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Contributed Paper

Physical Pretreatments for Improving Nutritive Value of Cyanobacterial Cells

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ABSTRACT

Cell wall is a physical barrier and an unavailable food or feed constituent in industrial applications. Various pretreatment methods (microwave irradiation, ultrasonication, gamma irradiation and electron beam irradiation) were used to improve the nutritive values of cyanobacterial cells. The pretreatment by microwave irradiation at least maintained (lipid, fiber, ash, nitrogen free extract and gross energy) or even improved (protein and neutral detergent fiber) the chemical composition, relative to other tested pretreatments. All pretreatments caused slight changes in the nutritive profile. Microwaving improved also various physicochemical properties in relation to hydrolytic capacity, namely turbidity, microstructure, thermal transition characteristics, and relative crystallinity. These effects contributed to a significant ($P < 0.05$) overall effect on *in vitro* carbohydrate digestibility. The findings indicate that microwaving improves the nutritive value and bioavailability of cyanobacteria, with a wide application potential in the pretreatment of food or feed prior to its administration.

Keywords: cyanobacteria, digestibility, microwave irradiation, physicochemical properties, pretreatment

1. INTRODUCTION

Cyanobacteria serve as an enriched source of nutrients in food or feed, in industrial applications. The main hindrance to nutritional bioavailability of cyanobacteria, as well as foodstuffs and feedstuffs from plants and algae, is the presence of cell walls [1, 2]. Cell walls are non-starch polysaccharide barriers mainly composed of cellulose and hemicelluloses, and they impair food utilization by encapsulating nutrients and by increasing the viscosity of intestinal contents [3]. Several pretreatments have been used to destroy the robust cell walls, so that digestive enzymes would gain access to the intracellular components [1, 2]. Effects on the nutritive value of the cells are the goal and motivation of such pretreatments.

Limited information is available on which pretreatment would be the best for cyanobacteria. Prior studies report a positive

effect on protein digestibility of algae by high pressure homogenization [1], and by ultrasonication [2]. Disruption of cyanobacterial cells in a Frence press can also increase the release of cytosolic proteins over pretreatments with high temperature, ultrasound and microwave [4]. In contrast, improving the carbohydrate utilization has not been studied much, while it may directly follow from the disruption of cell walls [5]. In other raw materials, successful pretreatments by physical methods have been applied widely to improve the nutritive value, these treatments including microwave irradiation [6], gamma irradiation [7] and electron beam irradiation [8]. The physical methods are quick to apply, do not require chemicals, and are fit for large scale applications.

The physical pretreatments improve the chemical composition of raw materials by inducing chemical reactions, and re-organize the infrastructure by aggregation or dissociation of macromolecules [9, 10]. Some changes in physicochemical properties can enhance enzymatic hydrolysis, which is related to turbidity, microstructure, thermal transition characteristics and relative crystallinity [5, 10, 11]. The effective changes depend on the nature of raw material as well as on the pretreatment method; the method should be matched to the material processed.

The goal of this study was to investigate the effects of various pretreatment methods (including microwave irradiation, ultrasonication, gamma irradiation, and electron beam irradiation) on the nutritive value, assessed from the chemical composition and the physicochemical properties. Cyanobacteria (*Nostoc commune*) used as the raw material can be successfully cultivated indoors, and appear to have potential for food and feed applications. A very sensitive technique, *in vitro* digestibility, using digestive enzymes extracted directly from reared animals, was used to assess treatment effects on bioavailability. Findings from the present study may help improve the nutritive value of cyanobacteria or other microalgae, contributing to their potential food and feed applications.

2. MATERIALS AND METHODS

2.1 Preparation and Pretreatment of Cyanobacteria

Dried cyanobacteria (*Nostoc commune* Vaucher ex Bornet & Flahault, 1888) were obtained from the Department of Bioscience, Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Province, Thailand. They had been cultivated in 250 mL Erlenmeyer flasks containing 100 mL BG-11 medium under 60 mmol photon m^{-2} s^{-1} illumination, 12 h dark and 12 h light photoperiod, and 150 rpm shaking at 28 ± 1 °C for 21 days. The drying was performed in a freeze dryer (Delta 2-24 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) over 48 h under dark conditions, prior to the pretreatment. The physical pretreatments of cyanobacteria were as follows. (1) Microwave irradiation. The dried cyanobacteria were placed in a 2 L beaker, mixed with 20-fold weight of distilled water, and then heated for 4 min at 800W in a microwave oven (MW 71B, Samsung, Selangor, Malaysia) under agitation and in the temperature range 90-95 °C. (2) Ultrasonication. A suspension of cyanobacteria was prepared as described above, and then sonicated continuously (sweep frequency 45 kHz and sonic power 300 W) in a 49.5 × 29.7 × 20 cm stainless steel ultrasonic bath (CP2600D, Crest Ultrasonics, Penang, Malaysia) at 50 °C for 15 min. (3) Gamma irradiation. Dried cyanobacteria

