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Research Paper

Protease inhibitors in groundnut varieties (Botanical Name: *Arachis hypogaea)*

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Abstract

The project is focused on studying "Protease Inhibitors in Groundnut Varieties (Botanical Name: *Arachis hypogaea)".* The present study is important because groundnut is called as the poor man's cashew nut. In developing country like India, due to marked geographical variation, it is important to consider local food preferences, availability and affordability by the people. Groundnut is rich in oil, protein and has high energy value. However, it contains protease inhibitor that limits the use of groundnut meal. Protease and Protease inhibitor interaction studies in variety of groundnut will reveal the groundnut variety having maximum nutritional value and minimum anti-nutritional component.

Keywords: Antinutritional component; Groundnut; Protease and Protease inhibitors.

Introduction

Protease is the digestive enzyme needed to digest protein.

FOOD GROUP	% OF DAILY DIET	DIGESTED BY
Protein	20-25 %	Protease
Carbohydrates	50-60 %	Amylase
Fat	20-30 %	Lipase

Every animal, including humans, must have an adequate source of protein in order to grow or maintain itself. Proteins, which yield amino acids, are the fundamental structural element of every cell in the body. Specific proteins are now recognized as the functional elements in certain specialized cells, glandular secretions, enzymes and hormones. Thus, protein well deserves its name, which is of Greek derivation, meaning "of first importance." Proteins, which are among the most complex organic compounds found in nature, are made up of nitrogen containing compounds known as amino acids. During the digestion of proteins, hydrochloric acid and proteolytic enzymes break down the intact protein molecule into amino acids which are then absorbed through the intestinal wall. If proteins are not properly broken down before they are absorbed, various health consequences may occur.

Classification

Standard Proteases are currently classified into six broad groups:

a. Serine proteases
b. Threonine proteases
c. Cysteine proteases
d. Aspartate proteases

e. Metalloproteases
f. Glutamic acid proteases

By optimal pH: Alternatively, proteases may be classified by the optimal pH in which they are active:

Acid proteases

Neutral proteases involved in type 1 hypersensitivity. Here, it is released by mast cells and causes activation of complement and kinins. This group includes the calpains.

Basic proteases (or alkaline proteases)

Trypsin and chymotrypsin, serine protease digestive enzymes Trypsin and chymotrypsin, like most proleotytic enzymes, are synthesized as inactive zymogen precursors (trypsinogen and chymotrypsinogen) to prevent unwanted destruction of cellular proteins, and to regulate when and where enzyme activity occurs. The inactive zymogens are secreted into the duodenum, where they travel the small and large intestines prior to excretion. Zymogens also enter the bloodstream, where they can be detected in serum prior to excretion in urine. Zymogens are converted to the mature, active enzyme by proteolysis to split off a pro-peptide, either in a sub cellular compartment or in an extracellular space where they are required for digestion.
Trypsin and chymotrypsin are structurally very similar, although they recognize different substrates. Trypsin acts on lysine and arginine residues, while chymotrypsin acts on large hydrophobic residues such as tryptophan, tyrosine and phenylalanine, both with extraordinary catalytic efficiency. Both enzymes have a catalytic triad of serine, histidine and aspartate within the S1 binding pocket; although the hydrophobic nature of this pocket varies between the two, as do other structural interactions beyond the S1 pocket.

The human pancreas secretes three isoforms of trypsinogen: cationic (trypsinogen-1), anionic (trypsinogen-2) and mesotrypsinogen (trypsinogen-3). Cationic and anionic trypsins are the major isoforms responsible for digestive protein degradation, occurring in a ratio of 2:1, while mesotrypsinogen accounts for less than 5% of pancreatic secretions. Mesotrypsin is a specialised protease known for its resistance to trypsin inhibitors. It is thought to play a special role in the degradation of trypsin inhibitors, possibly to aid in the digestion of inhibitor-rich foods such as soybeans and lima beans. An alternatively spliced mesotrypsinogen in which the signal peptide is replaced with a different exon 1 is expressed in the human brain; the function of this brain trypsinogen is unknown.

