Food Chemistry 111 (2008) 187–191

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Mode of inhibition of finger millet malt amylases by the millet phenolics

S. Chethan, Y.N. Sreerama, N.G. Malleshi *

Department of Grain Science and Technology, Central Food Technological Research Institute, Mysore 570020, India

ARTICLE INFO

Article history:
Received 17 December 2007
Received in revised form 14 March 2008
Accepted 18 March 2008

Keywords:
Finger millet
Eleusine coracana
Polyphenols
Amylase activity
Mode of inhibition

ABSTRACT

The effect of millet polyphenols on starch hydrolysis catalyzed by amylases developed during malting were investigated. The enzyme kinetic studies using Michaelis–Menten and Lineweaver–Burk equations showed the K_m remained constant (0.625%) but the maximum velocity (V_{max}) decreased in the presence of a crude extract of millet polyphenols, indicating mixed non-competitive inhibition. On the other hand, gallic acid, vanillic acid, quercetin and *trans*-cinnamic acid isolated from the polyphenol extract of the millet showed uncompetitive inhibition. Kinetic studies of amylase inhibition by phenolic compounds indicated the presence of secondary binding sites in malted finger millet amylases similar to other cereal amylases.

1. Introduction

Polyphenols are known to inhibit the activity of digestive enzymes such as amylase, glucosidase, pepsin, trypsin and lipases and the subject has been studied extensively (Rohn, Rawel, & Kroll, 2002). Synergy between phenolics may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of type 2 *Diabetes mellitus*, which is characterized by high blood glucose levels (Saito, Sakai, Sekihara, & Yajima, 1998; Toeller, 1994). Polyphenols may act as inhibitors of amylase and glucosidase (similar to acarbose, miglitol and voglibose) leading to a decrease in post-prandial hyperglycemia (Bailey, 2001). Finger millet or ragi, is one of the minor cereals and is a staple food in south India and parts of Africa. Germination has been suggested as an inexpensive and effective method for improving the overall nutritional quality of cereals and legumes, by enhancing their digestibility (Chavan & Kadam, 1989) and reducing the contents of anti-nutritional factors (Ghorpade & Kadam, 1989). Malting of cereals and germination of legumes is documented but very little has been reported on the effect of polyphenols on the activity of enzymes developed during germination. Millet malt is also a good source of α- and β-amylases (Chandrasekhara & Swaminathan, 1953) and malted millet is extensively used in weaning food, infant food and supplementary food formulations (Malleshi, 2005). The hydrolytic enzymes in the malt are largely confined to the endosperm fraction of the malted millet. The seed coat of the millet contains nearly 90% of the polyphenols and remaining 10% are distributed in the endosperm as constituents of the cell walls (Chethan & Malleshi, 2007). Millet is being used as a source of amylases in improving the nutrient density and texture of weaning food formulations. Although there are reports on the inhibitory activity of the polyphenols on the cereal amylases (Rohn et al., 2002), there are no reports on the inhibition of finger millet malt amylases by its polyphenols. In the present study, the inhibitory effects as well as the mode of action of the millet polyphenols on its malt amylases have been investigated through kinetic studies, using Michaelis–Menten and the derived Lineweaver–Burk (LB) equations.

2. Materials and methods

2.1. Materials

Finger millet (variety GPU 28) seeds were procured from the University of Agricultural Sciences, Bangalore. Phenolic standards: gallic, vanillic, ferulic, *p*-coumaric, *p*-hydroxy benzoic, syringic, *trans*-cinnamic, *p*-catechuic, quercetin and trifluoroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other reagents were of analytical and HPLC grade.

2.2. Malting of finger millet

The millet seeds (1 kg) were cleaned, steeped for 24 h and germinated on moist cloth at 25 °C in a BOD incubator (Alpha Scientific Co., Bangalore, India) up to 120 h. The seeds were withdrawn from the germination bed at intervals of about 24 h, dried at 50 °C in an air oven for 6–8 h and the vegetative growth

* Corresponding author. Tel.: +91 0821 2510843; fax: +91 0821 2517233.
E-mail address: malleshi@yahoo.com (N.G. Malleshi).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.03.063

was removed by gentle brushing. The devegetated seeds were powdered and used for the assay of amylases (Malleshi & Deshikachar, 1982).