were subjected to 50 kGy radiation dose from ^{60}Co source, in a carrier type gamma irradiator (JS 8900 IR-155, MDS Nordion, Ottawa, ON, Canada). (4) Electron beam irradiation. Dried cyanobacteria received a 30 kGy radiation dose from an electron accelerator (TT-200, IBA Co. Ltd., Louvain-la-Neuve, Belgium) set at 10 MeV. These selected pretreatment conditions for microwave irradiation [5], gamma irradiation and electron beam irradiation [8], and ultrasonication [12] were chosen based on achieving the highest digestibility in the cited reference, or reported breakage of cells. The pretreated cyanobacteria in aqueous suspension were dried again as describe above. All preparations were separated to three replicate samples (n = 3). Dry non-pretreated and pretreated samples were then packed in black polyethylene bags and kept in desiccators, for later analyses of chemical composition, physicochemical properties, and *in vitro* digestibility.

2.2 Chemical Composition

Non-pretreated and pretreated cyanobacteria samples were analyzed for proximate compositions, including crude protein, lipid, ash, fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF), following standard methods of AOAC [13]. Nitrogen free extract (NFE, g kg^{-1} of dry matter) and gross energy (GE, kcal kg^{-1}) were estimated from the measurements as NFE = 1,000 - (crude protein + crude lipid + crude fiber + crude ash), and GE = (crude protein × 5.6) + (crude lipid × 9.44) + (crude fiber × 4.1) + (NFE × 4.1). All the chemical compositions were analyzed in triplicate and are reported on dry matter basis.

2.3 Nutritive Profiles

Nutritional quality was characterized using Fourier transform infrared spectrometer (Equinox 55, Bruker, Karlsruhe, Germany). Sample discs were prepared by mixing 1 mg of freeze-dried cyanobacteria with 100 mg of KBr in mortar and then pressing the mixture at 10 MPa for 5 min. FTIR spectra were taken for each sample from 4,000 to 400 cm^{-1}. Identification and interpretation of the results followed prior publications [14-22].

2.4 Physicochemical Properties

2.4.1 pH

One gram of freeze-dried cyanobacteria was suspended in 25 mL of water at 25 °C and agitated for 10 min [23], and a measurement was taken with a pH meter (CyberScan 510, Eutech Instrument, Ayer Rajah, Singapore).

2.4.2 Turbidity

Turbidity of each freeze-dried cyanobacteria sample was analyzed as described by Thongprajukaew et al. [10]. The sample was suspended in distilled water (1% w/v), and kept at 90 °C for 1 h under 100 rpm agitation. The suspension was cooled to 30 °C and held for 1 h, and then stored at 4 °C for 48 h. The supernatant was collected and measured spectrophotometrically at 640 nm against a water blank.

2.4.3 Microstructure

Shape, fracturing, surface and roughness of cyanobacteria in each sample were studied using scanning electron micrographs (Quanta 400, FEI, Brno, Czech Republic)

at 300, 2,000 and 10,000× magnifications. Sample powder was mounted by double-sided adhesive tape on an aluminum stub, and coated with gold. A 20 kV acceleration potential was used for imaging.

2.4.4 Thermal transition properties

Thermal transition properties of freeze-dried cyanobacteria were determined with a differential scanning calorimeter (DSC7, Perkin Elmer, Waltham, MA, USA). A three milligram sample was placed in an aluminum pan, sealed, allowed to equilibrate at room temperature for 1 h, and then heated with comparison against an empty pan. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures, melting temperature range (T_c-T_o), and transition enthalpy (ΔH) were determined by scans from 40 to 400 °C at a rate of 10 °C min^{-1}.