There are two isoforms of pancreatic chymotrypsin, A and B, which are known to cleave proteins selectively at specific peptide bonds formed by the hydrophobic residues tryptophan, phenylalanine and tyrosine.

The Importance of Proteases

Proteases refer to a group of enzymes whose catalytic function is to hydrolyze (breakdown) proteins. They are also called proteolytic enzymes or proteinases.
Proteolytic enzymes are very important in digestion as they breakdown the the peptide bonds in the protein foods to liberate the amino acids needed by the body. Additionally, proteolytic enzymes have been used for a long time in various forms of therapy. Their use in medicine is notable based on several clinical studies indicating their benefits in oncology, inflammatory conditions, blood rheology control, and immune regulation.

Protease is able to hydrolyze almost all proteins as long as they are not components of living cells. Normal living cells are protected against lysis by the inhibitor mechanism.

Parasites, fungal forms, and bacteria are protein. Viruses are cell parasites consisting of nucleic acids covered by a protein film. Enzymes can break down undigested protein, cellular debris, and toxins in the blood, sparing the immune system this task. The immune system can then concentrate its full action on the bacterial or parasitic invasion.

Deficiency of Protease

Acidity is created through the digestion of protein. Therefore a protease deficiency results in an alkaline excess in the blood. This alkaline environment can cause anxiety and insomnia. In addition, since protein is required to carry protein-bound calcium in the blood, a protease deficiency lays the foundation for arthritis, osteoporosis and other calcium-deficient diseases. Because protein is converted to glucose upon demand, inadequate protein digestion leads to hypoglycemia, resulting in moodiness, mood swings and irritability. Protease also has an ability to digest unwanted debris in the blood including certain bacteria and viruses. Therefore, protease deficient people are immune compromised, making them susceptible to bacterial, viral and yeast infections and a general decrease in immunity.

Plant protease inhibitors are ubiquitous in plant kingdom. Most leguminous species contain high amounts of inhibitors in varying concentrations, ranging from zero to 20% by weight in their mature seeds, suggesting that they are not metabolically relevant (Fernanades et al, 1991). The molecular weights of plant protease inhibitors are mainly in the range of 3000 to 25000 Da. They have been most extensively studied in Leguminaceae, Graminae and Solanaceae families presumably because of the large number of species in these families, which are important food crops (Richardson, 1991).

Proteinaceous protease inhibitors (PIs) are generally categorized according to the class of proteases they inhibit. Four types of protease inhibitors have been identified as serine, cysteine, aspartic and metallo-protease inhibitor based on the type of protease they inhibit. Some plant serine protease inhibitors are bi-functional, typically possessing trypsin and chymotrypsin or trypsin or chymotrypsin and amylase activities; other PIs exist as multi-domain proteins in which each domain possesses functional PI activity (Koiwa et al., 1997).

Anti-nutritional and other factors in groundnut

Lusas (1979) has briefly reviewed various anti-nutritional factors present in groundnut. Trypsin inhibitor activity in groundnuts has been reported as one half to approximately one fifth of the activity found in soybeans. Trypsin inhibitor activity in groundnut is significantly enough to cause pancreatic hypertrophy in rats receiving 15 percent of protein intake from groundnuts. Lectins shown to possess a remarkable array of biological activities have been found in groundnuts. An interesting aspect of the lectins in groundnut is that, roasting of groundnut does not destroy the lectins. Oil seed proteins constitute the most highly allergenic food groups. Groundnuts also have been shown to be highly allergic and shown some hypersensitivity reactions in children. Groundnut oil has been attributed to contain potent anti-inflammatory compounds, however, Calloway, et al. (1971) observed that groundnuts are absolutely non-flatulent. Later on varietal differences were noticed for the differences exist in groundnut in their ability to cause flatulence. In this connection, the consumption of new raw groundnuts is not advisable; groundnuts always should be consumed fried, boiled or roasted. Plant breeding programmes should also focus on developing varieties of groundnut with minimal content of allergenic proteins and antinutritional factors.

Materials and Methods

The experimental material comprised of twenty different varieties of groundnut belonging to species *Arachis hypogaea.* The seeds were obtained from National research centre for Groundnut, Junagarh, Gujarat. These groundnut varieties were subjected for studying protease, protease inhibitors and their protein content.

