AN IN-VITRO EVALUATION FOR SOME NATURAL SEQUESTRANT RESOURCES

AHMED, A. I. S., SAHAR A. ARAFA AND DALIA M. EL-NAHAL

Special Food & Nutr. Dept., FTRI, ARC

(Manuscript received 27 July 2004)

Abstract

Since artificial sequestrants are administrated in chunk amounts and prescribed, in most cases, for long duration of time, an acute adverse effect may frequently be noticed. Taking the drug resin cholestyrmine as a 100 % binding bile acid, three groups of natural slowering for enterohepatic circulation exist. The strongest group, i.e. over 30 % the drug, has been shown to be black-eye pea, chickpea, dehulled lentil, kidney bean and mung bean. Soybean comes among some other beans as, or little less, the middle group of over than 25 to 30 % of the drug. Surprisingly, lupine, enzymatically processed, which was the weakest sequestrant, became one of the strongest when prepared at high pH. Hence, no remarkable changes have been obtained for chickpea and oat in that case, a noticeable drop has been seen for faba and mung beans. The degree of processing of proteolytic enzymes for soy extrudates has confirmed the major role of indigestible protein over the expected role of fiber type or content in manufactured drug from natural sources. In another words, as soy extrudate digests up to a limit of enzymatic action, the potential of indigestible in trapping the bile acid increases. This idea will be the main objective in designing new natural sequestrants. That topic will be investigated in near future. In this research, the more equipotent with less amount of prescribed drug, from naturals of valuable medicinal agent in certain forms, is being observed.

INTRODUCTION

Diet versus drug is incompletely understood, and the use of drugs to treat hyperlipemia should always be approached and conducted with caution, since this therapy can involve life-long administration of drugs whose long-term effects are poorly understood.

The common lipoproteinemia are 2_a, 2_b and 4 forms, in total of six categories, with lipid elevated TC, TG + TC and TG, respectively. These more incidence blood lipemia are due to high level in serum for LDL, VLDL + LDL and VLDL, respectively (Graig and Stitzed, 1982).

The drug clofibrate, for instance, treats the 3, 4 and 5 hyperlipoproteinemia. It's decrease mortality from coronary heart diseases (CHD) is still debated, but 25 –

35% nonfatal heart attacks over a 5 years period of treatment with 1.6 g/d was recorded (Graig and Stitzed, 1982). The adverse reaction includes mild nausea, diarrhea, and weight gain and gall bladder trouble owing to the ability of cholesterol concentrate in bile and coumarone anti-coagulants interaction (Graig and Stitzed, 1982).

Cholestyramin, another drug, is a resin exchanges CL^- for other anions. A gram of which binds 100-mg bile salts. Oral ingestion ranges from 100–200 mg/d to about 1 to 2 g/d (Graig and Stitzed, 1982). It is the choice to treat type 2 plasma lipoprotein. The adverse reaction may go from constipation, gall stones to mild steatorhea with the impairment of intestinal absorption of vitamin A, D and K. Moreover, it interacts many ionic drugs, e.g. digitoxin, wafarin, tetracycline, etc. Still however shown in very recent research studies that several health risk are associated with short or long runs of uncontrollable cholesterol (Ahmed *et al,* 2003).

On the other side, dietary therapy is effective in most cases with or without drug administration. A clear understanding of why the plant protein diet increases VLDL catabolism and production while leaving the pool size unaltered requires further investigation (Huff, 1984).

Technically, diet therapy may prove to be a kind of semimodified food or added bioingredient value, nutracutical or eventually may come to be of high rate to save real drug.

According to the above mentioned six types of hyperlipoproteinemia, complication of chronic therapy should be considered. Moreover, bile salt's sequestrant therapy may alter the absorption of nutrients and other drugs, e.g. folate and hypoprothrombinemia because of less absorbable vitamin K due to a drug such as cholestyramine, but bleeding as a complication is probably only significant when there is concurrent liver disease (Graig and Stitzed, 1982). In addition, this drug develops symptoms consistent with peptic ulcer diseases, vomiting, abdominal pain and the activation of duodenal ulcer.

The main target of this study was to determine the health potential of some foodstuffs, mainly beans, as natural sequestrant or more practically as source of saving cholesterol-lowering pharmaceuticals

MATERIALS AND METHODS

A simple chemical method was developed to measure the binding capacity of bile salts to some foodstuffs, mainly beans, found in local market). This *in-vitro* method is based on measuring the remaining light absorption at 302 nm of bile acid after incubation with samples at 37 °C/ 3 hrs and centrifugation. The following equation was used.

