

The biochemistry and nutrition group: 30 years of research in a developing country

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SUMMARY. The most relevant results of 30 years of research from the Group of Biochemistry and Nutrition are presented. Research was focused mainly around the identification and detection of the heat-labile toxic factors present in legume seeds of human consumption, namely protease inhibitors and lectins with especial emphasis on their isolation, molecular characterization, mechanistic and nutritional relevance of both protein groups. The antinutritional effect of the polyphenols, thermolabile compounds present in colored seeds, has also been studied as well as the impact of seed complex carbohydrates on the digestive process.

RESUMEN. El grupo de bioquímica y Nutrición: 30 años de investigación en un país en desarrollo. Se presentaron los principales aportes del Grupo de Bioquímica y Nutrición en 30 años de investigación. Ésta se ha centrado en la identificación y detección de los factores tóxicos termolábiles presentes en las semillas de leguminosas de consumo humano, tales como los inhibidores de proteasas y lectinas, con énfasis en el aislamiento, caracterización molecular, mecanística y relevancia nutricional de ambos grupos de proteínas. También se han estudiado los polifenoles compuestos termolábiles presentes en las semillas coloreadas, como agentes antinutricionales y el impacto de los carbohidratos complejos de las semillas en el proceso digestivo.

INTRODUCTION

After having found that raw black beans were toxic, while the cooked ones constituted the basic diet of most of the underdeveloped peoples of Latin America. In the sixties our research concentrated mainly around the identification and detection of the heat labile toxic factors in legume seeds. They turned out to be the inhibitors of digestive enzymes and the lectins. These two groups of proteins, their isolation, molecular, mechanistic, and nutritional characterization occupied our research efforts during two more decades.

Technics used in early studies on the structure of the active sites of the Bowman-Birk inhibitor from soybeans and its interaction with trypsin and chymotrypsin in Dr. Irvin Liener's laboratory in the University of Minnesota, were applied by Dinah Seidl to the determination of the sites on the six isoinhibitors purified from black beans [1].

All were double-headed and interacted independently and simultaneously with trypsin and chymotrypsin, as shown by the electrophoretic migration pattern on cellulose acetate of

their binary and ternary complexes with the enzymes. Their differences in electrophoretic migration were justified by their aminoacid composition, which were found to be between 51 for inhibitor IV and 83 for inhibitor I [1].

During specificity screening of black bean extracts an «odd» protein molecule was detected which inhibited microbial enzymes, such as subtilisin and proteinase K, but did not interact with either animal digestive or plant thiol enzymes [2]. The unusual characteristic of this inhibitor was its specific recognition of human leucocyte elastase. The so called «subtilisin inhibitors» (SI) were later found and purified, in addition to black beans, from broad beans, chick peas and Jack beans, (Table 1). Their molecular masses were around 9 KD and their isoelectric values between pH 4 and 6.3.

SI reacted with subtilisin by the «standard mechanism» proposed by Laskowski Jr. for the interaction of the trypsin inhibitor with trypsin [4]. In fact, SI molecules having split active sites were detected, and resynthesis of the bond was achieved [3].

Structural differences among the SI were detected mainly

in their stability under denaturing conditions and in immunochemical assays. Here, antibodies raised against Jack bean (*Canavalia ensiformis*) SI, which interacted with SI from other species and varieties of the *Canavalia* genus, did not recognize the purified inhibitors from either black beans, chick peas or broad beans [3].

TABLE 1
SUBTILISIN INHIBITORS CONTENT OF VARIOUS
LEGUME SEEDS

Species	Subtilisin Inhibitors (mg/100 g dry seeds)
Black beans (<i>P. vulgaris</i>)	7
Chick peas (<i>C. arietinum</i>)	16
Jack beans (<i>C. ensiformis</i>)	21
Broad beans (<i>V. faba</i>)	28

Modified from reference 3

The nutritional implications of the bean trypsin inhibitors were focused on by studying the varying degrees of sensibility of the digestive enzymes of several animal species including man, toward these molecules, which are not necessarily inactivated during cooking.

Original methods to detect inhibitors in single seeds [5], and others to determine toxicity in microquantities were developed as part of our research [6].

Studying lectins in 1968 we demonstrated for the first time the leucoagglutinating and mitogenic properties of Concanavalin A [7]. This was followed by the observation of the toxic effect of this lectin on cultured cat kidney cells. This was one of the first reports of lectin toxicity on cultured cells [8].