2.3. Polyphenol extraction

Native and malted millet flour from 24 to 120 h was extracted with 1% HCl–methanol by refluxing in a boiling water bath for 30 min. The refluxed material was filtered using Whatman No. 1, filter paper. The residue was again refluxed with fresh solvent and the process was repeated until the polyphenols were fully extracted. The extracts were pooled together and their polyphenol content was estimated using a gallic acid standard (Singleton, Orthofer, & Rosa, 1995).

2.4. Fractionation of millet polyphenols

An adequate quantity of sodium hydroxide was used to neutralize (pH 7) the polyphenolic extract from the native millet. Since, precipitate was formed on neutralizing the acid, the contents was centrifuged to separate the precipitate. Subsequently, the supernatant was concentrated and its polyphenols were fractionated into component phenolics using reverse phase analytical HPLC [(LC-10A, Shimadzu Corporation, Kyoto, Japan) equipped with CBM-10A system controller, SPD-M10 AVP photodiode array detector and a software Class LC-10 fitted with C-18 column (250 mm × 4.6 mm, 5 m; μ-Bondapak, Waters Corp., Milford, USA)]. The mobile phase consisted of a binary solvent system using water acidified with 0.1% trifluoroacetic acid (solvent A) and 100% methanol (solvent B) at a flow rate of 1.0 ml/min. The gradient programme initiated with 80% eluent A and 20% eluent B, ramped linearly to 60% solvent A and 40% solvent B within 40 min. This proportion was maintained for next 10 min and subsequently, the solvent gradient was reverted to initial conditions (80:20, v/v) within next 5 min. Detection and quantification of the eluted phenolics was performed at 295 nm. Individual peaks and phenolic acids were identified by comparing the retention time and spectra of each peak with known standards under identical conditions.

2.5. Extraction of amylases

The amylase of the malted millet were extracted from the whole meal of malt samples with 3 volumes (w/v) of 0.1 M sodium acetate buffer pH 4.8 by shaking for 2 h at 4 °C. The extract was centrifuged for 20 min at 6500*g* at 4 °C using refrigerated centrifuge. The supernatant was dialyzed against the extraction buffer.

2.6. Amylase activity

Amylase activity from the extract was assayed (Bernfeld, 1955). Gelatinized soluble starch (1%) in sodium acetate buffer (50 mM, pH 4.8) was incubated with appropriate aliquots of the enzyme extracts at 37 °C for 30 min. The reaction was terminated by adding 3, 5-dinitrosalicylic acid reagent and the absorbance was measured at 540 nm. One unit of enzyme activity was defined as one micromole maltose equivalent released per min under the assay conditions.

2.7. Amylase inhibitory activity

The inhibitory effect of polyphenols on amylases extracted from 96 h malted millet flour was determined (Maeda, Kakabayashi, & Matsubara, 1985). The extracted amylase was pre-incubated with polyphenols (μg/ml) at 37 °C for 10 min before addition of 1.0% gelatinized starch. Similarly, the inhibitory activities of individual phenolic acids found through HPLC were also determined against amylases extracted from 96 h malted millet. The enzyme activity was determined by measuring absorbance at 540 nm.

2.8. Amylase activity staining

The extract from 72 h and 96 h germinated samples were analyzed by non denaturing PAGE (10% T) using Davis buffer system (Davis, 1964). After, electrophoresis the gel was immersed in gelatinized and cooled starch solution (2%) in 50 mM sodium acetate buffer pH 5.0 and incubated at 45 °C for 30 min. The gel was then rinsed with distilled water and stained in a solution containing 3% iodine and allowed to stain for 10 min. The amylase bands were visualized as transparent bands on a dark blue background. Similarly, for determination of inhibitory effects of polyphenols on amylase activity, the gel after electrophoresis was pre-incubated with the inhibitor and then with the substrate and incubated at 45 °C for 30 min, and thereafter, the gel was stained with iodine reagent.