- 302 nm
- 50 µg bile salt

$$= 0.7 \text{ OD in water}$$

$$\text{Then, bile } \mu g/g = \frac{OD_{stock} - OD_{after\ incubation}}{0.7} \times \frac{50}{\text{sample wt in grams}}$$

Foodstuff samples were first dried and defatted then either digested with proteolytics according to Maliwal (1983) or alternatively incubated for the same both time and temperature (37 °C/ 3 hrs) with 0.1 *N* Na OH.

Soyprotein in defatted soy extrudate was periodically digested with a combination of proteolytic enzymes for 0, 10, 20, 40, 60, 80 and 120 min before discarding the supernatants. (Ahmed *et al,* 2003). The precipitates were lyophilized and treated for bile binding measurement as described above.

RESULTS AND DISCUSSION

Bile acid absorption was estimated at different wavelengths from 300 to 330 nm. The best absorption response with bile increment was executed at 302 nm (see Fig. 1).

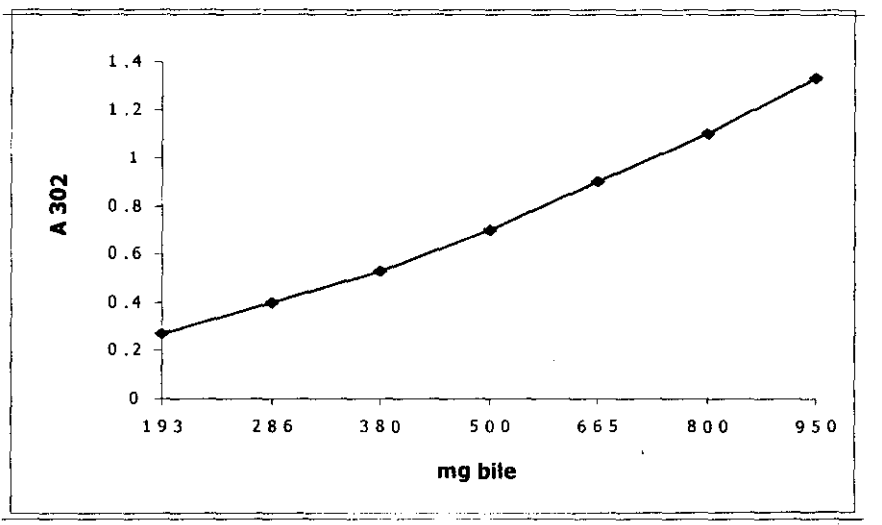

Fig 1 . Correlation between bile concentration and absorption at 302nm

Any reduction of absorption for the bile stock of 4 g/L after incubation was directly converted to bile absorption as mg/g sample using an 0.7 OD is equal to 50 mg bile equation.

Table 1. Some enzymatic processed natural sequestrants in comparison to a synthetic drug

Source	indigests mg bile/ g	Raw mg/g	Soybean %	Cholestyramin %	mmol/100g DM
Black-eye pea	37.26	29.82	129.0	32.25	3.48
Chickpea	36.78	29.33	126.9	31.75	3.43
De-hulled lentil	36.55	29.24	126.5	31.70	3.42
Kidney bean	36.15	28.91	125.1	31.25	3.38
Mung bean	33.34	26.67	115.4	28.85	3.12
Whole lentil	33.20	25.44	110.1	27.75	2.93
Faba bean	29.76	23.38	101.1	25.28	2.73
Soybean	28.83	23.11	100.0	24.60	2.70
Oat	26.78	21.42	92.3	23.10	2.49
Lupine	20.83	16.67	72.1	18.03	1.95

The natural sources for seqestrants were ranked according to its powerful properties as binding to bile salt as depicted in Table (1). Hence, three groups, i.e. over 30 %, between 25 to 30 % and less than 24 % of cholestyramine rate have been arranged. The strong natural sequestrants are about to be similar without an appearance remarkable range of binding affinities. The second group ranged between almost 29 to 25 % of cholestyramine. Finally, the weakest group among those sources undertaken may start with soybean of just less than 25 % to lupine of 18.03 % of drug power. It is obvious to remember that the only step of processing other than defatting was proteolytic action for three hours. That is why these groups most likely called the proteolytic indigest sequestrants. However, similar trend has been reported earlier (Kahlon and Qshao,2004). The ideas that these drugs act just near large intestine, more indigests as well as the less soluble matters are considered to be the main constituents to react with .

Table 2. Sequestrant parameters of some natural source insoluble at high pH.

