การดัดแปลงใยอาหารจากกากมันสำปะหลังเพื่อลดโลหะหนัก โดยการประเมินการยับยั้งชีวภาพพร้อมใช้

Modification of Dietary Fiber from Cassava Pulp to Reduce Heavy Metal by Assessing Their Heavy Metal Bioaccessibility Inhibition

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อหาวิธีที่เหมาะสมในการดัดแปลงใยอาหารจากกากมันสำปะหลังและผลต่อการยับยั้งชีวภาพพร้อมใช้ในโลหะหนัก การเตรียม MDF (ใยอาหารดัดแปลง) จากกากมันสำปะหลังเริ่มจากการแยกแป้งและโปรตีนด้วยเอนไซม์เพื่อเตรียมใยอาหารหยาบ (CDF) ด้วยแอลฟาอะมัยเลส 1% (w/v) อะมัยโลกลูโคซิเดส 0.1% (v/v) และ นิวเทรส 1% (v/v) จากนั้นดัดแปลง CDF ด้วย 4 วิธี ได้แก่ วิธีเอสเทอริฟิเคชั่น ฮาโลจีเนชั่น ออกซิเดชันและอีเทอริฟิเคชั่น ผลการทดลองพบว่าการดัดแปลง CDF สามารถปรับปรุงคุณสมบัติในการจับกับโลหะหนักได้ โดย MDF มีปริมาณ neutral detergent fiber (NDF) acid detergent fiber (ADF) acid detergent lignin (ADL) เซลลูโลส เฮมิเซลลูโลส โปรตีน ไขมัน ความชื้นและแป้งมากกว่าใน CDF นอกจากนี้คุณสมบัติเชิงหน้าที่ของ MDF มีค่าความสามารถในการอุ้มน้ำ ความสามารถในการจับกับน้ำมัน ความสามารถในการละลายน้ำ ค่าการพองตัว และกลุ่มคาบอกซิลมากกว่า CDF

นอกจากนั้นศึกษาผลของ MDF จาก 4 วิธี ต่อชีวภาพพร้อมใช้ของตะกั่วด้วยแบบจำลองการย่อยอาหาร พบว่า MDF จากทุกวิธีสามารถลดชีวภาพพร้อมใช้ของตะกั่วได้อย่างมีนัยสำคัญทางสถิติเมื่อใช้ MDF ปริมาณ 0-1000 mg ($p < 0.05$) โดยลดลง 25-80% ที่ 1000 mg ให้ผลดีที่สุด และ MDF จากวิธีอีเทอริฟิเคชั่นสามารถยับยั้งได้ดีกว่าวิธีอื่นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในทุกระดับของใยอาหารที่ใช้ จึงสรุปได้ว่า MDF ที่ได้จากการดัดแปลงด้วยวิธีอีเทอริฟิเคชั่นสามารถลดชีวภาพพร้อมใช้ของตะกั่ว และสามารถนำมาประยุกต์ใช้ในผลิตภัณฑ์อาหารเสริมและผลิตภัณฑ์เสริมอาหารได้

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คำสำคัญ: กากมันสำปะหลัง, ใยอาหาร, การดัดแปลงใยอาหาร, ชีวภาพพร้อมใช้, โลหะหนัก

ABSTRACT

The objectives of this study were to determine the optimal method to modify dietary fiber from cassava pulp and their effects on heavy metals bioaccessibility inhibition. The preparation MDF (modified dietary fiber) from cassava pulp was started from separating starch and protein from fiber through the application of enzyme in order to prepare crude dietary fiber (CDF) that could be derived from enzymatic digestion condition of 0.1% of α-amylase (w/v), 0.1% of amyloglucosidase (v/v) and 1% of neutrase (v/v), and modifying them with 4 methods that were esterification method, halogenation method, oxidation method and etherification method. The results showed that modification of CDF could improve heavy-metal-binding properties as the chemical composition of MDF shows neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, hemicelluloses protein, moisture, fat and starch more than CDF. Furthermore, the functional properties of MDF show a greater water holding capacity, oil binding capacity, water solubility index, swelling capacity and COOH content than CDF.

