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## **SORPTION OF BILE ACIDS AND CHOLESTEROL BY DIETARY FIBER OF CARROTS, CABBAGE AND APPLES**

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### **ABSTRACT**

Adsorption of common bile acids namely lithocholic (LCA), dexychoic (DCA) and cholic (CA) acids and cholesterol by fiber of carrots, cabbage and apples raw and after heat treatment, respectively, was estimated. It was shown that degree of bile acids adsorption depends on kind of the raw material, type of heat treatment and type of bile acids. Fiber carrots had higher sorption than cabbage and apples. Fiber of baked vegetable and apples had significantly higher sorption capacity in compare with boiled and steamed plants material. Lithocholic acid was adsorbed in higher degree than DCA and CA. The cholesterol was bound in the highest degree by fiber cabbage on compared to fiber carrots and apples. Heat treatment of plants material, especially baking, increased the sorption of cholesterol by fiber cabbage as well as carrots and apples.

**Key words:** dietary fiber, bile acids, cholesterol, heat treatment.

### **INTRODUCTION**

Products of plant origin like cereals and their processed products, fruit, vegetable and legumes are main sources of fiber for human. The content of dietary fiber and its fractional composition in plants is differentiated. Hemicelluloses dominate in cereals, while in fruit and vegetable pectins and cellulose [6, 15, 13, 21, 24]. In

consideration of heterogeneous structure and chemical composition of fiber substances, they are characterized by different physiological properties as increase of fecal weight, reduce transit time, decrease of glucose and cholesterol level in blood, bile acids binding, and functional properties as water holding capacity, cations exchange capacity, fat adsorption [5, 9, 12, 23]. Very important nutritional properties of dietary fiber are connected with adsorption bile acid and cholesterol. Bile acids salts may form with fibers complexes that are not adsorbed and are next excreted with fecal. Faster cholesterol metabolism and bile acid salts synthesis in liver as well as lowering cholesterol LDL fraction level in blood plasma, occurs at the same time [8]. Epidemiological studies have shown that the fecal bile acids, especially the secondary bile acids, are higher in populations that consume higher- fat / lower- fiber diets. It was shown also that the fecal ratio of LCA to DCA is high in patients with colon cancer. The ratio may be a marker of risk of cancer of the large bowel [17]. Dietary fiber, as a component of plant origin products, is subjected to various technological treatments, among other grinding and heat treatment. There are no univocal settlements considering influence on fraction composition and fiber functional properties of this treatments still [4, 14, 19, 20, 11]. Therefore, the aim of present study was examine the adsorption of bile acids and cholesterol by fiber of carrots, cabbage and apples, and evaluate the impact of various factors involved, including heat treatment, kind and concentration of bile acids.

## MATERIALS AND METHODS

The following study was conducted on both raw and after heat treatment of cabbage, carrots and apples. Carrots (Karatka cultivars), white cabbage (Kamienna Głowa cultivars) and apples (McIntosh cultivars) were experimental material. All products were bought on local market. Raw material was subjected to following heat treatment: boiling, steaming and baking.

Preliminary processing included washing and cleaning as well as removing seed nests (in case of apples) or cutting (in case of cabbage). Boiling was carried out by placing in the boiling water of raw plants material (in ratio 1:1). Steaming was done in "Rational" convection oven in temperature 100°C, and baking in the same place in temperature of 180°C. All material was lyophilized and after them grinded and sieved (0.6 mm).

**Adsorption measurements.** Adsorption of bile acids by dietary fiber was estimated from the change in bile acid concentration on exposure of the solution to the fiber [7]. Bile acids were obtained from Sigma Chemical Co. The concentration of bile acids was chosen as 1 and 6 mM as a typical concentration in the human terminal ileums. Bile acids were dissolved in 0.1 M phosphate buffer at pH 6.9. A 0.5 g of sample and 20 ml of buffered bile acids solution were placed in conical flask. Additionally, two samples were prepared: one contained bile acids solution without fiber, for use as a standard sample, and the other contained 0.5 g of plant material and 20 ml of buffer solution, as a blank. Samples were shaken in a shaker bath in temperature of 37°C for 2 hours and next the supernatant was cleared with filtration, and aliquots were taken for bile acid estimation.

**Bile acid estimation.** A 5 ml aliquot of the supernatant was mixed with 5 ml of 70% sulphuric acid. Two minutes later 1 ml of 25% furfural solution was added. A pink color appeared and took 5 min to develop to maximum intensity. Readings were made at the absorbance by wave of 510 nm.

