

## Screening of Bile Acid Binding Capacity of Some Synthetic Dietary Fiber

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**Abstract:** This study was conducted to investigate *in vitro* bile acid binding capacity (BABC) of some new synthetic dietary fiber (DF) sources based on dry matter (DM) and water holding capacity (WHC) properties. Pectin<sup>®</sup>, Vitacel<sup>®</sup> R 200, Vivapur<sup>®</sup> (Microcrystalline Cellulose, 101) (MCC), Arbocel<sup>®</sup> (RC FINE, R and RC) and Carboxymethyl Cellulose<sup>®</sup> 7M8/SF (CMC) were used as synthetic DF sources and sodium deoxycholate as bile acid sample. The MCC and CMC DF sources had the highest and lowest DM contents, respectively ( $P < 0.05$ ). Arbocel<sup>®</sup>-RC FINE and CMC DF sources had the highest and MCC as well as pectin DF sources had the lowest WHC ( $P < 0.05$ ). Moreover, *in vitro* BABC of used DF sources was  $< 11$  mg/g DM which the highest one was related to CMC DF source ( $P < 0.05$ ). Values obtained for DM and WHC ( $P < 0.05$ ) as well as DM and BABC ( $P < 0.01$ ) of DF sources showed significantly negative correlation. According to the results of present study it seems that *in vitro* BABC of tested synthetic DF sources is negligible. Moreover, their DM rather than WHC is correlated with *in vitro* BABC property of tested DF sources.

**Key words:** Dietary Fiber • Bile Acid Binding • Water Holding Capacity

### INTRODUCTION

Dietary fiber (DF) is defined as cell walls of plant tissues [1]. It has many health benefits that encouraged manufacturers to produce commercial products to develop their application in consumer diets. The intrinsic benefits of DF intake, which led to increased intake by consumers, raised many questions about their possible detrimental effects on mineral bioavailability and lipid metabolism which latter comes from fiber capacity to bile acid and cholesterol binding [2, 3].

Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, they are secreted into the duodenum [4]. Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism by which DF lowers cholesterol [5-7] and linked with lipid metabolism. By binding bile acids, food/feed DF fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids [8, 9]. The healthful or cholesterol-lowering properties of food/feed DF fractions could be predicted by evaluating their *in vitro* bile acid binding, based on positive correlations found between *in vitro* and *in vivo* studies [10, 11]. By reviewing the effects of DF bile acid

binding capacity (BABC) in previous literatures it found that BABC of various DF sources is not correlated by soluble fiber [11, 12], protein [13], total DF [2, 12-17], oil [11] and  $\beta$ -glucan contents [12, 18]. However, dry matter (DM) content [12-14, 19] and water holding capacity (WHC) [20] of DF sources have determinative roles in BABC and DM has basic role in WHC. On the other hand, the behavior of new synthetic DF sources is not clear in relation to *in vitro* BABC. Therefore, this study was carried out to evaluate the *in vitro* BABC of some new DF sources based on DM and WHC properties.

### MATERIALS AND METHODS

**Dietary Fiber Samples:** In this experiment, Pectin<sup>®</sup>, Vitacel<sup>®</sup> R 200, Vivapur<sup>®</sup> (Microcrystalline Cellulose 101) (MCC), Arbocel<sup>®</sup> (RC FINE, R and RC) and Carboxymethyl Cellulose<sup>®</sup> 7M8/SF (CMC) were used as synthetic DF sources. Pectin was prepared from HP, USA and other products from JRS RETTENMAIER and SOEHNE GmbH+Co KG, Germany, respectively. The DF source powders were kept in airtight plastic bags and stored in a desiccator at room temperature (24°C) prior to parameters determination.