In addition, to study the MDF (4 methods) affecting the heavy lead bioaccessibility was estimated by using in vitro digestion model. The result showed that MDF from all methods significantly reduced lead bioaccessibility in a dose dependent manner from 0-1000 mg of MDF ($p < 0.05$). Lead bioaccessibility was decreased by 25-80%. MDF 1000 mg showed the strongest effect on heavy metal bioacessibility. A method comparison suggested that MDF from etherification method significantly showed more inhibition than other methods ($p < 0.05$) for all the amounts used. In conclusion, this study suggested that MDF with etherification method could decrease lead bioaccessibility and could be applied in functional food and dietary supplement products.

Key words: cassava pulp, dietary fiber, modification of fiber, bioaccessibility, heavy metal

INTRODUCTION

Humans can be exposed to heavy metals through various pathways. Such as water irrigation, solid disposal, sludge applications, vehicular exhaust, and industrial activities. (Khan *et al.*, 2008). Many researchers have reported the transfer of heavy metals from polluted soils to various sources of food, such as vegetables (Bahemuk and Mubofu, 1999), rice (Fu *et al.*, 2008), wheat (Huang *et al.*, 2008) and chicken (Zhuang *et al.*, 2009), resulting in pollutant levels higher than those declared permissible for human consumption by the Food and Agriculture (Wang *et al.*, 2006). Heavy metals such as lead, cadmium, and mercury are non-essential nutrients, which are potentially toxic at very low concentrations due to their nonbiodegradable nature and prolonged biological half-life (Barbier *et al.*, 2005). Several reports have shown that the accumulation of heavy metals in humans can cause severe damage to kidney and liver as well as impair the immune and central nervous systems, resulting in vital pathological changes and functional abnormalities (cognitive and behavioral), gastrointestinal toxicity, and chronic renal failure (Sabolic, 2006).

Bioaccessibility is the maximum concentration of chemicals or nutrients that are released from the food matrix into aqueous fraction following simulated digestion, which are then available for absorption by the intestinal mucosa. The combination of *in vitro* digestion model and Caco-2 cell line is useful tool to measure bioaccessibility and bioavailability. In addition, this method can give information related to *in vivo* experiments (Courraud *et al.*, 2013).

Chelating agents could decrease the bioaccessibility of heavy metal to be absorbed or reabsorbed in the gastrointestinal tract. Ethylenediaminetetra acetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) 2,3-dimercaptopropanol (BAL), D-penicillamine (D-β, β-dimethylcysteine), deferoxamine dimercaptosuccinic acid (DMSA), penicillamine and 2, 3-dimercapto-1-propane sulfonate (DMPS) are used as chelating agents for the removal of heavy metal through inhibition of heavy metal bioaccessibility or intestinal re/absorption, however, many of these treatments have reported side effects and are thus not suitable for long term application. In recent years, many researchers have reported applying dietary fibers as adsorbents of heavy metal because of their nontoxic properties (Nawirska, 2005; Hu *et al.*, 2010; Zhuang *et al.*, 2009).

Cassava (*Manihot esculenta Crantz.*) is the third most important crop in Thailand. Cassava pulp, a by-product of cassava starch factory processing, contains a large quantity of starch, accounts for approximately 10-30% by weight (wet) of the original tubers. Therefore, the tapioca starch industry in Thailand is estimated to generate at least one million ton of cassava pulp annually from 10 million tons of fresh tubers (Kosugi *et al.*, 2009). Most of cassava pulp are used for feed. The above information shows that cassava pulp has high level of fiber. So, there is a potential of using cassava pulp for binding heavy metals and preventing their toxicity. The advantage of dietary fiber is that is can be absorbed/bound with heavy metal or other materials and carries them through the gastrointestinal tract because it is resistant to digestion by the human alimentary enzymes (Zhang *et al.*, 2011).

MATERAILS AND METHODS

1. Sample preparation

Cassava pulp were obtained from Sanguan Wongse Starch Co., Ltd. Drying at 60 °C for 8-12 hours leaves were ground with a grinder (High speed grinder, 3500 w, Simon, Inc., Foodmachine, China) until they were a fine powder. The extraction process of the CDF was obtained from enzymatic digestion condition of 0.1% of α-amylase (w/v), 0.1% of amyloglucosidase (v/v) and 1% of neutrase (v/v) (Kachenpukdee *et al.*, 2016).