At the same time we were interested in the inheritance of the factor or factors responsible for the haemagglutinating activity of *Phaseolus vulgaris* seeds. It was found that the genetic analysis pointed to a single dominant trait of inheritance of the phytohaemagglutinin (PHA) [9].

Jaffé, Brücher and Palozzo studying numerous genetically pure cultivars of *Phaseolus vulgaris* found that they could be classified into 4 groups according to their differences in haemagglutinating specificity, oral and intraperitoneal toxicity and mitogenicity. The simple haemagglutination test could then be used to predict toxicity in edible beans [10].

The lectins of each group were studied *in extenso* with Pedro Bonay. They were shown to be isolectins with similar but not identical biological properties [11,12]. Isolectins from the latex of *Hura crepitans* were also characterized and, interestingly, they are different from the one present in the seeds of the same plant [13].

The effect of PHA on the absorption and transport of glucose in the small intestine was also studied. The lectin from a toxic variety reduced both absorption and transport of

glucose [14]. Glucose metabolism as judged by lactate formation was not affected (Table 2). In other experiments, the inhibition of intestinal maltase and glycoamylase with a decrease of glucose absorption, was also demonstrated, Figure 1 [15].

TABLE 2
EFFECT OF PHA ON INTESTINAL GLUCOSE
TRANSACTIONS

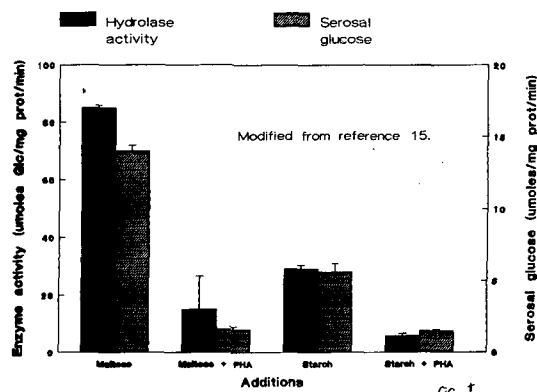
Lectin* µg/ml	Glucose		Lactate Production
	Absorption	Transport	
0	26.4 ± 2.2	2.9 ± 0.2 ^a	13.3 ± 0.5 ^a
50	19.3 ± 2.2 ^a	1.4 ± 0.2 ^a	12.9 ± 0.5 ^a
100	16.1 ± 1.6 ^a	0.8 ± 0.2 ^{ab}	12.5 ± 0.7 ^b
200	15.2 ± 1.5 ^{ab}	0.7 ± 0.2 ^a	12.1 ± 0.6 ^b

* Pre-incubation time: 15 minutes

Means sharing the same superscript are not statistically different (p<0.05)

Modified from reference 14

FIGURE 1
Effect of PHA on brush border maltase and glycoamylase



The effect of some plant polysaccharides on lectin activity was investigated [16]. Arabic gum enhances lectin haemagglutinating activity. The more glycosylated the lectin, the greater the stimulatory effect of the gum. The unglycosylated PHA retains the same agglutinating activity as the native, glycosylated lectin. However, the gum has no effect on the modified lectin, suggesting that the interaction between gum and lectin is of a carbohydrate-carbohydrate nature.

The activity of pancreatic α -amylase is inhibited by PHA. No such effect was observed with the unglycosylated lectin, suggesting again the importance of the sugar moiety in the biological properties of lectins [17].

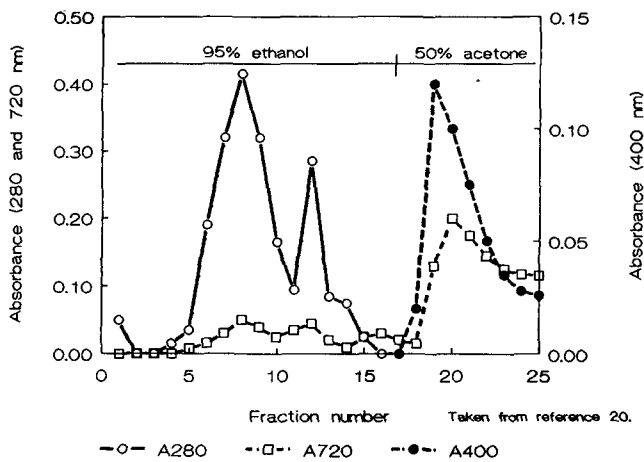
In addition, although heat treatment (90 min, 96 °C) inactivates PHA hemagglutinating activity, a remaining anti amylolytic effect is still observed (Figure 3). This finding stresses the importance of thermoresistance of some legume antinutritional factors.

