In-vitro Binding Capacity of Coconut Kernel Fibre Concentrates to Iron, Copper and Zinc at Different pH Levels

L. L. W. Chandi Yalegama[1,4*], D. Nedra Karunaratne[2,4], Ramiah Sivakanesan[3,4] and Chithrangani Jayasekara[1]

[1]*Coconut Research Institute, Lunuwila, Sri Lanka*

[2]*Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka*

[3]*Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka*

[4]*Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka*

[*]*Corresponding Author: cyalegama@yahoo.co.in*

ABSTRACT

High-fibre diets reduce the bioavailability of metal ions. Therefore, a study was carried out to investigate the metal ion binding capacity to fibre isolates obtained from coconut kernel. The by-product of virgin coconut oil (VOR) is a potential source of dietary fibre. The cell wall polysaccharides in defatted VOR were concentrated using combination of methanol (80 %), SDS (1%), mercapto ethanol and 90 % DMSO; ethanol (70 %) and 0.1M NaOH (0.1M) and ethanol (70 %) to isolate coconut cell wall polysaccharides, CCWP(1), CCWP(2) and CCWP(3) respectively.The binding affinity of fibres to Fe (II), Cu (II) and Zn (II) were investigated with increasing pH of the medium. The binding capacity of Cu (II) to all types of fibres at the strong acid conditions (pH 2) was 6-12.5 % and the binding capacity of Cu (II) to fibres was over 90 % at pH near neutral except for VOR. All types of fibres re-sorped Zn (II), which had already bound to fibres at strong acid conditions (pH 2). However, re-sorption of Zn (II) decreased with the increase of pH and increased binding capacity was shown by binding more than 95 % of Zn (II) in the medium. The binding of Fe (II) to fibres was 62-91 % at strong acidic conditions (pH 2-3). The binding affinity of metal ion to fibres increased when the VOR was concentrated except binding of iron to fibres at pH2.

Keywords: Coconut kernel, Fibre concentrate, Metal-binding capacity,

INTRODUCTION

A diet containing high-fibre content is associated with the role of lowering the risk of obesity, cardiovascular diseases and diabetics. Plant-based foods contain cellulose, hemicellulose, pectin, gums and mucilage as fibre in different contents depending on the source of plant. Dietary fibre shows variable physiological actions in the gastrointestinal tract due to physical and chemical composition of the fibre (Babio *et al*, 2010). The fibre molecules form linkages with the surrounding molecules by chemical and physical bonds. These linkages become stable, weaken or broken due to the action of enzymes and pH of the surrounding. Therefore, high-fibre diet may lower bioavailability of mineral nutrients due to the linkages formed between mineral ion and the fibre components. The binding sites of different components in the food bind minerals in varying capacities. Therefore, the absorption of mineral through the digestion system is affected, either through a positive effect or negative effect. However, the removal of toxic heavy metals like Pb, Cd or Hg from the diet is favourable and deficiency of essential mineral in the people consuming high-fibre diets have been reported (Elhardollou and Walker, 1999). Therefore, the information of binding affinities of the mineral to the food fibre is essential to food industries and nutritionists.

Coconut kernel fibre is obtained from the by-product of the coconut oil and coconut milk-producing industries. Coconut kernel fibre lowers the increase of serum total cholesterol, triacylglycerides and glucose concentrations in rats (Yalegama, 2012). It hasan ability to lower the weight gain of rats (Yalegama, 2012). Trinidad *et al.* (2006) observed that coconut flour prepared from the by-product of coconut milk could be fermented to produce butyrate, acetate and propionate. Coconut flour-incorporated foods show low glycemic index, which is good for control and management of diabetes mellitus and in maintenance of weight. Like other fibre-rich food products, it could also reduce serum total cholesterol, LDL cholesterol and triglycerides in moderately raise serum cholesterol levels in humans.According to Sindurani and Rajamohan (1998), faecal excretion of inorganic cation in rats was increased with increasing the levels of neutral detergent fibre in the diet. Therefore, coconut kernel fibre has a potential for development of value-added fibre-rich products.

The aim of the present study is to investigate the metal-binding capacity of coconut kernel fibre. The by-product of virgin coconut oil contains considerable amount of fibre; acid detergent fibre 14 %, neutral detergent fibre 33.1 % and hemicelluloses 17 % (Yalegama *et al.,* 2013). This fibre content was further concentrated by removing residual fat, protein and sugars. This study investigates on the *in-vitro* metal-binding capacity (iron, zinc and copper) of coconut kernel fibre at different pH levels.