2. Analytical Characterization of Fiber

The fiber was analyzed according to the crude protein, moisture, ash, fat, carbohydrate, acid detergent fiber (ADF), acid detergent lignin (ADL) and neutral detergent fiber (NDF). NDF (Van Soest *et al.*, 1991). The percentage of cellulose was calculated from ADF-ADL and the percentage of hemicellulose was calculated from NDF-ADF. Functional property analysis included water holding capacity (WHC) (Jasberg *et al.*, 1989), oil holding capacity (OHC) (Caprez *et al.*, 1986), solubility (AACC, 2000) method No. 44-19, swelling (Robertson *et al.*, 1999) and COOH content (United States Pharmacopeia, 1995).

3. Preparation of MDF

Modification of CDF could be developed with improving physiochemical and functional properties for binding with lead (Pb). Modified CDF with 4 methods such as esterification method (Type I) (Doczekalska *et al.*, 2014), halogenation method (Type II) (Aoki *et al.*, 1999), oxidation method (Type III) (O'Connell *et al.*, 2008) and etherification method (Type IV) (Saliba *et al.*, 2000). The MDF powder was then kept in a sealed container until further treatment.

4. *In vitro* digestion

Binding of heavy metals was investigated using the following model solutions: lead (Pb) 2.1 ppb lead (II) nitrate. Samples (after removal of sequential fractions) was weighed in 1 g portions and added into 300 mL conical flasks which will then be treated with 100 mL of appropriate model solution. After thorough mixing, the flasks were stored at room temperature. From each flask (after 30 min of storage) a 7 mL portion of the solution was taken and placed in the test-tube centrifuge (Nawirska, 2005) and mixed with MDF. One milliliter of saline (0.9% w/v sodium chloride; NaCl, Sigma-Aldrich) was added to the test tube and was homogenized twice by a cell disruptor at 20 kHz and 150-500 Watts for 30 s and was then mixed with MDF (0-1000 mg) prior to initiation of digestions.

The 2-stage *in vitro* digestion model used in the present study was originally described by Garrett *et al*. (1999) with modification. The gastric phase was initiated with additional porcine pepsin (3 mg/ml, Sigma Chemical Co., St. Louis, MO) and adjustment of the pH to 2-2.5 with 0.1 M HCl (Analytical grade, Sigma Chemical Co.). Samples were vortexed and flushed to the top of the tube with nitrogen gas (99.99%, Air Gas, Indianapolis, IN) and were then incubated at 37°C for 1 h in a shaking water bath at 150 rpm (VWR, Cornelius, OR). The intestinal phase was initiated by pH adjustment to 5.3 with 100 mM sodium bicarbonate solution (Sigma Chemical Co.) and addition of 9 mL of a bile extract/pancreatin/lipase mixture: pancreatin (0.4 mg/mL, Sigma Chemical Co., St. Louis, MO), lipase (0.2 mg/mL, Sigma Chemical Co.) and porcine bile extract (2.4 mg/mL, Sigma Chemical Co.); then, the pH was adjusted to 6.5-7.0 with 0.1 M NaOH (Analytical grade, Sigma Chemical Co.), and the solution was made up to 30 mL with 0.9% saline (pH 7). Samples were vortexed and flushed to the top of the tube with nitrogen gas and were incubated at 37°C for 1 h in a shaking water bath at a speed of 150 rpm. One sample tube was separated for digesta, and the other 3 sample tubes were centrifuged at 167,000 g for 35 min (Beckman L8-70M, Beckman Coulter, San Antonio, TX). Aliquots of raw materials, digesta, aqueous phases, and residual pellets were collected and stored at -80°C prior to analysis. Concentrations of metal ions was measured by atomic absorption spectrometry (AAS).

