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Microwave irradiation improves physico-chemical properties of soya meal for economic freshwater fish

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Abstract: The effects of microwave heating time on the chemical composition, physico-chemical properties and *in vitro* digestibility of soya meal are investigated. Heating time has no effect on the protein, carbohydrate, fibre and ash content ($P > 0.05$), but microwave irradiation reduces the lipid content significantly ($P < 0.05$). The hydrolytic properties are different between raw and microwave-irradiated soya meals. The irradiation increases the degree of starch gelatinisation and water solubility of nutrients. Diffraction patterns show the same characteristics for all treatments, but relative crystallinity decreases dramatically. For nutrient utilisation, microwave irradiation of soya meal improves the *in vitro* digestibility of protein and carbohydrate for three economic fish species (Nile tilapia, broadhead catfish and striped snakehead). The changes in all observed parameters indicate that microwave irradiation for an appropriate time improves the physico-chemical properties of soya meal including the enhancement of enzymatic hydrolysis of its protein and carbohydrate.

Keywords: soya meal, microwave irradiation, Nile tilapia, broadhead catfish, striped snakehead

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INTRODUCTION

The global utilisation of soya meal as a feed ingredient has progressively increased. Soya meal has been successfully used as a feedstuff for culturing various fish species. However, its nutritional content is limited due to a large amount of anti-nutritional compounds including goitrin, phytohaemagglutinins, lectins, non-starch polysaccharides, phytate, phytoestrogens, protein antigens, saponins and trypsin inhibitor [1, 2]. These compounds can play a significant role in suppressing the growth and development of animals. Conventional heat treatment is used to reduce the toxic effects of these compounds and promote the utilisation of nutrients from the feedstuff [1, 3].

Microwave heating has been used to treat various food and feed for industries. In comparison to the conventional heating processes, the microwaves provide rapid heating characteristics and reduce energy costs [4]. Microwave irradiation has been practically used for improving the digestibility of protein [3, 5-8] and carbohydrate [5-7, 9-11] in various feedstuffs as well as in fish diet [12]. Pre-treatment of raw feedstuffs using microwave irradiation can enhance the enzymatic digestion by altering their physico-chemical properties such as protein and starch degradability [7], starch gelatinisation and water solubility [10-12], lignocellulosic content [9, 13] and relative crystallinity [9-11, 14]. However, the beneficial alterations of appropriate properties specifically occur at some optimal heating time that varies with different feedstuffs [7, 8, 11, 12, 15].

The effects of microwave heating time on the nutritional utilisation of soya meal for specific aquatic animals have not been evaluated previously. The main purpose of this study is therefore to improve the feed quality of soya meal using microwave irradiation. Three species of fish of high economic importance, namely Nile tilapia, broadhead catfish and striped snakehead, are used as sources of digestive enzymes to determine the protein and carbohydrate digestibility *in vitro* of the treated feedstuff.

MATERIALS AND METHODS

Preparation of Microwave-Irradiated Soya Meal

One hundred grams of unprocessed soya meal (Phatthalung Livestock Co., Phatthalung, Thailand) were placed in a plastic box (20-cm diameter × 10-cm height), mixed with distilled water (400 mL) and heated at 700W in a 20-L microwave oven (Sanyo, Model EM-700T, 2450 MHz). The box was covered with a lid during the microwave process. Uncooked raw soya meal was used as control. The control and microwave-irradiated soya meals were dried with a freeze dryer (Heto FD3, Heto-Holten, Denmark) for 48 hr and then ground, sieved and kept in a desiccator prior to investigations of their chemical composition, physico-chemical properties and *in vitro* digestibility.

Chemical Composition, Gelatinisation and Water Solubility

Raw and microwave-irradiated soya meals were analysed for their protein, lipid, fibre and ash contents as described by AOAC [16]. Carbohydrate or nitrogen-free extract was obtained by subtraction. Gross energy values were calculated from the combined amounts of crude protein, crude lipid and nitrogen-free extract. The degree of starch gelatinisation was determined spectrophotometrically at 600 nm according to Guraya and Toledo [17]. Water solubility was measured gravimetrically according to the method of Chung et al. [18] by calculating from the ratio of the weight of the dissolved solids in the supernatant to the weight of dry solids in the original sample.