2.9. Kinetics of inhibition

The individual phenolics identified from the native finger millet were used for recording kinetic studies. The inhibition was measured with increasing concentrations of starch as a substrate (0.5–2.0%) in the presence of phenolics at different concentrations (30–700 ηM). The type of inhibition was determined by LB plot analysis of the data (Origin software ver. 6.1), which were calculated from the results according to Michaelis–Menten kinetics. The inhibitory constants (K_i) and dissociation constants of enzyme-substrate inhibitor complexes (K_i') were determined (Bowden, 1974; Dixon, 1953). All the experiments were repeated three to four times.

3. Results and discussion

3.1. Total polyphenol content

The native millet contained about 2.5% of polyphenols and nearly 44% of it was lost during the first 24 h of germination. Only 40% of the polyphenols were lost over the next 48 h of germination (Fig. 1). The polyphenols in the millet occur both in free and bound form and the initial decrease could be due to the synergistic effect of the millet polyphenols leaching during steeping beside hydrolysis during germination. It may also be due to the action of induced

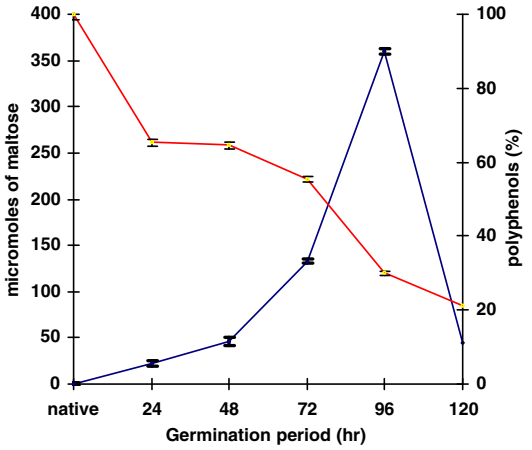

Fig. 1. Total phenolic contents (■) and amylase activity (▲) during the course of finger millet malting. Results are expressed as mean ± standard error of the mean ($n = 4$).

esterases activity on bound phenolics, which act on various phenolic acid esters linked either to arabinoxylans or other non-starch polysaccharides (Maillard, Soum, Biovin, & Berset, 1996). A twofold decrease in all the major phenolic acids after 96 h of malting of finger millet was observed (Subba Rao & Muralikrishna, 2001). A decrease in the polyphenol contents has also been reported during malting of sorghum (Chukwura & Muller, 1982).

3.2. Fractionation of polyphenols

Fractionation of the millet polyphenols on reverse phase HPLC revealed the presence of a number of phenolic compounds. Among these, only nine phenolics were identified, namely, benzoic acid derivatives (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid and syringic acid), cinnamic acid derivatives (ferulic acid, *trans*-cinnamic acid and *p*-coumaric acid) and a flavonoid compound (quercetin). Benzoic acid derivatives accounted for about 85% of the total phenolic compounds (Table 1). Since, the millet extract possesses different polyphenolic compounds, it may exhibit different biological properties.

3.3. Amylase activity

Millet malt, is a rich source of α-amylase besides being a good source of β-amylases. Various malting conditions including the duration of germination are important factors with respect to the amylase activity of cereals, as it is well known that in all the cereals, the amylase activity increases with the period of germination up to certain point and then decreases. The amylase activity of the millet increases rapidly during germination up to 96 h and subsequently decreases (Fig. 1). Hence, the amylases of 96 h germinated millet were quantitatively extracted and the activity was assayed (357 units/g). The extracted amylases were fractionated on non-denaturing polyacrylamide gel electrophoresis (PAGE). The activity stain assay indicated a single isoform of amylase (Fig. 2), in contrast to the three isoforms reported for the Indaf 15 variety of finger millet (Nirmala & Muralikrishna, 2003). Cereal amylases are known to exist in multiple isoforms in different varieties of some cereals (Beleia & Marston, 1981). Genetic diversities are one of the main reasons suggested for multiple isoforms of cereal amylases (Ainsworth & Gale, 1987; Muthukrishnan & Chandra, 1988).

