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Relative Bile Acid Binding Potential of Extruded Lentil Snacks

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ABSTRACT

Previously we have reported that extrusion significantly improved healthful potential of cereals. It was hypothesized that snacks produced by extrusion would be more healthful than their raw formulations. Bile acid binding has been reported to indicate cholesterol lowering and cancer risk reduction potential of food and fractions. Bile acid binding potential of five lentil snack raw formulations and their extruded snacks were evaluated. The raw formulations were 100% lentils (F01), 69% lentils (F02), 57% lentils + 12% supplement (F03), F03 with 125 µg/100g Chromium (F04), F03 with 536 µg/100g Chromium (F05), and their respective extruded (E) snacks E01, E02, E03, E04 and E05. The *in vitro* bile acid binding on an equal dry matter basis relative to cholestyramine, was F01 (0.5%), E01 (3.7%), F02 (0.6%), E02 (3.0%), F03 (1.6%), E03 (5.1%), F04 (2.0%), E04 (4.2%), F05 (0.8%) and E05 (3.6%). Replacing 12% lentils with high protein supplements (F02 vs. F03) resulted in significantly higher bile acid binding, suggesting that the supplement appears to have higher bile acid binding capacity than that of lentils. All the extruded lentil snacks had significantly higher bile acid binding compared with their raw formulations. Extruding with added chromium containing yeast resulted in significantly lower bile acid binding in a dose dependent manner. Most healthful lentil snacks were produced with the addition of high protein supplement without added chromium-containing yeast (E03). Data proved the hypothesis that lentil snacks produced by extrusion are significantly more healthful than their raw formulations.

KEYWORDS

Lentils; Raw Formulations; Extruded Snacks; Bile Acid Binding

1. Introduction

Atherosclerosis and cancer are the two leading causes of death and disability in the developed world and are increasing rapidly in the developing world [1]. These are the major human nutrition problems and are preventable with diet and physically active lifestyle. The healthful, cholesterol-lowering (atherosclerosis amelioration) potential of food fractions could be predicted by evaluating their *in vitro* bile acid binding, based on positive correlations found between *in vitro* and *in vivo* studies showing that cholestyramine (bile acid-binding, cholesterol-lowering drug) binds bile acids and cellulose does not [2-5]. Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, bile acids are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation [6]. Bile acids are needed for fat absorption and high fat diets are implicated in obesity as well as in raising plasma cholesterol. In response to lowered bile acid levels liver uses cholesterol to synthesize additional bile acids. Excreting bile acids is the major route for removal of cholesterol from the body [7]. Binding of bile acids and increasing their fecal excretion have been hypothesized as a possible mechanism by which food fractions lower cholesterol [8-10]. Secondary bile acids are known to be carcinogenic [11].

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Cholesterol lowering and cancer risk reduction potential of food fractions could be evaluated by their bile acid binding capacity. *In vitro* bile acid binding without the use of labeled isotopes is an economical method for screening various foods and food fractions to evaluate their healthful potential before initiating time and cost intensive animal and human studies. Some international plant breeding companies have been using *in vitro* bile acid binding procedure [12] in their seed selections to propagate more health promoting crops. Extruded wheat bran and other foods have been shown to lower blood cholesterol in humans [13]. Extruded wheat bran at low specific mechanical energy (SME) of 120 - 221 Wh/kg, has been reported to significantly lower plasma and liver lipids in hamsters compared with unextruded wheat bran [14,15]. Previously it has been observed that *in vitro* bile acid binding of wheat bran extruded at 120 and 177 Wh/kg SME was significantly higher than un-extruded wheat bran [16]. It was hypothesized that snacks produced by extrusion would be more cholesterol-lowering compared with their raw formulations, as determined by their bile acid binding potential. In the study reported herein five lentil raw formulations and their extruded snacks were evaluated for their healthful potential by determining their *in vitro* bile acid binding relative to cholestyramine.

2. Methods and Materials

2.1. Bile Acid Binding Procedure

Kritchevsky and Story [17] have described *in vitro* bile acid binding procedure where labeled isotopes of bile acids were used. The *in vitro* bile acid binding procedure described here does not use labeled isotopes, thus eliminates the radiation hazards and disposal costs of the isotopes. The *in vitro* bile acid binding procedure for food fractions has been established by Kahlon and Chow [12] and further fine-tuned by Kahlon and Woodruff [18,19].