Scanning Electron Microscopy and X-ray Diffraction

Micrographs were taken using a scanning electron microscope (Jeol JSM-5410W, Jeol Ltd., Japan) at magnifications of 750 and 3500, with the electric potential set at 15 kV. The soya meal samples were mounted on an aluminum stub using two-sided adhesive tape, with the sample then coated with gold. X-ray diffraction patterns were observed with an X-ray diffractometer (X'Pert MPD, Philips, Netherlands) operating at a 40 kV and 40 mA. Diffractograms were recorded for 2θ in the range of 4–35°, with a scanning rate of 1°/min. The relative crystallinity (%) was calculated from the ratio of the peak area to the total area (sum of peak areas and amorphous areas) of a diffractogram using Microsoft Excel 2007 (Microsoft Corp., USA).

Fish Preparation and *In Vitro* Digestibility

Fish preparation

Adult samples of the economic fish, namely Nile tilapia (*Oreochromis niloticus*), broadhead catfish (*Clarias macrocephalus*) and striped snakehead (*Channa striata*), were obtained from a farm in Songkhla province. The fish (n = 3) were acclimatised for 14 days in tanks (80-cm diameter × 40-cm height). The tank water was maintained at a temperature of 27.40 ± 0.30°C and a pH of 7.03 ± 0.07. The lighting was controlled at a diurnal cycle of 12-hr light/12-hr dark. All fish were fed *ad libitum* twice daily (08:00 hr and 18:00 hr) with commercial diet containing 20% crude protein (for Nile tilapia) and 37% crude protein (for broadhead catfish and striped snakehead). At the end of acclimatisation, all fish were starved for 24 hr prior to sampling. The fish were sacrificed by chilling in ice according to "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes" developed by the National Research Council of Thailand. The intestines of each fish were carefully collected and kept at –80°C until use.

Digestive enzyme extraction

The digestive enzyme from the small intestine of each fish species was extracted in 50 mM Tris-HCl buffer (pH 8) containing 200 mM NaCl (intestine: buffer = 1: 4) using a micro-homogeniser (Model THP-220, Omni International, USA). The homogenate was centrifuged at 15000*g* for 30 min. at 4°C. The lipid portion (the upper layer) of the supernatant was carefully removed and the supernatant was collected and kept at –80°C prior to *in vitro* digestibility test.

In vitro digestibility

Each enzyme extract was dialysed overnight against an extraction buffer (50 mM Tris-HCl buffer pH 8 containing 200 mM NaCl) a proportion of 1: 50 (enzyme extract: buffer). The protein and carbohydrate digestibility of the soya meals were then determined using the method described by Thongprajukaew et al. [12], with the reaction mixture containing the feedstuff (5 mg), 50 mM phosphate buffer pH 8.2 (10 ml), 0.5% chloramphenicol (50 μL) and the dialysed crude enzyme extract (125 μL). The protein and carbohydrate digestibility were determined by measuring the increase in the liberated reactive amino groups of cleaved peptides and the reducing sugar respectively after incubation with the digestive enzyme. The *in vitro* digestibility of protein and carbohydrate were expressed as mmol *DL*-alanine equivalent/g and μmol maltose/g respectively.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). All average values were calculated from triplicate observations (n = 3). A one-way ANOVA was used to evaluate the effects of microwave irradiation on the chemical composition, physicochemical properties and *in vitro* digestibility. Significant differences between the mean values were ranked using Duncan's Multiple Range Test at 95% significance level.