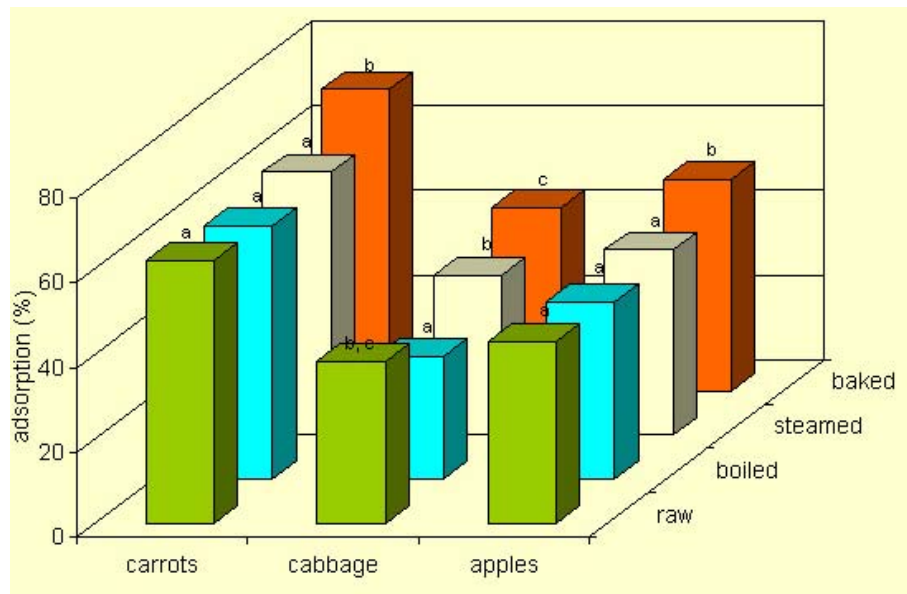
**Adsorption of cholesterol.** A 10 ml of emulsion containing 1.375% deoxycholic acid, 0.225% cholesterol and 1.00% lecithin was added to 1 g sample to estimate adsorption of cholesterol by dietary fiber [10]. Deoxycholic acid and cholesterol were dissolved in 10 ml propanol-1; similarly in the same volume of propanol-1 lecithin was dissolved. Both solutions were combined and completed with buffer pH 6.8. Dietary fiber capacity for binding cholesterol was indicated by enzymatic method, at use of P.O.Ch. Gliwice, Poland reagents kit.

Results of work were verified statistically, at use of Turkey's test on  $p = 0.05$ .

## RESULTS AND DISCUSSION

Carried out study shown, that adsorption of bile acids by dietary fiber of plants material depends on kind of fiber source, type of heat treatment and type of bile acids. The highest sorption, independently from type of heat treatment and type of bile acids, was observed at carrots (63.5%), while cabbage was characterized by the lowest one (37.1%) – [Fig. 1](#). High degree of bile acids sorption by carrots is probably correlated with different fraction composition of carrots in comparison with cabbage and apples. From investigations carried out earlier it results, that among the insoluble fractions, lignin has the strongest bile acids binding, while for cellulose the lowest sorption degree was found [1, 22]. Camire et al. [2] attribute considerable sorption properties also to pectins, but its properties increase with increment of esterification degree.

**Fig. 1. Bile acids adsorption by fiber of carrots, cabbage and apples depending on heat treatment**



a, b, c – various letters in the same cluster of fiber source indicate statistically significant differences ( $p < 0.05$ )

Food processing may change physico-chemical properties of dietary fiber, especially water holding capacity and adsorption other organic components. Through all heat treatments that have been applied, baking process significantly increased sorption of bile acid by dietary fiber, in compare with raw material (Fig. 1 and Tab. 1). Baked carrot bound circa 11% more LCA, while apple and cabbage circa 6% of this acid. During baking vegetables and fruits the content of fiber and its fractional composition changes. It is mainly correlated with products of Maillard reaction formation, that, as Ragot et al. [18] investigations have shown, posses bile acids adsorption capacity. Fiber of boiled vegetables and fruits bound bile acids in smaller degree in compare with raw plants material. However, the influence of boiling as well as steaming vegetables and fruit on sorption of bile acids was insignificant.

Bile acids adsorption degree has also been changing depended on type and concentration of bile acids (Table 1). Sorption degree among all used acids was in the following order: LCA > DCA > CA with 6 mM concentration, while sorption value was connected with kind of plant material.

**Table 1. Bile acids adsorption by dietary fiber of carrots, cabbage and apples raw and after heat treatment (%)**

Product	Type of bile acids					
	LCA		DCA		CA	
	1 mM	6 mM	1 mM	6 mM	1 mM	6 mM
Carrots	69.8ab	82.2a	65.0ab	76.3a	30.4a	37.2a
Raw	66.5a	80.7a	56.3a	74.3a	27.4a	35.8a
Boiled	70.1ab	82.1a	63.9ab	77.0a	30.3a	36.8a
Steamed	77.3c	92.2b	70.3c	85.9b	42.0b	44.6b
Baked						
Cabbage	40.8b	49.4a	36.6a	48.9bc	14.0a	23.6ab
Raw	28.7a	39.6b	26.9b	38.2a	13.1a	21.7a
Boiled	40.7b	49.6a	34.3a	44.0ab	16.3ab	22.0a
Steamed	49.7c	53.1a	45.8c	52.3c	19.7b	28.9b
Baked						
Apples	49.1a	53.6a	46.1bc	53.4b	19.0b	26.1b
Raw	49.0a	62.5b	39.4a	46.4a	15.7a	20.3a
Boiled	51.3ab	52.9a	44.9b	55.7bc	18.8ab	26.2b
Steamed	54.6b	60.9b	50.4c	60.3c	25.7c	31.8c
Baked						

a, b, c – different letters in columns show statistically significant differences ( $p < 0.05$ ).