2.4.5 X-ray diffraction pattern

The diffraction pattern of a pressed sample of freeze-dried cyanobacteria powder was obtained with an x-ray diffractometer (X' Pert MPD, Philips, Amsterdam, Netherlands). The device was operated at 40 kV and 30 mA. The operating conditions had 0.154 nm wavelength (CuK_α), 1.2 second time/step, and $2\theta = 0.04°$ step size. The diffractograms were recorded for 2θ from 4° to 35° with a scanning rate of 2° min^{-1}. The percent relative crystallinity was calculated from the ratio of peak area to the total area (sum of peak areas and amorphous areas) in each diffractogram using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA).

2.5 *In vitro* Digestibility

2.5.1 Enzyme extraction and preparation

Four-month-old Nile tilapia, *Oreochromis niloticus* (n = 3, 105-110 g body weight and 18.5-20.2 cm total length) were obtained from a private farm in Trang Province, Thailand. The fish were sacrificed by chilling in ice according to "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes", National Research Council, Thailand. Small intestines were collected and then extracted with 0.2 M phosphate buffer at pH 8 (1:4 *w*/*v*), using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenate was centrifuged at 15,000×*g* for 30 min at 4 °C, and the collected supernatant was dialyzed overnight against an extraction buffer. The dialyzed enzymes were kept as small aliquots at -20 °C until use.

2.5.2 *In vitro* carbohydrate digestibility

Carbohydrate digestibility of each cyanobacteria sample was determined using the method described by Thongprajukaew et al. [24]. The reaction mixture contained 5 mg of freeze-dried cyanobacteria, 10 mL of 50 mM phosphate buffer at pH 8.2, 50 µL of 0.5% chloramphenicol, and 125 µL of dialyzed enzyme extract, and was allowed to react at 25 °C for 24 h. Digestibility (µmol maltose g^{-1}) was determined from the increase in reducing sugar, detected by the dinitrosalicylic acid (DNS) method calibrated with a maltose standard curve.

2.6 Statistical Analysis

Completely randomized experimental design was used, and the data are reported as mean and SEM (n = 3). Significant differences between means were analyzed by One-Way ANOVA and by Duncan's multiple range test at 95% confidence level.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition

Pretreatments have been widely used to improve the nutritive values of various food or feed raw materials. Differences in chemical

composition were observed between the non-pretreated and the pretreated cyanobacteria (Table 1). Protein content was significantly improved by all actual pretreatments ($P < 0.05$), except for ultrasonication ($P > 0.05$), relative to non-pretreated control. The increased protein content in all irradiated cyanobacteria matches well the findings from 10 min microwave irradiation of gamma irradiation of palm kernel meal [5], soybean meal [6], and electron beam irradiation of dried *Amanita* mushrooms [25]. The irradiation could modify the molecular properties of protein, as well as other N-containing compounds, by forming covalent cross-linkages or conversions to higher molecular weight aggregates [9]. Such effects would increase the protein content as detected by the Kjeldahl method. Moreover, C-N bond scissions in the backbone of polypeptide chains, or physical changes like unfolding, can increase the availability of nitrogen atoms [25]. On the other hand, pretreatments without effects on protein quantity have also been reported [26].

Table 1. Chemical composition (g kg^{-1} of dry matter) and gross energy (kcal kg^{-1}) of non-pretreated and pretreated cyanobacteria. Data were calculated from triplicate determinations.

Chemical composition	Non-pretreated	Microwave irradiation	Ultrasonication	Gamma irradiation	Electron beam irradiation	*P* value
Crude protein	415.9 ± 0.4^{c}	422.1 ± 1.6^{b}	413.4 ± 0.2^{c}	429.8 ± 0.7^{a}	427.2 ± 1.0^{a}	< 0.001
Crude lipid	14.8 ± 1.0^{a}	17.8 ± 1.6^{a}	17.7 ± 1.3^{a}	6.7 ± 0.7^{b}	18.3 ± 1.5^{a}	0.005
Crude ash	21.9 ± 0.2^{b}	24.1 ± 0.8^{ab}	22.7 ± 0.1^{ab}	25.0 ± 1.1^{a}	25.1 ± 1.1^{a}	0.123
Crude fiber	3.9 ± 1.0^{b}	5.3 ± 1.5^{b}	9.8 ± 1.0^{a}	9.0 ± 1.0^{a}	1.8 ± 0.6^{c}	0.012
NDF	223.6 ± 5.8^{a}	158.0 ± 0.6^{b}	126.4 ± 0.6^{d}	160.4 ± 5.1^{b}	148.0 ± 0.7^{c}	< 0.001
NFE	543.5 ± 2.1	532.2 ± 0.4	536.3 ± 2.0	529.5 ± 0.3	527.6 ± 1.1	0.342
GE (kcal kg^{-1})	$4,713 \pm 6.5$	$4,736 \pm 1.7$	$4,721 \pm 5.5$	$4,678 \pm 6.5$	$4,736 \pm 0.7$	0.248

ADF, acid detergent fiber; NFE, nitrogen free extract; GE, gross energy.
Values with different superscripts in the same row are significantly different ($P < 0.05$).

