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Improving chemical composition, physicochemical properties, and in vitro carbohydrate digestibility of fish coconut meal

Sunetra Chumwaengwapee[a], **Sarisak Soontornchai**[a], **Karun Thongprajukeaw**[b,c,*]

[a] School of Health Science, Sukhothai Thammathirat Open University, Nonthaburi 11120 Thailand
[b] Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112 Thailand
[c] Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok 10900 Thailand

[*]Corresponding author, e-mail: karun.t@psu.ac.th

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ABSTRACT: Coconut meal (CM) is an industrial byproduct of coconut oil and coconut milk production which may be modified for use as fodder. Here we study the effects of the physical modification of CM. Four modification methods were tested: water soaking, microwave-, γ-, and electron-beam irradiation. We determined the CM chemical composition, physicochemical properties, and in vitro carbohydrate digestibility by digestive enzyme extracts from Nile tilapia (*Oreochromis niloticus*) and silver barb (*Barbonymus gonionotus*). CM modifications decreased the level of crude fibre but increased the available carbohydrates ($p < 0.05$). Furthermore, water soaking changed the physicochemical properties including pH, water solubility, microstructure, thermal properties, and crystallinity. Water soaking also increased the carbohydrate digestibility by the fish enzymes tested. The findings of this study therefore suggest that the quality of CM as a feedstuff can be improved by water soaking.

KEYWORDS: economic fish, feedstuff, irradiation

INTRODUCTION

Coconut meal (CM), a common industrial by-product of the production of coconut oil and coconut milk, serves as a feed ingredient for rearing terrestrial animals. For aquatic species, however, and especially for fish, limited information is currently available on the use of CM in diets. This may be due to the nutritional limitations of CM, such as deficiencies in important amino acids and low protein digestibility[1]. CM contains high dietary fibre, mainly in the form of mannan (26%), galactomannan (61%), and cellulose (13%)[2]. These components may impair the digestibility and utilization of nutrients, by encapsulating them or by increasing the viscosity of the intestinal contents. Decreasing the dietary fibre in CM may improve the utilization of available carbohydrates and indirectly that of other nutritional components.

The modification of food or feed ingredients can improve the physicochemical properties and the digestibility of the various raw materials. Soaked CM, can be used as a replacement for soya bean meal (30%) without any deleterious effect on the growth performance and nutrient utilization of fingerlings of Nile tilapia, *Oreochromis niloticus*[3]. Moreover, other physical procedures, including microwave irradiation (non-ionizing radiation)[4,5], γ-irradiation[6,7] and electron beam (ionizing radiation)[8,9], have also been used to improve the nutritional quality of raw materials. These methods can alter otherwise unavailable constituents by changing or cleaving chemical bondings. On the other hand, some conventional methods are frequently carried out under long term heating which can often lead to nutritional loss and have less effect on destructing unavailable carbohydrates.

This study aimed to study the effects of different methods of modifying CM to improve the level of available nutrients, particularly carbohydrates. Analysis of the chemical composition, physicochemical properties, and in vitro digestibility were the primary means used to evaluate the nutritional quality. Two freshwater fish with high economic values, and different feeding habits, namely the omnivorous Nile tilapia (*O. niloticus*) and the herbivorous silver barb (*Barbonymus gonionotus*), were used as sources of digestive enzymes for in vitro screening. The findings from present study might be used to improve the nutritional quality of CM as a feed ingredient for the production of aquatic animals.

MATERIALS AND METHODS

Modification of CM

CM from pressed coconut meat was obtained from three local markets in Hat-Yai, Songkhla, Thailand. Unprocessed CM was used as a control sample with another four samples being physically modified. The modification methods used were (1) water soaking; the CM was soaked in distilled water (1:10 w/v) for 12 h at ambient temperature; (2) microwave irradiation; 50 g of CM was placed in a beaker, mixed with distilled water (1:9 w/v), and then cooked at 800 W in a microwave oven (MW 71B, Samsung, Malaysia) under agitation for 5 min; (3) γ-irradiation; the CM sample was irradiated at a dose of 30 kGy using ^{60}Co from a γ-irradiator (JS 8900 IR-155, MDS Nordion, Canada); and (4) electron beam irradiation; CM was irradiated at a dose of 30 kGy at a fixed beam energy of 10 MeV using an electron accelerator (TT-200, IBA Co. Ltd., Belgium). For γ- and electron beam-irradiation, the processes were conducted at the Institute of Nuclear Technology (Public Organization), Thailand.