5. Data analysis

Relative bioaccessibility (%) = (µg/L of Pb in aqueous)/(µg/L of Pb in digesta) ×100

Absolute bioaccessibility (µg/g) = (% Bioaccessibility × ug of Pb starting material)/100

Uptake efficiency (%) = (Accumulation of Pb in cell (ng/well))/(Pb content in test media (ng/well)) ×100

Data are expressed as the mean ± standard error. For cellular uptake studies, a sample size of n=3 was used. Statistical analysis for each parameter assessed was performed using analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences among means were considered statistically significant at $p < 0.05$.

RESULT AND DISCUSSION

1. Characterization of crude dietary fiber (CDF) and modified dietary fiber (MDF)

Table 1 presents in percentages the physicochemical properties of CDF. From the analysis, CDF contained crude protein, ash, moisture, fat, starch, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and hemicelluloses by 1.21, 2.97, 6.57, 0.54, 10.20, 78.78, 72.16, 2.29, 69.87 and 6.62% respectively. The CDF shows high neutral detergent fiber (NDF), which included cellulose, hemicellulose and lignin. It has been reported that insoluble fiber can bind with heavy metal better than soluble fiber (Mertens, 1987). However, MDF shows more neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicelluloses and less crude protein, moisture, fat and starch than is contained in CDF after modified are that 0.83, 5.85, 4.32, 0.34, 1.09, 88.23, 81.19, 6.17, 75.02 and 7.04 % respectively.

The functional properties of the CDF shown in Table 2 are that water holding capacity is 5.97 %, oil binding capacity 4.87 g oil/g sample, water solubility index 3.18%, swelling capacity 7.80 ml/g DM and COOH content 4.84%. MDF are that water holding capacity is 8.32%, oil binding capacity 6.37g oil/g sample, water solubility index 7.58%, swelling capacity 11.43 ml/g DM and COOH content 7.96%. The functional properties of MDF show a greater water holding capacity, oil binding capacity, water solubility index, swelling capacity and COOH content than CDF.

This suggests that chemical modification of dietary fiber enables an improvement in the chemical composition and functional properties of fiber and used to evaluate the potential of dietary fiber to bind/absorb heavy metal. The functional properties that influence function along the gastrointestinal tract are a combination of water holding capacity, oil binding capacity, water solubility, swelling capacity and carboxyl group (-COOH) content. The biological effects of dietary fiber along the intestine and colon may be improved by absorption in the gut and an increase in stool weight (Eastwood and Morris, 1992)

Table 1 Characterization of crude dietary fiber and modified dietary fiber

Component	**% Content (Dried basis)**	
	Crude dietary fiber	**Modified dietary fiber Type IV**
Crude protein	1.21 ± 0.30^{a}	0.83 ± 0.34^{b}
Ash	2.97 ± 0.63^{b}	5.85 ± 0.46^{a}
Moisture	6.57 ± 0.98^{a}	4.32 ± 0.55^{b}
Fat	0.54 ± 0.23^{a}	0.34 ± 0.11^{b}
Starch	10.20 ± 0.47^{a}	1.09 ± 0.42^{b}
Neutral detergent fiber (NDF)	78.78 ± 0.87^{b}	88.23 ± 1.13^{a}
Acid detergent fiber (ADF)	72.16 ± 0.52^{b}	81.19 ± 1.64^{a}
Acid detergent lignin (ADL)	2.29 ± 0.92^{b}	6.17 ± 1.48^{a}
Cellulose[a]	69.87 ± 0.81^{ns}	75.02 ± 1.33^{ns}
Hemicellulose[b]	6.62 ± 0.93^{b}	7.04 ± 0.79^{a}

[a] ADF -ADL, [b] NDF-ADF

* Values in the same row with different alphabet designations are significantly different at $p < 0.05$.

Table 2 Functional properties of crude dietary fiber and modified dietary fiber

Functional properties	**Content**	
	Crude dietary fiber	**Modified dietary fiber Type IV**
Water holding capacity (WHC) (%)	5.97 ± 0.29^{b}	8.32 ± 0.34^{a}
Oil binding capacity (OBC) (g/g sample)	4.87 ± 0.65^{b}	6.37 ± 0.32^{a}
Water solubility index (WSI) (%)	3.18 ± 0.65^{b}	7.58 ± 0.53^{a}
Swelling capacity (SC) (mL/g dietary fiber)	7.80 ± 0.98^{b}	11.43 ± 1.49^{a}
COOH content (%)	4.84 ± 0.96^{b}	7.96 ± 0.89^{a}

* Values in the same row with different alphabet designations are significantly different at $p < 0.05$.

