Study On Use Of Pectinolytic Enzyme For Liquefaction Of Feed Substrates-Corn And Cottonseed

Hardeep Singh

Lecturer in Biology, Govt. Senior Secondary School, Singowal in Jind, Haryana (India)
Email: hssonadil@gmail.com

Abstract: Accumulation of reducing sugar and protein at different pectinase dose by Corn and Cottonseed spotted. In substrates weight loss is spotted but no major weight loss is found in corn seeds, In cottonseeds approximately 25% weight loss is spotted. In case of corn seeds maximum sugar and protein released with an enzyme dose. Table 1 in observation showed the amount of sugar and protein released with varying dose of enzyme. In case of sugar test optimum enzyme dose is found to be 1.5 ml while in case of protein test optimum enzyme dose is found to be 1 ml. The variation in results obtained for different substrates can be attributed to the cell wall composition of the substrates which might not be same. The significant amount of reducing sugars found in the reaction filtrates after enzymatic treatment proved the liquefaction of feed substrates. The amount of soluble protein released in the reaction mixture was also appreciable. The proteins are present in the cell walls bound by the strong and fibrous pectins, thus are not available as nutrients. By the action of pectinase these proteins became available and these soluble proteins can be easily estimated in the reaction mixture. This can be explained as incomplete degradation of cellulose resulted in release of large amount of proteins but less sugar. In this substrates were reacted with optimum enzyme dose. All substrates released highest amount of reducing sugar and protein at 45˚C, pectin becomes easily accessible to pectinase for release of more reducing sugar and protein. Results showed that pretreatment of forage prior to feeding can make significant differences. While, from the all reports and researches it was not clear whether the major benefit of enzyme application occur in prefeeding treatment or after the feed enters the rumen of the ruminants or cattles.

[Singh, H. **Study On Use Of Pectinolytic Enzyme For Liquefaction Of Feed Substrates-Corn And Cottonseed.** *Life Sci J* 2019;16(6):84-89]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 13. doi:10.7537/marslsj160619.13.

Keywords: Pectinolytic Enzyme, Seed, Corn, Cottonseed**.**

1. Introduction:

Mixed crop livestock production system constitute an important sources of livelihood to the majority of small holder farmers involved in agriculture production.[1] In developing countries the reason to use crop residues as the principle component of diet is there locally available and relatively cheap resources. [2] The crop residues constitute an important sources of low cost feeds supplying over 20% of the ruminants energy requirement. Since recent improvements in fermentation technology and biotechnology, Enzyme preparation with specific activity can be used to derive specific metabolic and digestive processes in the gastro intestinal tract and may increase natural digestive processes to improve the availability of nutrients and feed intake thereafter.[3] Currently, research has been focused on using exogenous fibrolytic enzymes to stimulate rumen digestion so that the energetic potential of these pectinase materials can be harnessed to economize the feeding.[4] Ruminant animals can be considered as the foundation of animal agriculture because they have served mankind all the way through many millennia. The microbial mode of digestion allows ruminants to better unlock the unavailable energy in the plant cell wall components than other herbivores.[5] This gave ruminant animals ability to convert low nutritive and resistant ligno-cellulosic biomass to milk, meat, wool and hides. However, most forage plants are high in cell walls and low nitrogen and energy content.[6]

Despite the importance of fibrous components in forages for salivation, rumen buffering and efficient production of ruminal end products only 10 to 35%of energy intake is available as net energy. [7] This is because the ruminal digestion of plant cell walls is not complete. Consequently, performance of ruminants fed such feedstuffs as major components of nourishment is often suboptimal because of their high lignin content. [8] As a consequence of a low nutritive value of forage at maturity, many strategies have been developed to improve the nutritional quality of forages used in ruminant systems. Despite its demonstrated role in ruminant nutrition crop residues are however low in metabolizable energy and crude protein. [9] But the major problem arises due to the susceptibility of ligno-cellulose to hydrolysis due to crystallinic structure. Due to this structural complexity limits the digestibility of conventional feed substrate in the animal gut. Removal of lignin from lignocellulose reduction of crystallinity of cellulose to loosen the cellulose structure increase the effective contact area of the cellulose with beneficial micro organisms. [10]

Degradation of lignin by means of biological treatment has got the potential to upgrade the quantity of straw. Considerable research effort has gone into improving their nutritional value through crop management breeding and physical, chemical and biological treatment of residues as well as supplementation through high protein oil cakes. [11] Several factors have been cited to influence the adaptation and utilization of crop residues in different countries. These include availability, quality, price, labour costs and capital investment in processing. [12] For increasing the nutritive value and protein content of crop residues, various microorganisms and fibrolytic enzymes (cellulose and pectinase) are used. Increase in milk yield has been reported in daily cows. [13] The objective of this research was to study the pretreatment of commonly used substrate (Corn and cottonseed), different parameters (time, temperature, enzyme dose) were also optimized for this treatment process under laboratory conditions. [14]

2. Materials and Methods:

Substrates: The agro-residues conventionally used in northern India as feed substrates were selected for the present study. They were named as C1 Corn (Zeamays) and CS1 Cottoseed (Gossypium spp.). They were washed, chopped and dried in the hot air oven overnight at 50 degree Celsius.