Decreased lipid content was only observed with gamma irradiation (Table 1). Changes in the lipid content mainly occur from effects at the double bonds of unsaturated fatty acids. The loss of lipids by irradiation may result from the formation of free radicals, enabling the molecules to react with conjugated systems, and free radicals are often considered initiators of lipid oxidation [27]. Even though irradiation can cause significant changes in lipid content and fatty acid profiles [28], both microwave and electron beam irradiation gave unchanged lipid content while gamma irradiation changed it. Such effects may depend on irradiation time and temperature [29] as well as intensity.

Ash content was significantly higher with both gamma and electron beam irradiation than with microwave irradiation, ultrasonication, or in non-pretreated sample (Table 1). Chumwaengwapee et al. [11] postulated an increase in ash content due to a chelating reactions induced by microwave or electron beam irradiation. This increase appears dose dependent in pretreatments by electron beam irradiation [25].

No significant treatment effects were observed on NFE or GE (Table 1). All actual treatments had the positive effect of reducing

NDF. The ADF was below detection limit in all cases. The presence of NDF (cellulose, hemicellulose and lignin) and absence of ADF (cellulose and lignin) indicates that cyanobacterial cell walls are hemicellulose based. The NDF can be various affected by ionizing or non-ionizing radiation [30, 31]. Disruption of the hemicelluloses dramatically decreased the crude fiber content in cyanobacteria pretreated by electron beam irradiation. This pretreatment appears to destroy cell wall constituents, based on interpreting FTIR spectra [5]. Generally, ionizing radiation (gamma and electron beam) has sufficient energy to break chemical bonds. Increased crude fiber in ultrasonication and gamma irradiation treatments might be due to the subsequent re-organization of the broken polymers.

3.2 Nutritive Profiles

The FTIR spectra in Figure 1 for the range from 4000 to 400 cm^{-1} exhibited at least nine bands (2960, 2924, 2852, 1652, 1542, 1454, 1411, 1258 and 1050 cm^{-1}), indicating the presence of protein, lipid, carbohydrate and deoxyribonucleic acid (Table 2). Generally all the spectra had similar characteristics but differed in peak heights and intensities. The crystalline/amorphous peak intensity ratio (1429/893 cm^{-1}) was significantly reduced by any actual treatment (0.724 ± 0.002, 0.754 ± 0.003, 0.784 ± 0.002 and 0.798 ± 0.002 for electron beam irradiation, gamma irradiation, ultrasonication and microwave irradiation, respectively), relative to non-pretreated control (0.888 ± 0.003). Similarly, little changes were also found at 1047/1022 cm^{-1}, giving the values 0.936 ± 0.003, 0.941 ± 0.002, 0.961 ± 0.001, 0.959 ± 0.003 and 0.983 ± 0.004 in the same order. These findings indicate that all actual treatments expanded the amorphous component, which could increase the bioavailability of nutrients under *in vitro* conditions [5]. The experimental findings indicate only minor treatment effects on nutritive value, but dramatic increases in amorphous content. Based on observed changes in the chemical composition and the nutritive profiles, microwave and electron beam irradiation treatments had no negative effects but appeared to improve the nutritive values of cyanobacteria.

