optimised processes for preparing healthy and added value food ingredients from lupin kernels, the European protein-rich grain legume

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FOREWORD

The existence of the Healthy-Profood project depends on some unpredictable coincidences. About 28 years ago within a few months, prof. Paolo Cerletti’s group published the first paper on white lupin and prof. Cesare R. Sirtori’s group the first paper on the hypocholesterolemic activity of soybean protein. Both were working in the University of Milan, the former in the Faculty of Agriculture and the latter in the Faculty of Pharmacy. Probably, if I had never happened to become professor of Functional Foods in the Faculty of Pharmacy, after having worked for 15 years in the Faculty of Agriculture, these two groups would have continued to work separately and we would continue to know nothing about the hypocholesterolemic and hypotensive activity of lupin protein.

C.R. Sirtori and I started to collaborate on soybean proteins in 1999 and immediately I realised that, being the structural similarities between soybean and lupin proteins so large, the hypothesis that also lupin may be hypocholesterolemic may be quite reasonable.

While we were discussing how to organise the experimentation, immediately it appeared that it was a very difficult task, because the commercial availability of lupin protein isolates necessary for the experimental studies was very scarce and the lupin food products necessary for the clinical studies were practically non-existent. We had, therefore, to build a well balanced consortium with different competencies and to find a proper grant. Since both were impossible inside our country, a European network and European funds were necessary. It took many months of continuous work to create an efficient network and about two years to get a grant within the Fifth Framework Programme of the European Union. Eventually the Healthy-Profood project (QLRT 2001-002235) started on January 1st 2003.

Reading all the manuscripts sent for this book, I am myself astonished at all the works we have done and how much exciting the results are.

Three years ago lupin protein isolates were very difficult to find, now there are two pilot processes that permit to produce some hundreds Kg of protein isolates or concentrates, which may be easily scaled up.
These materials have been thoroughly characterised and used as ingredients for preparing a great variety of innovative foods, such as lupin-enriched pasta, biscuits, muffins, beverages with different flavours, emulsified creams, imitation meat products etc. All these foods have been evaluated for their sensory characteristics, also involving a consumer federation belonging to the consortium. I have eaten some and I can assure that most of them are really good tasting.

But for sure, the most innovative results of the project are in the area of the health benefits. When we planned this project, only two papers dealing with the potential hypocholesterolemic properties of lupin had been published: one by Rahman and co-workers in 1996 and the other by Chango and co-workers in 1998. The former was on rats fed a cholesterol-free diet containing semi-purified protein fractions from *Lupinus angustifolius*, the latter was on rats fed a hypercholesterolemic Nath’s diet containing *Lupinus albus* meal.

Based on two well-established animal models (hypercholesterolemic rat and rabbit) and a clinical study with a lupin drink, the Healthy-Profood project has demonstrated that indeed lupin protein may be useful for controlling cholesterol and that the mechanism of action may be different from that of soybean proteins. In addition the clinical study gave a completely unexpected result, i.e. a clear indication of an anti-hypertensive activity. This is the first observation in vegetable proteins of such activity, which in the past had been reported only for milk peptides.

The safety issue was addressed very carefully and with different techniques, since there are several subjects allergic to other legumes, such as peanut and soybean, and there are a few scattered reports also on lupin. The main results indicated a possible cross-allergy mostly between peanut and a specific lupin fraction, i.e. $\gamma$-conglutin. With the agreement of the consortium, as coordinator of the project, I have insisted because the European Union decided to include lupin in the list of food allergens for labelling purpose. The Scientific Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority is currently working on this topic and its scientific opinion is expected for the first months of 2006. In the meantime, within the project, analytical methods to assure the traceability of lupin proteins have been developed.
This book contains also some external contributions, in particular a paper from UK dealing with an idiopathic anaphylaxis, which subsequently was attributed to lupin, and a paper from Australia dealing with the health benefits of lupin fibre.

At the end of this foreword, I want to thank all my co-workers who have worked indefatigably and with great competence for the preparation of this book, i.e. G. Boschin, A. D’Agostina, D. Locati, and D. Resta, and E. Citterio for the secretary work.
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THE HEALTHY-PROFOOD PROJECT: OPTIMISED PROCESSES FOR PREPARING HEALTHY AND ADDED VALUE FOOD INGREDIENTS FROM LUPIN KERNELS, THE EUROPEAN PROTEIN RICH LEGUME

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This three-year project was planned during 2001-2002 and finally financed by the European Union starting from 2003 January 1st. The consortium is made of 5 academic and 1 private research centres, 4 companies, the AEP - European Association of Grain Legume Research, and 4 consumer federations.