Dry seeds were used to study protein content in groundnuts. Protein and protease inhibitors were extracted by defatting the seeds and then used for activity detection using spot test, detection of protease inhibitors isoform i.e. separation using electrophoresis (gel x-ray film contact print method) and for interaction studies.

Protein Studies

Preparation of the sample

Twenty different samples were prepared by making use of the seeds obtained from National Research Centre for Groundnut, Junagarh, Gujarat. 10 to 15 seeds of each variety were decorticated and then ground using pestle and mortar. The powdered seeds were then given washes of Chloroform: Methanol (3:1) and hexane so as to remove the fat from them. The samples were air dried and then used for protein extraction.

Extraction of protein and protease inhibitor from seeds

100mg of each sample (as prepared above) was taken in 1.5 ml micro centrifuge tube.600ul of distill water containing 1% PVP was added to the same tube. *For all the groundnut varieties 1:6 (w/v) dilution was done (100mg of defatted powder in 600µl distilled water containing 1% PVP)* The diluted sample in 1% PVP was then kept overnight at 4℃ followed by centrifugation at 6℃, 10,0000 RPM, 15mins the next day. The clear supernatant that was obtained was then transferred into the fresh micro centrifuge tubes and then as a source of protein and protease inhibitors.

Following were the groundnut samples that were used as protein and protease inhibitor sources: **Accession number**6255,145, SB-X1, 3672,Kudiri-3,T-28,4738,JL 24,GG 4,2458,ICGV 86590,12053,RG-141,9877,2460,ICGS-1,J-11,GG-2,M 13,MH-1

Dot Blot Assay / Spot Test

Procedure

Spot test was carried out in order to check out the inhibition activity present in all the different groundnut varieties. Spot test is the preliminary test.

Spot test for Non-Heated samples using Trypsin/Chymotrypsin

The strip of an (undeveloped) X-ray film (gelatin coated on X-ray film was used as a substrate for trypsin, chymotrypsin) was used for activity detection of sample extracted from groundnut varieties. 20 µl of proteases i.e. trypsin and chymotrypsin and 20 µl of distilled water (control) were loaded on to X-ray film strip and was left undisturbed for twenty minutes at room temperature and the strip was then washed with running tap water so as to observe digestion of gelatin over X ray film. The presence of trypsin and chymotrypsin will lead to the digestion of gelatin coated on X-ray film and will lead to the development of transparent blue color and the spot containing distilled water will not lead to any change.

Small size blue colored undeveloped x-ray film *(*gelatin coated on X-ray film was used as an substrate for trypsin, chymotrypsin) was taken and a drop of 20ul was added of each i.e. Distill water, Trypsin and Trypsin : Trypsin inhibitor in varying ratio of (1:1, 1:3, 3:1). These spots were allowed to remain on to the film for 20 minutes after which they were washed off with running tap water and the changes in the strip were observed. 20 ul of trypsin and 20 ul of distilled water were used as control. Sample containing protease inhibitors lead to the digestion of gelatin coated X-ray film.

For ***chymotrypsin*** also the procedure remains the same. Small size blue colored undeveloped x-ray film(gelatin coated on X-ray film was used as an substrate for *trypsin, chymotrypsin*)was taken and a drop of 20ul was added of each i.e. Distill water, Chymotrypsin and chymotrypsin: chymotrypsin inhibitor in varying ratio of (1:1, 1:3, 3:1). These spots were allowed to remain on to the film for 20 minutes after which they were washed off with running tap water and the changes in the strip were observed. 20 *ul* of chymotrypsin and 20 *ul* of distilled water were used as control. Sample containing protease inhibitors lead to the digestion of gelatin coated X-ray film.

Protein Estimation

Protein in the extracts was estimated using Folin-Lowry's method with bovine serum albumin as

standard (500ug/ml). All the observations were taken using UV-VIS spectrophotometer.