The stock bile acid mixture was formulated with glycocholic bile acids providing 75% and taurine-conjugated bile acids 25% of the bile acids based on the composition of the human bile [20,21]. This stock solution contained glycocholic acid (9 mmol/L), glycochenocholic acid (9 mmol/L), glycodeoxycholic acid (9 mmol/L), taurocholic acid (3 mmol/L), taurochenocholic acid (3 mmol/L) and taurodeoxycholic acid (3 mmol/L) in pH 6.3, 0.1 M phosphate buffer. A stock solution of 36 mmol/L was stored in a freezer maintained at −20˚C. Prior to each assay, working solution 0.72 or 2.88 µmol/mL was prepared from the stock solution, for test samples or cholestyramine, respectively. Cellulose, a non-bile acid binding fiber, was the negative control and cholestyramine, a bile acid binding anionic resin, was the positive control. Cholestyramine is a drug that lowers cholesterol by binding bile acids. Eight replicate incubations, consisting of six substrates with bile acid mixture, one substrate blank without bile acid mixture and one bile acid mixture without the substrate, were run for each of the ten treatments and two controls. The treatment replicates were weighed into 16 × 150 mm glass, screw-capped tubes. Diagram of the bile acid binding procedure is given in **Figure 1**. Each supernatant sample was analyzed in triplicate for unbound bile acids. Values were determined from a standard curve obtained by analyzing Trinity Biotech bile acid calibrators (No. 450-11) at 5, 25, 50, 100 and 200 µmol/L. Individual blank substrates were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank).

2.2. Lentil Snack Formulations

Lentil flour was purchased from a local wholesale distributor. Composition of lentil snack formulations is given in **Table 1**. The raw formulations and their extruded snacks were milled using 0.5 mm screen in a Cyclone mill (Udy Corp., Fort Collins, CO., USA), and stored in air-tight glass jars at room temperature for further analysis.

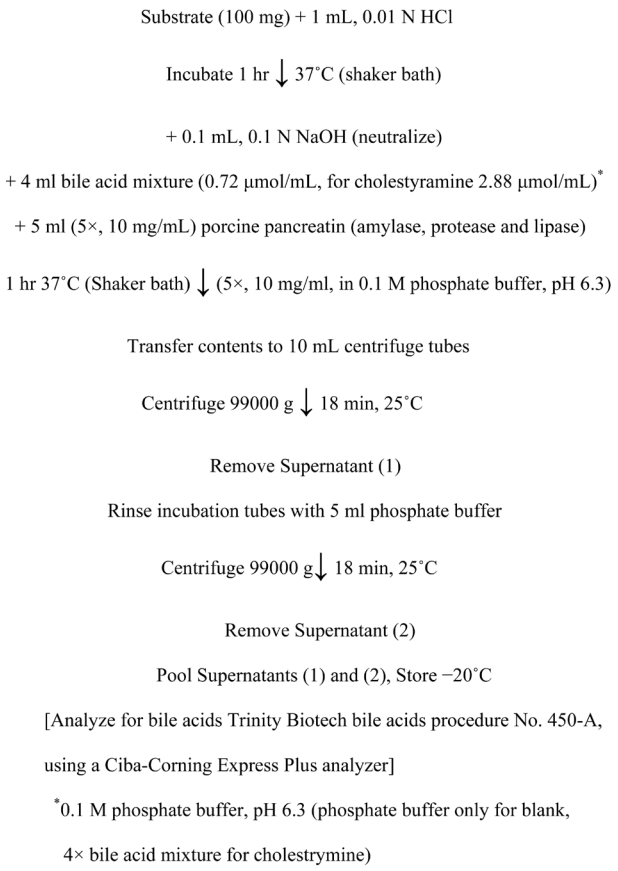

Figure 1. Diagram of the bile acid binding procedure.

Table 1. Composition of lentils snack raw formulations, % (as is)[a].