Source	mg bile/g insoluble	mg/g raw	Soy bean %	Cholestyramine %	m mol/ 100Gdm
Chick pea	34.91	34.04	117.84	29.00	3.20
Lupine	25.40	23.83	85.72	21.10	2.32
Oat	24.44	23.80	82.52	20.30	2.20
Mung bean	11.11	10.84	37.50	9.23	1.01
Faba bean	9.53	9.30	32.15	7.91	0.87

The data tabulated in Table (2) may spotlight the variability of bile binding sequestrants, or in direct way foodstuffs, in more than one base. In another way, binding condition seems to be of special importance. In more details, proteolytic insoluble lupine is one of the weakest agent in this concern, it's insoluble in high pH extract became stronger than oat, mung bean and faba beans. As a matter of fact, the falling down of both mung and faba bean insoluble much more down their indigestible assuming the effect of condition on the real work of sequestrants. We all belief that substituting artificial drug with natural ones, even though they might be much more little efficient, is one of the modern biological theme. However, protein binds the widest range of legends in metabolic pathways in order to carry out the most important biological reactions. Most metabolic pathways are based on those specific properties. In our opinion, indigestible protein at large intestine acts more frequently than type or amount of fiber. The binding properties in the later case are less specific and weaker

Looking at Table (3), soybean was both extruded and proteolytically digested. The digestion time goes from zero to 120 min. The capabilities of binding bile acid have consistently improved up to level of digestion.

In brief, Table (3) and illustration in Fig (2) prove the importance of processing in facilitating the natural raw matter to act as enteroheptic circulation slowering agent. The protein, again, seems to play a major role. Protein design and protein engineering may become an important pharmaceutical system.

It is worth to notice that processing can move soy as sequestrant to group one whose effect as raw belongs to group three. A plan to produce this natural sequestrant is on progress and will publish soon.

Table 3. Effect of enzymatic processing on soy extrudate as sequestrant

Degree of soy extrudate hydrolysis	Extrudate mg/g	Soy %	Cholestyramin %	Mmol/ 100 DM
0	26.68	93.0	22.9	2.91
10	19.30	67,0	16.5	1.81
20	21.45	77.5	19.1	2.10
40	26.70	94.0	22.9	2.51
60	32.72	113.5	27.9	3.10
80	33.69	120.0	29.5	3.25
120	33.68	120.0	29.5	3.25

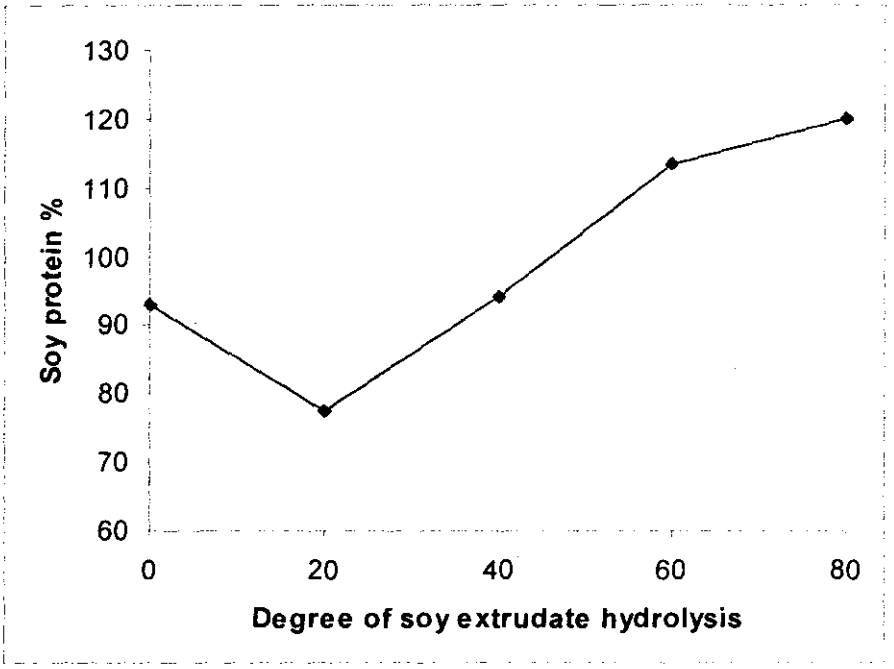

Fig 2. Soy protein extrudate correlated with enzymatic action as sequastrants

However, the overall data obtained here suggests that some beans, in a particular condition, may compensate the one third of favorable action of cholestyramine without any connection to the drug adverse actions. For example, Asian countries have a notably low risk of CHD, presumably due to lower intake of animal protein and higher intake of various beans. The 110 g/ week of Asian beans consumption to only 9 g for Americans is most probably the reason (Lucer *et al*, 2000)

More precisely, beans consumption four times or more per week compared with less than once a week have been associated with 22% lower risk of CHD (Bazzano *et al*, 2001). As clear, beans proteins or fiber either after digestion or in high pH, or both together may reach a comparable slowering action for enterohepatic circulation. This information stated the importance of incorporation of beans in dietary patterns, which should be encouraged. Soyprotein, for instance, has bean shown to significantly reduce hepatic cholesterol and increase bile acid excretion (Wright & Salter, 1998). That is why we considered these beans as reference for other beans.