The Michealis–Menten constant (K_m) and maximum velocity (V_{max}) for the extracted millet amylases were calculated from double reciprocal plots. The K_m and V_{max} were found to be 0.62% and 201 units/g respectively, when rice starch was used as a substrate. Millet malt amylase has a higher apparent affinity for starch compared to sorghum malt amylases (1.55%), whereas, wheat amylases have high affinity for starch (0.23–0.53%) compared to other cereal amylase (Kumar, Singh, & Rao, 2005). The K_m for barley amylase is reported to be 0.21, 79.3 and 213 μM for amylose, maltodextrin and maltoheptaose respectively (Oudjeriouat et al., 2003). The kinetic constants for amylases vary with different substrates. The millet malt amylases have comparable V_{max} to other cereal amylases.

Table 1
Inhibitory activity of HPLC fractionated phenolic compounds on finger millet malt amylase

Phenolic compounds[a]	Retention time (min)[b]	Concentration (μg/g)[b]	Inhibition (%)[c]
Gallic acid	3.1	30	67.7
Protocatechuic acid	6.1	405	64.4
p-Hydroxybenzoic acid	7.4	370	61.3
p-Coumaric acid	11.9	41	62.5
Vanillic acid	15.0	20	71.9
Syringic acid	20.1	10	55.8
Ferullic acid	22.3	41	65.6
Trans-cinnamic acid	32.9	100	79.2
Quercetin	43.1	3	73.5

[a] Phenolic compounds were separated and purified by reverse phase HPLC as described in Section 2.
[b] Calibration curves of respective standard phenolic compounds were used to determine the retention time and concentration of phenolic compounds.
[c] Inhibitory activity was measured as described in Section 2.

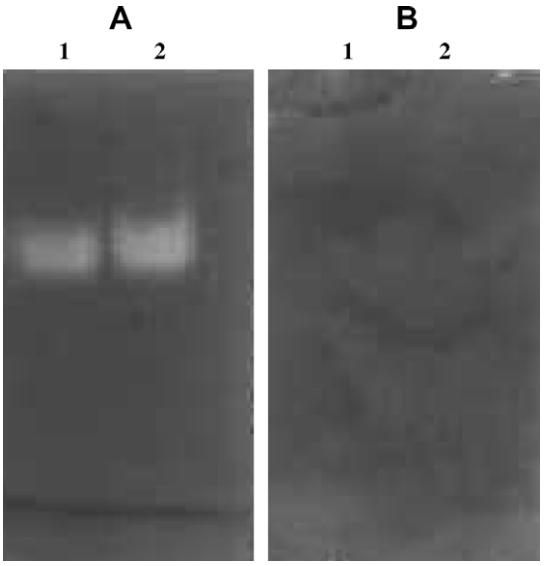

Fig. 2. Native PAGE of the crude extracts from malted finger millet stained for amylase activity, without inhibitor (A) and with inhibitor (B). Lanes 1 and 2 are extracts from 72 h and 96 h malted finger millet, respectively (~1 μg of protein was loaded in each lane). The amylase activity staining shows clear bands against dark blue background.

3.4. Inhibition of the amylase activity

The extent of inhibition of the fractionated individual phenolic acids against amylase is presented in Table 1. Among the phenolic acids tested, gallic acid (67.7%), vanillic acid (71.9%), the flavonoid quercetin (73.5%) and *trans*-cinnamic acid (79.2%) were potent inhibitors of the millet amylases. *Trans*-cinnamic acid exhibited a higher degree of inhibition as compared to other phenolic compounds and syringic acid was found to be a weaker inhibitor (~56% inhibition). Even though protocatechuic acid and *p*-hydroxybenzoic acid are the main derivatives of benzoic acid in the millet polyphenol extract, they were less effective at inhibiting the amylase activity. Depending on the structure, the phenolics react with proteins/enzymes and alter various properties of biopolymers such as the molecular weight, solubility and *in vitro* digestibility (Rohn et al., 2002). It has also been shown that the decrease in enzyme activity depends on the concentration as well as the number and position of hydroxyl groups of the phenolics (Rohn et al., 2002). The mode of inhibition is also dependent on the substrate specificity of the enzymes. Acarbose, a synthetic amylase inhibitor has been reported to exhibit uncompetitive, mixed and noncompetitive types of inhibition when amylose, maltodextrin and maltoheptaose were used as respective substrates (Oudjeriouat et al., 2003).

