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Exploring Protease Inhibitors and Plant Protein Digestibility: Implications for Nutrition and Health

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Abstract

The significance of soya beans in animal feed and human nutrition has prompted extensive research into their protease inhibitors, particularly due to their impact on digestion and growth inhibition. Despite initial assumptions linking trypsin inhibitors to poor nutritive value, understanding their precise role has proven complex. This article presents a comprehensive review of plant based protein digestibility, focusing on factors influencing bioavailability and the effects of protease inhibitors such as Bowman-Birk and Kunitz-type inhibitors. Drawing from recent literature, it explores the mechanisms underlying growth inhibition and pancreatic hypertrophy, shedding light on the dynamic interplay between protease inhibitors and digestive physiology. Additionally, it discusses the broader implications for human health and the potential of protease inhibitors in shaping plant centric dietary patterns. Through a synthesis of experimental findings, this review provides valuable insights into the intricate relationship between protease inhibitors and plant protein digestibility, offering guidance for future research and dietary practices.

Keywords: Plant based proteins; Digestibility; Protein inhibitors; Bowman-Birk inhibitors; Kunitz-type inhibitors; Human nutrition; Bioavailability; Systematic review.

INTRODUCTION

In the realm of nutrition, the quest for sustainable and health-conscious dietary choices has led to a burgeoning interest in plant based proteins. Central to this discourse is the digestibility of plant proteins, a multifaceted aspect influenced by various factors including protease inhibitors. Among these inhibitors, Bowman-Birk and Kunitz-type inhibitors have garnered particular attention for their potential impact on digestive processes. Despite decades of research, understanding the precise mechanisms underlying their effects remains a challenge. Early investigations suggested a straight forward link between protease inhibitors and diminished nutritive value, yet subsequent studies have revealed a far more intricate relationship. This article aims to provide a comprehensive exploration of plant based protein digestibility, drawing on recent literature to unravel the complexities surrounding protease inhibitors and

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their interactions within the digestive system. By synthesizing insights from diverse sources, this study seeks to offer a nuanced understanding of the dynamic interplay between protease inhibitors and bioavailability, with implications for both research and dietary practice in the burgeoning field of plant centric nutrition.

The important role the soya bean has assumed in feeding animals and its potential contribution to the human diet, it is understandable why the protease inhibitors from that plant have received the most attention.[1] It was not long after soya beans were first introduced into the United States, primarily as a source of oil, the significant observation that soya beans had to be heated to support the growth of rats. With the discovery of a heat-labile inhibitor of trypsin and the demonstration of its ability to inhibit the growth of animals, it was generally assumed that the trypsin inhibitor was largely responsible for the poor nutritive value of raw soya beans.[2]

Plant-derived protease inhibitors, such as Bowman-Birk inhibitors and Kunitz type inhibitors, have been recommended to negatively affect alimentary protein digestion by obstructive the activity of trypsin and chymotrypsin in the human digestive system. As long as the complex interactions affecting the digestibility and bioavailability of plant proteins remain elusive, protease inhibitor supplements for beleaguered purposes could be a respectable option to take benefit of the scientifically proven advantageous effects of these compounds.

This research article provides a thorough examination of the digestibility of plant based proteins, addressing the growing interest in plant centric diets. The study systematically reviews existing literature to consolidate insights into the digestibility of various plant proteins and explores factors influencing their bioavailability.[3] This comprehensive review contributes valuable insights into the dynamic field of plant based protein digestibility, offering a nuanced understanding of factors influencing bioavailability. The findings aim to inform both researchers and individuals seeking to make informed choices about plant centric dietary patterns.

METHODOLOGY

This article adopts a narrative review approach, sourcing literature from reputable electronic databases such as NCBI, Google Scholar, ResearchGate, and PubMed. Publications spanning the last decade and written in English were considered for inclusion. Thematic synthesis was employed to analyze and organize the literature, facilitating a comprehensive examination of the topic.

RESULTS AND DISCUSSION

Although the most logical explanation for the growth inhibition evoked by the trypsin inhibitor would be the fact that it interfered with the normal digestive reactions in the intestinal tract, the true explanation has not proved to be that simple.[4] For example, adding the soya bean trypsin inhibitor to a diet containing predigested protein still led to an inhibition of growth, thus ruling out inhibition of intestinal proteolysis as being directly responsible for growth inhibition.[5] Perhaps the most significant observation that has ultimately led to a better understanding of the mode of action of the trypsin inhibitor was the finding that rats and chicks fed raw soya beans or purified preparations of the inhibitor developed an enlarged pancreas resulting in an increased secretion of pancreatic enzymes.