Preparation of CM

The samples of raw and modified CMs were dried using a freeze dryer (Delta 2–24 LSC, Germany) for 48 h, ground, and sieved. All the prepared samples were packed in polyethylene bags covered by black plastic and then kept in desiccators for later analysis of their chemical composition and physicochemical properties.

Chemical composition

The chemical composition of the CM samples including their protein, lipid, ash, and fibre content were analysed according to standard methods of the AOAC[10]. The nitrogen free extract was calculated based on the difference. All the chemical compositions are reported on dry matter basis.

Physicochemical properties

One gram of CM was suspended in 25 ml of water at 25 °C and agitated for 10 min[11]. The measurement of pH was conducted using a pH meter (Cyber Scan 510, Eutech Instrument, Singapore). The water solubility was determined according to the method of Chung et al[7]. Briefly, 1 g of CM was mixed with 10 ml of water, gently stirred for 1 h at room temperature and centrifuged at 1500g for 10 min. The solubility of the CM was calculated from the ratio between the dissolved solid weight in supernatant and the dried solid weight in the original sample.

The CM samples were mounted by double-sticky tape on an aluminium stub and coated with gold. Microscopic pictures of the CM samples were produced using a scanning electron microscope (Quanta 400, FEI, Czech Republic) at 50, 250, and 1500$\times$ magnifications. The energy potential during micrography was 15 kV.

The diffraction patterns of the CM samples were determined with an X-ray diffractometer (X′Pert MPD, Philips, Netherlands) operated at a voltage of 40 kV and a current of 40 mA current. Diffractograms were recorded between 4° and 35° (2θ) with a scanning rate of 1°/min.

The thermal properties of the CM samples were measured using a differential scanning calorimeter (DSC7, Perkin Elmer, USA). Approximately 3 mg of a freeze-dried sample was placed in an aluminium pan, and sealed, then allowed to equilibrate at room temperature for 1 h, then heated from 40–180 °C at a rate of 5 °C/min. The thermal parameters, onset ($T_{\rm o}$), peak ($T_{\rm p}$) and conclusion ($T_{\rm c}$) temperatures, and transition enthalpy (ΔH), were recorded automatically.

Determination of in vitro carbohydrate digestibility

One year old Nile tilapia (n = 3, 800–900 g body weight and 33–36 cm total length) and six months old silver barb (n = 3, 400–450 g body weight and 29–30 cm total length) were randomly collected from a farm in Hat-Yai, Songkhla, Thailand. The fish were fed *ad libitum*, twice daily (08:00 and 18:00 h) with a commercial diet (20% crude protein). The preparation of fish for killing was conducted based on "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes (Sections 1.4 and 4.5.3)", National Research Council, Thailand. The intestines of all the fish were carefully collected, kept in ice and then transported to the Department of Applied Science, Faculty of Science, Prince of Songkla University. Subsequently, the samples were homogenized in 50 mM Tris-HCl buffer (pH 8) containing 200 mM NaCl (1:4 w/v) using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). Centrifugation of the homogenate was carried out at 15 000g for 30 min at 4 °C. The supernatant was collected and then kept at -20 °C.

The crude enzyme extracts were dialysed overnight against the extraction buffer. The in vitro reaction was performed according to the method described in Thongprajukaew et al[12]. The carbohydrate digestibility was determined by quantitative analysis of the liberated maltose after incubation. The digestibility values were calculated,

Table 1 Chemical composition and physicochemical properties of raw and modified CM samples. Data were calculated from triplicate determinations and are expressed on dry matter basis.

