Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, No 1, pp. 69–73

ADSORPTION OF BILE ACIDS AND CHOLESTEROL BY DRY GRAIN LEGUME SEEDS*

Danuta Górecka, Józef Korczak, Ewa Flaczyk

Department of Human Nutrition Technology, A. Cieszkowski Agricultural University, Poznań

Key words: legumes, fibre, functional properties, bile acids, cholesterol

The aim of the study was to estimate sorption degree of selected bile acids (lithocholic acid - LCA and cholic acid - CA) with 1 mmol/L and 6 mmol/L concentration as well as that of cholesterol by legumes. It was shown that bile acids sorption degree depended on the variety of seeds (the highest sorption ability had pea, while lentil showed the lowest one), grinding degree (bile acids were bound better by big particles) and the kind of acid (LCA acid with 6 mmol/L concentration was bound to a greater extent, independently on the variety of seeds and grinding degree). Next, the best binding ability of cholesterol was reported for bean, while the lowest one for soybean. Development of sorption surface of the investigated seeds significantly influenced extension of cholesterol binding ability.

INTRODUCTION

Considerable interest in fibre influence on lipid metabolism has been noticed for many years. The first scientist who showed the influence of wheat bran on lipid metabolism was Eastwood [1969]. Hypocholesterolemic effect of cereal fibre is often attributed to β-glucans [Lupton & Yung, 1991; Kahlon & Chow, 1997]. Bile acids and cholesterol are bound in their gel structures. Binding and extension of bile acids excretion with faeces causes transferring of cholesterol to bile acids synthesis. This way the quantity of cholesterol accessible for lipoprotein synthesis decreases, which next causes decrease of its concentration in plasma [Johnson, 1990; Huang & Dural, 1995; Bartnikowska & Lange, 2000]. It is also possible to inhibit endogenous cholesterol biosynthesis by short-chain fatty acids, which are produced by colon bacteria during fermentation process of soluble fibre [Johnson, 1990; Rieckhoff *et al.*, 1999]. Cholesterol and bile acids binding by fibre prevents their transformation into other compounds, like those of cancer-causing character. It is thought that lithocholic and deoxycholic acids (secondary bile acids) may have mutagenous properties [Hallmans *et al.*, 1997]. Not only fruit, vegetables and cereals but also leguminous plants are a rich source of dietary fibre. Because of heterogeneous structure and chemical composition of dietary fibre originating from the mentioned sources, it is characterized by different physiological and technological properties [Schneeman, 1986]. Functional properties of dietary fibre depend most of all on the kind of fraction, pH and environmental temperature, as well as technological processing, size of particles of investigated material, and the kind of bile acid [Mongeau & Brassard, 1985; Korczak *et al.*, 1995; Górecka *et al.*, 1996; Thebaudin *et al.*, 1997].

That is why the aim of the study was to estimate sorption of selected bile acids and cholesterol by seeds of leguminous plants with different degree of grinding.

MATERIAL AND METHODS

Green lentil, white small bean, yellow pea as well as yellow soybean bought in shops were used for investigations. All legumes were ground, and divided into individual fractions: 1.0 mm; 0.63 mm and 0.2 mm, with the help of suitable sieves. Two acids with different number of hydroxyl groups – lithocholic acid (LCA) and cholic acid (CA) were selected for investigations. Moreover, in the selection of bile acids, their physiological characteristic was also taken into account. Cholic acid is a primary acid, while lithocholic is secondary one, formed during transformation processes of primary acids. Selection of bile acids concentration (1 mmol/L and 6 mmol/L) was based on the fact that in the intestine these acids occur in concentration varying between 1 mmol/L and 12 mmol/L [Huang & Dural, 1995].

Bile acids adsorption measurements. Bile acids binding ability by legumes was determined with the use of colorimetric method [Huang & Dural, 1995]. Factor causing colourful reaction in this method is furfural water solution. Rule of measurement is based on determination of bile acids concentration in supernatant after incubation at a temperature of 37°C and reaction characteristic for intestinal juice. That is why 0.5 g of the investigated substance as well as 20 mL of bile acids were dissolved in phosphoric buffer (pH 6.9) and placed in conical flask. Additionally two samples were prepared: one containing bile acids without plant material, as a standard sample, and a second one containing 0.5 g of plant material and 20 mL of buffer, as a blank. Samples were shacked in a shaker bath at 37°C for 2 h, and next the supernatant was cleared with filtration. A 5 mL aliquot of the supernatant was mixed with 5 mL of 70% sulfuric acid. Two minutes later 1 mL of 25% furfural solution was added (a pink colour appeared and it took 5 min to develop its maximum intensity). Readings were made at the absorbance by wave length of 510 nm.