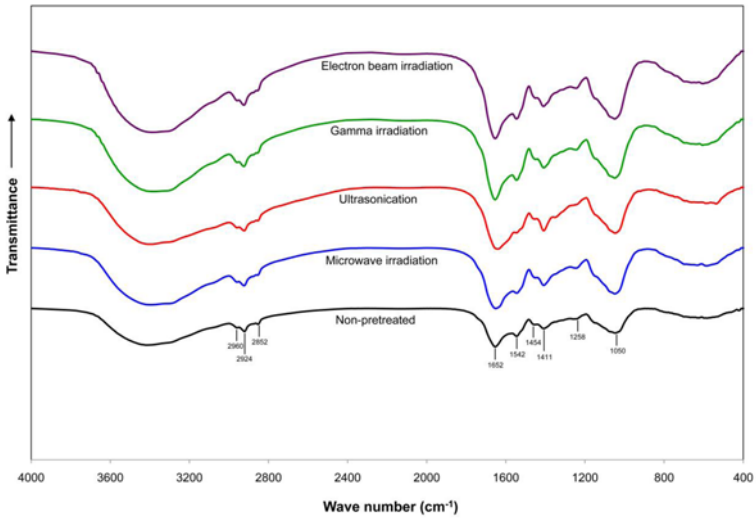

Figure 1. FTIR spectra of non-pretreated, microwave-irradiated, ultrasonicated, gamma-irradiated and electron beam-irradiated cyanobacteria.

Table 2. Tentative assignment of FTIR spectral peaks found in non-pretreated and pretreated cyanobacteria.

Wave number (cm^{-1})	Tentative band assignment	Macromolecule	References
2960	$\nu_s(CH_2)$ stretching of methyl	Lipid	[17]
2924	$\nu_{as}(CH_2)$ stretching of methylene	Lipid	[15, 17]
2852	$\nu_s(CH_2)$ stretching of methylene	Lipid	[15, 17]
1652	ν_s(C=O) stretching of amide I	Protein	[19]
1542	δ(N-H) bending and ν (C-N) stretching of amide II	Protein	[17]
	Double bond vibrations of bases	Deoxyribonucleic acid	[16]
1454	$\delta_{as}(CH_2)$ bending of methyl $\delta_{as}(CH_2)$ and	Lipid	[15, 17]
	$\delta_{as}(CH_3)$ bending of methyl	Protein	[18]
1411	ν_s (COO^-) stretching of amino acid salt	Protein	[21]
	ν_s (C=O) stretching vibrations of carboxylate	Carbohydrate	[22]
1258	N(C=O) stretching and δ(C-OH) bending of deprotonated amino acid	Protein	[20]
1050	ν (C-O-C) stretching of polysaccharide	Carbohydrate	[14]

3.3 Physicochemical Properties

3.3.1 pH

Macromolecule breakdown after the pretreatments could be observed by the direct measurement of pH. The pretreatments had a significant effect in reducing the pH. The lowest pH-value was observed in those cyanobacteria pretreated by ultrasonication (6.71 ± 0.03), followed by microwave irradiation (7.07 ± 0.03), and then electron beam (7.32 ± 0.03) and gamma irradiation (7.25 ± 0.02), and finally non-pretreated control (7.45 ± 0.04). In the case of carbohydrates, the breakdown of starch by actions of free radicals can induce the formation of carboxyl groups, resulting in a lower pH [7]. This phenomenon has been similarly reported in gamma and electron beam irradiated coconut meal [11].

3.3.2 Turbidity

There were significant differences in turbidity between the pretreatments of cyanobacteria. The lowest turbidity was observed in microwave-irradiated cyanobacteria (0.277 ± 0.011); significantly differing from the group of ultrasonicated (0.423 ± 0.015), gamma-irradiated (0.381 ± 0.021) and electron beam-irradiated (0.397 ± 0.028) samples; which further differed from the non-pretreated control (0.543 ± 0.017). Decreased turbidity would be nutritionally advantageous in food or feed raw materials. This is because the reflection or scattering of light relates to the interactions between leached amylose and amylopectin chains [32], to starch granule remnants and swelling, and to the chain lengths of leached amylose and amylopectin [33]. Reduced turbidity might then indicate a reduction in the number of leached molecules, or that the molecules are smaller in size; and elevated turbidity may indicate the strong aggregation of molecules [10]. Thus, the decreased pH and turbidity of pretreated cyanobacteria suggest improved digestibility due to cleaved molecules.