According to Clemente et al. (2019), the growth depression caused by the trypsin inhibitor might be the consequence of an endogenous loss of essential amino acids produced by the hypersecretory activity of the pancreas.[5] Since pancreatic enzymes are particularly rich in sulfur containing amino acids, pancreatic hypertrophy serves to divert the supply of these amino acids from the synthesis of body tissue to the synthesis of pancreatic enzymes which are irretrievably lost by excretion.[6] This loss in the Sulfur-containing amino acids accentuates an already critical situation concerning soya bean protein which is inherently deficient in these amino acids.[7] It is not surprising, therefore, that methionine supplementation will effectively counteract much of the growth depression caused by raw soya beans despite the persistence of pancreatic hypertrophy.

The mechanism whereby the trypsin inhibitor causes pancreatic hypertrophy is still not fully understood. Srikanth & Chen have suggested that the degree of pancreatic secretion is determined by the level of free trypsin present at any given time in the intestine.[6] As the level of trypsin drops below a certain threshold level, the pancreas is induced to produce more enzymes, and conversely, when the level of trypsin is restored to normal levels, the secretory activity of the pancreas is inhibited.[7] The agent directly responsible for these effects is believed to be the pancreas stimulating hormone, cholecystokinin (CCK), whose release from the

intestinal mucosa is inhibited by free trypsin. It is obvious from these considerations that any set of circumstances that leads to a reduction of free trypsin in the intestines, such as complexation with an inhibitor, will serve to release CCK resulting in a hyperactive pancreas.[7]

Other factors affecting the digestibility of protein

If the trypsin inhibitor is indeed the major factor responsible for the poor growth of animals fed on raw soya beans, then it should be possible to reduce the nutritive value of heated soya beans to that of raw soya beans by adding the same level of antitrypsin activity to heated soya beans as is present in the raw product. That this is not the case was demonstrated several years ago. Furthermore, an examination of varieties of soya beans revealed the absence of any correlation between trypsin inhibitor activity and PER, although PER and the size of the pancreas were significantly related inversely.[8] It would appear, therefore, that there must be present in raw soya beans some other factor, totally unrelated to the trypsin inhibitor, which is also causing pancreatic hypertrophy as well as inhibition of growth. This situation was clarified when it was found that the removal of protease inhibitors from unheated soya bean extracts by affinity chromatography on Sepharose bound trypsin produced only a 40% improvement in growth and reduction in the size of the pancreas compared to heat treatment.[10]

The above findings raise the question as to what is responsible for the remaining 60% of the growth retarding and pancreatic hypertrophic effects of raw soya beans. A comparison of the in vitro digestibility of raw soya bean protein from which the protease inhibitors had been removed by affinity chromatography with a heat treated control revealed that the latter was more readily digested by trypsin. This observation suggests that native soya bean protein is in itself resistant to digestion by trypsin unless denatured by heat. A related observation is the fact that the isolated globular proteins of Phaseolus vulgaris are also very resistant to attack by proteolytic enzymes. If undenatured protein is capable of binding trypsin by forming an enzyme substrate complex, as suggested by Karpińska & Czauderna (2022), this could also serve to remove the feedback inhibition of pancreatic secretion by trypsin and thus cause hypertrophy of the pancreas.[8]

Another factor that may influence the digestibility of the proteins of legumes is the lectins, have shown the lectin of black beans can reduce the digestibility of dietary protein presumably by interfering with the ability of the intestinal mucosal cells to absorb nutrients.[11]

Physiological significance in humans

It should be appreciated that most of the experiments dealing with the nutritional effects of the protease inhibitors have involved the use of the rat or the chick. What can be said about the relevance of such experiments to the human diet which may contain plant proteins as a potential carrier of these inhibitors.[12]