Author's address for correspondence: Department of Human Nutrition Technology, A. Cieszkowski Agricultural University, ul. Wojska Polskiego 31, 60-624 Poznań, tel. (48 61) 848 74 30, fax (48 61) 848 74 31, e-mail: gordan@owl.au.poznan.pl

Adsorption of cholesterol. A 10 mL of emulsion containing 1.375% deoxycholic acid, 0.225% cholesterol dissolved in phosphate buffer at pH 6.9 and 1.00% of lecithin were added to 1 g sample to estimate adsorption of cholesterol by legumes [Kmita-Głażewska & Kostyra, 1999]. 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. Legumes ability for binding cholesterol was indicated by enzymatic method, at use of P.O.Ch. Gliwice, Poland reagents kit.

The results of the study were verified statistically with Tukey test at $\alpha = 0.05$ significance level.

RESULTS AND DISCUSSION

The study carried out in a model system showed that adsorption of bile acids by plant material depends on variety of legumes, and grinding degree of seeds, as well as that the type of bile acid and its concentration have significantly differentiated bile acids sorption (Table 1). The highest sorption, independently from particle size and type of bile acids, was observed at pea (52.5%), while soybean and bean bound bile acids much less, 37.5% and 28.3% respectively, while lentil cabbage was characterized by the lowest sorption (19.25%) – Figure 1. This differentiated sorption ability of legumes is probably correlated with different fractioning of dietary fiber [Górecka *et al.*, 1998]. From earlier investigations, it results that lignin has the strongest ability to bind bile acids [Story & Kritchevsky, 1976]. It was confirmed by Anderson *et al.* [1994]. Camire *et al.* [1993] and Dongowski [1997] showed that pectins are also characterized by considerable sorption properties, but pectin-bile acid interaction intensification increases with increase of pectin methylation degree. The highest sorption of bile acids by pea showed in the present study is probably correlated with higher lignin content (3.79% d.m.) as compared to bean (2.34% d.m.) and soybean (2.56% d.m.) [Górecka *et al.*, 1998].

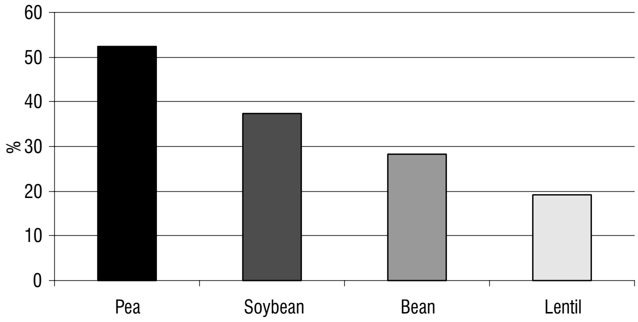

FIGURE 1. Bile acids adsorption by legumes independently of type and concentration of acid.

Bile acids adsorption by fibre also depends on grinding degree of material (Figure 2). It was shown that the smallest particles were characterized by the highest sorption abilities. Sorption value in the case of particles of 1 mm diameter (independently on the type of acid and concentration) for pea, soybean, bean and lentil was 40; 27.5; 17.8 and 11.3% respectively, while for the same seeds but smaller particles (0.2 mm) it was 68; 48.3; 38.5 and 28% respectively. It is probably connected with increase of total adsorbing surface as particles reduce [Mongeau & Brassard, 1985; Huang & Dural, 1995; Thebaudin *et al.*, 1997]. Story and Kritchevsky [1976] noticed that during material grinding process fibre matrix may be destroyed. It leads then to change of fibre functional properties. Moreover, during grinding of seeds percentage of individual fractions in these seeds fibre is changing. Probably particles of smaller size contain more lignin fraction, and that is why they posses higher sorption abilities.

The adsorption of bile acids by legumes also depended on type and concentration of bile acids. Among all investigated acids lithocholic acid was bound better than cholic acid. It is connected with different number of hydroxyl groups that are contained in them - the more hydroxyl groups the smaller binding ability of bile acid [Amarowicz *et al.*, 1993; Camire *et al.*, 1993; Huang & Dural, 1995;

TABLE 1. Adsorption of bile acids by legumes depending on type of bile acid, its concentration and particle size [%].