3.3.3 Microstructure

There were significant changes in the microstructure of cyanobacteria caused by the treatments (Figure 2). The general features at a low magnification (left panel) were irregular and fragile. Disruptions of cell wall, as indicated by surface roughness, were observed at higher magnifications (middle and right panels). The gamma (Figures 2J-L) and electron beam (Figures 2M-O) irradiation treatments induced damaged characteristics with scrubbed surface and shallow grooves. Concave surfaces appearing molten were observed in the cyanobacteria pretreated by microwave irradiation (Figures 2D-F). Chumwaengwapee et al. [11] reported that microwave irradiation gave porous and concave surfaces to raw materials, while damaged surfaces with shallow grooves were observed after pretreatments by gamma or electron beam irradiation. Also Thongprajukaew et al. [5] reported similar pretreatment effects. This agrees with our current work, and prior studies have linked the effects to *in vitro* digestibility. Ultrasonication (Figures 2G-I) appeared to have intermediate effects between those caused by non-ionizing (microwave irradiation) and ionizing radiation (gamma and electron beam irradiation). Kotopoulis et al. [12] applied ultrasound to crack cyanobacterial cells, causing the strands to sink. The microscopy observations in the current study suggest that ultrasonication damaged the cell walls efficiently. Among the four pretreatment methods, microwave irradiation gave the strongest apparent effects on the surface structure, and positively impacted the hydrolytic rate. Moreover, based on the melting characteristics the microwave pretreatment probably caused gelatinization, conferring nutritional advantages.

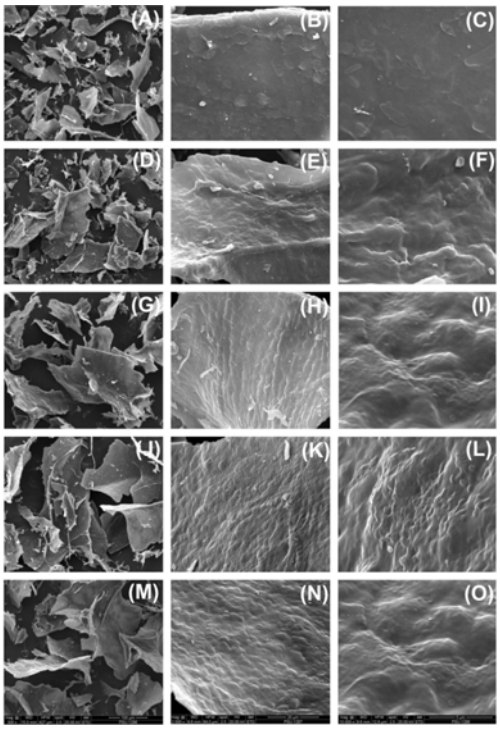

Figure 2. Microscopic structures of non-pretreated (A-C), microwave-irradiated (D-F), ultrasonicated (G-I), gamma-irradiated (J-L), and electron beam-irradiated (M-O) cyanobacteria. Magnifications of photographs were 300× (left), 2,000× (middle) and 10,000× (right).

3.3.4 Thermal transition properties

There were differences in thermal transition properties (T_o, T_p, T_c, T_c-T_o and ΔH) between the five treatments of cyanobacteria (Table 3). Two transition peaks were observed within the studied temperature range from 40 to 400 °C. Peak 1 was designated to available nutrients and peak 2 to unavailable cell wall constituents, these peaks spanning the temperature ranges 41.7-118.1 °C and 266.4-310.2 °C, respectively. The low temperature peak 1 showed the following patterns. Microwave irradiation treatment gave the narrowest

T_c-T_o among all treatments, while a dramatic increase in this characteristic was caused by gamma irradiation that strongly increased T_c. These two treatments also had the lowest ΔH values. Peak 2 at higher temperatures gave somewhat similar patterns. All actual treatments decreased T_c-T_o relative to non-pretreated control, while electron beam irradiation gave the lowest ΔH, followed by microwave irradiation.

Thermal transitions at relatively low temperatures indicated the presence of substances that are easily gelatinized or denatured. Focusing on carbohydrates, a decrease in T_p could result from weakening the starch granules [34], while a narrow T_c-T_o range indicates a narrow chain length distribution of the polymers cleaved by a pretreatment [10]. Positive changes in both these parameters occurred with microwave treatment of cyanobacteria. These effects tend to lower the ΔH by forming a high fraction of partially transformed macromolecules. Disruptions of the cell walls, generally observed through the NDF content, were also detected by the DSC at high temperatures. Significant changes in the thermal transition parameters (T_o, T_p, T_c, T_c-T_o and ΔH) indicated that the four pretreatments effectively disrupted cell walls. In terms of these characteristics, microwave irradiation appeared to have a strong effect on the thermal transition properties of both available and unavailable feed constituents in cyanobacteria.

Table 3. Thermal transition properties of non-pretreated and pretreated cyanobacteria. Data were obtained by triplicate observations.