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RESULTS AND DISCUSSION

Chemical Composition

No significant difference was found in the contents of protein, carbohydrate, fibre and ash in the raw and microwave-irradiated soya meals (Table 1). This is similar to the results obtained after microwave pre-treatment of leguminous seeds such as Bengal gram, green gram and horse gram [6] and canola seed [19] as well as fish diet [12]. However, the available carbohydrates were relatively higher after heating times of 15-20 min., and crude fibre was relatively higher after a 5-10-min. heating time when compared to the control ($P > 0.05$). This shows that microwave irradiation of soya meal could partially disrupt unavailable components in feedstuff, especially cell wall constituents, that to some extent govern the quality of nutrient utilisation [10, 11].

The lipid content of the microwaved soya meals significantly decreased after all heating times when compared with the control (Table 1). Similar reduction in fatty acid content have also been observed in soya bean and olive oil and found to depend on irradiation time and temperature [20, 21]. Irradiation activates the release of free unsaturated fatty acids and causes the formation of secondary oxidation products, decreasing the level of total fatty acids [20]. However, microwave treatment has been reported to have no effect on the crude lipid content of rice bran [11] or the saturated fatty acids in fish diet [22].

Physico-chemical Properties

Gelatinisation and water solubility

Microwaved soya meal had a significantly higher degree of gelatinisation than did the unmodified meal while the water solubility of the irradiated soya meal slightly increased (Table 1). These properties are important for starch digestibility and utilisation of feeds and feedstuffs [12, 22, 23], and contribute to the hydrolytic capacity of digestive enzymes, as observed by Thongprajukaew et al. [12], Chung et al. [18] and Lee et al. [24]. Increased gelatinisation and solubility of the microwave-treated soya meal may therefore improve digestion in animals.

Microstructure

With a small amount of starch, the macrostructure of soya meal during heating was coarse but not clotting. As for the microstructure, a rough surface was observed in the control (Figures 1a-b) and the microwaved samples with heating time of 5-10 min. (Figures 1c–f), while a smooth surface was mainly observed for the meal heated for 15-20 min. (Figures 1g-j). These findings show that the microwave treatment of soya meal has changed the architecture of the feedstuff. Similar observations have been reported with increased hydrolytic properties of the feedstuffs [25, 26].

Diffraction pattern

The diffraction patterns of raw and microwave-irradiated soya meals have similar characteristics in terms of position and strength of their peaks (Figure 2). Similar findings were reported by Thongprajukaew [22] and by other researchers in other raw and modified feedstuffs [18, 25, 27]. This indicates that microwave irradiation mainly affects the amorphous regions of the starch. As seen in Table 1, relative crystallinity decreased dramatically: 3.9- and 4.7-fold after 10- and 15-min. heating respectively. This probably occurred through a partial disruption of the crystalline regions after microwave irradiation. Therefore, the decrease in crystallinity of the

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Table 1. Chemical composition and physico-chemical properties of raw and microwave-irradiated soya meals with different heating times. Average values were obtained from triplicate determinations and are expressed on a dry-matter basis.

Composition/Property	Raw soya meal	Microwave-irradiated soya meal at different heating times				Pooled SEM
		5 min.	10 min.	15 min.	20 min.	
Chemical composition (%)						
Crude protein	45.28	45.81	45.82	45.28	44.61	< 0.001
Carbohydrate	35.53	35.04	35.89	36.55	37.43	< 0.001
Crude lipid	1.15[a]	0.70[b]	0.59[b]	0.72[b]	0.83[b]	0.004
Crude fibre	10.84	11.59	11.28	10.82	10.28	< 0.002
Crude ash	7.20	6.86	6.42	6.63	6.85	< 0.001
Gross energy (kJ/g)	18.37	18.22	18.34	18.38	18.42	< 0.001
Physico-chemical properties (%)						
Degree of gelatinisation	46.29[a]	52.70[b]	52.53[b]	50.75[b]	53.70[b]	0.960
Water solubility	7.74	7.83	8.79	8.61	8.28	0.200
Relative crystallinity	29.25	16.28	6.61	5.54	19.77	–

Note: Relative crytallinity was calculated from only one sample of each condition.
Values with different superscripts in the same row indicate significant difference ($P < 0.05$).