The present study was carried out by various methods such as Lowry Protein Assay[15], Miller DNSA for sugar[16], Sugar test by DNS methods[17] and Protein test by Lowery method[21].

Methods: Lowry Protein Assay- The lowry protein assay is a biochemical assay for determining the total level protein concentration is exhibited by a color change of the sample solution in proportion to protein to concentration, which can then be measured using colorimetric techniques.

Miller DNSA for sugar: estimation- 3,5-Dintrosalicylic acid (DNSA) is used extensively in biochemistry for the estimation of reducing sugars. It detects the presence of free carbonyl group (c=o) of reducing sugar. This involves the oxidation of the aldehyde functional group and the ketone functional group.

Preparations: Preparation of buffer-

Requirements: Sodium citrate, Citric Acid, Distilled water

Procedure: First take 10.29gm of sodium citrate in 350 ml of distilled water, On the other hand, take approximately 2 and half t-spoons of Citric acid and mix it in 100 ml of distilled water. Now, we have to set the pH at 5 by adding citric acid in sodium citrate. Preparation of DNSA for sugar test.

Requirements: 1g of Dinitrosallicylic acid,30g of sodium potassium Tartarate, sodium hydroxide (2M) 20mls.

Procedure: To prepare 100mls of DNSA, We need to mix 30g of sodium potassium tartarate with 1g of DNSA AND 20MLS OF NaOH. Then we make it up to 100mls with distilled water. We can scale it up if your assay demands it and you can still scale it down as well.

Preparation of Reagent-C for protein test.

For preparing reagent-C we need reagent-A and reagent-B.

By adding 2ml of B and 100ml of A it will constitute to form Reagent-C.

Procedure: Performing Enzyme Dose Action On Feed Substrates-

- As I have taken two substrates i.e. Corn and Cottonseed, So first we have to take 14 flasks,7 for each substrate.

1. Take 10ml buffer in all flasks. Number flasks from 0-6.
2. Add substrates to flasks (corn=0.5gm and cottonseed=1gm).
3. Now, add enzyme dose in flasks, No enzyme dose in flask zero,0.5 ml in flask 1,1ml in flask 2,1.5 ml in flask 3,2 ml in flask 4,2.5 ml in flask 5,3ml in flask 6. Do this for flasks of both substrates.
4. Now place the flasks in shaker for one hour of duration.
5. Now, filter the substrates with the help of filter paper.
6. After filtering, Place solid substrate in oven for drying for measuring the weight after enzyme action and keep samples in storage tubes numbering them 0-6.
7. Now we have to perform sugar test and protein test on them.

Sugar Test By Dns Method-

Add 2ml of sample and 3ml of DNS in all test tubes. Now, Place it in water bath incubation for 10 minutes.

Now our sample are ready for taking readings on spectrophotometer. For sugar test absorbance is set on 575.

Protein Test By Lowry'S Method-

Add 0.4ml of sample and 4ml of Reagent-C in test tubes. Now incubation for 10 minutes. After incubation 0.4ml of Follin's reagent is added, again incubated for half an hour and taken for readings on spectrophotometer. For protein test absorbance is set on 660.

Effect Of Temperature On Feed Substrates

As I have taken 2 substrates i.e. Corn (1gm) and Cattle feed (1.5gm)

1. Take four flasks, two for each substrate, Mark flasks zero and 1.

2. Add substrates to flasks and 10ml buffer. Now, optimum dose of enzyme should be added, No enzyme dose swill be added to flasks numbered zero of both substrates. Optimum dose is which we found from the readings of sugar test and protein test of Enzyme action.

i.e. 1.5ml for sugar test and 1ml for protein test.

3. Now, flasks are kept in shaker at 35 degree Celsius for one hour of duration and then readings are taken on spectrophotometer.

4. Process is repeated for different temperature ranges i.e. 40,45,50,55.

Results and Observation: Effect Of Enzyme On Cattle feed

-Here it is the table for reading of enzymes action on both the feed substrates i.e. Corn and Cottonseed. Where S is sugar (mg/m) & P is protein (mg/m)

Table 1: Effect of enzyme on cattle feed.