Parameter	Unmodified	Water soaking	Microwave irradiation	γ-irradiation	Electron beam
Chemical composition (%)					
Crude protein	4.63 ± 0.05^{a}	4.60 ± 0.00^{ab}	4.70 ± 0.05^{a}	4.68 ± 0.00^{a}	4.46 ± 0.04^{b}
Crude lipid	31.00 ± 0.01^{b}	30.08 ± 0.10^{c}	30.08 ± 0.03^{c}	32.14 ± 0.25^{a}	31.44 ± 0.22^{b}
Ash	1.03 ± 0.00^{c}	1.26 ± 0.06^{a}	1.09 ± 0.04^{bc}	1.17 ± 0.01^{ab}	1.17 ± 0.02^{ab}
Crude fibre	36.89 ± 0.05^{a}	33.02 ± 0.41^{b}	28.74 ± 0.10^{c}	24.38 ± 0.61^{d}	25.49 ± 0.34^{d}
Nitrogen free extract	26.45 ± 0.07^{d}	31.04 ± 0.43^{c}	35.39 ± 0.12^{b}	37.63 ± 0.66^{a}	37.44 ± 0.41^{a}
Physicochemical properties					
pH	5.61 ± 0.01^{c}	6.84 ± 0.01^{b}	6.95 ± 0.01^{a}	5.34 ± 0.02^{d}	5.27 ± 0.00^{e}
Water solubility (%)	5.78 ± 0.58^{b}	8.69 ± 0.62^{a}	6.09 ± 0.30^{b}	5.50 ± 0.53^{b}	5.19 ± 0.16^{b}
Relative crystallinity (%)*	5.7	3.9	4.4	5.2	5.6

* Relative crystallinity was calculated from only one sample.
Values with different superscripts in the same row indicate significant difference ($p < 0.05$).

standardized with equal amylase activity, and are expressed as μmol maltose/g.

Statistical analysis

Data are reported as mean ± SE from triplicate observations. The significant differences between the means were analysed by Duncan's multiple range test at 95% confidence levels.

RESULTS

Chemical composition

Physical modification had the effects on the chemical composition of the CM samples shown in Table 1. A significant decrease in protein content was found in the CM modified by electron beam irradiation ($p < 0.05$) whereas the protein content of the samples modified by the other methods were not statistically different when compared with that of the control sample ($p > 0.05$). The lipid content increased in the γ-irradiated CM, and decreased in the water-soaked and microwave-irradiated CM samples. The ash content increased significantly in the water-soaked sample and higher values were found in the samples modified by other methods. The crude fibre decreased progressively after all the modifications whereas the available carbohydrate increased inversely.

Physicochemical properties

Significantly increased in pH values were found in the water-soaked and microwave-irradiated CM samples, whereas a decrease in pH was observed in the CMs modified by γ- and electron beam-irradiation ($p < 0.05$, Table 1). The water solubility of the nutrients had the highest value in the samples pretreated by soaking (Table 1), with, similar solubility being detected between the control sample and the samples modified by other methods.

Modification changed the microscopic structure of the CM samples (Fig. 1). Porous and concave surfaces were similarly found in the CM samples modified by water soaking (Fig. 1d–f) and microwave irradiation (Fig. 1g–i). The area of rough surface was, however, higher in the sample pretreated by soaking as compared to that of the microwave-irradiated sample. Damaged surfaces with shallow grooves were observed after modification by γ- (Fig. 1j–l) and electron beam-irradiation (Fig. 1m–o).

The diffraction patterns of the control and modified CM samples were similar (Fig. 2). The main peaks were found at diffraction angles of 15.9°, 20.1°, 23.6°, and 25.2° (2θ). The calculated relative crystallinity (RC) significantly decreased in the water-soaked (4% RC), microwave-irradiated (4% RC), and γ-irradiated (5% RC) CM samples whereas similar values were found in the unmodified (6% RC) and electron beam-irradiated (6%) CM samples (Table 1).