MATERIALS AND METHODS

Fully-mature coconuts were obtained from *Bandirippuwa* Estate of the Coconut Research Institute of Sri Lanka for the preparation of virgin coconut oil residue (VOR) and coconut cell wall polysaccharides (CCWPs).

Virgin Coconut Oil Residue (VOR):

The shells of fully-mature and seasoned coconuts were removed and the brown testa was peeled off. The white kernels were disintegrated and dried in a cabinet dryer (Wessberg, Martin, Germany) at 70°C until it reached a moisture content of 2-3 %.

The dehydrated coconut kernels were fed to virgin coconut oil extraction machine (Cold press; Komet DD85, Germany). The oil was expelled at 65 °C. The residue obtained in this process was ground in a domestic grinder (LG, Korea) to obtain coconut flour from virgin coconut oil residue (VOR).

Coconut cell wall polysaccharides (CCWP):

The VOR was defatted using petroleum ether (1:5, w/v) overnight. The CCWP (1) was concentrated using methanol (80 %), SDS (1 %) and mercapto ethanol mixture and DMSO (90 %); the CCWP (2) was concentrated using ethanol (70 %) and CCWP (3) was concentrated using NaOH (0.1M) and ethanol (70 %) at 80 °C (Yalegama *et al*, 2013).

Determination of metal-binding capacity

The metal-binding capacity was determined using the method reported by Yalegama *et al.*, 2013).The sample (100 mg) each was weighed into a 30 ml centrifuge tubes. The samples were washed in 10 ml of glucose saline solution (GSS) by occasional shaking for 20 minutes followed by centrifuging for 20 minutes at

11,000 rpm. The supernatant was discarded and the residue was added to 10ml of GSS containing 1mM sodium acetate, 0.5mM imidazole, 0.02mMHCl and the test mineral (2.0 ppm). The mixture was mixed well in a vortex mixer for 1 minute and the pH was adjusted to 2. The final volume was made up to 14 ml with GSS. The contents were mixed again for 1 minute and left for 20 minutes. The tubes were centrifuged at 11,000 rpm for 20 minutes. The supernatant was collected after filtering through Whatman 42 filter paper. The concentration of metal ions in the filtrate, i.e., the amount of metal ion (not bound by the fibre) in the medium was measured using Atomic Absorption Spectrophotometer (GBC 904AA, Australia) with Hollow Cathode Lamps Fe, Cu and Zn. The experiment was repeated at different pH values.

The percentage of metal ion bound by the fibre was calculated as follows.

% of metal ion bound by the fibre =

$$\frac{\left(\begin{array}{c}\text{Initial concentration}\\ \text{of metal iron}\end{array}\right) - \left(\begin{array}{c}\text{Concentration of the metal iron}\\ \text{in the supernatant}\end{array}\right)}{\left(\begin{array}{c}\text{Initial concentration}\\ \text{of the metal iron}\end{array}\right)} \text{ x } 100$$

RESULTS AND DISCUSSION

Binding capacity of Fe (II) at different pH

Table1 shows the metal-binding capacity of Fe (II) to coconut kernel fibre. Binding of Fe (II) to CCWP (1) and CCWP(3) increased with increasing pH, and showed similar binding capacities to Fe (II).The binding of Fe (II) to CCWP (2) and VOR decreased with increasing pH. VOR had lower affinity to bind Fe (II) compared to the binding affinity of CCWP (2) to Fe (II) from pH 2 to 6.Therefore, the availability of Fe (II) was higher with VOR than that with CCWP (2). The composition of the four fibre types was different due to the solubility of residual matter with the solutions.

The CCWPs were concentrated from VOR which is the dehydrated defatted coconut kernel. Therefore, VOR contains higher residual matter than CCWPs (Yalegama *et al.*, 2013). The percentage yields were 35.5±2.2 %, 43.2±2.5 % and 39.1±1.8 % for CCWP (1), CCWP(2) and CCWP(3) respectively (Yalegama, 2012). CCWP (3) had the lowest residual matter (Protein 11.9 %, Fat 0.4 % and Sugar 0.1 %) while CCWP (2) had

Table 1 Binding capacities of Fe (II) to fibre concentrates at different pH levels