Thermal parameter	Non-pretreated	Microwave irradiation	Ultrasonication	Gamma irradiation	Electron beam irradiation	*P* value
Peak 1						
T_o (°C)	42.33 ± 0.51	43.93 ± 0.62	41.67 ± 0.53	44.47 ± 1.20	41.58 ± 0.34	0.125
T_p (°C)	71.75 ± 0.32^a	64.08 ± 0.61^b	71.00 ± 0.20^a	69.50 ± 0.42^a	65.25 ± 0.38^b	0.007
T_c (°C)	102.90 ± 0.12^c	91.04 ± 0.57^d	105.87 ± 0.21^b	118.10 ± 0.56^a	106.90 ± 0.32^b	< 0.001
T_c-T_o (°C)	60.57 ± 0.04^c	47.11 ± 0.58^d	64.20 ± 0.41^b	76.63 ± 0.25^a	65.32 ± 0.62^b	< 0.001
ΔH (J g^{-1})	47.97 ± 0.24^b	38.38 ± 0.51^c	54.45 ± 0.21^a	28.12 ± 0.22^d	47.93 ± 0.49^b	< 0.001
Peak 2						
T_o (°C)	266.37 ± 0.42^b	272.09 ± 1.22^a	268.30 ± 0.52^{ab}	271.67 ± 1.05^b	275.59 ± 2.10^a	0.012
T_p (°C)	284.75 ± 0.81	284.75 ± 0.42	280.83 ± 1.40	285.75 ± 0.16	285.33 ± 0.27	0.214
T_c (°C)	310.18 ± 0.40^a	305.00 ± 0.14^b	302.65 ± 0.28^b	304.11 ± 0.19^b	313.51 ± 0.65^a	0.021
T_c-T_o (°C)	43.81 ± 0.25^a	32.91 ± 0.34^c	34.35 ± 0.22^c	32.44 ± 0.15^c	38.16 ± 0.12^b	< 0.001
ΔH (J g^{-1})	52.53 ± 0.15^a	25.70 ± 0.21^d	43.88 ± 0.27^b	34.79 ± 0.10^c	13.82 ± 0.05^e	< 0.001

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; T_c-T_o, melting temperature range; DH, transition enthalpy.

Values with different superscripts in the same row are significantly different ($P < 0.05$).

3.3.5 Diffraction patterns

No differences in diffraction patterns or diffraction peaks were observed between the treatments (Figure 3). This finding is in agreement with prior studies in palm kernel meal [5] and coconut meal [11]. Only one diffraction peak (19.6°) was observed within the 2θ range from 4 to 35°, and checking the range up to 90° did not change this observation (data not shown). Kaur et al. [35] reported a negative correlation coefficient between relative crystallinity and the *in vitro* digestibility of rapidly and slowly digestible starches in Indian lentils. Hence a significant decrease in this parameter may cause an increase in digestible starch. Calculated relative crystallinity of cyanobacteria was significantly reduced by ultrasonication (34.74 ± 0.04%), electron beam irradiation (35.56 ± 0.04%), and microwave irradiation (36.40 ± 0.03%), but not by gamma irradiation (37.09 ± 0.03%), relative to non-pretreated control (37.02 ± 0.02%). This finding is also supported by the DSC transition properties and FTIR (intensity ratios of 1429/893 cm^{-1} and 1047/1022 cm^{-1}), suggest disruption of the crystalline regions and expansion of the amorphous regions.