In 1973 Jaffé reported that the digestibility of colored cooked beans was lower than that of white seeds, and suggested that this difference could be attributed to the larger polyphenol content of the red and black beans. Later on, works from the Instituto Nacional de Nutrición de Venezuela, revealed that black bean broth impaired the weight gain of animals fed casein [19].

At that time Andrés Carmona joined our Research Group and started a new line aimed to characterize the black bean polyphenols, selecting the best conditions to extract, purify and estimate them. The antinutritional effect of purified bean tannins was also evaluated.

The first outcome was the clear separation of bean tannins by adsorption chromatography resulting in an elution pattern similar to that reported for sorghum grain, Figure 2 [20]. The in vitro inhibitory α -amylase activity of the condensed tannins was not eliminated by autoclaving, however it was abolished by the polyphenol complexing agent polyvinyl polypyrrolidone [20].

FIGURE 2
Adsorption chromatography of black
bean polyphenols

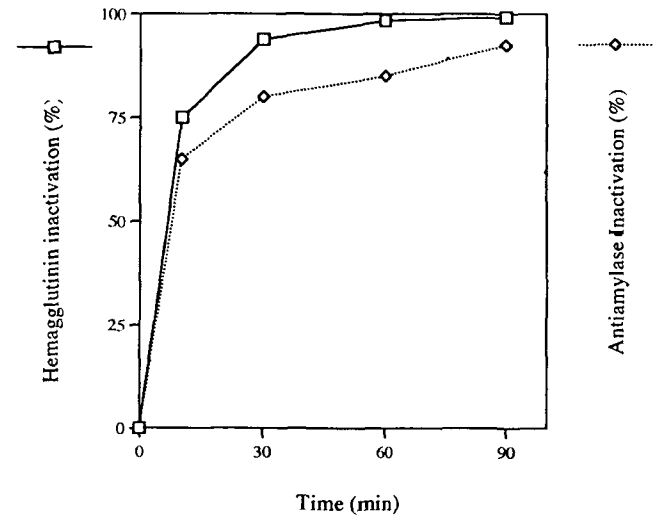


A great deal of work has been put by our Research Group into the determination of the specificity and selectivity of the most commonly used spectrophotometric assay procedures for tannin estimation. Those based on reductive power (Prussian Blue and Folin-Denis) did not distinguish between tannins and non-tannin polyphenols. This could be accomplished by using the vanillin reaction if corrected by subtracting the colour contributed by the seed extracts [21,22].

Recently some of the shortcomings of the traditional vanillin reagent, such as the interference from non-tannin

polyphenols present in the raw bean extracts, have been overcome by using acetic acid as solvent. This procedure increases sensitivity and decreases background noise [23].

FIGURE 3
Effect of heat treatment on hemagglutinating and
antiamylase activities of PHA



Modified from (17)

The incorporation of Gina Borges in 1991 allowed us to explore in detail the interaction of tannins with several components of the digestive machinery, both in vivo and in vitro. Apparently, the inhibition of pancreatic and brush border carbohydrate hydrolases is stronger than that of glucose absorption.

From the pioneering work of David Jenkins it is known that legume seeds elicit a low glycemic response in humans [24]. This prompted us to go into a new research field, now headed by Juscelino Tovar.

It was demonstrated that in legume seeds the indigestible residue represents a larger fraction than that chemically estimated. Therefore, enzymatic methods allow for a more precise analytical calculation of the fiber content [25]. These observations must be taken into account when the physiological implications of legume intake are to be considered.

Lately it was found that the indigestible residue of black beans (*P. vulgaris*) inhibits the in vitro activity of trypsin and pancreatic α -amylase [26]. The starch limited hydrolysis could account in part for the low glycemic response.

It is also of interest that thermic processing of pulses produces indigestible fractions in greater proportion than in other amylaceous food stuffs [17]. These changes decrease polysaccharide bioavailability.

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