Procedure

0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard solution were pipette out into series of test tubes in duplicates and 10 microlitre of sample extract were pipette out in test tubes in duplicates. These volumes were adjusted to 1ml using distilled water, followed by addition of 5ml of solution C in all the tubes and mixed well and were incubated at room temperature for 10 minutes. 0.5ml of Folin-Ciocalteu reagent was added in all the tubes and mixed well followed by incubation at room temperature in dark for 30mins. The optical density was measured at 660 nm on a double beam spectrophotometer. The protein contents in the experimental samples were determined by comparing their optical densities with those on the standard curve.

Solution Assays

Protease inhibitor activity was determined by using three different synthetic substrates Azocasein (for determining total protease inhibitor activity), benzoyl 1-arginyl 1-p-nitro-anilide (BApNA) (for determining trypsin inhibitor activity) (Erlanger et al.,1961) and n-glutaryl 1-phenylalanine p-nitroanilide (GLUPHEPHA) (for determining chymotrypsin inhibitor activity) (Muller and Weder, 1989).

Suitability Assay for AZOCASEIN as a Substrate for Trypsin

(Brock, R.M. Forsberg, C.W. and Buchanan-Smith, J.G. 1982. Proteolytic activity of rumen microorganisms and effect of proteinase inhibitors. Applied Envionmental Microbiology. 44: 561-569)

Trypsin
Azocasein + H2O -------> Colored Reaction Products

Conditions: T = 37°C, pH 7.8, A450nm
Method: UV-VIS spectrophotometer
Procedure:
Note: For all the above mentioned assay it was the reaction mixture that was prepared first and incubated at room temperature for 30 minutes.
With the help of micropipette the following reagents were added into 2ml micro centrifuge tubes:
Duplicates of each tube were prepared.

Test Blank:
Azocasein 400ul
Buffer 160ul

Protease:
Trypsin 80ul
Buffer 80ul
Azocasein 400ul
Duplicates of both the tubes were mixed by swirling and then equilibrated to 37°C for 15 minutes.

Reaction Mixture:
Trypsin 240ul
Extracted sample 240ul

The reaction mixture was co-incubated for 30 minutes.
160ul of the above co-incubated reaction mixture and 400ul of azocasein was then placed in the 2ml micro centrifuge tubes. Duplex were prepared for the same and then equilibrated to 37°C for 15 minutes.

Reaction termination and Reading

The reaction was terminated exactly after 15 minutes with 30% acetic acid followed by tubes

centrifugation at 10,000 RPM for 10 minutes. Supernatant was then taken into fresh eppendorf tubes and about 500ul of 1N NaOH was added and O.D. was then taken at 450nm.
Note: For heated samples the procedure remained the same only the samples were heated at 82C for 20 minutes in water-bath.

Suitability Assay for Bapna as a Substrate for Trypsin Erlanger et al., 1964).

N-a-benzoyl-DL-arginine-p-nitroanilide (BAPNA) is a simple colourless ester which is recognised by trypsin as a substrate (trypsin is an **esterase** as well as a protease).

Conditions: T = 37°C, pH 7.8, A410nm
Method: UV-VIS spectrophotometer
Procedure:
With the help of micropipette the following reagents were added into 2ml micro centrifuge tubes:
Duplicates of each tube were prepared.

Test Blank:
Bapna 560ul
Buffer 400ul

Protease:
Trypsin 80ul
Buffer 480ul
Bapna 400ul
Duplicates of both the tubes were mixed by swirling and then equilibrated to room temperature for 15 minutes.

Reaction Mixture:
Trypsin 240ul
Extracted sample 240ul
The reaction mixture was co-incubated for 30 minutes.
160ul of the above co-incubated reaction mixture and 400ul of bapna along with 400ul of Tris-Cl buffer was then placed in the 2ml micro centrifuge tubes. Duplex were prepared for the same and then equilibrated to room temperature for 15 minutes.

Reaction termination and Reading

The reaction was terminated exactly after 15 minutes with 30% acetic acid and O.D. was then taken at 410nm.
Note: For heated samples the procedure remained the same only the samples were heated at 82C for 20 minutes in water-bath.

Suitability Assay for Gluphepa as a Substrate for Chymotrypsin (Muller and Weder, 1989)

Method: UV-VIS spectrophotometer
Procedure:
With the help of micropipette the following reagents were added into 2ml micro centrifuge tubes:
Duplicates of each tube were prepared.

