·100 $g_{DRB}{}^{-1}$. In contrast, this study employed milder conditions (121.1 °C, 35 min, and 1 atm of pressure) and achieved higher results, with an average of 48 g_{CHO}·100 $g_{DRB}{}^{-1}$ and 78.30% of efficiency.

Table 8. Validation of the study performed for carbohydrate extraction from DRB by hydrothermal treatment using a sequential strategy of experimental design.

Run	Carbohydrates ($g_{CHO} \cdot 100\ g_{DRB}^{-1}$)	Efficiency (%)
1	47.03 ± 0.54^{b}	76.73 ± 0.88^{b}
2	50.74 ± 0.72^{a}	82.77 ± 1.18^{a}
3	$48.15 \pm 1.22^{a,b}$	$78.55 \pm 1.99^{a,b}$

Average ± standard deviation. Different lowercase letters in the same column mean statistical differences ($p < 0.05$).

Antunes et al. [14] applied the optimized conditions from this study in subsequent research. They obtained similar results for carbohydrate extraction, with approximately 51 $g_{CHO} \cdot 100\ g_{DRB}^{-1}$ and an efficiency of 83.11% using hydrothermal treatment.

3.4. Functional Group Identification for the Extracted Carbohydrates

The FTIR spectrum of the carbohydrates extracted from DRB via the hydrothermal method was compared with the spectra of standard sugar, including fructose, xylose, maltose, raffinose, ribose, and stachyose (Figure 3). The carbohydrates from DRB exhibited characteristic infrared absorption bands typical of polysaccharides [16,49].

A band at 3319 cm^{-1} corresponds to the O–H vibrational stretching, indicative of strong inter- and intramolecular hydrogen bonding within the polysaccharide chains [16]. Symmetry at this wavenumber aligns with the patterns observed for maltose and stachyose, which correspond to the same functional group.

Modest bands observed at 3000–2500 cm^{-1} are attributed to the symmetric and asymmetric stretching vibrations of skeletal CH and CH_2 in polysaccharides [50]. A specific band at 2921 cm^{-1} exhibits symmetry with the other analyzed sugars.

The band at 1649 cm^{-1} corresponds to the vibrational stretching of C=O, indicative of the strong bonding in carbonyl groups. Similar stretching was observed in the stachyose and raffinose samples. However, Mohd Nor et al. [29] reported C=O vibrational deformations at 1747.3 cm^{-1}, potentially indicating the presence of esters.

Bands in the 1175–1140 cm^{-1} region are likely due to glycosidic bonds in polysaccharides. Distinctive conformations of glycosidic bonds often result in variations in the 1000–920 cm^{-1} region [50]. A broad stretching band at 1006 cm^{-1} is characteristic of C-O vibrations, commonly observed in the 900 to 1300 cm^{-1} range, which is typical for alcohols.

No bands were detected at 1640 cm^{-1} or 1530 cm^{-1}, which are characteristic of amide I and II. According to Mohd Nor et al. [29], peaks at these wavenumbers indicate the presence of protein. The absence of such bands suggests that the extracted carbohydrates are likely free of residual amides, demonstrating the efficiency of the process in avoiding protein contamination.

The FTIR spectrum for the extracted carbohydrate from DRB resembles those reported in other studies [14,16,49]. The symmetry observed in comparisons of DRB carbohydrates with different sugars suggests the presence of mono-, di-, and oligosaccharides. However, this analysis cannot definitively confirm their presence.