INTRODUCTION

The lupin seed is produced in pods which develop on the main stem of the lupin plant. Pods contain between three and seven seeds and these seeds vary in size, colour, appearance and composition according to the species of lupin. Three species are cultivated: white lupin, yellow lupin and narrow-leafed (or blue) lupin.

Seeds of white lupin are the largest, measuring 8-14 mm in diameter and with a 1000-grain weight of 300 g. They have a circular flattened shape and are cream in colour. Narrow-leafed lupins have round seeds which are more like those of soybeans. Yellow lupins have round, beige or brown speckled seeds, but these are smaller and lighter than those of blue lupins.
Species of wild and partially domesticated lupins were grown thousands of years ago both in the Mediterranean region (white lupin) and in the South American Andes before the Incan Empire. The cultivation of white lupin was well known to the ancient Greeks and Romans and its cultivation has been mentioned by early writers including the poet Virgil (70-19 BC) and Pliny the Elder (c. AD 23-79). Lupins were grown for a variety of uses including soil improvement, grazing, human consumption and medicinal purposes. However, the nutritious seeds contained bitter alkaloids, which were unpalatable and toxic, and these had to be removed by repeated boiling and soaking before they were consumed.

It was not until the twentieth century that the old bitter types of lupin were replaced by ‘sweet’ low-alkaloid types. Before this major development, bitter lupins spread in southern Europe and North Africa and were introduced in northern Europe when Frederick the Great of Prussia sent for lupin seeds from Italy in 1781 to improve the poor soils in north Germany. Yellow lupins were grown widely on the sandy acid soils of the Baltic Coastal Plain in the 1860s and also became important in the Saxony Merino wool industry until lupinosis poisoning caused sheep deaths.

The German scientist, Reinhold von Sengbusch (1898-1986), who had developed a simpler and quicker method of screening large plant populations for alkaloids, selected the first low-alkaloid cultivars of yellow lupin and narrow-leafed lupin from 1928. These were mutants of the bitter types, contained only 0.05% alkaloids, were palatable, safe to eat and had non-shattering pods and soft seeds. These were the requirements for a commercial crop and this period marked the beginning of breeding and improvement of lupins as crop plants.

Sweet varieties of narrow-leafed lupin were gradually introduced in Australia after World War 2nd. The crop became highly successful with exports to Europe and an area of 120,000 ha grown in Western Australia in the mid-1970s. By 1979 it was also widely grown in South Australia, Victoria and New South Wales.

Generally plant proteins are increasingly used as food ingredients because they improve nutritional profile, stabilise the texture and optimise recipe costs. Analyses of nutritional values of sweet lupin have shown, that the bio-availability of the constituents are comparable to those of processed soybeans [1]. In contrast to other
leguminous plants (peas, soy, beans), lupins contain extremely low amounts of trypsin inhibitors, lectins, isoflavones, saponins and cyanogens.

Lupins and lupin products have traditionally formed part of the human diet. The requirements with regard to chemical composition, nutritional value and product safety were laid down by the Advisory Committee on Novel Foods and Processes (ACNFP) in 1996 for certified lupins (sweet lupins) and on the strength of that these products were recommended as foodstuffs and food ingredients (e.g. lupin flours for baked goods).

Currently, there are only few companies in Europe that produce lupin protein ingredients for food use. The products available are toasted and non-toasted lupin flour, lupin grits, lupin granulates, lupin fibre and lupin protein concentrates from non-defatted seed [2]. Lupins and lupin products (flours) were considered to be traditional foods even before the introduction of the Novel Food Decree (1997). Food products available on the market are lupin snacks, lupin pasta, lupin bread and cookies, lupin coffee and some vegetarian instant meals [2].

In 2003 sweet lupin varieties from white, narrow-leafed, and yellow lupin have been cultivated in several areas of Australia (1,43 Mio. t/a), South America (54,450 t/a) and Europe (53,178 t/a) for feed and food applications [3]. According to FAO the main lupin cultivating countries in Europe are France (24,000 t/a), Italy (5,000 t/a) and Spain (9,800 t/a) [1]. In France, the lupin cultivation increased from 6,321 t/a in 1996 to 24,000 t/a in 2003, which demonstrates the growing interest for lupin as food and feed source. In Germany, not included in the FAO statistics, the cultivation area of lupins used for food and feed use in 2002 reached 40,000 ha (cf. France 13,600 ha) equal to an estimated lupin production of 100,000 t/a [2]. The amount of lupins used for food applications, which is not available in official statistics, is estimated to be around 5 to 10% of the total lupin production [2].

