Original Research Article https://doi.org/10.20546/ijcmas.2020.911.362

Substrate Specific Enzyme Mixture for Tapioca Flour

D. Uma Maheswari[1*], V. Balakrishnan[2] and C. Valli[2]

[1]*Department of Animal Nutrition, RIVER, Pondicherry, India*
[2]*Department of Animal Nutrition, MVC, Chennai, India*

*Corresponding author

A B S T R A C T

A study was carried out to customize enzymes specific to tapioca flour to improve its nutritive quality. Tapioca flour was rich in crude fibre ($15.45\pm7.22\%$) and soluble carbohydrate ($78.27\pm7.01\%$) content. Hemicellulose was higher in tapioca flour. The per cent glucan, xylan and arabinan in tapioca flour were 27.67 ± 1.62, 29.40 ± 0.45 and $24.19\pm1.78\%$, respectively. An experiment was done to fix the required level of enzymes for tapioca flour (first experiment) with six replicates in each stage of experiment. The respective levels of *cellulase*, *xylanase*, *glucanase*, *pectinase* and *amylase* identified for tapioca flour were 21.0, 18.0, 0.6, 16.0 (U/g) and 12000 (IU/g). As the enzymes used are found to have associated enzyme activities and in particular the *xylanase* enzyme was found to be effective in the release of monomer, only *xylanase* enzyme at the concentration of 0.016 g/g of tapioca flour as selected level of enzyme was used in further experiments. In the second experiment, five treatments *viz.,* selected level, two levels lower (5% and 10%) and two levels higher (5% and 10%) than the selected level, each with six replicates in completely randomised design were tested to arrive at the "customized enzyme mixture" for tapioca flour. Significantly ($p<0.05$) highest release of monomers per g of tapioca flour were noticed in 5% and 10% lower than the selected level, therefore 10% lower level may be used to evolve "Customized enzyme mixture for tapioca flour".

Introduction

The need to meet the rising challenge for better production in the livestock sector with available feeds, has opened new avenues in the field of additives, specially enzymes. Enzymes have been used in animal feeds for the past two decades. The successes of feeding enzymes to mono gastrics have been well documented as against the ruminants.

Scientists have proved in their study that fibrolytic enzymes improved productive performance and proved to be economically effective in buffaloes on enzyme supplementation (Gaafar *et al.,* 2010). Several studies do show that there is a potential in supplementing exogenous fibrolytic enzymes to improve the productivity in ruminants (McAllister *et al.,* 2001). Though conflicting results over the use of enzyme

supplementation in cattle feed were observed over the decade, the main reasons for failure of enzymes in ruminants may be attributed to cocktail of enzyme usage, which is not specific for feed ingredients. Hence this study was aimed to customise enzymes specific for tapioca flour a common choice of the farmers here in Puducherry for the cereal source of the ruminant diet.

Materials and Methods

The entire study was carried out through five stages, excluding preliminary investigations like chemical analysis and enzyme activity. The first experiment was to customize the enzymes specific to tapioca flour which was carried out in three different stages. In stage one the approximate quantity of exogenous enzymes (*cellulase, xylanase, glucanase, pectinase and amylase*) needed for maximum sugar release was done using individual enzymes at a range starting from minimum to a higher level with broader interval. In the second stage of the experiment, the levels of the individual enzymes were narrowed down and the exact level of enzymes needed were fixed and, assigned as selected enzyme level. The third stage of experiment was carried out, wherein associated enzyme activity of individual enzymes was determined by assessing the maximum sugar release when incubated with tapioca flour. The "selected levels of the enzymes" *viz., cellulase, xylanase, glucanase, pectinase* and *amylase* identified in the previous experiments were used in this experiment. The enyzme which had shown better associated enzyme activity was added at five levels *viz.,* selected level, two levels at lower (5% & 10%) and two levels at higher (5% & 10%) than the selected level to arrive at customize enzyme level.

Six tapioca flour samples were collected from different shops at Puducherry for this study. The proximate analyses and acid insoluble ash of tapioca flour samples were estimated as per AOAC (2000) and the results were expressed on dry matter basis
.
The fibre fractions namely neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose and lignin were estimated for the six tapioca flour samples as per the method of Goering and Van Soest (1970). The non-starch polysaccharide contents (total NSP, soluble NSP and insoluble NSP) of tapioca flour samples were also estimated as per the procedure of Englyst (1989).

Enzyme activity for individual pure enzymes

The pure enzymes chosen for the study *viz. Cellulase, Xylanase, Pectinase, Glucanase* and *Amylase* were procured from a commercial firm and assessed for their individual and associative enzyme activity.

