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# Cholesterol-lowering effect of whole lupin (*Lupinus albus*) seed and its protein isolate

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ABSTRACT

This study describes the hypocholesterolaemic effect of whole lupin and its protein in hamsters. The diets were: casein (control group HC), lupin protein isolate (group HPI) and whole lupin seed (group HWS). Diets from HPI and HWS promoted a significant reduction of total cholesterol and non-HDL cholesterol in the hamsters' plasma as compared with HC. The true digestibility of HPI and HC groups were similar and differed significantly from the HWS one, which in turn showed a significant difference in total sterol excretion as compared to the former groups. Histological analysis of the liver revealed that animals fed on HPI and HWS diets presented a low level of steatosis (level 1) as compared to the ones fed on HC diet (level 4). Our findings demonstrate that protein isolate from *Lupinus albus* from Brazil has a metabolic effect on endogenous cholesterol metabolism and a protector effect on development of hepatic steatosis.


## 1. Introduction

Currently, the implications of hypercholesterolaemia and cardiovascular disease such as atherosclerosis, are the main problems facing the public health system and deserve more attention as dietary intervention is a very important procedure for preventing and even controlling this disease (Johnson, Chua, Hall, & Baxter, 2006; Kerckhoffs, Brouns, Hornstra, & Mesink, 2002). An unbalanced diet can have an impact of over 30% on the development of cardiovascular diseases, 35% in the development of cancer and 50% in the development of obesity (Holm, 2003).

Epidemiological studies and *in vitro* and *in vivo* tests in animals and humans show that diets based on the consumption of vegetables can have a hypocholesterolaemic effect and reduce the risk of chronic diseases (Anderson & Major, 2002; Craig, 1997; Frota, Mendonça, Saldiva, Cruz, & Arêas, 2008; Jones, 2002; Macarulla et al., 2001), especially for cardiovascular diseases. This action is exerted by biologically active substances such as proteins, oils, dietary fibres, phytosterols and saponins (Duranti, 2006; Potter, 1995; Roy, Boye, & Simpson, 2010; Sirtori, Galli, Anderson, & Arnoldi, 2009).

Among the legumes, soya bean and its protein fractions 7S and 11S, are those that are most studied because of their effect in reducing total serum cholesterol and LDL-c through the modulation of genes related to the lipid metabolism (Anderson, Johnstone, & Cook-Newell, 1995; Fukui, Tachibana, Fukuda, Takamatsu, & Sugano, 2004; Reynolds et al., 2006). Other seeds such as peas, lentils, chick peas, beans and lupin are also being investigated due to their chemical composition and great potential in the prevention of lipid disorders (Duranti, 2006; Sirtori et al., 2004; Smith et al., 2006). However, no study has been undertaken to demonstrate the cholesterol-lowering effect of the whole lupin (*Lupinus albus*) and/or its protein isolate using hamsters, the best type of animal for use in experiments involving a lipid metabolism (Frota et al., 2008).

Among legumes, lupin is the one that has the highest protein content in its composition apart from being a good source of fibres (Martínez-Villaluenga, Frías, & Valverde, 2006), giving it great potential for consumption. The lupin protein has two major fractions, albumins and globulins in a ratio of 1:9, respectively. The globulin is characterised by two dominant classes, fractions 7S globulin and 11S globulin, followed by fractions of α and β conglutinins, respectively (Nadal, Canela, Katakis, & ÓSullivan, 2011), similar to the same fractions present in soybeans that exert physiological features.

The amount of lipids present in the grain varies between the species, from 6% to 15%, with a high concentration of polyunsaturated

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fatty acids (Beneytout, Desmaison, Najid, & Rigud, 1987; Musquiz, Burbano, Rey, & Cassinello, 1989). The fractions of soluble and insoluble fibre range from 30% to 40%, practically double that of soybean (21.7%), peas (18%) and faba beans (19%) (Van Barneveld, 1999).

Considering the potential cholesterol-lowering effect of lupin due to its constituents and the lack of tests using an experimental model of the lipid metabolism similar to that of humans, the objective of this work was to investigate whether the whole lupin and its protein isolate have an effect on the reduction of cholesterol in hypercholesterolaemic hamsters fed on a diet containing high levels of saturated fats and cholesterol and which possible mechanisms were involved in this process. Our group, using the same protocol, has already reported different mechanisms for these effects in the reduction of cholesterol and has been undertaking studies of bioavailability to show which fractions could be responsible for them.

## 2. Materials and methods

### 2.1. Materials

Mature seeds of a sweet variety of white lupin were obtained from IAPAR (Agronomic Institute of Paraná), Londrina, PR, Brazil. Alkaloids were removed by soaking the seeds in water at 50 °C, three times a day, for five days. Afterwards, the seeds were oven dried at 50 °C and pulverised in a hammer grinder with a 0.4 mm sieve (whole flour). The dried alkaloid free seeds were manually decorticated, pulverised in a hammer grinder with a 0.4 mm sieve, defatted with hexane (1:5 w/v) for 4 h, under constant shaking, and oven dried at 50 °C to remove the solvent residues. The resulting lupin seed flour (LSF) was finally homogenised, stored in polyethylene bags and frozen (−18 °C) until analysis.