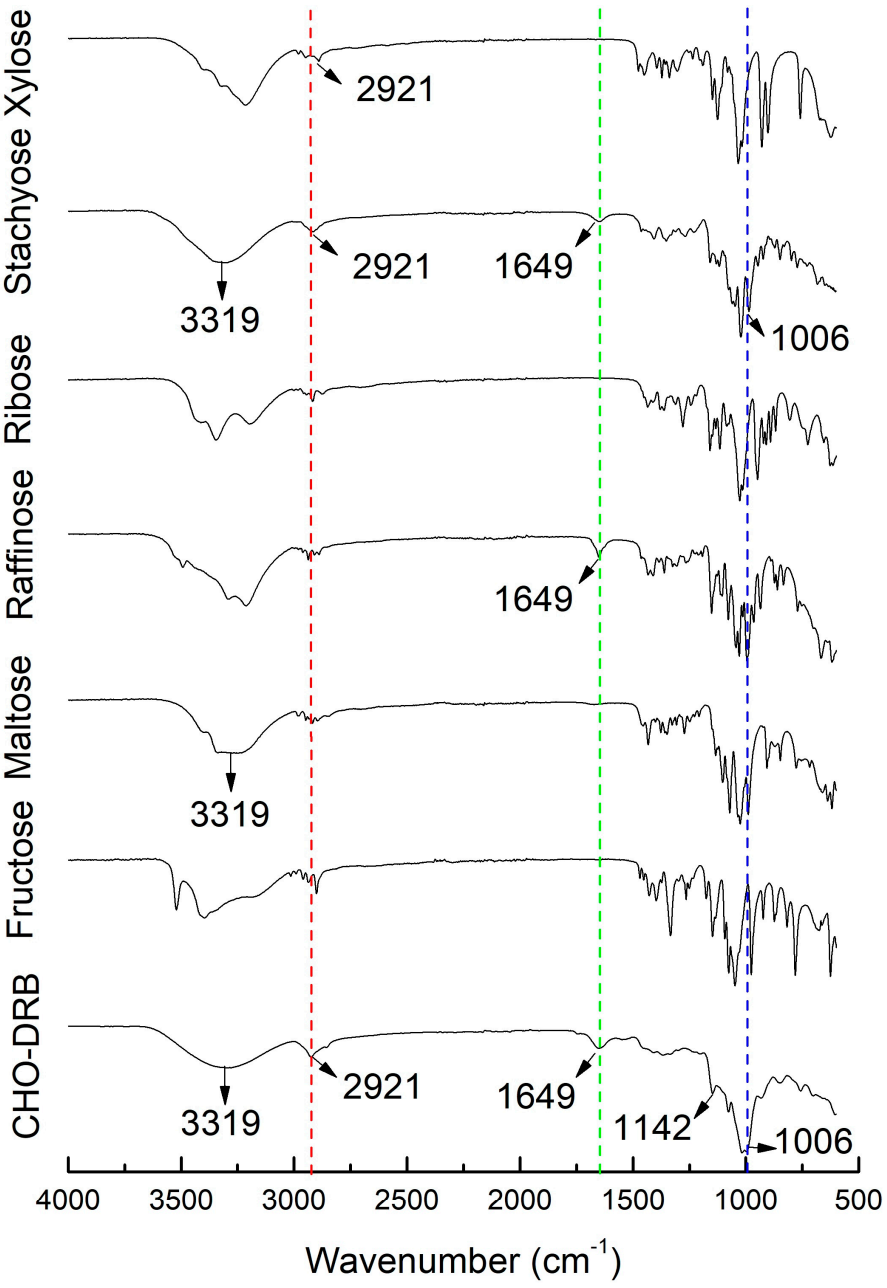


Figure 3. FTIR spectra of standard monosaccharides, disaccharides, oligosaccharides, and DRB carbohydrates (CHO-DRB) extracted.

3.5. Comparison Between the Methods

An analysis of the final yield from each extraction method revealed that UAE (60.26 g_{CHO}·100 $g_{DRB}{}^{-1}$ and 98.30% yield) provided a higher yield than hydrothermal treatment (average 48 g_{CHO}·100 $g_{DRB}{}^{-1}$ and 78.30% yield). Antunes et al. [14], under similar extraction conditions, also reported higher yields with UAE (59 g_{CHO}·100 $g_{DRB}{}^{-1}$ and 96.72 of efficiency) compared to hydrothermal treatment (50 g_{CHO}·100 $g_{DRB}{}^{-1}$ and 83.11% efficiency) for carbohydrate extraction from DRB.