Fibrolytic enzymes activities

The activities of fibrolytic enzymes (*cellulase, xylanase, pectinase* and *glucanase*) were determined using di nitro salicylic acid (DNSA) reducing sugar method (Miller, 1959).

In vitro assay to study the associated activity of enzyme

The pure fibrolytic enzyme *cellulase* was cross tested for associated fibrolytic enzymes *viz., xylanase, glucanase* and *pectinase* activities as per the method indicated above.

Amylase activity

Estimation of *amylase* activity was done based on the method of Smith and Roe (1949). One IU of amylase is defined as the amount of enzyme required to produce 10 per

cent fall in the intensity of the blue colour of starch-iodine complex under the assay condition.

Results and Discussion

Proximate composition and acid insoluble ash and fibre fractions of tapioca flour

The proximate composition and acid insoluble ash content of tapioca flour analyzed in the present study is presented in table 1.

Tapioca flour was high in nitrogen free extractives and its chemical composition is comparable to that of cereal byproducts with low protein content.

The fibre fractions of tapioca flour analyzed in the present study is presented in table 2. Tapioca flour has a relatively high content of hemicellulose and its lignin content is found to be low.

Non starch polysaccharide (NSP) content in tapioca flour

The results (Table 3) of the non-starch polysaccharide content of tapioca flour analysed in the study showed an increased amount of total non-starch polysaccharide in tapioca flour with a less amount of soluble NSP and high insoluble NSP.

Enzyme activity for individual pure enzymes

The results of the enzyme activity of the various fibrolytic enzymes *viz., cellulase, xylanase, pectinase* and *glucanase* in the pure enzymes purchased from the open market was found to be 1660.67, 1410.67, 6000 and 830.33 U/g, respectively. The *amylase* activity was 198250 IU/g. Based on this, the minimum levels of the enzymes required for maximum monomer release was arrived by

incubating the feed ingredients at 42°C for 2 hours and is presented in table 4.

Cellulose and hemicellulose are the major plant structural polysaccharides and account for approximately 70% of plant biomass. In addition to the maintenance of the structural integrity of plants, cellulose and hemicellulose, serve as major source of nutrients for herbivores and also serve as substrate for the production of food, animal feed as well as textiles (Gilbert and Hazlewood, 1993; Beguin and Aubert, 1994). Cellulose being a linear polymer of glucose linked by β-1,4-glycosidic bonds contains cellobiose as its repeating unit (Bhat and Hazlewood, 2001). The chain length has varied glucose which goes to form micro fibrils. The micro fibrils consist of highly ordered crystalline regions interspersed by more disordered amorphous regions. The crystalline regions of cellulose are rigid and not easily accessible to endo-acting *cellulase*s while the amorphous regions are easily attacked by dilute acid, endo*glucanase*s or exo*glucanase*s (Sinitsyn *et al*., 1990). Therefore, the level of cellulase, which was determined to be slightly higher than xylanase required to release the maximum level of monomer in this study may be attributed to the nature of glucose polymer in tapioca flour which may be more of amorphous in nature.

Based on the main sugar present in the polymer structure, hemicelluloses can be termed xylans, glucomannans, galactans or arabinans. Xylans and glucomannans are generally considered to be two main types of hemicelluloses (Viikari *et al*., 1993). Though the tapioca flour showed considerably higher hemicellulose content than the cellulose, their requirement for the level of xylanase enzyme is found to be lesser than the level of *cellulase*. This may be due to the type of xylan. The hemicellulose of tapioca flour is reported to be arabinoxylan category which is

supported by the higher level of *pectinase* enzyme requirement (16U/g) as determined in this study. Jayani *et al.* (2005) reports that pectic substances mainly consist of galacturonans and rhamnogalacturonans wherein, the C-6 carbon of galactose is oxidized to a carboxyl group; the arabinans and the arabinogalactans. The higher level of *pectinase* usage is advantageous as it ensures reduced feed viscosity, increasing absorption of nutrients, either by hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers, and reduces the amount of faeces (Hoondal *et al.,* 2000).

The need for *amylase* as high as 12000 U/g for hydrolyzing the tapioca flour is justified as the level of soluble starch in tapioca flour is found to be high as indicated by the level of nitrogen free extract.

In vitro assay to study the associated activity of the enzymes

The result indicated that each enzyme exhibited different levels of associated activities among them. Though *xylanase activity* was predominant in *xylanase* it also showed activities of *cellulase* and *glucanase* which were almost equal in distribution. Similarly *pectinase* had associated activities of *xylanase* and *glucanase* in equal terms.