3.5. Mode of inhibition

The millet polyphenols may affect the amylases in several ways, for instance by competing with the substrate to bind to the active site of the enzyme or by disrupting irreversibly the catalytic process. Kinetic studies were performed using the Michaelis–Menten

and LB derivations to identify the mode of inhibition of millet phenolics. In the presence of crude phenolic extract, slope of the straight lines in double reciprocal plot increased with increasing concentrations of polyphenols. The straight lines were intercepted at a single point in the second quadrant indicating mixed non-competitive inhibition (Fig. 3A). The heterogeneity of phenolics having different structural features in crude extract may be the reason for the observed mode of inhibition. However, the concentrations of the millet polyphenols affect both the slope and the vertical axis intercept of LB plot. The crude extract has an inhibitory constant, K_i value of 66.7 μg (Fig. 3B).

The mode of inhibition of most potent individual phenolic compounds from millet (*trans*-cinnamic acid, quercetin, vanillic acid and gallic acid) were investigated by Michaelis–Menten and LB equations. In contrast to the crude polyphenol extract, the four isolated polyphenols, when tested individually they showed an uncompetitive type of inhibition. The presence of inhibitor in the reaction mixture resulted in no intersection of the straight lines (i.e., lines are parallel) in the LB plot (Fig. 3C). In this case, the inhibitor only binds to the enzyme substrate complex resulting in a change in K_m and V_{max}.

The mixed non-competitive inhibition exerted by crude polyphenol extract on malted millet amylases indicates that the phenolics can bind to *E* or to the ES complex other than catalytic site, decreasing the V_{max} proportionate to the concentration of phenolic compounds. The kinetic data indicated that two abortive complexes could be produced: the amylase–phenolic complex (EI) resulting from binding of the phenolic compound to the active site