	Enzyme Dose (u/ml)											
	3.75		7.5		11.25		15		18.75		22.5	
Sub.	S	P	S	P	S	P	S	P	S	P	S	P
Corn	.76	0.44	0.94	0.57	1.08	0.66	0.90	0.54	0.72	0.43	0.64	0.38
Cotton Seed	.70	0.50	0.87	0.62	1.0	0.71	0.88	0.58	0.68	0.46	0.60	0.41

Here it is the table for effect of different range of temperatures on feed substrates i.e. Corn and Cottonseed.

Where S is sugar (mg/m) & P is protein (mg/m)

Here are the graphs of effect of enzyme dose and effect of temperature on feed substrates i.e. corn and cattle feed.

Table 2: Effect of temperature on cattle feed.

	TEMPERATURE									
Substrate	35		40		45		50		55	
Corn	1.32	0.80	1.39	0.85	1.47	0.88	1.40	0.84	1.36	0.79
Cottonseed	1.26	0.87	1.34	0.89	1.43	0.91	1.37	0.88	1.33	0.85

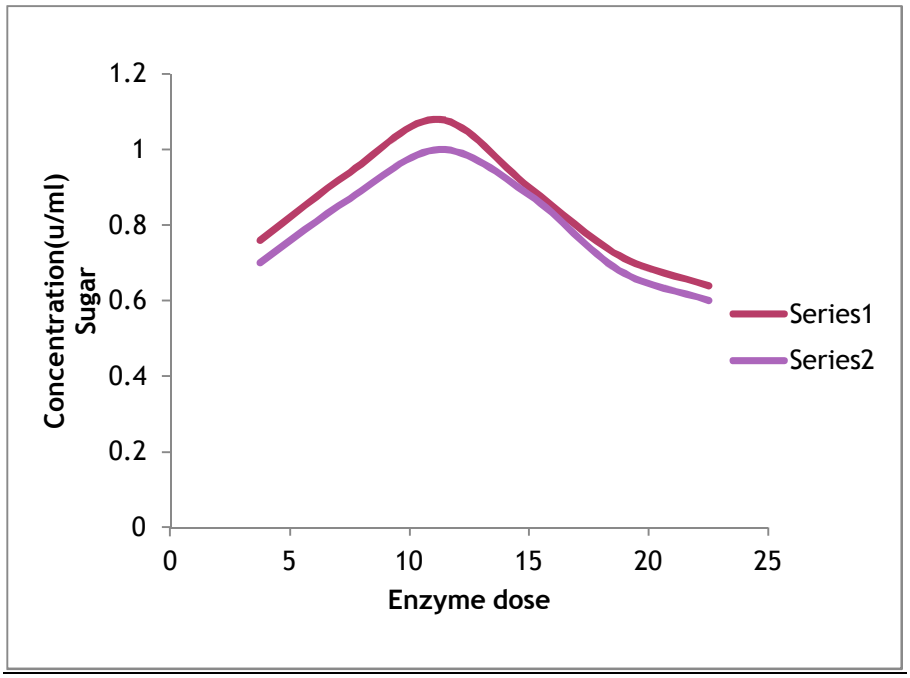

Fig. 1. Effect of enzyme dose on sugar concentration.

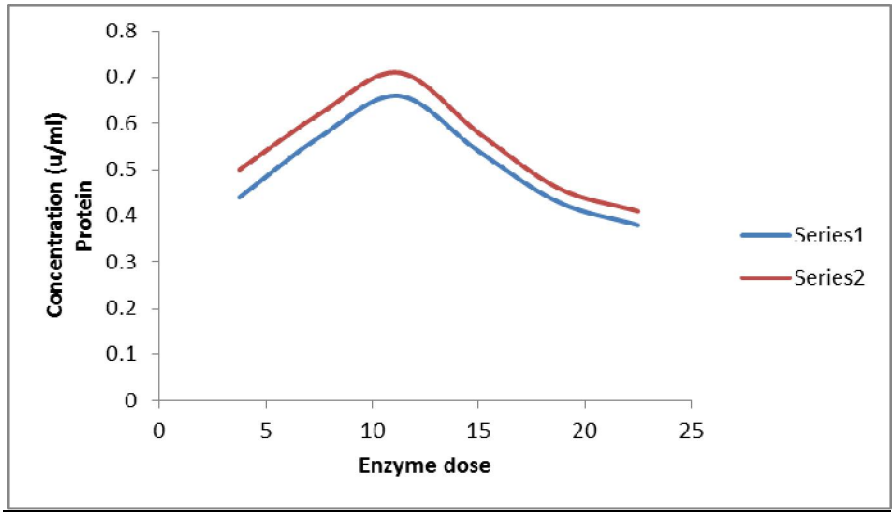

Fig. 2. Effect of enzyme dose on protein concentration.