Fibre concentrates	Percentage bound				
	pH 2.0	pH 3.0	pH 4.5	pH 6.0	pH 7.5
CCWP(1)	62.03^{c}	77.30 ^{b}	82.09^{b}	83.30^{a,b}	91.40^{a}
CCWP(2)	90.73^{a}	88.73^{a}	88.33^{a}	75.27^{c}	38.50^{d}
CCWP(3)	58.33^{c}	76.20^{b}	83.87 ^{b}	87.23^{a}	92.00^{a}
VOR	78.80^{b}	78.90^{b}	77.87^{c}	50.47^{d}	46.38 ^{c}

Values are means of triplicate analysis. Different superscripts in a column are significantly different at $p<0.05$. One-way ANOVA was performed to determine levels of significance. Means were compared with LSD.

the lowest residual matter (Protein 11.9%, Fat 0.4% and Sugar 0.1%) while CCWP (2) had protein 29.9 %, fat 0.9 % and sugar 0.03 % and CCWP (1) contained protein 16.2%, fat 0.5% and sugar 0.34%. VOR contained protein 12.6%, fat 9.2% and 13.7%. Similar binding capacities of Fe (II) to CCWP (1) and (3) at all pH values were observed although the residual protein content was significantly different.

The binding on Fe (II) to coconut kernel fibre isolates was studied previously and showed binding capacity of Fe (II) to CCWP (1) and VOR at pH 6.5 was similar and was higher than the binding capacity of CCWP (3) in the presence of 0.5 – 2 ppm of Fe (II). However, the binding capacity was similar at 4 ppm of Fe (II) for all fibre types (Yalegama *et al*, 2012).

Reinhold *et al.* (1981) observed that Fe (II) remained dissolved in the solution until its pH approached 7.0 after which it precipitated. They further observed that concentration of Fe (II) in the solution decreased (bound percentage increased) as pH increased from 2.0 to 7.0 due to formation of Fe (II) fibre complexes in solutions containing wheat NDF, maize NDF, wheat ADF and maize ADF and celluloses. MahaLaksmi and Sumathi (1997) also observed that binding of Fe (II) to NDF obtained from sorghum and ragi increased with increasing pH from 4.5 to 6.5. Nearly 40-80 % of Fe (II) was bound to sorghum and ragi NDF. These observations are similar to the results of the present study with CCWP (1) and CCWP (3). Lee and Gracia Lopez (1985) observed increased binding of Fe (II) to NDF and ADF extracted from cooked pinto beans with pH increasing from 2 to 7 similar to CCWP(1) and CCWP(3). Thomson and Weber (1979) observed a portion of the iron bound to brans, hulls and cellulose was solubilised at acidic pH. They observed that Fe (II) was rebound to the fibres (in an insoluble form) when the systems were brought back to pH conditions approaching neutrality (pH 6.8). Although CCWP(1) and CCWP(3) conform to these observations as they contain less residual fat, sugar and protein compared to CCWP(2) and VOR. NDF and ADF are more concentrated fibre than the fibres in our study; the coconut fibres contain higher amount of residual components which can alter the interaction with metals. Therefore CCWP (2) and VOR showed different metal-binding characteristics to CCWP (1) and CCWP (3) probably due to the difference in compositions.

Binding capacity of Cu (II) at different pH levels

The binding capacity of coconut fibre concentrates to Cu (II) increased steadily with increasing pH 2 to 6 significantly ($P<0.05$) and then remained constant up to 7.5. Under strong acidic conditions, the binding was very low (6.7-12.5 %). The bound percentage increased to 62-85 % at pH 4.5 and to 75-97 % at pH 7.5.

Table 2 indicated that the binding capacities of Cu (II) to fibre concentrates were significantly different among different types of concentrates within pH range 2 to 7.5 ($p<0.05$). The binding of Cu (II) to CCWPs were similar at low acidic and neutral conditions (pH > 4). CCWPs bound 82 - 97 % Cu (II) at pH 4.5 – 7.5. As indicated previously, the CCWPs has less residual component than VOR. Therefore, the porous structure of CCWPs can absorb more metal ions than VOR. This is clearly seen in the results as VOR has lower binding capacity of Cu (II) throughout the working pH range. Although the difference was not highly significant at pH 2 and 3, it was significant at pH4.5 and beyond.