2. Bioaccessibility of lead (II) nitrate (Pb) is impacted by co-digestion with MDF

Table 3 Modified dietary fiber (MDF) in amounts of 0-1000 mg. Total lead (Pb) 2.1 ppb (lead (II) nitrate) in each phase and the relative bioaccessibility following *in vitro* digestion.

Fiber	**Fiber (mg)**	**Total Pb (10^{-2} µg)**		**Relative Bioaccessibility**[1] **(%)**
		Digesta	**Aqueous**	
CDF (Control)	0	19.5 ± 0.05^{a}	19.5 ± 0.05^{a}	$100 \pm 0.00^{a,1}$
	50	13.79 ± 0.12^{d}	10.84 ± 0.09^{b}	$78.60 \pm 0.62^{b,2}$
	100	12.06 ± 0.11^{e}	9.01 ± 0.08^{c}	$74.71 \pm 0.87^{c,2}$
	500	13.88 ± 0.05^{c}	8.93 ± 0.04^{c}	$64.32 \pm 0.84^{d,34}$
	1000	13.95 ± 0.09^{b}	8.90 ± 0.02^{c}	$63.78 \pm 0.61^{d,34}$
MDF Type I	0	19.5 ± 0.05^{a}	19.5 ± 0.05^{a}	$100 \pm 0.00^{a,1}$
	50	11.40 ± 0.10^{c}	8.29 ± 0.10^{b}	$72.67 \pm 1.22^{b,23}$
	100	10.92 ± 0.09^{d}	7.21 ± 0.04^{bc}	$65.98 \pm 0.70^{c,3}$
	500	10.72 ± 0.04^{e}	5.14 ± 0.03^{c}	47.92 ± 0.89^{d}
	1000	19.02 ± 0.04^{b}	5.08 ± 0.01^{d}	$26.70 \pm 0.94^{e,67}$
MDF Type II	0	19.5 ± 0.05^{a}	19.5 ± 0.05^{a}	$100 \pm 0.00^{a,1}$
	50	12.98 ± 0.08^{d}	8.89 ± 0.08^{b}	$68.45 \pm 0.92^{b,3}$
	100	14.43 ± 0.12^{c}	7.56 ± 0.08^{bc}	$52.38 \pm 0.99^{c,4}$
	500	16.18 ± 0.06^{b}	5.74 ± 0.05^{c}	$35.47 \pm 0.86^{d,6}$
	1000	19.32 ± 0.05^{a}	5.02 ± 0.07^{d}	$25.98 \pm 0.73^{e,7}$
MDF Type III	0	19.5 ± 0.05^{a}	19.5 ± 0.05^{a}	$100 \pm 0.00^{a,1}$
	50	11.76 ± 0.08^{e}	9.08 ± 0.10^{b}	$77.17 \pm 1.02^{b,2}$
	100	12.73 ± 0.19^{d}	8.17 ± 0.04^{bc}	$64.14 \pm 0.87^{c,34}$
	500	15.67 ± 0.07^{c}	7.14 ± 0.03^{c}	$45.55 \pm 0.68^{d,5}$
	1000	17.65 ± 0.09^{b}	5.08 ± 0.01^{d}	$28.60 \pm 0.56^{e,67}$
MDF Type IV	0	19.5 ± 0.05^{a}	19.5 ± 0.05^{a}	$100 \pm 0.00^{a,1}$
	50	11.31 ± 0.10^{d}	7.14 ± 0.12^{b}	$63.12 \pm 1.02^{b,34}$
	100	8.87 ± 0.09^{e}	4.13 ± 0.07^{c}	$46.54 \pm 0.67^{c,5}$
	500	12.17 ± 0.04^{c}	3.83 ± 0.04^{cd}	$31.45 \pm 0.67^{d,6}$
	1000	16.49 ± 0.04^{b}	2.08 ± 0.02^{d}	$12.61 \pm 0.75^{e,8}$

[1] Relative bioaccessibility is defined as the % of lead recovered in digesta.