However, it was observed that the *xylanase* had fairly good activity of other enzymes *viz., cellulase, pectinase* and *glucanase* (25% *cellulase*, 27% *xylanase,* 25% *glucanase* and 23% *pectinase* as per percentage of associative activity of other enzymes)*,* which means supplementing *xylanase* in the feed will also provide fair amount of *cellulase, pectinase* and *glucanase.* Hence, *xylanase* was chosen to supplement. As one gram of tapioca flour requires 21 U of *cellulase,* 18 U of *xylanase,* 0.6 U *glucanase* and 16 U of pectinase and the amount of xylanase required to be supplemented to provide above levels of

activity was calculated to be 0.016 g, which will provide 20.9 U of *cellulase,* 22.57 U of *xylanase,* 20.9 U *glucanase* and 19.2 U of *pectinase.* In addition to 0.016 g of *xylanase* each gram of tapioca flour also required 0.06 g of *amylase* (12000/198250=0.06) for effective hydrolysis of NFE (soluble starch content as reflected by the nitrogen free extractives is a measure for the *amylase* content of the feeds) present in the tapioca flour. At this selected level of enzymes, the level of *glucanase* is four times its actual requirement which may be on the higher side, since both *cellulase* and *glucanase* share the same substrate for action as indicated by the monomers released it could be argued that glucanase does not affect degradability adversely (Table 5).

The *in vitro* experiment was designed with two graded level of 5% and 10% higher level than the above selected level of *xylanase* supplemented to tapioca flour and another two graded level of 5% and 10% lower level than the selected level of *xylanase* supplemented to tapioca flour.

Significantly highest (p<0.05) release of monomers/g of tapioca flour were noticed in the reaction where 5% lower and 10% lower than the selected level of enzyme (control) were used. Beauchemin *et al.* (2003) in their review on exogenous enzyme supplementation in ruminants observed that high levels of enzyme addition can be less effective than low levels and the optimal supplementation may depend on the diet nature. The results of this experiment are also in par with this observation. Among the 5% and 10% lower levels, numerically higher monomer release was achieved at 10% lower levels of enzyme supplementation which is also significantly higher than the control. Therefore this level is recommended to be "Customized enzyme mixture for tapioca flour".

Table.1 Per cent proximate composition and acid insoluble ash (Mean* ± SE) of tapioca flour used in this experiment (DMB)

S. No.	Nutrients (%)	Tapioca flour
1.	Moisture	10.76±0.56*
2.	Dry matter	89.23±0.56*
3.	Crude protein	2.71±0.38*
4.	Crude fibre	15.45±7.22*
5.	Ether extractives	0.37±0.03*
6.	Total Ash	3.2±0.19*
7.	Nitrogen free extractives	78.27±7.01*
8.	Acid insoluble ash	0.70±0.29*

*Mean of 6 samples each

Table.2 Per cent fibre fractions (Mean* ± SE) of tapioca flour used in this experiment (DMB)

S. No.	Fibre Fractions (%)	Tapioca flour
1.	NDF	38.59±1.95
2.	ADF	17.59±0.99
3.	Cellulose	14.72±0.95
4.	Hemicellulose	21.0±0.95
5.	ADL	2.87±1.945
*Mean of 6 samples each		

Table.3 Per cent Non Starch Polysaccharide content (Mean* ± SE) of tapioca flour (DMB)

Ingredient	Soluble NSP			Insoluble NSP			Total NSP		
	Glucan	Xylan	Arabinan	Glucan	Xylan	Arabinan	Glucan	Xylan	Arabinan
Tapioca flour	8.9 ±0.20	10.66±0.61	11.49 ±1.24	18.78±1.73	18.74±0.63	12.70±2.27	27.67±1.62	29.40±0.45	24.19±1.78

*Mean of 6 samples each

Table.4 Minimum activity levels of *cellulase* (U/g), *xylanase* (U/g), *glucanase* (U/g), *pectinase* (U/g) and *amylase* (IU/g) required to hydrolyze tapioca flour to release maximum level of monomers on incubating at 42°C for 2 hours

Enzyme	U/g Tapioca flour
Cellulase	21.0
Xylanase	18.0
Glucanase	0.6
Pectinase	16.0
Amylase	12000 (IU/g)

Table.5 Carbohydrate monomers released / g of tapioca flour in various treatments due to different enzymes on incubating at 42 °C for 2 hours