The higher binding capacity of CCWP (1), CCWP (2) and CCWP (3) to Cu (II) at higher pH may be due to the formation of insoluble Cu (II) carbohydrate and protein complexes at neutral pH as indicated by (Mod *et al.*, 1981). The three fibres contain lower fat content but higher carbohydrates and proteins which can form chemical binding to metal ions. VOR contains lower amount of carbohydrates and proteins than CCWPs, and due to compact structure, reaching of metal ions can be hindered.

Table 2 Binding capacity of Cu (II) to fibre concentrates at different pH levels

Type of fibre/ Substrates	Percentage binding				
	pH 2.0	pH3.0	pH4.5	pH 6.0	pH7.5
CCWP(1)	6.67 ^{b}	47.00 ^{c}	82.07 ^{a}	95.83 ^{a}	96.53 ^{a}
CCWP(2)	12.50 ^{a}	53.20 ^{b}	83.10 ^{a}	94.57 ^{a}	92.63 ^{a}
CCWP(3)	10.00 ^{ab}	61.5 ^{a}	83.93 ^{a}	92.93 ^{a}	94.53 ^{a}
VOR	7.00 ^{b}	44.67 ^{c}	67.43 ^{b}	74.46 ^{b}	75.30 ^{b}

Values are means of triplicate analysis. Different superscripts in a column are significantly different at $p<0.05$. One-way ANOVA was performed to determine levels of significance. Means were compared with LSD.

This could be the reason for the significant lower binding affinities ($p<0.05$) of metal to VOR. Even though, the binding pattern shown by VOR was similar to the pattern shown by CCWP (1), (2) and (3), it showed lower binding capacities.

Binding capacity of Zn (II) to fibre concentrates at different pH values

The binding capacity of Zn (II) to fibre concentrates increased significantly with increasing pH ($P<0.05$) (Table 3). The binding capacity of fibres to Zn (II) remained constant between pH 2 and 3, and steadily increased up to pH 7.5 where it showed 90-100 % binding of added Zn (II). The binding pattern of Zn (II) to CCWP (3), VOR and MR was totally different from that of Cu (II) and Fe (II). The results indicated that Zn (II) was resorbed from the matrix to the solution at strong acid conditions. The binding of Cu (II) and Fe (II) to fibre concentrates showed only adsorption but they did not show re-sorption. The re-sorption of Zn (II) from CCWPs, VOR and MR was similar at pH 2 and 3.

The binding capacities of CCWPs, and VOR to Zn (II) were significantly different at pH 2 to 7.5 at $p<0.05$ (Table 3). The re-sorption reduced when pH was increased from 2 to 3 with the highest re-sorption of VOR. The re-sorption of Zn (II) from CCWP (1) and CCWP (2) was not significantly different at pH 3. When the pH increased from 3 to 4.5 CCWPs, and VOR adsorbed Zn (II) rather than re-sorption. This absorption pattern was observed up to pH 7.5. The binding of Zn (II) to CCWP (3) was the highest at pH 4.5 followed by VOR, CCWP (2) and CCWP (1). CCWP (1) and CCWP (2) bound similar amounts while CCWP (1), CCWP (3) and VOR bound the highest percentage of Zn (II) at pH 6. The binding capacity of substrates further changed at slightly alkaline pH (7.5). Almost all the Zn (II) added bound to CCWP (1). CCWP (2), CCWP (3) and VOR showed similar binding capacities, and it was significantly lower than binding capacity of CCWP (1).

MahaLaksmi and Sumathi (1997) observed that binding of Zn (II) to NDF fibre obtained from ragi increased with increasing pH from 4.0 to 6.5. This observation agrees with the results of the present study. Laszlo (1989) observed that more Zn (II) was extracted from the fibre matrix in the pH range from 0.7 to 3.2. This was true for soy hull and corn bran and also with the result of the present study where re-sorption of Zn (II) was observed under the strong acidic conditions. In high acidic condition, Zn (II) ion is liberated from the fibre. When the pH increases Zn (II) become less soluble. Therefore, re-sorption was not observed at higher pH.

Table 3 Binding capacity of Zn (II) to CCWPs and VOR at different pH levels

Type of fibre/ Substrate	Percentage bound				
	pH2.0	pH3.0	pH4.5	pH6.0	pH7.5
CCWP(1)	-22.33 ^{c}	-14.67 ^{bc}	20.83 ^{c}	83.07 ^{a}	99.26 ^{a}
CCWP(2)	-16.17 ^{b}	-13.17 ^{b}	23.00 ^{c}	71.83 ^{b}	97.03 ^{b}
CCWP(3)	-23.33 ^{c}	-6.67 ^{a}	50.43 ^{a}	83.70 ^{a}	96.63 ^{b}
VOR	-26.50 ^{c}	-18.33 ^{c}	29.5 ^{b}	86.07 ^{a}	96.53 ^{b}

Values are means of triplicate analysis. Different superscripts in a column are significantly different at $p<0.05$. One-way ANOVA was performed to determine levels of significance. Means were compared with LSD.