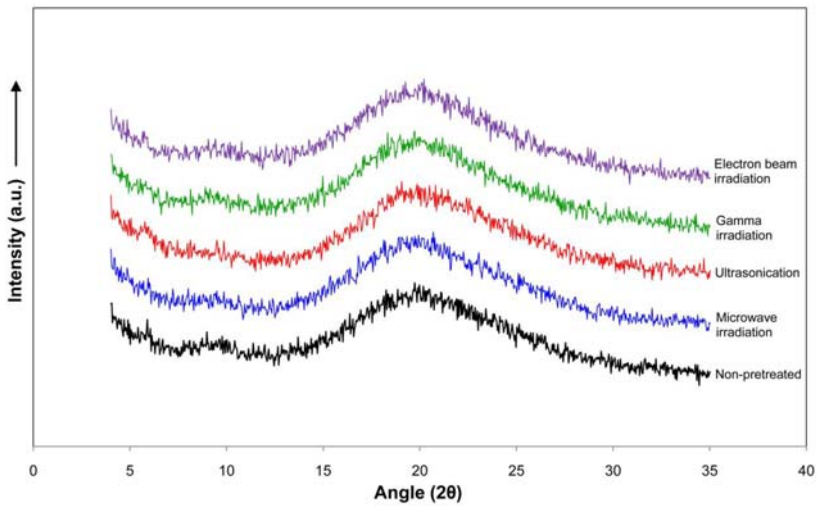

Figure 3. X-ray diffractograms of non-pretreated, microwave-irradiated, ultrasonicated, gamma-irradiated and electron beam-irradiated cyanobacteria.

3.4 *In vitro* Carbohydrate Digestibility

The treatments significantly affected carbohydrate digestibility (Figure 4). The highest digestibility was obtained by microwave irradiation significantly ($P < 0.05$) differing from non-pretreated control, followed by gamma and electron beam irradiation without such significance ($P > 0.05$). The improved *in vitro* carbohydrate digestibility of microwave pretreated cyanobacteria, strongly suggesting positive effects on *in vivo* digestibility. Improvements in carbohydrate digestibility by microwave irradiation have been reported for various raw materials, and have been linked to physicochemical changes [5, 6, 11]. Ultrasonication as pretreatment can disrupt cell walls and facilitate extracting various active ingredients [36]. However, a negative effect observed in the current study was the significant decrease in carbohydrate digestibility. Optimization of sonication to improve the physical properties of starch has been reported [37]; and the physical

parameters are sufficiently informative for improving carbohydrate digestibility [35]. Study reports tend to emphasize positive effects, but also the negative side effects of pretreatments are of similar importance. Ultrasonication and other pretreatment methods may provide dominantly positive outcomes, but only when applied at specified conditions. Therefore, some process parameters of each pretreatment method, such as heating time, ratio of feedstuff to water, and temperature and power, should be optimized experimentally to improve the positive effects and reduce the negative effects; such assessments would warrant several future research projects.

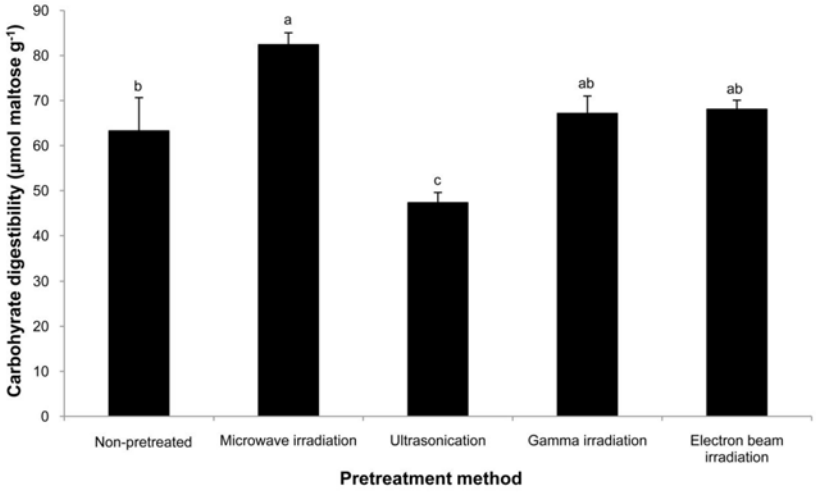

Figure 4. *In vitro* carbohydrate digestibility of non-pretreated, microwave-irradiated, ultrasonicated, gamma-irradiated and electron beam-irradiated cyanobacteria, using digestive enzyme extracts from Nile tilapia (n = 3). Data with different superscripts are significantly different ($P < 0.05$).

4. CONCLUSIONS

Improved chemical composition (protein and neutral detergent fiber) and physicochemical properties were achieved by microwave irradiation of cyanobacteria. This treatment contributed to the bioavailability by disrupting the cell walls and transforming the infrastructure. The hydrolytic rate was enhanced based on *in vitro* digestibility in an aquatic animal model. Pretreatment by microwave irradiation has a fast heating rate and a short processing time compared to conventional heating. The remarkable acceptance by the food and feed industries also supports this concept. Optimizing the microwave irradiation conditions could further improve the results, and may be explored in a future study. The effects of microwave pretreatment on physicochemical properties in relation to protein digestibility of cyanobacteria are currently under work.

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