**Dry Matter and Water Holding Capacity Measurement:**

The moisture (DM) was determined as the weight loss for 16 h at 105°C followed 2 h at 130°C at air-oven drying [21]. The WHC of DF samples was measured by the modified centrifugation method [22]. In brief, 20 mL of ultrapure water was added into a centrifuge tube containing 200 mg of DF samples. Subsequently, the centrifuge tubes were incubated in a 25°C shaking water bath for 24 h. After centrifuged (14,000 g for 30 min at 25°C), the supernatant was discarded and the moisture content of pre-weighed pellet was determined after dehydration in an air-oven for 2 h at 120°C. The WHC of each DF sample was expressed as the weight of water held by one gram of corresponding DF samples.

**Binding of Bile Acid:** The BABC of DF sources was determined by colorimetry [12, 19]. The factor triggering the colour reaction in this method is a 5% aqueous solution of furfural (Merck, Germany). Sodium deoxycholate (SD) (Fluka, 30970) was selected for analysis at a concentration of 1 mM. The measurement principle was to determine concentrations of SD in the supernatant after incubation at a temperature of 37°C. The analytical sample of SD (0.423 mg) was dissolved in 25 mL ethanol, using ultrasounds and next made up with phosphate buffer of pH 6.3. The analysed sample of 0.5 g and 20 mL SD dissolved in the phosphate buffer was placed in a conical flask and shaken in a water bath (37°C, 2h). Additionally a blank test was prepared containing 0.5 g analysed DF samples in 20 mL phosphate buffer, the standard sample containing SD dissolved in the phosphate buffer and the proper sample, containing SD dissolved in the phosphate buffer and the tested material. Samples were shaken in a water bath at 37°C for 2 h, after which they were filtered. The amount of 5 mL supernatant was collected for analysis and mixed with 5 mL 70% sulphuric acid. Two minutes later 1 mL 5% furfural solution was added (after 5 min pink colouring appeared) and next absorbance was measured. The amount of SD absorbed by DF sources was determined based on the difference of concentrations before and after incubation. The concentration of SD in the tested samples was determined based on the standard curve for a given acid within the range of concentrations 0.1 to 0.8 mM. Absorbance was measured using a spectrophotometer at a wavelength of 510 nm.

**Statistical Analysis:** All data were analysed for normal distribution using the NORMAL option of the UNIVARIATE procedure and for homogeneity of variances for treatment means through the Levene's Test,

using the HOVTEST option of GLM procedure of SAS [23]. All determinations were performed in triplicate and were analysed as a completely randomized design by the GLM procedure of SAS [23]. Significant differences were compared by Duncan tests ( $P < 0.05$ ). All differences were considered significant at  $P \leq 0.05$ . Moreover, correlation coefficients were calculated between measured parameters.

**RESULTS AND DISCUSSION**

The results of measured parameters related to tested DF sources are showed at Table 1. The MCC and CMC DF sources had the highest and lowest DM contents, respectively ( $P < 0.05$ ). In general, range of moisture (3.50-11.5 %) led to have significant differences in DM between DF sources. This difference in moisture content suggests water-DF interactions, which could influence other characteristics of DF sources. The moisture content was calculated as weight loss at 105°C overnight and 130°C for 2 h. This method of DM determination resulted in accurate estimation rather than weight loss at 105°C overnight [21]. Differences in moisture contents may be due to differences in type, processing or storage conditions of DF sources [24]. Since the DF sources were stored in the same conditions only differences in intrinsic nature and processing conditions of DF sources could involved in this variation. Moreover, except CMC the calculated moisture content is <10%, which are agrees with the results of other studies for various DF sources [24, 25].

Arbocel®-RC FINE and CMC DF sources had the highest and MCC as well as pectin DF sources had the lowest WHC ( $P < 0.05$ ). Associated water with DF sources play important role in emerges of DF properties. Such water would affect DF metabolic activity across GIT. Water soluble and insoluble portions of DF sources influence WHC of DF sources. It is indicated that DF sources with high water insoluble portion induce lower WHC [26-28]. So, WHC of DF sources is attributed to water insoluble fiber, while other ascribed it to high acid uronice content [29, 30]. However, there is general agreement that WHC depends on experimental conditions (temperature, pH, time and characteristics of centrifuge), particle size and processing [22, 29, 31].