Mod *et al.* (1982) and (1981) investigated the binding of rice hemicelluloses with trace minerals and their releasing characteristics by digestive enzymes (hemicelluloses, pepsin and trysin). According to them, both enzymes and pH released considerable amounts of bound minerals to different degrees. Therefore, the minerals are available for re-sorption *in-vivo*. We observed lower binding percentages of Zn (II) and Cu (II) to all fibres at pH 2 and 3 indicating possible availability for re-sorption *in-vivo*. However, Fe (II) did show such re-sorption.According to Mod *et al.*(1981), the availability of metal nutrients was reduced by fibre. However, highly acidic conditions prevailing in stomach can release metal ions. Protease and hemicellulose release Fe (II), Cu (II) and Zn (II) from fibre, and this could result in the re-sorption of the metals and increase in bioavailability although it is strongly bound in *in-vitro* conditions.

The fibre concentrates with lower residual matter showed high-binding affinity. Therefore, the raw form of fibre has higher bioavailability of micro nutrients over corresponding fibre concentrates. The removal of residual matter reduces the binding capacity of metal ion to fibre isolates resulting in increased bioavailability of metal ions. Therefore, further investigations are necessary to concentrate fibre isolates from VOR which has higher bioavailability of essential metal ion.

ACKNOWLEDGEMENT

Funding by National Research Council (Grant No. 09/15) is greatly acknowledged.

REFERENCES

- Babio, N., Balanza, R., Basulto, J., Bullo, M., Salas – Salvado, J. (2010).Dietary fibre: influence on body weight, glycemic control and plasma cholesterol profile. *Nutricion Hospitalaria.*, 25(3): 327-340.

Cho, S., Jonathan,W., Devries and Leon Broskey. The definition of dietary fibre in Dietary fibre analysis and applications.1-10. AOAC International, 481 North Fredric Avenue Suite 50, Gaithersbury, Maryland 20877-2417, USA.

Elhardallou, S.B. and Walker, A.F. 1999. Binding of Ca by three starchy legumes in the presence of Ca alone or with Fe, Zn, Mg and Cu. Food Chem,. 52: 379-384.

Fry, S.C. 1988. The growing plant cell wall: Chemical and metabolic analysis. Longman, Essex, UK.

Laszlo, J.A. 1989.Effect of gastrointestinal conditions on the mineral – binding properties of dietary fibre. Advances in Experimental Medicine and Biology, 249:133-145.

MahaLaksmi, R. and Sumathi, S. 1997. Binding of iron, calcium and zinc by fibre of sorghum and ragi. Food Chem., 60(2): 293-217.

Mod, R.R.,Ory, R.L., Morris, N.M., and Normand, F.L. 1981.Chemical properties and interaction of rice hemicellulose with trace mineral in vitro. J.Agri.Food Chem., 29:449-452.

Mod, R.R.,Ory, R.L., Morris, N.M., and Normand, F.L. 1982.In vitro interaction of rice hemicellulose with trace mineral and their release by digestive enzymes. Cereal Chem., 59(6): 538-542.

Platt, S.R., Cladesdale, F.M. 1984. Binding of iron by cellulose, lignin, sodium phytate and beta-glucan, alone and in combination, under stimulated gastrointestinal pH conditions. J.Food Sci., 49:531-535.

Reinhold, J.G. and Gracia- Lopez, J. 1981. Binding of iron by fiber of wheat and maize. Amer. J. ClinicalNutri., 34:1384-1381.

Yalegama, L.L.W.C. 2012. Characterization and nutritional effects of cell wall polysaccharides from coconut kernel. PhD thesis submitted to the Post Graduate Institute of Peradeniya, University of Peradeniya, Sri Lanka.

Yalegama, L.L.W.C., Karunarathne, D. N., Sivakanesan, R. and Jayasekara, C. 2013. Chemical and functional properties of fibre concentrates obtained from by- products of coconut kernel. Food Chem., 141: 124-130.