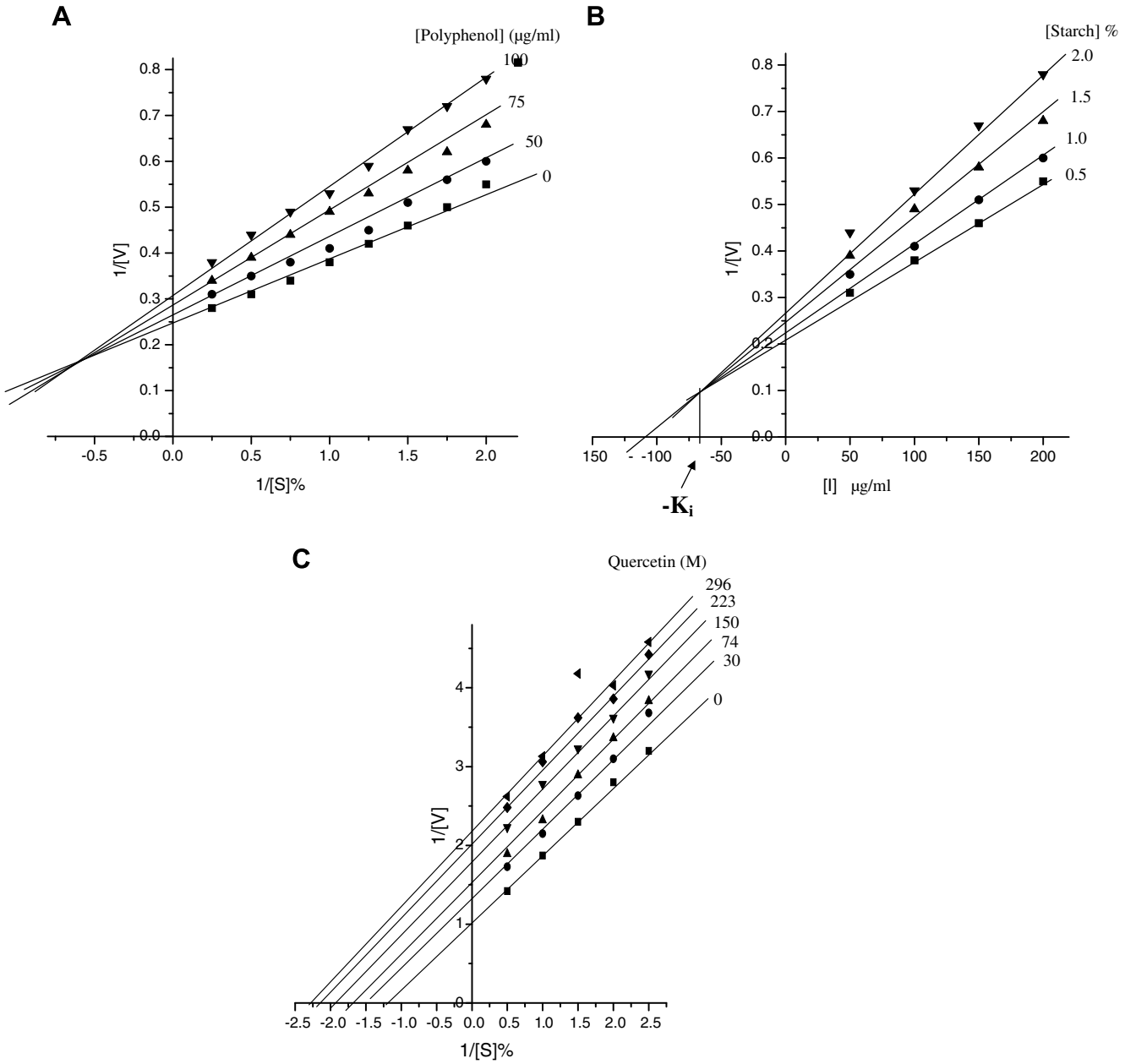

Fig. 3. Lineweaver–Burk and Dixon plots of finger millet malt amylase in the presence of phenolic compounds. A, LB plot of the amylase hydrolysis reaction with variable starch concentrations (0.25–2.0%) and at fixed concentration of crude phenolic compounds as indicated on the right side of the graph. B, Dixon plot of the amylase hydrolysis reaction with starch at a fixed substrate concentration as indicated on the right side of the graph and with variable concentrations phenolic compounds. C, LB plot of the amylase hydrolysis reaction with variable starch concentrations (0.5–2.5%) and at fixed concentration of quercetin as indicated on the right side of the graph. Other phenolic compounds also represent similar graphs and are omitted here for reasons of clarity. LB and Dixon plots were calculated from the values of mean of three independent determinations.

Table 2
Dissociation constants (K_i') of phenolic compounds against finger millet malt amylase

Phenolic compound	K_i'[a]
Gallic acid	4.60×10^{-7} M
Vanillic acid	5.06×10^{-7} M
Quercetin	4.63×10^{-7} M
Trans-cinnamic acid	7.30×10^{-7} M

[a] Determined from secondary plots of s/v vs. I (Bowden, 1974).

and the amylase–starch–phenolic complex (ESI) in which phenolic compound is bound at a secondary binding site other than the active centre, which is accessible only after starch binding has occurred at the active centre. In contrast, the individual phenolic compounds purified by HPLC showed uncompetitive inhibition of starch hydrolysis. In this case, binding of starch at the catalytic site may have modified the confirmation of amylase, making the putative inhibitor binding site available. The kinetic constants K_m and V_{max} decreased upon binding of phenolic compounds.