Test Blank:
Gluphepa 400ul
Buffer 560ul

Protease:
Chymotrypsin 80ul
Buffer 480ul
Gluphepa 400ul
Duplicates of both the tubes were mixed by swirling and then equilibrated to room temperature for 1 hour.

Reaction Mixture:
Chymotrypsin 240ul
Extracted sample 240ul
The reaction mixture was co-incubated for 30 minutes.
160ul of the above co-incubated reaction mixture and 400ul of Tris-Cl buffer and 400ul of Gluphepa was then placed in the 2ml micro centrifuge tubes. Duplex were prepared for the same and then equilibrated at room temperature for 1 hour.

Reaction termination and Reading

The reaction was terminated exactly after 1hour with 30% acetic acid i.e. 200ul.Tubes were then centrifuged at 10,000 RPM for 10 minutes. Supernatant was then taken into fresh eppendorf tubes and O.D. was then taken at 405nm.

Note: For heated samples the procedure remained the same only the samples were heated at 82^{0}C for 20 minutes in water-bath and were then used for assay.

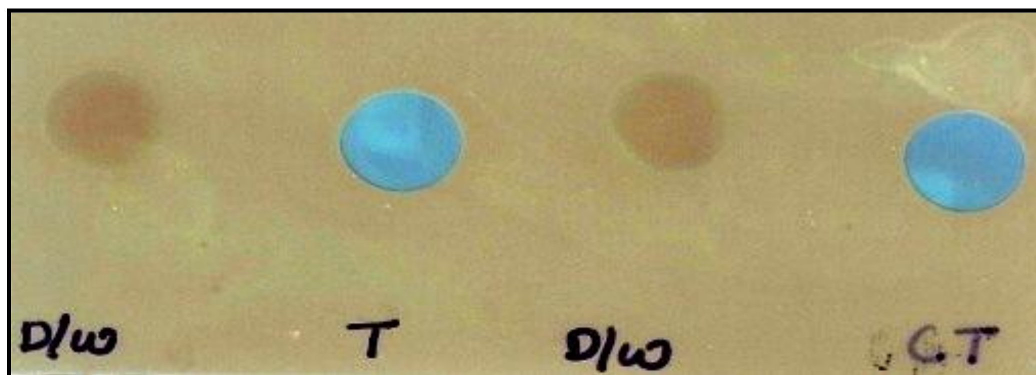

Figure 1: spot test of protease with different groundnut variety on x-ray film

Samples Accession no.	**20 MINUTES INCUBATION (Non Heated Samples)** **With trypsin and chymotrypsin**
6255	Non heated sample with trypsin Non heated sample with chymotrypsin

Figure 2: Control

Table 1: Inhibition Activity for Nonheated samples

Accession no.		1:1	1:3	3:1
6255	For trypsin	+++	+++	+++
	For chymotrypsin	++	+	++
145	For trypsin	+++	+++	+++
	For chymotrypsin	++	+	++
SB-X1	For trypsin	+++	+++	+++
	For chymotrypsin	++	+	+++
3672	For trypsin	+++	+++	+++
	For chymotrypsin	+	+	++
Kudiri-3	For trypsin	+++	+++	+++
	For chymotrypsin	+	+	++
T-28	For trypsin	+++	+++	+++
	For chymotrypsin	++	++	++
4738	For trypsin	+++	+++	+++
	For chymotrypsin	++	+	++
JL 24	For trypsin	+++	+++	+++
	For chymotrypsin	+	+	+
GG 4	For trypsin	+++	+++	+++
	For chymotrypsin	++	+	++
2458	For trypsin	+++	+++	+++
	For chymotrypsin	++	+	++
ICGV 86590	For trypsin	++	+	++
	For chymotrypsin	+++	+++	+++
12053	For trypsin	+	+	+
	For chymotrypsin	+++	+++	+++
RG-141	For trypsin	++	+	+
	For chymotrypsin	+++	+++	+++
9877	For trypsin	++	+	+
	For chymotrypsin	++	++	++
2460	For trypsin	+	++	+
	For chymotrypsin	+++	++	+
ICGS-1	For trypsin	+	+	++
	For chymotrypsin	++	++	++
J-11	For trypsin	+	+	+
	For chymotrypsin	++	+++	+++
	For trypsin	+	+	+