Particle size [mm]	Type and bile acid concentration [mmol/L]	Pea	Soybean	Bean	Lentil	$X^{1)}$
1.00	LCA 1	29 ± 0.08^{b}	14 ± 0.09^{a}	15 ± 0.18^{a}	10 ± 0.03^{a}	17.00
	LCA 6	62 ± 0.15^{d}	49 ± 0.05^{c}	26 ± 0.03^{b}	16 ± 0.17^{a}	38.25
	CA 1	22 ± 0.05^{b}	11 ± 0.10^{a}	11 ± 0.02^{a}	7 ± 0.01^{a}	12.75
	CA 6	47 ± 0.13^{c}	36 ± 0.14^{b}	19 ± 0.06^{a}	12 ± 0.03^{a}	28.50
$X^{2)}$		40.0	27.5	17.8	11.3	
0.63	LCA 1	36 ± 0.05^{c}	26 ± 0.22^{b}	20 ± 0.04^{ab}	14 ± 0.14^{a}	24.00
	LCA 6	77 ± 0.06^{d}	58 ± 0.11^{c}	45 ± 0.10^{b}	28 ± 0.17^{a}	52.00
	CA 1	27 ± 0.14^{b}	19 ± 0.11^{ab}	15 ± 0.02^{a}	11 ± 0.01^{a}	18.00
	CA 6	58 ± 0.07^{d}	44 ± 0.15^{c}	34 ± 0.03^{b}	21 ± 0.03^{a}	39.25
$X^{2)}$		49.5	36.8	28.5	18.5	
0.20	LCA 1	69 ± 0.12^{c}	40 ± 0.18^{b}	28 ± 0.05^{a}	24 ± 0.20^{a}	40.25
	LCA 6	86 ± 0.05^{d}	70 ± 0.17^{c}	60 ± 0.05^{b}	40 ± 0.04^{a}	64.00
	CA 1	52 ± 0.08^{c}	30 ± 0.15^{b}	21 ± 0.03^{ab}	18 ± 0.01^{a}	30.25
	CA 6	65 ± 0.05^{d}	53 ± 0.04^{cb}	45 ± 0.02^{b}	30 ± 0.02^{a}	48.25
$X^{2)}$		68.0	48.3	38.5	28.0	

a, b, c, d – values in lines shown by different letters indicate statistically significant differences (α=0.05); $X^{1)}$ – mean value independent of variety of legumes; $X^{2)}$ – mean value independent of type and concentration of acid; LCA – lithocholic acid; CA – cholic acid.