Differences in transition temperatures (T_o, T_p and T_c), enthalpy (ΔH), and the degree of gelatinization (DG) were observed in the CM samples modified by different methods (Table 2). Notable changes in thermograms were detected within the temperature range of 46.5–152.2 °C. The γ-irradiated and electron beam-irradiated CM samples had higher transition temperatures whereas the water-soaked and microwave-irradiated CM samples had higher T_o but lower T_p and T_c, when compared with that of the control sample. The melting temperature range was broader after γ-irradiation, and was narrower after modification by the other methods, when compared with that of the

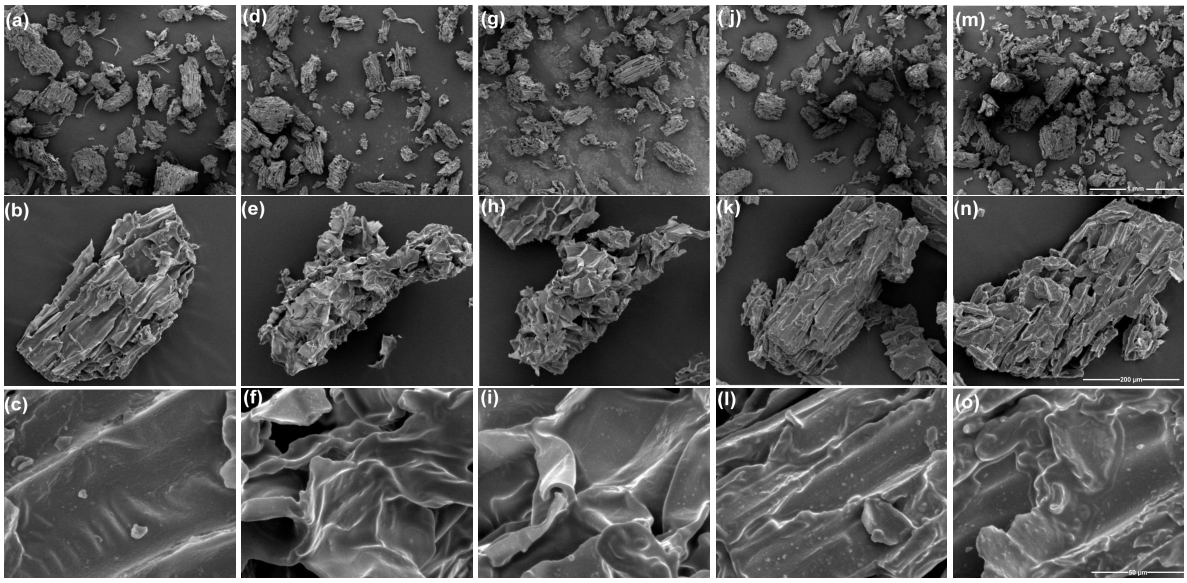

Fig. 1 Microstructures of (a–c) raw and (d–o) modified CM samples: (d–f) water soaking, (g–i) microwave irradiation, (j–l) γ-irradiation, and (m–o) electron beam. Magnifications of photographs were recorded at 50× (top panel), 250× (middle panel) and 1500× (bottom panel).

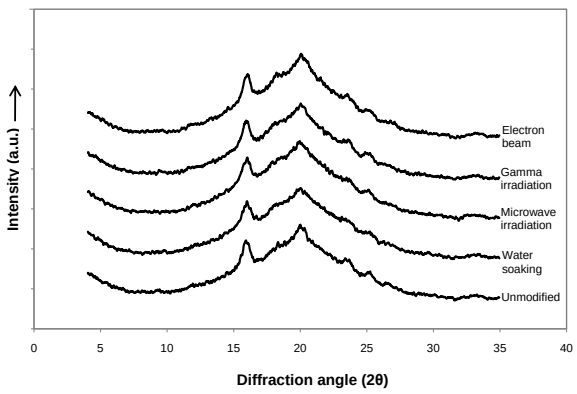

Fig. 2 Diffractograms of CM samples modified by different physical methods. Diffraction patterns were detected between 4° and 35° (2θ).

control sample. The ΔH of the modified CM samples had lower values than in the control sample. The highest DG value was observed in the water-soaked CM, followed by that modified by electron beam, γ-, and microwave irradiation, respectively.