The WHC of a DF sources measures the amount of water retained by DF sources after subject to a stress such as centrifugation [32]. It is an important hydration property of a DF that needs to be measured before their incorporation on the diets. The range of measured WHC values of tested DF sources fall in the range other DF sources (wheat bran: 6.40-6.60 g/g [33, 34]; oat bran:

5.50 g/g [35]; AGIOLAX: 6.60 g/g [36]; wheat bran: 2.6 g/g, corn bran: 2.5 g/g and soy bran 2.4 g/g [37]). The WHC determined by centrifugation method in the present study represented all three types of waters (water bound to the hydrophilic polysaccharides, held within the fiber matrix, trapped within the cell-wall lumen) associated with the DF sources [38]). Numerous factors could influence the DF water related parameters. Although raw source of DF is determinant factor for its chemical composition and structure, also, final microstructure (fiber length, particle size and porosity) and processing conditions is important [32]. The microstructure of DF sources is believed to have more profound effect that it's chemical composition on the WHC [39]. In the present study, differences in WHC between DF source samples (be significantly higher in Arbocel®-RC FINE and CMC) is not clearly obvious. However, smaller particle size of water insoluble portions might be loss insoluble fiber [35] and increase surface area for exposure of their hydrophobic regions and might cause to higher WHC value. On the other hand, CMC with lower DM led to higher WHC and MCC with higher DM led to lower WHC. This observation could emphasize that some correlation exist between DM and WHC values of some DF sources which the importance of DF structure with water related parameters is critical [40].

The highest BABC was obtained by CMC DF source ( $P < 0.05$ ), although did not differ significantly with Arbocel®-R and Arbocel®-RC DF sources. It seems that the ability of non-absorbable dietary compounds to *in vitro* BABC was approved many years ago [41]. The results of *in vitro* determinations of BABC to DF sources, given in the literature, are not comparable in each case, because several modifications are used. For example, some authors have preferred to digest samples in the presence of bile acid, simulating the conditions in the stomach and small intestine [42]. Other authors have used pre-digested starch- and protein-rich DF samples [43]. For removal of the un-bound proportion of bile acid, different techniques were applied, e.g., filtration [44], pressure filtration [45] or centrifugation [11]. Most authors have preferred to test only one bile acid per experiment [46]; other authors used bile acid mixtures [2]. Furthermore, the concentrations of bile acid and DF differed strongly between the studies. Finally, different methods of bile acid analysis were applied, including HPLC [43], colorimetry [44], determination of radioactivity [47] or enzymatic methods [42]. However, BABC based on DM (rather than cholestyramine, 100% bound) was reported for various bran (rice bran 25%, wheat bran 20%, oat bran 5% and corn bran 3%) [11], various raw vegetables (collard greens, kale and mustard

greens, 13%; broccoli, 10%; Brussels sprouts and spinach, 8%; green bell pepper, 7%; and cabbage, 5%) [48], various grains (wheat 9.4%; oats 8.6%; rice 2.8%; and corn 2.1%) [12] and some vegetables (okra, beets, asparagus, eggplant, turnips, green beans, carrots and cauliflower 1-16%) [49]. The concentrations of bound bile acids in the present study are in accordance with the results of most of the other binding studies.