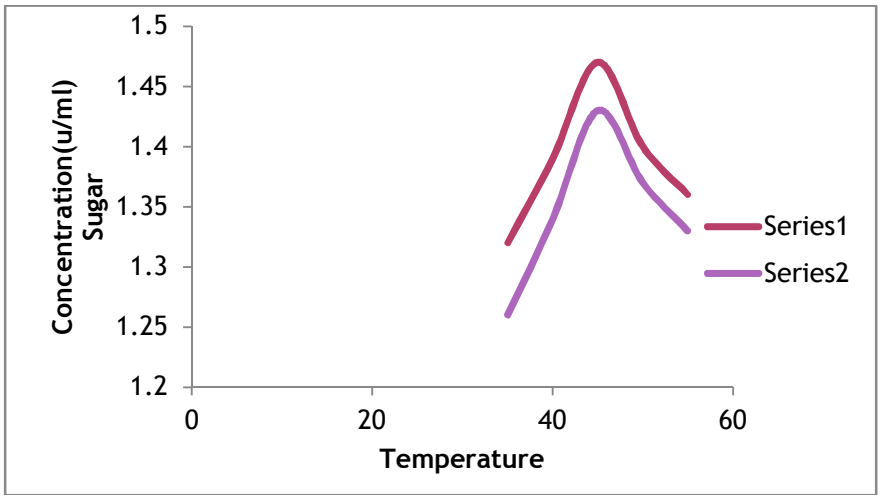

Fig. 3. Effect of temperature on sugar concentration.

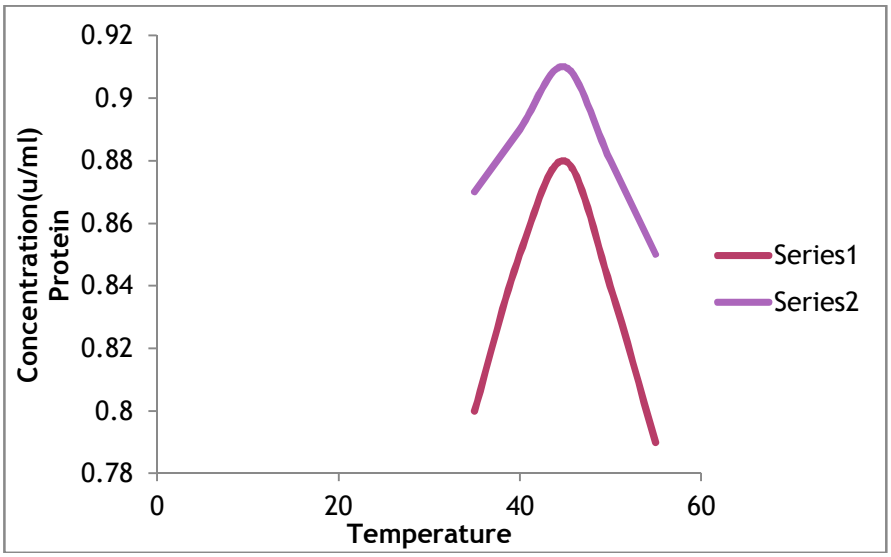

Fig. 4. Effect of temperature on protein concentration.

Results And Discussions

Effect of Enzyme dose: Accumulation of reducing sugar and protein at different pectinase dose by Corn and Cottonseed spotted. In substrates weight loss is spotted but no major weight loss is found in corn seeds, In cottonseeds approximately 25% weight loss is spotted. In case of corn seeds maximum sugar and protein released with an enzyme dose. Table 1 in observation showed the amount of sugar and protein released with varying dose of enzyme. In case of sugar test optimum enzyme dose is found to be 1.5 ml while in case of protein test optimum enzyme dose is found to be 1 ml. The variation in results obtained for different substrates can be attributed to the cell wall composition of the substrates which might not be same.

The significant amount of reducing sugars found in the reaction filtrates after enzymatic treatment proved the liquefaction of feed substrates. The amount of soluble protein released in the reaction mixture was also appreciable. The proteins are present in the cell walls bound by the strong and fibrous pectins, thus are not available as nutrients. By the action of pectinase these proteins became available and these soluble proteins can be easily estimated in the reaction mixture. This can be explained as incomplete degradation of cellulose resulted in release of large amount of proteins but less sugar.