* Data represent mean +/- SEM from n=3 independent *in vitro* digestion experiments

* Presence of different letters indicate significant differences between treatments as determined by a Tukey's post hoc test ($p < 0.05$) a, b, c... is fiber dose effect and 1, 2, 3...is fiber type effect.

The CDF and MDF were assessed for their effect on Pb bioaccessibility effect using the *in vitro* digestion model. The Pb that remained in the aqueous fraction was defined as evaluate potential of fiber to reduce Pb. The first step involved studying the effects of the amounts of CDF and MDF on Pb bioaccessibility. Table 3 show the effects of fiber in the form of CDF and MDF from cassava on Pb bioaccessibility. Both forms of dietary fiber showed significantly reduced Pb bioaccessibility in a dose dependent manner from 0-1000 mg of CDF and MDF ($p < 0.05$). Pb bioaccessibility was decreased by 63-68% for CDF and 12-77% for MDF compared to control (Not added fiber) with inclusion of CDF and MDF up to 1000 mg per digestive reaction. MDF 1000 mg showed the strongest effect on Pb bioacessibility. A comparison of 5 fibers suggests that MDF by etherification method (type IV) showed significantly more Pb inhibition than other types ($p < 0.05$).

All type of MDF showed that significantly stronger binding with Pb than CDF might be due to the chemical composition and functional properties of MDF. MDF showed more neutral detergent fiber; NDF (Cellulose, Hemicellulose, Lignin), acid detergent fiber; ADF (lignin, cellulose), acid detergent lignin, and ADL (lignin) than CDF. These are insoluble fibers (cellulose, hemicellulose and lignin). It has been reported that insoluble fiber is able to easily bind with heavy metal than soluble fiber (Mertens, 1987).

However, MDF from etherification method (Type IV) showed the highest effect on Pb bioacessibility because etherification method is directly modifying cellulose by grafting a second polymer as a long branch on the molecule that give cellulose new properties such as hydrophilic or hydrophobic character, improved elasticity, water absorbent and ion-exchange capability. Esterification and halogenation process could increase carboxylic content of the fiber surface and oxidation method could oxidize approximately 70% of cellulose to insoluble fiber. These suggest that grafting polymer to long branch molecule that helped to absorbed metal ion in the fiber structure better than absorb on fiber surface. As a result, MDF Type IV showed more reduced Pb than other types.

Bioaccessibilities can vary depending on factors such as the composition of the food matrix, pH, shaking time and enzyme conditions. However, the bioaccessibilities can be very different (38-83%) with the same method (Torres-Escribano *et al.*, 2010).

The present results suggest that the addition of dietary fiber may impact on Pb bioaccessibiilty potentially by the binding of Pb. The mechanism for Pb reduction is likely to be a combination of physical and chemical adsorption. Physical sorption is nonspecific, which does not include the sharing or transfer of electrons. Therefore, these mechanisms are the absorption of heavy metal in the fiber matrix while the adsorbed molecules of heavy metal are free to cover the surface of the adsorbent (dietary fiber). Chemisorption is the connection between the fiber matrix of phenolic groups from lignin and carboxyl groups from uronic acid (Zhang *et al.*, 2011), which is specific and dependent on the formation of covalent bonds (sharing of electrons) between the adsorbate and a specific fiber surface site.

CONCLUSION

This study confirmed the positive effects of MDF from 4 methods. The MDF for removal heavy metal on inhibiting lead bioaccessibility by using an *in vitro* digestion model. These results suggest that MDF might act as a chelating agent for reducing lead bioaccessibility, which could reduce Pb exposure to the human body. The result further demonstrate the usefulness of an *in vitro* digestion model as a rapid and cost-effective alternative for evaluating the impact of ingredients on bioaccessibility of heavy. Subsequent *in vivo* studies are needed to expand on the applicability of these results

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หมายเหตุ: บทความนี้มาจากงานประชุมวิชาการระดับชาติมหาวิทยาลัยเทคโนโลยีราชมงคล ครั้งที่ 10 ปี พ.ศ. 2561