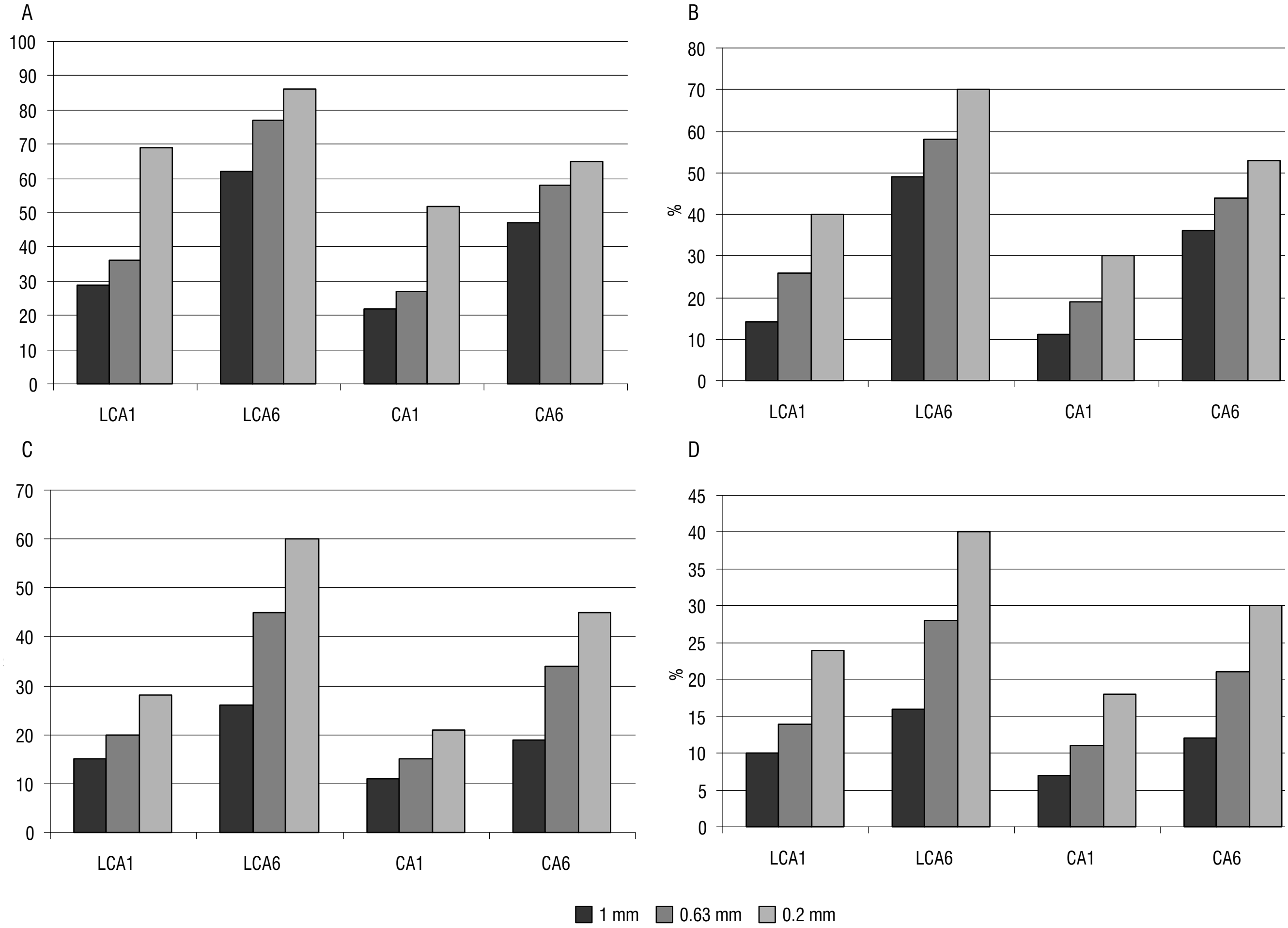

FIGURE 2. Bile acids adsorption by legumes depending on particle size: A – pea, B – soybean, C – bean, D – lentil.

Dongowski, 1997]. Cholic acid is tri-hydroxyl acid, while lithocholic acid is mono hydroxyl. That is why cholic acid sorption was considerably smaller than that of lithocholic acid. Moreover, cholic acid is a primary acid, while lithocholic acid is a secondary one formed during transformations of primary acids. Mutogenous properties are attributed to secondary acids; this is the reason why adsorption by dietary fibre seems to be especially important from the nutritional point of view [Nair, 1998].

Bile acids adsorption depended on their concentrations (1 mmol/L and 6 mmol/L). These concentrations are based on typical concentration in the human proximal and terminal ileums, ranging 1 mmol/L to 12 mmol/L [Huang & Dural, 1995]. Acid with higher concentration (6 mmol/L), independently on the kind of product and grinding degree was much better adsorbed. Legumes bound almost twice more lithocholic acid of 6 mmol/L concentration than of 1 mmol/L concentration.

Cholesterol adsorption by legumes was differentiated depending on the kind of sorption material and grinding degree (Table 2). Cholesterol was in the highest degree bound by bean (97.7%), less by lentil and pea, 78.3% and 62.7%, respectively, and in the lowest degree by soybean (56.7%). The cholesterol adsorption was in the following order: bean > lentils > pea > soybean. Many authors connect hypocholesterolemic effect with the content of different fibre fractions. This is attributed (among others) to β-glucans contained in oat products [Anderson *et al.*, 1994; Kahlon & Chow, 1997, Rieckhoff *et al.*, 1999]. Also

TABLE 2. Cholesterol adsorption by legumes depending on particle size [%].