Effect of Incubation Temperature: In this substrates were reacted with optimum enzyme dose. All substrates released highest amount of reducing sugar and protein at 45˚C, pectin becomes easily accessible to pectinase for release of more reducing sugar and protein. Results showed that pretreatment of forage prior to feeding can make significant differences. While, from the all reports and researches it was not clear whether the major benefit of enzyme application occur in prefeeding treatment or after the feed enters the rumen of the ruminants or cattles.

Conclusions:

Forage pretreatment either by enzyme is effective. Using pectinase from Pseudozyma sp. SPJ showed promising results with all the commonly used feeds (rice straw, cottonseed, wheat straw, corn seeds and sorghum) for their upgradation. This is a environmental friendly process used to improve quality or to upgrade the feed substrates.

Corresponding Author:
Hardeep Singh
Lecturer in Biology, Govt. Senior Secondary School, Singowal in Jind, Haryana (India)
Email: hssonadil@gmail.com
Phone No. -91+9728706261

References:

1. Abdullah LAG, Sulaiman NM, Aroua MK and Noor MMMJ (2007) Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. J. Food Eng, 81, 65e71.
2. Abe F, Minegishi H, Miura T, Nagahama T, Usami R, Horikoshi K (2006) Characterization of cold and high-pressure-active polygalacturonases from a deep-sea yeast, Cryptococcus liquefaciens strain N6. Biosci Biotechnol Biochem, 70: 296–9.
3. Abeles FB, Morgan PW, Saltveit Jr ME (1992) Ethylene in plant biology, 2nd edn. San Diego: Academic Press.
4. Brummell DA and Harpster MH (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 47: 311–340.
5. Buenrostro M and Lopes-Munguia A (1986) Enzymatic extraction of avocado oil. Biotechnol. Lett. 8:505-506.
6. Buldini PL, Ricci L and Sharma JL (2002) Recent applications of sample preparation techniques in food analysis. J Chromatogra 975, 47–70.
7. Campbell I (1973) Computer identification of yeasts of genus Saccharomyces. J. Gen. Microbiol. 77, 127−135.
8. Cao J, Zheng L and Chen S (1992) Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of rammie. Enz. Microbiol. Technol. 14, 1013-1016.
9. Caffall KH and Mohnen D (2009) The structure function and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. res, 344(14), 1879-1900.
10. Carpita N and Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. The Plant Journal, 3:1-30.
11. Margetts B and Buttriss (2003) Epidemology linking consumption of plant foods and their constituents with health, In: Plants: Diet and Health. Goldberg, G (Ed) Blackwell publishing. Oxford, UK, pp 49-64.
12. Marquina D, Peres C, Caldas FV, Marques JF, Peinado JM, Spencer-Martins (1992) Characterization of the yeast population in olive brines. Lett. Appl. Microbiol. 14, 283–297.
13. Martel MB, Leètoublon R and Feévre M (1998) Purification and characterization of two endo

polygalacturonases secreted during the early stages of the aprophytic growth of Sclerotinia sclerotiorum. FEMS Microbiol. Lett. 158, 133-138.
14. Martín MA, Fernández R, Serrano A and Siles JA (2013) Semi-continuous anaerobic codigestion of orange peel waste and residual glycerol derived from biodiesel manufacturing. Waste Manage 33(7):1633–1639.
15. Marti-Renom MA, Madhusudhan MS, Fiser A, Rost B, Sali A (2002) Reliability of assessment of protein structure prediction methods. Structure (Camb) 10:435-440.
16. Vincken JP, Schols HA, Oomen RJFJ, Beldman G, Visser RGF, Voragen AGJ (2003) In: Voragen AGJ, Schols HA, Visser RGF (eds) Advances in pectin and pectinase research. Kluwer Academic Publishers, Dordrecht, pp 47–60.
17. Yu X, Hallet SG, Sheppard J and Watson AK (1997) Application of the Plackett-Burman experimental design to evaluate nutritional requirements for the production of Colletotrichum coccodes spores. Appl. Microbiol. Biotechnol. 47: 301-305.
18. Zandleven J, Sorensen SO, Harholt J, Beldman G, Schols HA, Scheller HV, Voragen AGJ (2007) Phytochemistr 68(8):1219.
19. Zhang CH, Ma YJ, Yang FX, Liu W and Zhang YD (2009) Optimization of medium composition for butyric acid production by Clostridium thermobutyricum using response surface methodology. Bioresour Technol 100, 4284–4288.
20. Zheng L, Heupel RC and Dellapenna D (1992) The β subunit of tomato fruit polygalacturonase isoenzyme 1: isolation, characterization, and identification of unique structural features. The Plant Cell, 4:1147-1156.

6/17/2019