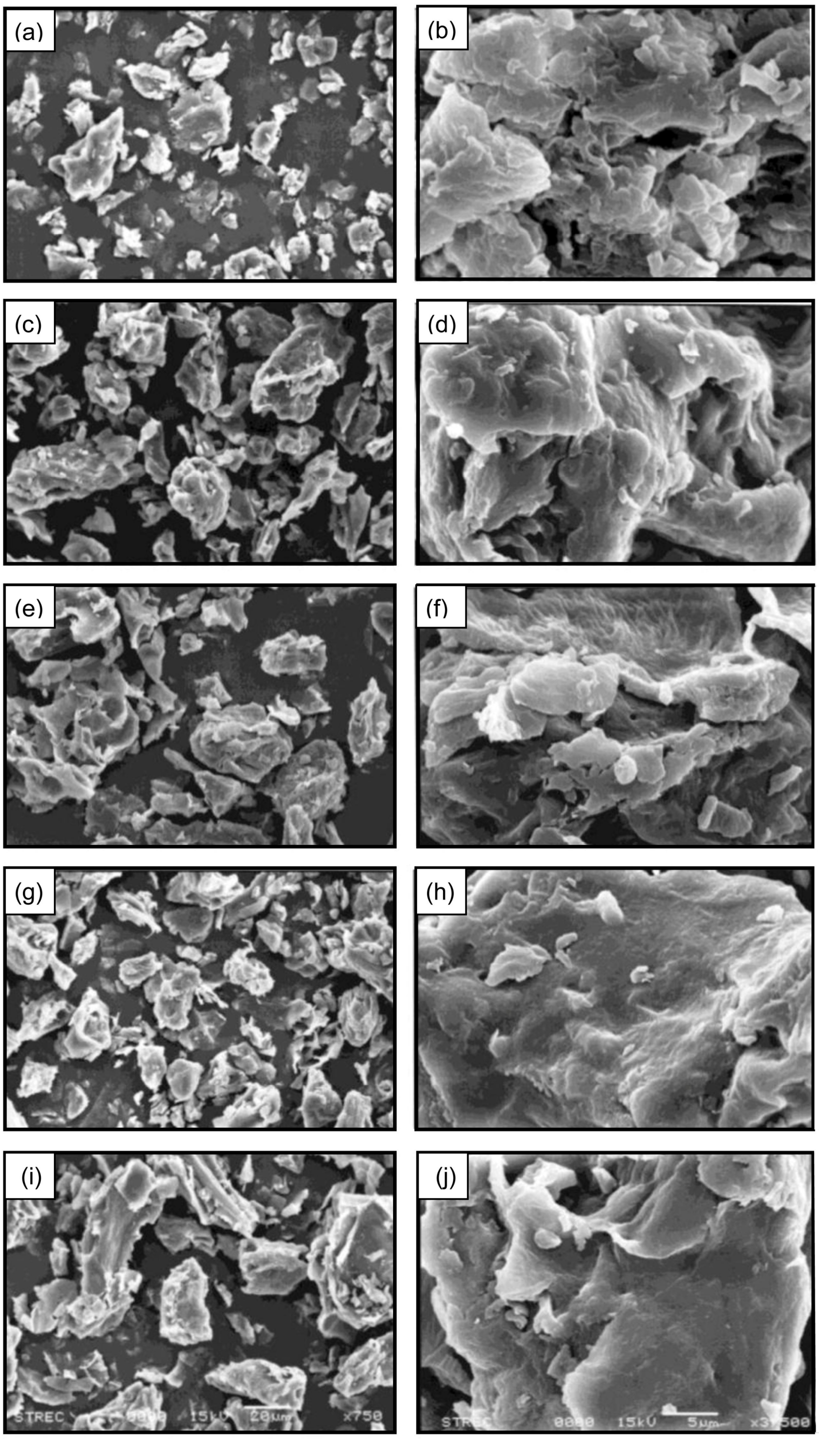

Figure 1. Scanning electron micrographs of raw soya meal (a and b) and soya meal microwave-irradiated for 5 min. (c and d), 10 min. (e and f), 15 min. (g and h) and 20 min. (i and j). The magnifications are 750× (left panel) and 3500× (right panel).