**OBJECTIVES OF THE HEALTHY-PROFOOD PROJECT**

In this scenery, the three main objectives of the project were:

1. To optimise economically competitive and environmentally sustainable processes for the preparation of food ingredients with optimal technological, sensory, and nutritional characteristics, based
on lupin kernels (*Lupinus albus* and *Lupinus luteus*) that may also be potentially beneficial for health.

2. To obtain protocols for the preparation of food items based on lupin proteins. The food items were mostly ready-to-serve foods (in particular vegetable drinks, biscuits, snacks, extruded products, creams) and were selected taking into account the requirements of the EU food industry for protein ingredients as well as trends of consumers for their sensory and nutritional characteristics and industrialists regarding novel food items.

3. To promote lupin based ingredients and food items in Europe through the involvement of consumer associations.

The food ingredients were expected to have the following features:

1. Optimal technological features to be exploited in different food items, such as solubility in water at different pH values, foaming activity and foam stability, emulsifying properties, interfacial properties, water and/or fat binding capacity, gelling properties, and a convenient shelf life.

2. Neutral taste, flavour, and colour in order to prepare food items with satisfactory sensory characteristics (having for example the neutral features of milk proteins as a model).

3. Good nutritional properties, relatively high sulphur and micronutrient contents, optimal digestibility (>85%), optimal bioavailability of essential amino acids (at least 80%) and very low allergenic potential, in comparison with other common food ingredients, such as milk, egg, soybean, and peanut proteins.

4. Potential health benefits, namely hypocholesterolemic properties (10% or more reduction of LDL-cholesterol in hypercholesterolemic individuals), suitability for dietary use in weight reducing regimens, in diabetes (10% or more reduction of glycemia, insulin resistance) and hypertension (4 mmHg or more systolic blood pressure reduction in hypertensives). Mild processes should allow products to retain their anti-oxidant properties in the experimental animals and in the clinic.

5. Reduced amounts of antinutritional or unpleasant components, all matters of concern to the consumer, such as hydrolase inhibitors,
lectins, phytates, alkaloids, oligosaccharides of the raffinose family, isoflavones, and saponines.

6. Traceability when these food ingredients are used in the preparation of food items.

The work packages of the project were the followings:

1. Process development for preparing food ingredients from lupin kernels.
2. Chemical and biochemical characterisation and traceability.
3. Preparation of model food based on lupin proteins.
4. Evaluation of the nutritional quality and allergenic potential.
5. Health benefits for the consumer.
6. Analysis of food market and consumer demands and evaluation of consumer acceptance of model foods.

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Why lupin and not any other seeds?

Lupin seed is very rich in protein (34–43% of dry matter), has an important percentage of oil (5.4–10.0%), and contains very little starch (0.7–2.2%). The percentage of crude fibre is high (14–16.5%). In addition the seeds of lupin species contain small amounts of other components, such as phytates, oligosaccharides and trypsin inhibitors. Traditionally these were known as antinutritional factors, but increasingly they are thought to be favourable nutritionally-active factors because of their potentially beneficial effects in pharmacological, medical, cosmetic and food applications. The high protein content and relatively high oil content of lupin seed, especially that of white lupin which is similar to soybeans, makes the lupin crop interesting for human consumption as well as for animal feed.

Health benefits for the consumers.

The investigation of the nutritional and nutraceutical properties of lupin proteins was one of the main targets of the project, with particular regards to cardiovascular disease prevention. Very exciting results have already been obtained by the Healthy-Profood Project in the area of possible nutraceutical applications. The experimental evaluation of the cholesterol lowering effect of white lupin proteins [4] was carried out on a well established animal model, i.e. rats consuming a casein diet with cholesterol-cholic acid. They were treated with a daily dose of 50 mg lupin protein per animal, which resulted in a relevant
reduction of total and low density lipoprotein cholesterol. This amount of protein is equivalent to 250 mg/kg, a dose frequently used for lipid lowering medications, for example fibrates. It is also important to underline that these animals showed a normal weight gain over the treatment period, which confirm the nutritional value of lupin protein.

A pilot clinical study based on a lupin drink confirmed the first experimental results and gave some indication of an anti-hypertensive effect. These very preliminary data need confirmation both at experimental level and in the clinic, however the structural similarity of lupin proteins and soybean protein, which has been approved by the US Food and Drug Administration as useful for reducing the risk of coronary disease [5], permits to be very optimistic that lupin may become a novel therapeutic tool for hypercholesterolemia. This may results, in the near future, in a growing interest for lupin production in Europe and in other countries, such as Australia.

Safety
This topic has been carefully examined in the project. Preliminary results of the investigation of the allergenic potential using suitable animal models indicate a very limited antigenic potential of specific protein fractions. Some cross-reactivity with other legume proteins (peanut and soybean) has been observed.

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