In the past *in vitro* efforts to found relationship between portion (s) of DF sources and the amount of BABC it clear that BABC of various DF sources is not correlated by soluble fiber [11, 12], protein [13], total DF [2, 12-17], oil [11] and  $\beta$ -glucan contents [12, 18]. However, DM content [12-14, 19] and WHC [20] of DF sources have determinative roles in BABC. The differences in BABC between various tested DF sources may relate to their different in anionic-cationic structure and physico-chemical nature [18], DF sources composition, hydrophobicity, or active binding sites [48]. However, it was shown that the degree of bile acids adsorption is depends on the kind of raw material and the type of bile acids [50]. Fewer than 10% BABC of various DF sources, in literature, were considered less important and are not perceived stimulatory effect on binding bile acids and compromised lipid metabolism. As Table 1 shows that although BABC were significantly differ between tested DF sources but these difference is not broad and likely their effects on *in vivo* BABC is low.

**Correlation Coefficients:** According to researches [20] there is a correlation between the taurocholate binding capacity of some wild leafy vegetables and WHC. The correlation coefficients of measured parameters for DF sources were illustrated at Table 2. Negative correlation coefficients were found between DM and WHC ( $P < 0.05$ ) and DM and BABC ( $P < 0.01$ ) of tested DF sources. Moreover, no significant correlation was observed between WHC and BABC of tested DF sources. These observations disagreed with other [20]. Type of DF sources seems discrepancy cause in this respect. This suggested that hydrogen bond and hydrophobic interaction mediated in the binding of bile acid to alcohol insoluble solids. It is suggested that variations in BABC values were based on the variation in WHC values [50]. This finding not supported in present study. But, the results of current study illustrated that DM contents of DF sources is negatively correlated with WHC and BAB. Therefore, other physiochemical factors, meant DM, other than WHC might be responsible for the variation BABC values.

Table 1: Dry matter, water holding capacity and bile acid binding of tested dietary fiber sources

Treatments	DM (%)	WHC (g/g)	BAB (mg/g DM)
Pectin <sup>®</sup>	93.30 <sup>c</sup>	2.79 <sup>d</sup>	9.15 <sup>bc</sup>
Vitacel <sup>®</sup> R 200	95.32 <sup>b</sup>	3.45 <sup>c</sup>	8.56 <sup>c</sup>
MCC	96.57 <sup>a</sup>	2.62 <sup>d</sup>	9.93 <sup>b</sup>
Arbocel <sup>®</sup> -RC FINE	95.46 <sup>b</sup>	5.33 <sup>a</sup>	8.83 <sup>c</sup>
Arbocel <sup>®</sup> -R	90.70 <sup>d</sup>	4.28 <sup>b</sup>	9.99 <sup>ab</sup>
Arbocel <sup>®</sup> -RC	90.50 <sup>d</sup>	4.16 <sup>b</sup>	10.10 <sup>ab</sup>
CMC 7M8/SF	88.51 <sup>e</sup>	5.47 <sup>a</sup>	10.94 <sup>a</sup>
P value	<0.0001	<0.0001	0.0010
SEM	0.63	0.24	0.20

DM: Dry matter; WHC: Water holding capacity; BAB: Bile acid binding; MCC: Vivapur<sup>®</sup> (Microcrystalline Cellulose, 101); CMC: Carboxymethyl Cellulose; SEM: Standard Error of the Means; Means with different superscripts in same column are significantly different (P<0.05)

Table 2: Correlation coefficients of measured parameters for tested dietary fiber sources

Parameters	DM	WHC	BAB
DM	1	-0.51 <sup>*</sup>	-0.65 <sup>**</sup>
WHC	-0.51 <sup>*</sup>	1	0.26 <sup>n.s</sup>
BAB	-0.65 <sup>**</sup>	0.26 <sup>n.s</sup>	1

DM: Dry matter; WHC: Water holding capacity; BAB: Bile acid binding; \*: Significant P<0.05; \*\*: Significant P<0.01; n.s: Not significant

### CONCLUSION

The results of current study have demonstrated that bile acid binding capacity of tested synthetic dietary fiber sources is relatively low and could be expected their inhibition effects on *in vivo* lipid absorption be low. Furthermore, no significant correlation was observed between water holding capacity and bile acid binding capacity of tested synthetic dietary fiber sources.

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