microwave-modified soya meal was due to an increase in the amorphous regions, which matches the observed increase in its starch gelatinisation and water solubility. This allows water to penetrate and increase the rate of hydrolytic reaction. On the other hand, crystallinity sharply increased again after a 20-min. heating. This result is similar to that found in rice straw after modifications by an electron beam [28]. It is possible that prolonged microwave treatment might have induced a molecular reorganisation or transformation of cell wall constituents, especially cellulose, which is crystalline in nature.

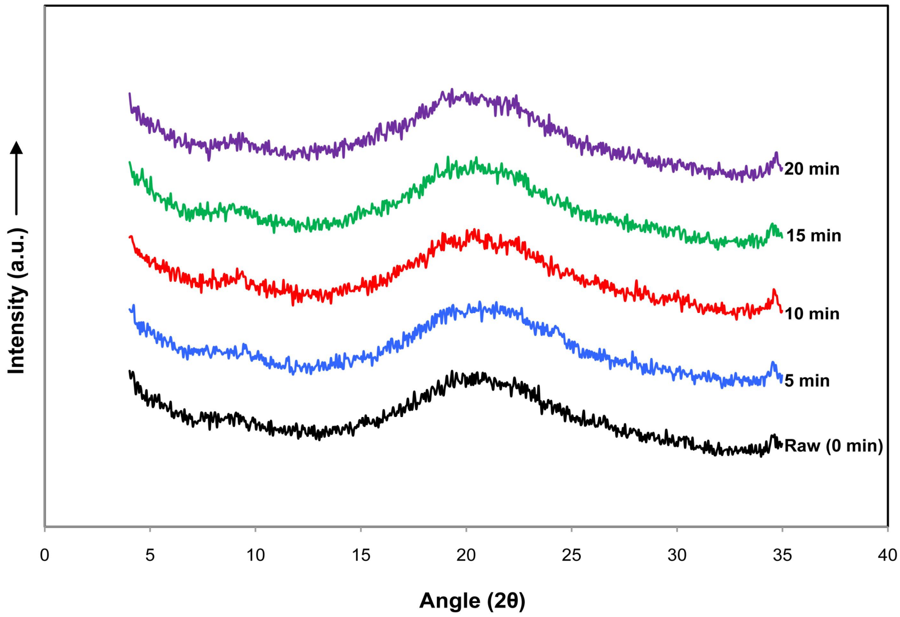

Figure 2. Diffraction patterns of raw and microwave-irradiated soya meal at different heating times

In Vitro **Digestibility**

Protein digestibility

Improved protein digestibility of various feedstuffs after microwave irradiation has been reported previously [3, 5, 6]. In this study, no significant difference was observed in the protein digestibility of treated and untreated soya meals for Nile tilapia (Table 2, $P > 0.05$), although a significant increase ($P < 0.05$) in digestibility of the treated meals was observed for both of the carnivorous fish, i.e. the broadhead catfish and the striped snakehead. Protein digestibility also tended to increase with heating time. Digested products for both species at the optimal heating time (15 min.) increased 1.17-fold on average when compared with the control. Differences in heating time for optimal protein digestibility have been reported [8, 12, 19]. They seem to be influenced by the method of material preparation including microwave intensity and substrate concentration [15], as well as by the source of digestive enzymes tested [8, 10]. Increased digestibility might stem from the unfolding of the protein, which exposes hydrophobic amino acids that act as active sites for pepsin and trypsin activities [29].