It has long been a practice to determine the inhibition constants of competitive inhibitors by the Dixon method. This plot does not distinguish unambiguously between competitive and mixed inhibitors (Schlamowitz, Shaw, & Jackson, 1969) and, for mixed or uncompetitive inhibitors, it provides no measure of the dissociation constant of the ESI complex (K_i'). Therefore, a new plot, which is similar to the Dixon plot but complementary to it, was used since it provides a simple way of measuring (K_i') rather K_i (Bowden, 1974). The dissociation constant (K_i') derived from the plots of s/v vs. I for gallic acid, *trans*-cinnamic acid, quercetin and vanillic acid are summarized in Table 2. Gallic acid and quercetin had the highest affinity for the enzyme-substrate complex with (K_i') of 4.6×10^{-7} M. However, *trans*-cinnamic acid had a low affinity for the ES complex of finger millet amylases (K_i' of 7.3×10^{-7} M).

The kinetics as well as difference spectral studies on porcine pancreatic α-amylase inhibition by α-, β- and γ-cyclodextrins indicated the involvement of secondary binding site including a tryptophan residue (Koukiekolo, Desseaux, Moreau, Mouren, & Santimone, 2001). The inhibitory effects of cyclohepta amylose on starch granule hydrolysis exerted by cereal α-amylases suggested the presence of a non-catalytic binding site in the enzyme molecule (Koukiekolo et al., 2001). Based on the observations on the kinetics of amylases in the presence of inhibitors which is in line with the various X-ray crystallographic studies (Qian, Haser, & Payan, 1995; Weselake & Hill, 1983), it may be inferred that finger millet malt amylases have more than one binding site similar to other amylases.

4. Conclusions

The crude polyphenolic extract from finger millet exerts mixed non-competitive inhibition whereas the individual phenolic compounds isolated from the extract exhibit uncompetitive inhibition of starch hydrolysis by malted finger millet amylases. Thus, we have described edible plant-based amylase inhibitors for modulation of carbohydrate breakdown and regulation of glycemic index of foods. Our results provide a scientific rationale for the use of finger millet as a low cost nutrient useful in reducing the chronic pathologies such as diabetes mellitus.

Acknowledgements

The authors acknowledge with thanks the support received from Dr. V. Prakash, Director, CFTRI, Mysore. One of the authors (S.C.), thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, India for the award of Senior Research Fellowship.

References

Ainsworth, C. C., & Gale, M. D. (1987). Enzymes structural genes and their exploitation in wheat genetics and breeding. In J. E. Kruger, D. R. Lineback, & C. E. Stautter (Eds.), *Enzymes and their role in cereal technology* (pp. 53–82). St. Paul, Minnesota, USA: AACC.

Bailey, C. J. (2001). New approaches to the pharmacotherapy of diabetes (3rd ed.. In J. C. Pickup & G. William (Eds.). *Text book of diabetes* (Vol. 2, pp. 73.1–73.2). UK: Blackwell Science Ltd..

Beleia, A., & Marston, V. E. (1981). Pearl millet amylases. I. Properties of partially purified α-amylase. *Cereal Chemistry, 58*, 433–437.

Bernfeld, P. (1955). Amylases, α and β. In S.P. Colowick, N.O. Kalpan, (Eds.), *Methods in enzymology* (Vol. 1, pp. 149–158).

Bowden, A. C. (1974). A simple method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. *Biochemical Journal, 137*, 143–144.

Chandrasekhara, M. R., & Swaminathan, M. (1953). Enzymes of ragi (*Eleusine coracana*) and ragi malt: I-amylases. *Journal of Science Industrial Research, 12B*, 51–56.

Chavan, J. K., & Kadam, S. S. (1989). Nutritional improvement of cereals by sprouting. *CRC Critical Review of Food Science Nutrition, 28*, 401–437.

Chethan, S., & Malleshi, N. G. (2007). Finger millet polyphenols: Optimization of extraction and the effect of pH on their stability. *Food Chemistry, 105*, 862–870.

Chukwura, E. N., & Muller, H. G. (1982). Effect of tannic acid on a low tannin African sorghum variety in relation to carbohydrate and amylase. *Journal of Food Science, 47*, 1380–1381.

Davis, B. J. (1964). Disc electrophoresis – Methods and application to human serum proteins. *Annals of the New York Academy of Sciences, 121*, 404–429.

Dixon, M. (1953). The determination of enzyme inhibitor constants. *Biochemical Journal, 55*, 170–171.