### 2.2. Preparation of protein isolates

Lupin protein isolates (PI) were obtained from lupin seed flour (LSF) as described by Liadakis, Tzia, Oreopoulou, and Thomopoulos (1995), with modifications to the extraction step. LSF was extracted (in the proportion 2:40, w/v) with 0.5 M NaCl at pH 10.0. The suspension was stirred for 30 min at room temperature and then centrifuged for 30 min at 10,000*g*. The supernatant volume obtained was further subjected to ultrafiltration (5 kDa pore size) and the permeate was discarded to reduce the initial volume to a few millilitres. Next, the supernatant was adjusted with 0.1 M HCl, to pH 5.0, for the isoelectric precipitation (pI) of protein, and centrifuged at 10,000*g* for 30 min. The protein pellet was re-suspended in water, adjusted to pH 7.0 with 0.5 M NaOH, freeze-dried and homogenised. This procedure guaranteed that all the protein fractions present in the whole grain were also in the isolated protein, verified by SDS-PAGE, and with less damage possible to its structure, confirmed by DSC (Fontanari et al., 2011).

### 2.3. Lupin seed and protein isolate composition analysis

The official methods of AOAC (1995) were used for proximate analysis. Water and ash content were determined gravimetrically, total protein by means of the micro-Kjeldahl method (N × 6.25), fat by diethyl ether extraction in a Soxhlet apparatus, crude fibre by an enzymatic–gravimetric method (Prosky, Asp, Schweizer, De Vries, & Furda, 1988), and carbohydrates by difference calculation.

### 2.4. Animals, diets and feeding procedures

Four-week-old male Golden Syrian hamsters ($n = 32$) were purchased from the animal house of the School of Medicine, University of São Paulo, São Paulo, Brazil. They were housed individually in stainless steel mesh cages under controlled conditions: temperature 23 ± 1 °C; 12-h periods of darkness and light (lights on from 8:00 a.m. to 8:00 p.m.); as well as free access to water and food. Preliminary tests were performed to assess all the methodologies employed in the animal assay. Thus, we decided to increase the group size and to sacrifice some animals for the baseline of cholesterol and fractions. After 7 days of adaptation time to a commercial diet (Nuvilab CR1, Brazil), 4 animals were killed to determine the basal levels of blood lipids. The hamsters (84.3 ± 7.4 g) were fed for 3 week *ad libitum* on a diet rich in saturated fatty acids (13.5%) and cholesterol (0.1%), containing 20% casein, to induce hypercholesterolaemia. At the end of this period, 4 animals were killed to check whether hypercholesterolaemia had been achieved. The remaining ($n = 24$) were randomized and assigned to 1 of 3 groups receiving the following diets *ad libitum* for 4 week: the casein group ($n = 8$), which was kept on the hypercholesterolaemic casein diet (HC); the hypercholesterolaemic whole seed group (HWS) ($n = 8$), which received a diet rich in saturated fatty acids and cholesterol but containing whole lupin seed; and the hypercholesterolaemic protein isolate (HPI) group ($n = 8$), which received a diet rich in saturated fatty acids and cholesterol but containing the lupin protein isolate.

The diets were formulated based on seed and protein isolate composition analyses (Table 1), and were designed to be isocaloric and identical in composition (including dietary fibre content) except for the protein source. The compositions of the experimental diets (HC, HWS, and HPI) are shown in Table 2.

Food intake was monitored daily and body weight, weekly. The food efficiency ratio (FER) was calculated as the ratio between body weight gained during the 4 week of experimental diets and the amount of food consumed over the same period. All the experimental protocols and procedures were approved by the Research Ethics Committees of the School of Pharmaceutical Sciences from São Paulo State University (Research Protocol N° 16/2009) and the Institute of Tropical Medicine (Research Protocol N° 54/2009), within the University of São Paulo, where this trial was performed.

### 2.5. Sample collection

During the last week of the feeding period (5 consecutive days), faecal samples were collected from the hamsters, which were housed in wired-bottomed cages. The samples were then weighed, dried at 50 °C overnight, weighed again and ground into a fine powder. At the end of the study, the hamsters were subjected to overnight fasting (14 h) and then their blood was withdrawn by cardiac puncture under anaesthesia, using ketamine (85 mg $kg^{-1}$ of animal weight) and xylazine (8.3 mg $kg^{-1}$ of animal weight) and the animals sacrificed. The blood samples were collected into heparin-moistened syringes, and plasma was obtained after centrifugation at 1500*g* for 15 min. The liver was excised, weighed, and washed with cold saline solution (9 g NaCl $l^{-1}$), and was kept in buffered formol. The animals were sacrificed under anaesthesia by hypovolemia.

**Table 1**
Composition of *Lupinus albus* whole grain and it protein isolate (g/100g on dry basis).

| | Whole grain | Protein isolate |
|---|---|---|
| Moisture | 5.53 ± 0.10 | 8.41 ± 0.45 |
| Ash | 1.17 ± 0.03 | 1.91 ± 0.07 |
| Protein | 36.47 ± 0.2 | 92.41 ± 0.4 |
| Lipids | 12.37 + 0.19 | 0.18 ± 0.4 |
| Dietary fibre | 49.99 ± 0.1 | n.a. |

n.a. not analysed.