Ingredient	F01	F02	F03	F04	F05
Lentils	100.0	68.75	56.75	56.75	56.75
Apple pomace	0.0	2.5	2.5	2.5	2.5
Wheat bran	0.0	2.5	2.5	2.5	2.5
Hylon V	0.0	20.0	20.0	20.0	20.0
SaFlavor plus	0.0	0.0	12.0	12.0	12.0
Salt	0.0	1.25	1.25	1.25	1.25
Sugar	0.0	5.0	5.0	5.0	5.0
Cromium	0.0	0.0	0.0	125 µg	536 µg

[a]All values are g/100g, except for Cromium µg/100g. Hylon V, high amylase (55%) corn starch. SaFlavor plus, % as is: protein 51.29%, carbohydrate 41.31%, ash 2.81%; fat 1.06, moisture 3.53, with added yeast.

2.3. Extrusion Conditions

A twin-screw extruder with co-rotating, inter-meshing screws, six barrel sections (128 mm each), screw diameter 32 mm, L/D ratio 24 and 50 kg feed/h (Clextral EVOL HT32-H) was used. The last barrel section and die temperature was maintained at 160˚C ± 1˚C. Screws were driven by a 74.8 kW variable speed drive, Model ACS600 (ABB Automation Inc., New Berlin, WI, USA). The screw speed was maintained constant at 500 rpm. A combination of feeding, transporting, compression, and kneading elements was used to provide a moderate shear screw configuration. The mixture was metered into the feed port by a twin-screw, loss-in-weight gravimetric feeder, Model LWFD5-20 (K-Tron Corp., Pitman, NJ, USA) at a rate of 25 kg/h (wwb). Water was supplied to the extruder by a triplex variable stroke piston pump with 12 mm plungers, Type VE-P33 (Bran and Luebbe, Wheeling, IL, USA) to provide a final moisture content of 17%. The lentil snack formulations were extruded through two circular dies each with 3.5 mm diameter openings. Pressure at the die was determined using a pressure transducer, Type PT412-5M (Dynisco Instruments, Sharon, MA, USA). A PLC+ Industrial computer (Allen-Bradley, Milwaukee, WI, USA) using Intouch software FITSYS PLUS ver. 1.23 was used to collect extruder parameter data at 1 s intervals for a total of 5 min. Data were collected approximately 10 min after the operation conditions of torque and pressure were at steady state.

2.4. Statistical Analysis

Data are presented as means ± SEM. Before accepting analysis of variance results, Lavene's test was used to check for homogeneity of variances among treatments. Since variances were considered homogenous from test results, analysis of variance was used to test for significant differences among treatments. Dunnett's one-tailed test was computed with treatments compared to cholestyramine as a positive control and compared to cellulose as a negative control. For comparing non-control treatments to each other, Tukey's test for comparison of all possible pairs of means was used. A value of $P \leq 0.05$ was considered the criterion of significance.

3. Results and Discussion

Extruding lentil snack formulations resulted in significant reduction in fat content, non-significant reduction in protein content, protein in the extruded snacks had significantly higher digestibility (Table 2). Cholestyramine resulted in significantly higher bile acid binding and cellulose significantly lower values than raw and extruded snack formulations tested. Cholestyramine resulted in 98% bile acid binding. This is consistent with previously reported observations and same physiological conditions [12,18,19]. Extruded lentil snacks resulted in significantly higher bile acid binding (0.43 - 0.67 µmol/100g DM) compared with their raw formulations (0.15 - 0.32 µmol/100g DM) Figure 2. Data suggest that extrusion would improve cholesterol-lowering (healthful potential) of all the snack formulations tested.

The mean binding value of cellulose (negative control) was deducted from all treatments and the positive control values. Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding values on a dry matter basis for the raw formulations values ranged from 0.52% - 2.04%, and for extruded snacks values ranged from 3.02% - 5.1% (Figure 2). Bile acid binding values for F03 (57% lentils and 12% SaFlavor) and F04 (F03 + 125 ug cromium) were significantly higher than those for F01, F02 and F05. Data suggest that replacing high amylase corn with SaFlavor with or without low level of cromium resulted in significant increase in health promoting potential. Relative bile acid binding significantly improved with extrusion for all the formulations tested compared with raw formulations (3.02 - 5.10 vs. 0.52% - 2.04% of cholestyramine). The highest relative bile acid binding values (5.10%) were for extruded snacks formulation E03 (57% lentils and 12% Saflavor). A relative bile acid binding value of 5% - 9% on dry matter basis for oat bran, oat bran ready to eat cereals and barley fractions (cereals with US-FDA and UK-Joint Health Claim initiative) approved for a label health claim for lowering cholesterol have been reported [22,23]. Data suggest that extruded snack E03 has the potential to qualify for the label US-FDA and UK-Joint Health Claims. Bile acid binding values for E03 were significantly higher that all other extruded snacks tested. Data suggest that E03 snack had the most health promoting potential. Rel-

Table 2. Proximate analysis of raw formulation (F) and extruded (E) snacks, % as is.