Ghorpade, U. M., & Kadam, S. S. (1989). Germination. In *CRC Handbook of World Food Legumes: Nutritional Chemistry. Processing Technology and Utilization* (Vol. 3, pp. 165–206). Boca Raton, FL: CRC Press.

Kumar, R. S. S., Singh, S. A., & Rao, A. G. G. (2005). Thermal stability of (α-amylase from malted jowar (*Sorghum bicolor*). *Journal of Agricultural Food Chemistry, 53*, 6883–6888.

Koukiekolo, R., Desseaux, V., Moreau, Y., Mouren, G. M., & Santimone, M. (2001). Mechanism of porcine pancreatic α-amylase. Inhibition of amylose and maltopentaose hydrolysis by α-, β- and γ-cyclodextrins. *European Journal of Biochemistry, 268*, 841–848.

Maeda, K., Kakabayashi, S., & Matsubara, H. (1985). Complete amino acid sequence of an α-amylase inhibitor in wheat kernel (0.10-inhibitor). *Biochemica Biophysica Acta, 828*, 213–221.

Maillard, M. N., Soum, M. H., Biovin, P., & Berset, C. (1996). Anti-oxidant activity of barley and malt: relationship with phenolic content. *Lebensmittel-Wissenschaft Und-Technologic, 29*, 238–244.

Malleshi, N. G. (2005). Finger millet (ragi), The Wonder Grain, IFIS., http://www.ieis.org/fsc.ixid13110.

Malleshi, N. G., & Deshikachar, H. S. R. (1982). Formulation of a weaning food with low hot paste viscosity based on malted ragi (*Eleusine coracana*) and green gram (*Phaseolus radiatus*). *Journal of Food Science Technology, 19*, 193–197.

Muthukrishnan, S., & Chandra, G. R. (1988). Regulation of the expression of hydrolase genes in cereal seeds. *Advances in Cereal Science and Technology*. Minnesota: Inc. St. Paul (pp. 129–159).

Nirmala, M., & Muralikrishna, G. (2003). Three amylases from malted finger millet (ragi, *Eleusine coracana*, Indaf 15) – Purification and partial characterization. *Phytochemistry, 62*, 21–30.

Oudjeriouat, N., Morean, Y., Santimone, M., Svensson, B., Moureu, G. M., & Desseaux, V. (2003). On the mechanism of α-amylase. Acarbose and cyclodextrin inhibition of barley amylase isozymes. *European Journal of Biochemistry, 270*, 3871–3879.

Qian, M., Haser, R., & Payan, F. (1995). Carbohydrate binding sites in a pancreatic α-amylase-substrate complex, derived from X-ray structure analysis at 2.1-Å resolution. *Protein Science, 4*, 747–755.

Rohn, S., Rawel, H. M., & Kroll, J. (2002). Inhibitory effects of plant phenols on the activity of selected enzymes. *Journal of Agricultural Food Chemistry, 50*, 3566–3571.

Saito, N., Sakai, H., Sekihara, H., & Yajima, Y. (1998). Effect of an α-glucosidase inhibitor (voglibose), in combination with sulphonilureas, on glycemic control in type 2 diabetes patients. *Journal of International Medical Research, 26*, 219–232.

Schlamowitz, M., Shaw, A., & Jackson, W. T. (1969). Limitations of the Dixon plot for ascertaining nature of enzyme inhibition. *Texas Reports Biology Medicine, 27*, 483–488.

Singleton, V. L., Orthofer, R., & Rosa, M. L. (1995). Analysis of total phenols and other oxidation substrate and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–171.

Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2001). Non-starch polysaccharides and bound phenolic acids from native and malted finger millet (ragi, *Eleusine coracana*, Indaf – 15). *Food Chemistry, 72*, 187–192.

Toeller, M. (1994). α-Glucosidase inhibitors in diabetes: Efficacy in NIDDM subjects. *European Journal of Clinical Investigation, 24*, 31–35.

Weselake, R. J., & Hill, R. D. (1983). Inhibition of α-amylase catalysed starch granule hydrolysis by cycloheptaamylose. *Cereal Chemistry, 60*, 98–101.