Product	Particle size [mm]			X
	1.0	0.63	0.2	
Bean	96 ± 0.06^{a3}	98 ± 1.20^{a3}	99 ± 0.1^{a2}	97.67
Lentil	66 ± 0.8^{a2}	77 ± 1.13^{b2}	92 ± 0.69^{c2}	78.33
Pea	49 ± 0.43^{a1}	57 ± 0.85^{a1}	82 ± 0.39^{b1}	62.67
Soybean	45 ± 0.36^{a1}	50 ± 0.59^{a1}	75 ± 0.38^{b1}	56.67

a, b, c, d – values in lines shown by different letters indicate statistically significant differences (α=0.05); 1, 2, 3 – values in columns shown by different numbers indicate statistically significant differences (α=0.05); X – mean value independent of particle size.

polysaccharides contained in guaran gum or pectins decrease the cholesterol level in humans and animals [Anderson *et al.*, 1994; Terpstra *et al.*, 1998; Bartnikowska & Lange, 2000]. High cholesterol sorption degree by bean is probably connected with different fibre fraction composition of these seeds, especially with different proportion of hemicelluloses to lignin, ranging 1: 0.4 for bean, and 1: 2.5 and 1: 3 for pea and soybean, respectively [Górecka *et al.*, 1998]. Taking into consideration grinding degree, it may be observed, that the smallest particles (0.20 mm) were characterized with the highest ability of cholesterol binding, but in the case of bean grinding degree did not have any significant influence on the sorption value. Statistically significant relationship between the size of bean and pea particles (1 mm and 6 mm diameter, respectively) and cholesterol adsorption was not observed. However this kind of relationship was noticed for the most and the least ground pea, soybean and lentil seeds. Cholesterol adsorption by particles of these seeds of the highest grinding degree (0.20 mm) was higher by about 33%, 30% and 26%, respectively, in comparison with the least ground particles, like in the case of bile acids adsorption.

CONCLUSIONS

Legumes were characterized with differentiated bile acids and cholesterol adsorption ability. Pea bound bile acids in the highest degree; while lentil bound them in the lowest degree. Grinding degree influenced significantly bile acids sorption. The most ground particles were characterized with the highest sorption properties. Lithocholic acid with higher concentration appeared to be the most susceptible to binding, while cholic acid with lower concentration was the least susceptible. Cholesterol was bound by bean in the highest degree, by soybean in the lowest one. Decrease of particles size significantly influenced increasing of cholesterol binding. The results suggested that legumes may though be recommended in lipid disorders prophylactic. However their properties may differ depending on the variety of seeds, technological processing, for example grinding degree and dehulling.