In vitro carbohydrate digestibility

The method of modification had some effect on the carbohydrate digestibility in both fish species. Soaking and microwave irradiation produced significantly increased digestibility in Nile tilapia, as did, to a lower degree, irradiation by electron beam, when compared with the control sample ($p < 0.05$, Fig. 3a). No significant changes in digestibility were found in any of the treatments for silver barb ($p > 0.05$, Fig. 3b). Relatively higher levels of digestibility were obtained, however, after modification by water soaking and microwave irradiation.

DISCUSSION

Chemical composition of CM samples

The protein contents were unchanged after modification by water soaking, microwave irradiation and γ-irradiation and this finding is in agreement with earlier research using γ- and microwave-irradiated fish feed mixtures[12], and γ-irradiated soy flour[13]. This indicates that the three modification processes had no effect on the quantity of protein. The increased lipid content after γ-irradiation probably arises as a result of the transformation of unsaturated fatty acids into saturated fatty acids, which is a normal factor affecting lipid rancidity. On the other hand, decreased lipid content in the CM might be due to the peroxidation of saturated fatty acids, providing hydroperoxides and other secondary oxidation products. This interpretation correlates with the decreased polyunsaturated fatty acids found after feed mixtures were exposed to microwave irradiation[14]. The significant increase in ash content might be due to a chelating reaction induced by soaking in water and irradiation by microwaves and electron beam.

Table 2 Thermal transition properties of raw and modified CMs observed from DSC thermograms.

Thermal parameter	Unmodified	Water soaking	Microwave irradiation	γ-irradiation	Electron beam
T_o (°C)	46.5	54.1	47.9	61.2	59.2
T_p (°C)	82.8	81.7	80.3	97.3	89.2
T_c (°C)	119.7	110.9	113.9	152.2	125.9
ΔH (J/g)	95.7	46.7	67.9	60.2	49.0
DG (%)*	0.0	51.2	29.0	37.1	48.8

* Degree of gelatinization (DG) was calculated from $1 - (\Delta H$ of modified CM)/(ΔH of unmodified CM)

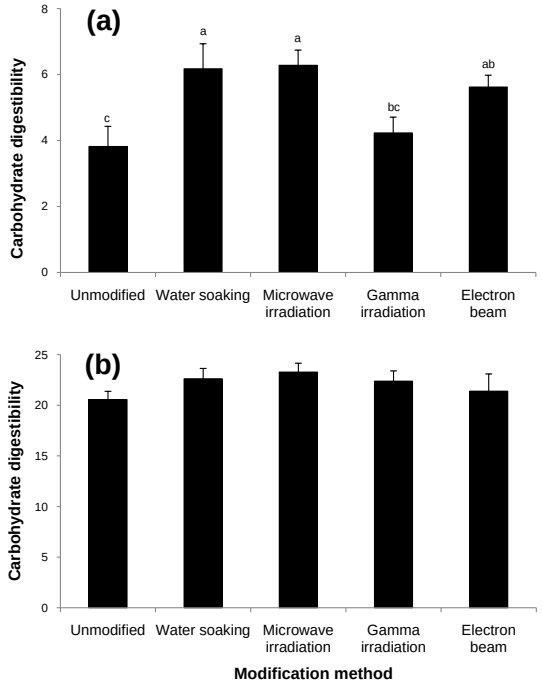

Fig. 3 In vitro carbohydrate digestibility (μmol maltose/g) of raw and modified CM samples, using digestive enzyme extracts (amylase activity = 1000 U) from (a) Nile tilapia and (b) silver barb. Data with different superscripts are significantly different ($p < 0.05$).