GG 2	For chymotrypsin	++	+++	++
M-13	For trypsin	+	+	+
	For chymotrypsin	+	+	+
MH-1	For trypsin	+	+	+
	For chymotrypsin	++	+	++

Table 2: Protein Estimation

S. No.	Accession no.	Protein (mg/gm of seed powder)	Azocasein (% inhibition)
1.	6255	3±0.02	94.62 ± 0.02
2.	145	3.48±0.04	93.34 ± 0.06
3.	SB-X1	5.4±0.03	95.56 ± 0.77
4.	3672	9±0.07	96.43 ± 0.47
5.	Kudiri - 3	9.6±0.03	95.56 ± 0.94
6.	T-28	7.2±0.01	90.31 ± 0.39
7.	4738	6±1.0	74.71 ± 0.67
8.	JL 24	7.8±0.02	94.35 ± 0.67
9.	GG 4	6.48±0.06	96.74 ± 0.10
10.	2458	6.50±0.09	78.90 ± 1.53
11.	ICGV 86590	9.6±0.05	74.07 ± 1.76
12.	12053	6.00±0.03	70.55 ± 1.31
13.	RG-141	3±0.02	73.74 ± 1.65
14.	9877	8.88±0.06	78.35 ± 0.76
15.	2460	11.4±0.08	79.78 ± 0.75
16.	ICGS-1	11±0.04	82.63 ± 0.31
17.	J-11	4.8±0.02	75.27 ± 0.54
18.	GG-2	9±0.05	80.55 ± 0.80
19.	M 13	12±0.03	88.02 ± 0.76
20.	MH-1	11±0.03	80.55 ± 0.33

Results shown as mean +/- SE n=3
% inhibition of protease inhibitor extract using three different substrates:
Azocasein for total protease inhibitor activity
Bapna for trypsin inhibitor activity
Gluphepha for chymotrypsin inhibitor activity