REFERENCES

1. Amarowicz R., Okubo K., Kmita-Głażewska H., Adsorption of bile salts by buckwheat fibre. Die Nahrung, 1993, 1, 66–68.
2. Anderson J., Jones A.E., Riddel-Mason S., Ten different dietary fibres have significantly different effects on serum and liver lipids of cholesterol – fed rats. J. Nutr., 1994, 124, 1, 78–83.
3. Bartnikowska E., Lange E., Znaczenie dietetyczne przetworów owsianych i ich wpływ na stężenie cholesterolu w osoczu oraz poposiłkową glikemię. Żywność. Nauka. Technologia. Jakość, 2000, 1, 22, 18–36 (in Polish).
4. Camire M.E., Zhao J., Violette D.A., *In vitro* binding of bile acids by extruded potato peels. J. Agric. Food Chem., 1993, 41, 12, 2391–2394.
5. Dongowski G., Effect of pH on the *in vivo* interactions between bile acids and pectins. Lebensm. Unters. Forsch., 1997, 205, 5, 185–192.
6. Eastwood M., Dietary fibre and serum lipids. Lancet, 1969, 2, 1222.
7. Górecka D., Sperra L., Janitz W., Wpływ obróbki cieplnej na właściwości funkcjonalne błonnika pokarmowego w warunkach zbliżonych do środowiska przewodu pokarmowego. Roczniki AR w Poznaniu, Technol. Żywn., 1996, 282, 20, 35-42 (in Polish).
8. Górecka D., Staszak W., Gancarek A., Szeląg L., Charakterystyka błonnika pokarmowego nasion roślin strączkowych. XXIX Sesja Naukowa KTiCHŻ PAN, Olsztyn, 1998, 281–282 (in Polish).
9. Hallmans G., Zhang J.X., Lundin E., Landström M., Åman P., Adlercreutz H., Härkönen H., Bach Knudsen K.E., Influence of rye bran on the formation of bile acids and bioavailability of lignans. Cereal Foods World, 1997, 42, 8, 696–701.
10. Huang C.M., Dural N.H., Adsorption of bile acids on cereal type food fibres. J. Food Process Engin., 1995, 18, 243–266.
11. Johnson I.T., The biological effects of dietary fibre in the small intestine, 1990, *In*: Dietary Fiber: Chemistry and Biological Aspects, (ed. D.A.T. Southgate). Royal Society of Chemistry, Cambridge, pp. 151–161.
12. Kahlon T.S., Chow F.I., Hypocholesterolemic effects of oat, rice, and barley dietary fibers and fractions. Cereal Foods World, 1997, 42, 2, 86–92.
13. Kmita-Głażewska H., Kostyra H., Adsorpcja cholesterolu przez nierozpuszczalne frakcje błonnika pokarmowego kapust. XXX Sesja Naukowa KTiChŻ PAN, Kraków, 1999 (in Polish).
14. Korczak J., Górecka D., Janitz W., Bornikowska A., Fabrycka A., Właściwości fizyko-chemiczne błonnika pokarmowego w symulowanych warunkach przewodu pokarmowego. Roczniki AR Poznań, Technol. Żywn., 1995, 270, 19, 2, 51–59 (in Polish).
15. Lupton J.R., Yung K.Y., Interactive effects of oat bran and wheat bran on serum and liver lipids and colonic physiology. Cereal Foods World, 1991, 36, 827.
16. Mongeau R., Brassard R., Dietary fiber and fecal characteristics in rats: Effects of level and particle size of bran. J. Food Sci., 1985, 50, 654–656.
17. Nair P.P., Role of bile acids and neutral sterols in carcinogenesis. Am. J. Clin. Nutr., 1988, 48, 768.
18. Rieckhoff D., Trautwein E.A., Malkki Y., Erbersdobler H.F., Effects of different cereal fibres on cholesterol and bile acid metabolism in the Syrian Golden Hamster. Cereal Chem., 1999, 76, 5, 788–795.
19. Schneeman B.O., Dietary Fiber: Physical and chemical properties, methods of analysis and physiological effects. Food Techn., 1986, 40, 2, 104–110.
20. Story J.A., Kritchevsky D., Comparison of the binding of various bile acids and bile salts *in vitro* by several types of fiber. J. Nutr., 1976, 106, 9, 1291-1294.
21. Terpstra A.H.M., Lapre J.A., De Vries H.T., Beynen A.C., Dietary pectin with high viscosity lowers plasma and liver cholesterol concentration and plasma cholesterol ester transfer protein activity in hamsters. J. Nutr., 1998, 128, 11, 1944–1949.
22. Thebaudin J.Y., Lafabvre A.C., Harrington M., Bourgeois C.M., Dietary fibres: nutritional and technological interest. Trends in Food Sci. & Techn., 1997, 8, 2, 41–48.

* Paper presented on XXXIII Scientific Session of the Committe of Food Chemistry and Technology of Polish Academy of Sciences, 12–13 September 2002, Lublin, Poland.

Received June 2002. Revision received and accepted September 2002.

ADSORPCJA KWASÓW ŻÓŁCIOWYCH I CHOLESTEROLU PRZEZ SUCHE NASIONA ROŚLIN STRĄCZKOWYCH

Danuta Górecka, Józef Korczak, Ewa Flaczyk

Katedra Technologii Żywienia Człowieka, Akademia Rolnicza im. Augusta Cieszkowskiego, Poznań

Celem badań było określenie stopnia sorpcji przez nasiona roślin strączkowych wybranych kwasów żółciowych (kwasu litocholowego – LCA i cholowego – CA) o stężeniu 1 mmol/L i 6 mmol/L oraz cholesterolu. Wykazano, że stopień adsorpcji kwasów żółciowych zależy od rodzaju nasion (największymi zdolnościami sorpcyjnymi charakteryzował się groch, najmniejszymi zaś soczewica), stopnia rozdrobnienia (większe cząstki w mniejszym stopniu wiązały kwasy żółciowe) oraz rodzaju kwasu (w większym stopniu wiązany był kwas LCA o stężeniu 6 mM, niezależnie od rodzaju nasion i stopnia rozdrobnienia). Z kolei cholesterol w największym stopniu wiązany był przez fasolę, w najmniejszym zaś przez soję. Rozwinięcie powierzchni sorpcyjnej badanych nasion wpłynęło w istotny sposób na zwiększenie zdolności wiązania cholesterolu.