Decreased fibre after modification has previously been reported in γ-irradiated wheat straw, cotton seed shell, peanut shell, soya bean shell, extracted olive cake, and extracted unpeeled sunflower seeds[15], and pretreated, delignified and steam-exploded rice straws[16]. This decrease occurred concurrently with a significant increase in available carbohydrate. Both findings indicate that modification may destroy physical barriers, which in CM would probably be lignocellulosic constituents. Hence the results found in this study may indicate that there is more available carbohydrate in modified CMs than in unmodified material.

Physicochemical properties of CMs

The increased pH in the water-soaked and microwave-irradiated CM samples might have arisen as a result of the release of hydroxyl groups from lignocellulosic degradation. On the other hand, decreased pH values in the CM samples modified with ionizing radiations (γ and electron beam-irradiation), are probably due to the breakdown of starch molecules due to the action of free radicals, inducing the formation of carboxyl groups. This tendency has been similarly reported in γ-irradiated corn[17] and potato and bean starches[18]. Water solubility is an important characteristic governing the hydrolytic properties of feedstuff due to digestive enzymes[12]. Significantly increased solubility after soaking suggests that the modification method may improve this property in CM.

As regards to the microstructure, soaking and microwave irradiation can cause an increase in the surface structure of CM. This was also observed in fish diet and wheat straw after modification[14,16]. On the other hand, the smooth and denser surface found in the other treatments is similarly in accord with previous findings relating to γ-irradiated corn and potato starches[17,18], when compared with non-treated starch.

Differences in RC and the strength of diffraction peaks were observed in water-soaked and microwave-irradiated CM samples when compared with the control sample. This suggests that the crystalline region might be disturbed, causing an increase of the amorphous region, after the modification of the raw material. Kaur et al[19] reported a negative correlation coefficient between RC 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 in CM. Moreover, Cooke and Gidley[20] suggested that the change of melting enthalpy primarily reflects a loss of double helical order rather than a loss of crystalline order. This suggests that the alteration in the modified CM occurs at both the double helical and crystalline levels.

With regard to melting temperature range, Bao and Corke[21] suggested that the value might be increased directly by the heterogeneity of the starch crystallites. Hence the narrowness in the temperature range of the water-soaked CM is probably due to the homogeneity in the cleaved length of amylose and amylopectin chains after modification. Decreased melting enthalpy probably contributes to partial gelatinization of starch during processing[7]. Hence the lowering of the enthalpy in this study was sufficient to gelatinize the soaked CM, leading to the higher DG. This characteristic is important for improving the carbohydrate digestibility in feedstuffs[12].

In vitro carbohydrate digestibility of CM

The increased carbohydrate digestibility was correlated with the observed physicochemical properties as described above. However, digestibility also depends on other properties of starch, such as its particle diameter and amylose content[19]. Although microwave irradiation improved the digestibility in fish, this method requires high energy and equipment costs as compared to soaking in water, which produced similar digestibility. Moreover, some anti-nutritional compounds, i.e., polyphenol, tannin, phytic acid, and α-amylase inhibitor can be reduced by soaking pretreatment[22,23]. The increased carbohydrate digestibility after soaking in water found in this study is in agreement with previous findings in moth beans, black grams, and chick peas[24,25]. Moreover, in an in vivo experiment, Hossain et al[26] reported that a supplementation of soaked Sesbania seeds in the diet of common carp (*Cyprinus carpio*) improved growth performance and feed utilization, and Sotolu and Faturoti[27], reported a similar finding relating to the use of soaked leucaena seed in African catfish (*Clarias gariepinus*).

CONCLUSIONS

The method of modification had significant effects in respect of decreasing the crude fibre level and increasing the available carbohydrate as well as changing physicochemical properties. These results indicate that this method of modification is effective in increasing amorphous structure, thus enhancing enzymatic hydrolysis and promoting better feedstuff utilization in animals. The study of in vitro digestibility indicates that water soaking increased carbohydrate digestion in both fish species. Thus the preparation of CM by soaking in water is an appropriate method to enable its use as an aqua feedstuff.

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