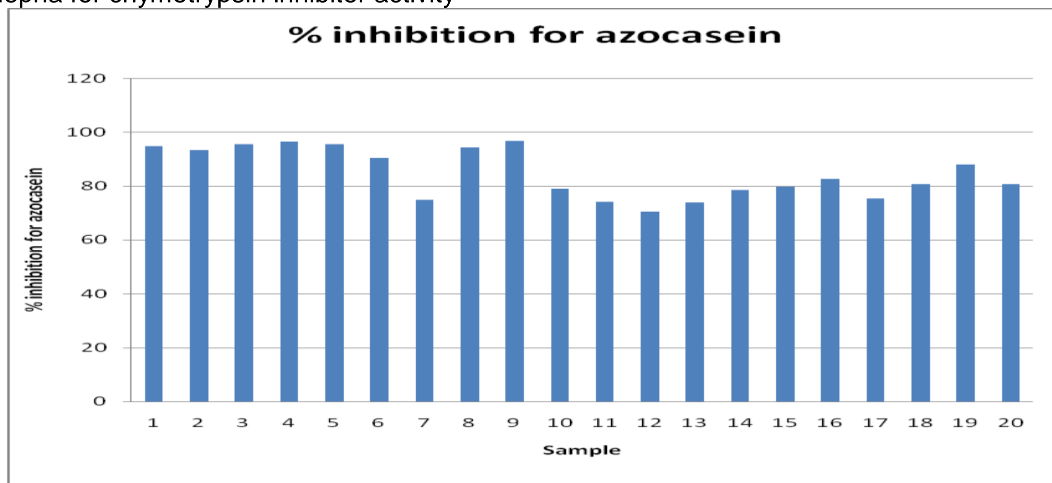

Figure 3: % inhibition for azocasein

Table 3: Bapna and Glupepha % inhibition

S. No.	Accession no.	Bapna (% inhibition)	Glupepha (% inhibition)
		Nonheated	**Nonheated**
1.	6255	62.29 ± 0.57	60.654 ± 4.19
2.	145	58.29 ± 1.43	62.79 ± 3.13
3.	SB-X1	35.43 ± 0.01	68.33 ± 0.43
4.	3672	36.29 ± 2.00	67.05 ± 0.72
5.	Kudiri - 3	24.58 ± 3.43	70.46 ± 1.14
6.	T-28	9.15 ± 0.57	69.04 ± 0.85
7.	4738	5.15 ± 0.55	65.35 ± 1.42
8.	JL 24	21.15 ± 1.82	68.19 ± 0.57
9.	GG 4	------------------	49.85 ± 0.99
10.	2458	73.43 ± 0.85	71.87 ± 0.02
11.	ICGV 86590	39.15 ± 1.15	63.36 ± 0.30
12.	12053	-------------------	63.79 ± 0.79
13.	RG-141	53.43 ± 0.57	75.97 ± 0.00
14.	9877	14.86 ± 0.57	63.97 ± 0.00
15.	2460	69.15 ± 1.43	65.06 ± 1.42
16.	ICGS-1	4.00 ± 4.00	75.97 ± 0.00
17.	J-11	32.86 ± 0.86	63.64 ± 0.16
18.	GG-2	78.29 ± 3.14	60.52 ± 0.29
19.	M 13	67.72 ± 0.00	64.78 ± 0.57
20.	MH-1	91.42 ± 0.28	59.14 ± 1.14

Results shown as mean +/- SE n=3
% inhibition of protease inhibitor extract using three different substrates:
Azocasein for total protease inhibitor activity
Bapna for trypsin inhibitor activity
Gluphepha for chymotrypsin inhibitor activity

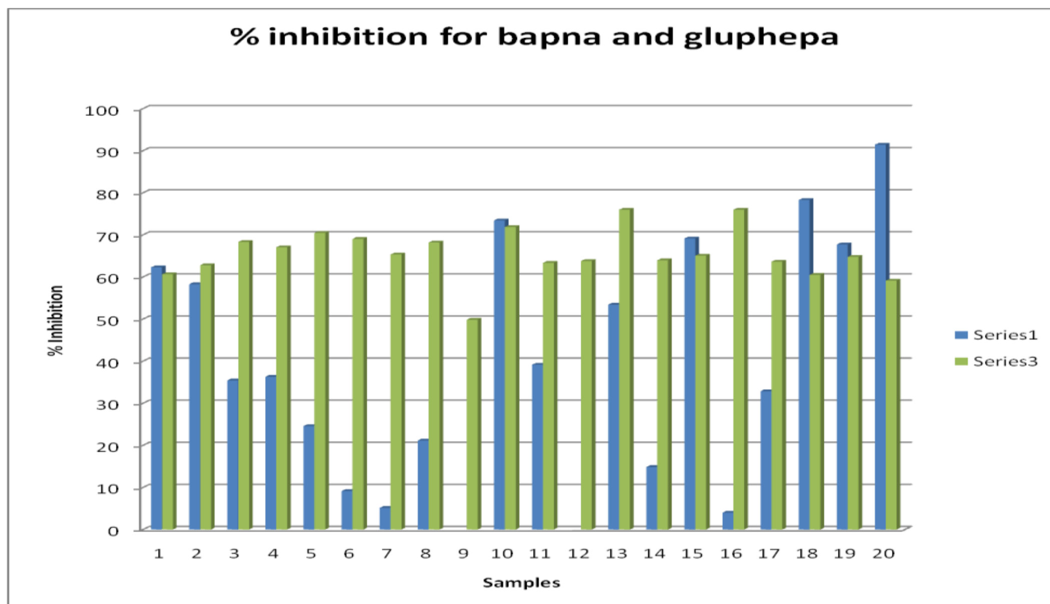

Figure 4: % inhibition for Bapna and Gluphepa

Discussion

Spot test for all the 20 goundnut varieties was performed so as to determine the level of inhibition

present in them. Spot test is the preliminary step and it was observed that most of the groundnut varieties when not heated had inhibition in all the three ratios i.e. 1:1, 1:3 and 3:1. But there were some exceptions such as variety 12053, J-11, GG-2, M-13 and MH-1 had very less inhibition in all the three ratios with trypsin. 9877, 2460, ICGS-I had very less inhibition in 1:3, 3:1 and slightly less in 1:1. ICGV 86590 and RG-141 had very less inhibition in 1:3 and slightly less in 1:1 and 3:1 ratio.