Formulation	Moisture	Fat	Ash	Protein	Carbohydrate	IVPD[a]
F01	8.12	1.81	3.15	27.82	58.39	80.08
E01	9.59	0.78	3.23	25.14	60.53	82.13
F02	8.38	1.43	3.60	20.10	65.72	79.84
E02	9.16	0.27	3.49	17.96	68.20	87.74
F03	8.55	1.75	3.93	23.59	61.38	84.37
E03	8.36	0.46	3.80	20.30	66.51	88.65
F04	7.97	1.90	3.96	22.94	62.54	81.95
E04	8.74	0.50	3.87	20.18	65.74	87.26
F05	7.94	1.80	3.86	22.87	62.85	81.77
E05	9.30	0.41	3.80	20.74	64.79	88.77
F (mean)	**8.19**	**1.74[a]**	**3.70**	**23.46**	**62.18**	**81.60[b]**
E (mean)	**9.03**	**0.48[b]**	**3.64**	**20.86**	**65.15**	**86.91[a]**

[a]IVPD, *in vitro* protein digestibility (by Method Hsu *et al*. 1977). Values within a column with different superscript letter differ significantly ($P \leq 0.05$). n = 3, for mean F or E n = 15.

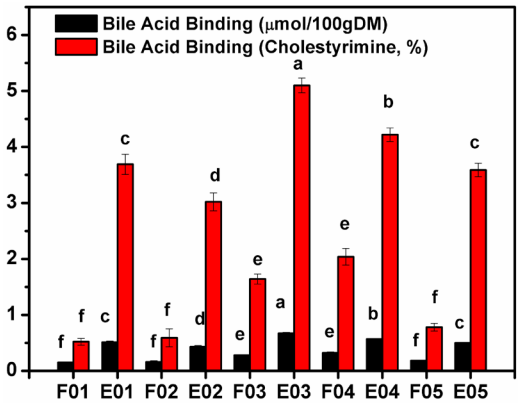

Figure 2. *In vitro* bile acid binding of lentil snack raw formulation (F01-05) and extruded formulations (E01-05); F01, 100% Lentils; E01, extruded F01, 128 WH/kg; F02, 69% Lentils, 0% SF; E02, extruded F02, 155 WH/kg; F03, 57% Lentils, 12% SF; E03, extruded F03, 145 WH/kg; F04, F03 + 125 µg Cr; E04, extruded F04, 140 WH/kg; F05, F03 + 536 µg Cr; E05, extruded F05, 140 WH/kg. Graph bars within µmol/100g DM or Cholestyramine %; that do not share a common superscript differ significantly ($P \leq 0.05$), n = 6. SF (Safalvor Plus), balance of the ingredients for each formulation are given in Table 1.

ative healthful potential of extruded lentil snacks were E03 > E04 > E01 = E05 > E02. Extruded lentil snacks with added chromium E04 (4.2%) and E05 (3.6%) with (125 and 536 µg chromium/100g, respectively) resulted in lower bile acid binding in a dose dependent manner. Significantly lower bile acid binding values were observed when 31% lentils were replaced with 20% high amylase corn and other ingredients (E01 vs. E02). These results suggest that added ingredients had lower healthful potential than lentils.

4. Conclusion

The study proved the hypothesis that lentil snacks produced by extrusion have higher cholesterol-lowering (health promoting) potential relative to their raw formulations. High protein Saflavor plus appeared to have higher health promoting potential than lentils. Adding cromium to E03 formulation resulted in lowered healthful potential in a dose dependent manner. The extruded formulation E03 was most desirable to promote human nutrition and health.

5. Future Considerations

Evaluating other pulses in addition to lentils in the extruded snack formulations would be warranted. Supplementing lentil and other legume snacks with additional levels of Safalvor plus or similar high protein supplements to obtain the maximum healthful effects needs to evaluated.

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