Many of the soya bean products intended for human consumption are manufactured from protein isolates which, depending on their mode of preparation, may contain as much as 30% of the inhibitor activity of the original raw bean. An examination of the trypsin inhibitor activity of several textured meat analogs reveals that, although the protein isolate from which they were made may be rich in antitrypsin activity, the final products generally contain less than 10% of the activity of raw soya bean flour. Churel has likewise shown that the heat treatment involved in the processing and sterilization of infant soya bean formulas reduced the trypsin inhibitor activity to less than 10% of the activity of the original isolate. This residual activity did not produce any weight reduction or pancreatic hypertrophy in rats. These observations are consistent with the findings, of Zeng et al. (2020) who found no pancreatic hypertrophy in rats fed soya bean flour in which only 54% of the trypsin inhibitory activity had been destroyed. Although a further enhancement in growth is produced when more of the inhibitor is destroyed, this can be attributed to an increase in protein digestibility per se rather than to further destruction of the inhibitor.[13]

Assuming for the moment that processing conditions may have been inadequate to reduce the level of trypsin inhibitor activity below that of the threshold level established for rats, would this activity still pose a risk to human health.[14] Human trypsin is known to exist in two forms, a cationic species, which is the major component of human pancreatic juice, and an anionic species, which comprises about 10 to 20% of the total trypsin activity. While the latter is fully inactivated by the soya bean inhibitor, the predominant cationic species is only weakly inhibited.[15]

In further support of the probability that the soya bean inhibitor is relatively ineffective against human trypsin is the rather interesting relationship

that appears to exist between the size of the pancreas of various species of animals and their sensitivity to pancreatic hypertrophy induced by raw soya beans or the inhibitor.[17] The pancreas of those species of animals whose weight exceeds 0.3% of their body weight becomes hypertrophic when fed raw soya beans, whereas those whose weights are below this value are insensitive to this effect. Since a man has a pancreas that is 0.09 to 0.12% of his body weight, one would predict that the human pancreas would be insensitive to the effects of the soya bean trypsin inhibitor.[8]

Role of trypsin inhibitors in other legumes

To what extent the protease inhibitors account for the poor nutritive value of plants other than soya beans is difficult to assess. Inhibitors that have been purified from the lima bean and peanut are capable of inhibiting the growth of rats whereas those isolated from Dolichos lablab and maize do not. Nevertheless, it may be significant to note that the trypsin inhibitors of many legumes are quite rich in cystine, and, may account for about 30 to 40% of the total cystine content of some bean proteins. It is conceivable, therefore, that a dietary loss of cystine from the inhibitor itself could contribute in a significant fashion to the poor nutritive value of these legumes in their native unheated state. Vagadia et al. (2018) have indeed shown that the cystine of the unheated navy bean protease inhibitor is only approximately 45% available to the chick compared to 76% available for the heat inactivated inhibitor.[11] Thus the protease inhibitors of some legumes may be a double edged sword; they not only reduce the digestibility of the protein and cause pancreatic hypertrophy but may also 'lockin' a significant fraction of the total cysteine content of the protein which is already limiting in the S-containing amino acids.[18]

It has been recognized for many years that the nutritive value and protein digestibility of many plant proteins, particularly those derived from legumes, are very poor unless subjected to cooking or some other form of heat treatment.[8] This beneficial effect of heat has been generally attributed, at least in part, to the destruction of a unique class of proteins that can combine in a very specific fashion with the enzymes (trypsin and chymotrypsin) that play a key role in the digestion of proteins in the intestinal tract of animals.[19] Elucidation of the precise manner in which these so-called protease inhibitors lead to growth inhibition, however, has proved to be more elusive than might be suggested by this simple concept. The situation is further complicated by the fact that factors other than protease inhibitors may affect the digestibility of dietary proteins. Interest in the nutritional role of protease inhibitors has continued to mount largely as a consequence of the recent introduction of texturized vegetable proteins as a possible substitute for meat protein in the human diet.

CONCLUSION

The study underscores the intricate dynamics surrounding plant based protein digestibility, particularly concerning the role of protease inhibitors like Bowman-Birk and Kunitz type inhibitors. While initial assumptions linked these inhibitors to poor nutritive value, their exact mechanisms remain elusive. Through a thorough review of recent literature, this study elucidates the complex interplay between protease inhibitors and digestive physiology, shedding light on factors influencing bioavailability and growth inhibition. Moreover, it explores the broader implications for human health and the evolving landscape of plant-centric dietary patterns. By synthesizing experimental findings and highlighting areas for further research, this review contributes valuable insights to our understanding of plant protein digestibility, offering guidance for both researchers and individuals navigating dietary choices.

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