Upon prolonged heating, however, protein digestibility tended to decrease (Table 2). A decrease in *in vitro* protein digestibility after a 6-min. microwave irradiation of cottonseed meal when compared with a 4-min. irradiation has also been observed [8]. This phenomenon could be an effect of prolonged heating on the protein molecules, which results in, among other transformations, the formation of cross-linkage to a higher molecular weight aggregate and subsequent indigestibility. For soya meal, for example, a 6-min.-irradiated sample containing two subunits of β-conglycinin has been found to be more resistant to ruminal

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Table 2. *In vitro* digestibility of protein (mmol *DL*-alanine equivalent/g) and carbohydrate (µmol maltose/g) of raw and microwave-irradiated soya meals using digestive enzyme extracts from Nile tilapia (100 U trypsin and 2000 U amylase), broadhead catfish (100 U trypsin and 1000 U amylase) and striped snakehead (100 U trypsin and 100 U amylase)

Digestibility	Raw soya meal	Microwave-irradiated soya meal at different heating times				Pooled SEM
		5 min.	10 min.	15 min.	20 min.	
Protein digestibility						
Nile tilapia	53.51	55.57	56.26	52.19	52.55	1.29
Broadhead catfish	16.29^{c}	18.29^{ab}	17.09^{bc}	18.49^{a}	17.41^{abc}	0.12
Striped snakehead	63.37^{b}	67.17^{a}	73.34^{ab}	75.46^{a}	68.15^{ab}	10.51
Carbohydrate digestibility						
Nile tilapia	57.82^{bc}	53.16^{c}	76.15^{ab}	90.37^{a}	54.99^{c}	27.67
Broadhead catfish	180.14^{bc}	134.08^{c}	208.80^{ab}	240.52^{a}	197.54^{ab}	160.82
Striped snakehead	50.74^{b}	46.39^{b}	86.50^{a}	88.43^{a}	76.83^{a}	15.09

Note: Different superscripts within the same row indicate a significant difference ($P < 0.05$).

degradation than the one undergoing 4-min. irradiation [30]. This valuable characteristic increases the escape of protein from the rumen to the intestine for microbial fermentation [7, 8, 30]. In contrast, for monogastric animals, this change has a negative effect on the protein digestion along the alimentary tract.

Carbohydrate digestibility

The use of microwave irradiation for increasing the apparent digestibility of carbohydrates has been reported for various leguminous seeds such as moth bean [5], green gram, Bengal gram and horse gram [6]. From Table 2, carbohydrate digestibility of the treated and untreated soya meals was statistically different in the three fish species ($P < 0.05$). The highest values of digestion were observed in samples heated for 15 min., which increased the digestibility 1.56- and 1.34-fold on average for Nile tilapia and broadhead catfish respectively when compared with control samples. For striped snakehead, carbohydrate digestibility was unchanged in the range of 10–15 min. heating time ($P > 0.05$). On average, heating improved digestibility 1.72-fold when compared with control. Decreased digestibility of all samples subjected to prolonged heating (20-min.) may be associated with increased relative crystallinity (Table 1).

In a previous study of Nile tilapia, improved carbohydrate digestibility was observed for a 5-min. microwave-irradiated coconut meal (feedstuff: water = 1: 9) [10] as well as a 10-min. microwave-irradiated rice bran (feedstuff: water = 1: 3) [11], in comparison with the raw material. Similar results for catfish and striped snakehead were also reported using a 4-min. microwave-irradiated palm kernel meal (feedstuff: water = 1: 2) [9]. These differences in optimal irradiation time were due to the differences in type of feedstuff, ratio of feedstuff to water and source of digestive enzymes.

CONCLUSIONS

Microwave irradiation of soya meal for economic freshwater fish seems to have no significant effect on most of its chemical composition or its gross energy, except for a reduced lipid content of the meal. Irradiation for an appropriate time can improve its physico-chemical properties in such a way that its enzymatic hydrolysis is enhanced. These findings show that microwave irradiation of soya meal can improve nutrient utilisation, although the irradiation time should be optimised for each fish species. The use of microwave irradiation to modify soya meal for fish in aquaculture therefore appears to have significant potential.

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