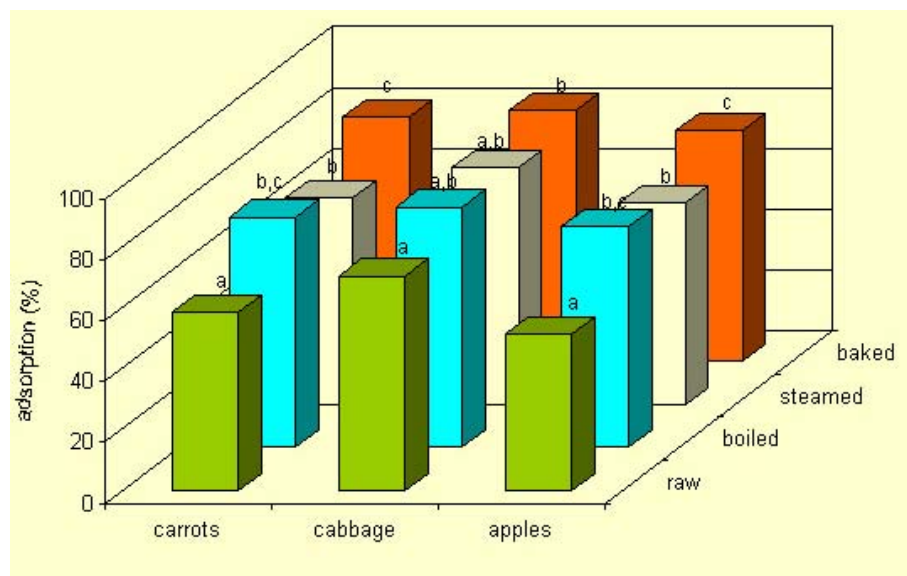
Abbreviations: LCA – lithocholic acid, DCA – deoxycholic acid, CA – cholic acid, 1 mM and 6 mM – concentrations of bile acids.

Cholic acid is a primary acid, however deoxycholic and lithocholic acids are secondary one, formed through transformation of primary acids. Mutagenic properties are attributed to secondary acids that are why its capacity for being adsorbed by dietary fiber seems to be particularly very important [16]. Investigations indicated hydrophobic character of bile acids sorption by dietary fiber [2, 3, 7]. The authors of researches have shown that bile acids sorption degree decrease with hydroxyl group increment in steroid ring structure. It is confirmed by this investigations, in which cholic acid (three hydroxyl acid) was the less adsorbed, however lithocholic acid (mono hydroxyl acid) was the best adsorbed.

Cholesterol adsorption by dietary of plants material was differentiated and depended on kind of material. Cholesterol was bounded by cabbage fiber in the highest degree (77.3%), by carrots fiber in lower degree (70.2%) and by apples fiber least of all (66.5%).

Not without any meaning was applied vegetables and fruit heat treatment. Fiber of baked carrots, cabbage and apples (Fig. 2) adsorbed cholesterol in the highest degree. Baked apples bound 47% more cholesterol (in compare with raw apples), while cabbage only about 17%. Probably, formatted products of nonenzymatic browning posses cholesterol binding capacity, similarly as in bile acids case. Heat treatment (boiling) of plants material increased cholesterol sorption about 29% in case of carrots and 41% in case of apples in compare with raw material. The increase of pectins content or changes in proportion of insoluble fraction – lignin participation increase and cellulose decrease, were probably the reason of adsorption increase. The fiber of steamed carrots, cabbage and apple has the lowest cholesterol binding capacity in compare with fiber of raw and boiled and baked plants material. Kmita-Głażewska and Kostyra's [10] investigations shown that insoluble fiber fraction of Italian cabbage may bind circa 70% of cholesterol, while white cabbage can bind 86% of cholesterol. From earlier investigations results that dietary fiber of examined vegetables and fruits contained much more insoluble fraction what may confirm that the insoluble fiber fraction is responsible for cholesterol binding.

**Fig. 2. Cholesterol adsorption by dietary fiber of carrots, cabbage and apples depending on heat treatment**



**a, b, c – various letters in the same cluster of fiber source indicate statistically significant differences ( $p < 0.05$ )**

## CONCLUSIONS

1. The adsorption of bile acids by fiber carrots, of cabbage and apples was depended on kind of plants material as well as type of heat treatment and type of bile acids. Among all examined plants material carrot's fiber had the highest sorption, while cabbage's fiber showed the lowest one.
2. Dietary fiber of backed carrots and apples adsorbed the most bile acids, however boiled plants material showed the lowest these properties.
3. The adsorption was correlated with hydrophobicity of the bile acids. Among examined bile acids, the most susceptible on sorption was lithocholic acid (mono hydroxyl acid), while the least one was cholic acid (three hydroxyl acid).

4. The cholesterol was adsorbed in the highest degree by fiber of cabbage and the lowest by apple's fiber. Fiber of plants material after heat treatment bound cholesterol in higher degree than raw plants material. The cholesterol was adsorbed in the highest degree by fiber of baked carrots, cabbage and apples.

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