Native and Associated Enzyme activities of Xylanase	Carbohydrate monomers released / g of tapioca flour in various treatments				
	"Selected level of Xylanase supplemented to tapioca flour"	Lower Level than the selected level		Higher level than the selected level	
	Control	5%	10%	5%	10%
Cellulase	15181.16[a] ±2286.02	22151.04[b] ±2159.06	22420.63[b] ±1794.99	14583.33[a] ±1720.08	15483.33[a] ±1398.99
Xylanase	15871.21[a] ±2389.93	22151.04[b] ±2374.96	26885.96[b] ±1983.94	15217.39[a] ±1794.86	16128.47[a] ±1457.29
Glucanase	15181.16[a] ±2286.02	22151.04[b] ±2159.06	22420.63[b] ±1794.99	14583.33[a] ±1720.08	15483.33[a] ±1398.99
Pectinase	13966.67[a] ±2103.14	26653.08[b] ±2065.19	20492.42[b] ±1713.40	13461.54[a] ±1587.76	14336.42[a] ±1295.37
Amylase	57240.44[a] ±861.94	15053.94[b] ±848.20	16496.91[b] ±698.05	54687.50[a] ±645.03	57773.63[a] ±522.01

*Mean of 6 samples each.
Mean values bearing different superscripts within a row differ significantly ($p<0.05$).

In conclusion, the minimum level of enzyme activity required for the maximum release of monomer from one gram tapioca flour were determined to be 21 , 18 , 0.6 , 16 U/g and 12000 IU/g for *cellulase, xylanase, glucanase, pectinase* and *amylase,* respectively. The assay of above enzymes for associate activities in *in vitro* trial revealed that no enzyme was pure and found to be associated with the other fibrolytic enzyme activities. Xylanase which showed effective associative fibrolytic enzyme activity was chosen and supplemented at 0.016 g/g of tapioca flour which found to provide 20.63 U of *cellulase,* 19.25 U of *xylanase*, 20.63 U of *glucanase* and 20.0 U of *pectinase* and 10800 IU of *amylase*. On further experimentation, the amount of xylanase, 10% lower than the above level was found to release the monomer from tapioca flour, significantly. Therefore, based on the above results and also taking the economics into consideration from farmers view point, 10% lower than the selected level

of xylanase with the associate enzyme activity which had yielded maximum hydrolysis can be taken as "Customized enzyme mixture for tapioca flour".

References

AOAC. 2000. Official methods of analysis 15th ed. of the Association of Official Analytical Chemists, 15th edition, Arlington, VA, USA.

Anonymous. 2003. Cassava products for animal feeding. Integrated cassava project www.cassavabiz.org/postharvest/lvstoc k_1.htm

Beauchemin, K. A, D. Colombatto, D. P. Morgavi and W. Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *J. Anim. Sci.* 81(2): E37–E47.

Beguin, P. and J. P. Aubert, 1994. The biological degradation of cellulose.

FEMS Microbiology Review. 13: 25-58.

Bhat, K. M. and G. P. Hazlewood. 2001. Enzymology and other characteristics of cellulases and xylanases. In: Enzymes in farm animal nutrition. Bedford, M. R. and Partridge, G. G. (eds). Wiltshire, UK. pp. 11-50.

Englyst, H. 1989. Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology.* 23: 27-42.

Goering, H. K. and P. J. Van Soest. 1970. Forage and fibre analysis, Agricultural handbook No.379. Agricultural research service, US, Department of Agriculture. Washington D, C. pp.1-20.

Hoondal, G. S., R. P. Tiwari, R. Tiwari, N. Dahiya and Q. K. Beg. 2000. Microbial alkaline pectinases and their applications: a review. *Appl. Microbiol.Biotechnol*. 59: 409–418.

Jayani, R. S., S. Saxena and R. Gupta. 2005. Microbial pectinolytic enzymes a review. Process Boochem. 40: 2931-2900

McAllister, T. A., A. N. Hristov, K. A. Beauchemin, L. M. Rode and K. J. Cheng. 2001. Enzymes in ruminant diets. In: Enzymes in farm animal nutrition. Eds. Bedford M. R. and G. G. Partridge. CAB inter. pp. 273-298.

Miller, G. L. 1959. Use of dinitrio saliyclic acid reagent for determination of reducing sugars. *Anal. Chem*. 31(3): 426-428.

Smith and Roe. 1949. A photometric method for the determination of α- amylase in blood and urine with the use of the starch-iodine colour. *J. Biological Chem.* 179: 53-59.

Viikari, L., M. Tenkanen, J. Buchert, M. Ratto, M. Bailey, M. Siika-Aho and M. Linko. 1993. Hemicellulases for industrial applications. In: Bioconversion of forest and agricultural plant residues. Saddler, J. N. (Ed.). CAB International, Wallingford. pp. 131-182.