In proposed mechanisms of action, bile acid binding by beans shows their health- promoting potential related to their phytochemical, e.g. flavonoid, tannins, estrogenic content or anionic, cationic, physical and chemical structure, composition of

metabolites, or their interaction with active binding sites (Kahlon and Qshao, 2004). Contrary, cholestyramine gives Cl, as mentioned before, to interact bile acids. In more details, guar gum inhances the cholesterol lowering value that appears to be mediated by an accelerated fecal excretion of sterols and a rise in the intestinal pool and biliary production of bile acid. Liver HMG CoA reductase and cholesterol 7á hydroxylase are induced in parallel but it is not sufficient to compensate for fecal steroid losses (Moundras *et al,* 1997)

In spite of the 11 fold increase caused by cholestyramine to that level of 5 or 4 times expected for the black-eye beans, the real endogenous action of beans, other than the only appeared exogenous action for the drug, could be the reason behind the proper action of beans against lipemia as all but not only the cholrsterol as in case of the cholestrolemia. The hypocholestrolemic action of drug leading to an up-regulation of hepatic bile acid synthesis is proposed too (Trautwein *et al,*1999). In contrast, the proposed mechanisms of action of sequestrates, e.g. soluble fiber, is by increasing expression of ilealapical sodium dependent bile acid transporter mRNA coordinately with does-responsive changes in bile acid metabolism (Buhman *et al,* 2000). As a matter of fact, different food constituents or sort of drugs have variable mechanisms of action are essentially based on more than one metabolic pathways (Xu *et al,* 2002).

In fact, changes in homeostatic metabolic situation can lead to severe abnormalities including uncontrollable cholesterol or general lipemia. This may refer to either hormonal imbalance or oxidative stress or both together. A theory about the hormonal and oxidative balance of diet was published (Ahmed *et al,* 2003). Again this deep endogenous metabolic action for drug drived from food may not available in the artificial one. However, a role of hormonal changes in the antilipogenic effect of oligofruetose included insulin, glucagon-like peptide, glucose dependent insulinotrophic polypeptide (GIP), IGF-1 as putative modulators of the hypolipidemic effectors is suggested (Kok *et al,* 1998). We believe that specially insulin and its antagonistic or promoter factors are playing a great role in this concern.

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تقييم مخفضات لمستوي الكوليستيرول فى الدم من مصادر طبيعية

على ابراهيم أحمد ، سحر أحمد عرفه ، داليا محمد النحال

قسم الاغذيه الخاصه و التغذيه – معهد بحوث تكنولوجيا الاغذيه - مركز البحوث الزراعيه- الجيزة

لما كانت المخفضات الدوائيه لمستوى كوليستيرول الدم و التى تعتمد على دفع حلقه أفراز الصفراء من الكبد وإبطاء أعاده أمتصاصها بالأمعاء لها الكثير من الاثار الجانبيه الضاره فلقد عنى الباحثون بالبحث عن وجود مصادر طبيعيه كبديل لمثل هذه الادويه مع معرفه أن لبعض البذور و الحبوب القدره على أداء هذا الدور الاكلينيكي. و لقد تم تقسيم مجموعه من عشر أنواع من الحبوب و البذور مقارنتا ببعض الادويه الشائعه و وجد أن أفضلها تبلغ فى قوتها أكثر من ٣٠ % من هذا الدواء شائع الاستخدام (وهو المصنع من مواد غير طبيعيه) مثل اللوبيا و الحمص او اقل من ٢٥% كالصويا و الترمس

ولقد أستخدمت طرق مختلفه لتجهيز هذه المصادر و ثبت أن دور البروتين الغير مهضوم فى معظم الاحيان أفضل من تأثير الالياف وحدها. كما ثبت ان طريقة الاعداد تحدد قوة المنتج حيث يتبادل الترمس المواقع مع الفول البلدى اذا استبدلت الانزيمات بالاستخلاص فى وسط قلوى

وسوف يتابع الباحثون البحث فى مصادر أخري و طرق أخرى لتجهيز هذه الادويه الطبيعيه حتى يتم تقليل مخاطر أستخدام الادويه المصنعه و التى تستخدم بكميات كبيره و لفترات طويله مما يؤدى الى ظهور هذه الاثار الجانبيه الضاره بالجسم.