When comparing the results from Table 1 (FDD 2^{4-1}) to Table 3 (CCRD 2^3) for UAE, a 24.6% increase in carbohydrate concentration was observed, leading to a corresponding increase in yield. A similar trend was noted for hydrothermal treatment. These findings indicate that the experimental design strategies and the modified variable ranges used in

this study effectively enhanced DRB carbohydrate extraction. These results underscore the value of experimental design in optimizing process conditions.

Regarding extraction time, both methods exhibited relatively short durations. However, the UAE time (20 min) was faster than that of the hydrothermal treatment (35 min). Wen et al. [19] also noted that sonication amplified yields while reducing extraction time. Additionally, the UAE operated at a lower temperature (maximum at 50 °C) compared to hydrothermal treatment (121.1 °C), reducing energy costs in the UAE process.

In this study, UAE was compared with hydrothermal treatment on the extraction of carbohydrates from DRB, and the results indicate that UAE is more efficient. These findings are consistent with other studies on extraction methods. For example, Antunes et al. [14] also concluded that the UAE outperforms hydrothermal treatment for carbohydrate extraction from DRB. Similarly, Surin et al. [46] reported that UAE achieves a higher carbohydrate yield compared to hot water extraction.

The superior efficiency of UAE compared to other extraction methods for various compounds has also been reported. In a study on protein extraction from DRB, Carmo [51] demonstrated that UAE was more effective than enzymatic extraction. Likewise, for carotenoid extraction from cashew peduncle, UAE proved to be more efficient, faster, and more cost-effective than conventional solvent extraction with agitation [52].

4. Conclusions

Carbohydrate extraction from a by-product such as DRB valorizes a low-commercial-value material traditionally used as animal feed. This study successfully optimized carbohydrate extraction from DRB using UAE and hydrothermal treatments.

Optimal conditions for UAE were a DRB-to-water ratio of 65 $g \cdot L^{-1}$, ultrasonic power of 350 W, a temperature of approximately 50 °C, and an extraction time of 20 min. Under these conditions, a yield of 60.26 $g_{CHO} \cdot 100\ g_{DRB}{}^{-1}$ (98.30%) was achieved. In comparison, hydrothermal treatment, under a DRB-to-water ratio of 100 $g \cdot L^{-1}$, a pH of 6.0, and extraction time of 35 min yielded 48 $g_{CHO} \cdot 100\ g_{DRB}{}^{-1}$ (78.30%).

UAE proved more effective than hydrothermal treatment, delivering higher yields, shorter extraction times (20 min vs. 35 min), and lower operating temperatures (~50 °C vs. 121 °C). Moreover, both methods eliminated the need for organic solvents, avoiding residue treatment and promoting environmentally friendly processing.

FTIR analysis revealed spectral similarities between standard sugars and the carbohydrates extracted from DRB, suggesting the presence of mono-, di-, and polysaccharides in the extract.

This research underscores the potential of experimental design strategies to enhance carbohydrate extraction efficiency from DRB, paving the way for its application in value-added products.

Author Contributions: Conceptualization, E.C., D.A.D., A.S. and I.J.B.; formal analysis, A.L.F. and B.P-S.; writing—original draft preparation, E.C. and B.P.-S.; writing—review and editing, E.C., B.P.-S. and D.R.M.; supervision, E.C., D.A.D. and I.J.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors thank IRGOVEL (Indústria Rio Grandense de Óleos Vegetais—Pelotas, RS, Brazil) for providing the DRB used in this study. Appreciation is also extended to Central Analítica Multiusuário de Medianeira (CEANMED) of Universidade Tecnológica Federal do Paraná, Campus Medianeira, Paraná, Brazil, for conducting the analytical assays.

Conflicts of Interest: Author Adreano Spessato was employed by the company Irgovel. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The Irgovel had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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