ICGV 86590, 12053 and RG-141 were found to be having extreme level of inhibition in all the three ratios with chymotrypsin whereas minimm in M-13, MH-1 and JL-24. Groundnut variety 6255, 145 and SB-XI had minimum level of inhibition in 1:3 and 3672, Kudiri-3 had minimum in 1:3 and 1:1.

Protein estimation was performed and it was observed that groundnut sample 1 (6255) and 13 (rg-141) had minimum amount of protein content i.e. 3.0 mg/gm whereas sample 19 (m-13) had the maximum amount of protein content i.e 12 mg/gm followed by sample 15 (2460), 1`6(icgs-1) and 20 (MH-1) having 11.4 mg/gm and 11 mg/gm respectively.

In solution assays Azocasein was used to determine total protease inhibitory activity, total protease inhibitory activity was found to be maximum with sample 9 (GG-4) and sample 4 (3672) each having about 97% inhibition whereas sample 12 (12053) showed the least as compared to other varieties. i.e. approx 70%.

BApNA was used to determine trypsin inhibitory activity and it was seen that sample 20(MH-1) showed 91% inhibition. Sample 16 (ICGS-1) showed minimum inhibition 4%.

GLUPHEPA was used to determine chymotrypsin inhibitory activity minimum amount of chymotrypsin inhibitory activity was present in sample 9 (GG-4) WHERE ONLY 49% inhibition was observed whereas maximum was seen in sample 16 (ICGS-1) WITH 75% inhibition.

BAPNA, GLUPHEPA and Azocasein

This work is carried out to understand the efficacy of groundnut PIs against bovine trypsin and chymotrypsin have been used while BAPNA, gluphepa and azocasein are used as synthetic substrates for trypsin, chymotrypsin and total protease activity respectively. Percentage inhibition calculated is the percent difference in the activities in presence and absence of protease inhibitors from groundnut. It is calculated based on the difference between the absorbace using specific substrates for trypsin, chymotrypsin and for both trypsin and chymotrypsin..

Summary

The present work is focussed on studying interaction of proteases and protease inhibitors from groundnut variety. Proteases were prepared freshly and protease inhibitors were extracted from different groundnut variety. Interaction studies were performed using methods such as Spot Test, Electrophoresis (Gel x-ray film contact print technique) and Solution assays. Sample containing proteases will lead to the digestion of gelatin coated on X-ray film and will lead to the development of transparent blue colour.

Spot test was used as a screening method for trypsin inhibitory, chymotrypsin inhibitory activity. For conformation of these activities solution assays were performed.

Solution assays was performed for estimation of protease inhibitory activity. Three synthetic substrates were used to determine protease inhibitory activity; azocasein was used as a synthetic substrate to determine total protease inhibitory activity, BApNA to determine trypsin inhibitory activity, GLUPHEPA to determine chymotrypsin inhibitory activity. The present study is important because various feed ingredients have high quantity of protease inhibitors and remain intact even after processing. Thus because of inhibition, availability of protein decreases even after utilization of these ingredient with higher protein content thereby affecting the growth of humans adversely. Protease and Protease inhibitors interaction studies will reveal suitability of groundnut variety that can be utilized in the diet.

Future Prospects

Groundnut which is known as poor man’s cashew nut has good amount of nutritional level. Groundnut

is a source of protein, carbohydrate, minerals, amino acids, fats, oil etc. And therefore it becomes extremely important that we come to know about the nutritional level in groundnut related products too.

Groundnut and its flour is consumed in our country in some or the other way and groundnut oil has huge demand too so checking the level of protein in the commercially available processed food preparation will help us understand that up to which level they can be consumed by humans so as to obtain maximum health benefits.
Also, the tests we performed were carried out with the help of bovine trypsin and chymotrypsin and not with human trypsin and chymotrypsin therefore, this can further be checked by performing dietary assays using rats.

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