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Polyphenols extraction from Niger seed meal by enzyme application.

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ABSTRACT

Plant polyphenols exhibit effective antioxidant activities. The effect of endo-xylanase and endo-mannanase on the extraction of polyphenols from Niger seed meal is reported in this study. The enzyme assisted extraction process was further optimized for different parameters such as enzyme concentration and duration of alkaline hydrolysis. Total polyphenols were determined by application of the Folin–Ciocalteau method. 1gm of defatted Niger seed meal when treated with 4000.0 IU/g of endo-xylanase yields 7.855 mg/g of GAE which was 24.89% greater than control and when 1gm of defatted Niger seed meal was treated with 60.0 IU/g of endo-mannanase showed 9.4 mg/g GAE release which was 68.0% greater than control. The synergistic interaction of endo-xylanase (5000 IU/g) and endo-mannanase (90 IU/g) resulted in higher release of polyphenols i.e., 11.05 mg/g GAE which is 45.62% greater than control. The obtained results strongly support the idea of using cell-wall degrading enzymes as an effective means for recovering polyphenols from defatted Niger seed meal.

Keywords: Polyphenols, antioxidant, endo-xylanase and endo-mannanase, Niger seed meal.

INTRODUCTION

Polyphenolic compounds possess numerous biological activities, such as anti-microbial, anti-inflammatory, prebiotics and antioxidant properties. They are found in all parts of higher plant, such as roots, barks, stems, leaves, fruits, and flowers (Schwarz et al., 2009). In recent years, considerable attention has been paid to polyphenols which have been found to be strong antioxidants for human health benefits (Kim, Tsao, Yang, & Cui, 2006; Schwarz et al., 2009). Reports have suggested that natural phenols specifically have excellent properties as food preservatives (Valenzuela, Nieto, Cassels, & Speisky, 1992) as well as having an important role in the protection against a number of pathological disturbances, such as atherosclerosis, brain dysfunction and cancer (Gordon, 1996).

Niger seed (*G. abyssinica)* is drawing commercial and academic attention because of its nutritional value, biological activities and excellent antioxidant properties. India and Ethiopia are major producers of Niger seed. It is a dicotyledonous herb, which is moderately to well branched, and attains up to two meters of height (Getinet and Teklewold, 1995). Niger seed belongs to the *Compositae* family and there are six species of which *G. abyssinica* is the only cultivated species (Baago, 1974). The utilization of Niger seed proteins in human food is very limited due to the presence of high fiber content and an unacceptable dark colour (Seegeler, 1983). Niger meal contains approximately 30% protein and 23% crude fiber after oil extraction (Getinet and Teklewold, 1995). Defatted Niger seed and its extracts can provide a natural source of antioxidants (Shahidi et al., 2003).

In higher plant cell, phenols may be classified as cell-wall phenols, which are bound to polysaccharides, and non-cell-wall phenols which are associated with vacuoles and the cell nucleus (Pinelo, Arnous, & Meyer, 2006). The retention of

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phenols in the cell-wall depends on compositional and structural characteristics. Cross-linking of cell wall polysaccharides is the main barrier for the release of intracellular substances. Enhanced release of these phenols from plant cells by cell disruption and extraction through the cell wall can be optimized using enzyme preparations either alone or in mixtures. Nowadays, enzyme assisted extraction has enticed considerable interest among researchers for the extraction of various kinds of compounds.

Materials and Methods

Materials:

Tri-Sodium Citrate, Sodium carbonate, Folin-Ciocalteau reagent, Potassium dihydrogen phosphate, Magnesium Sulphate, Calcium chloride, Starch, Ammonium sulphate, Sodium dihydrogen phoshphate (monohydrate), Sodium hydroxide, Aluminium chloride, Acetic acid, Methanol, Ethanol, Ethyl acetate were purchased from Merck ltd. Mumbai-India. Ammonium sulphate and Hydrochloric acid was procured from Rankem Laboratories, India. All the reagents were of analytical grade.

Xylanase producing *Bacillus sp*. PKD-9 was procured from laboratory culture collection. endo-xylanase was produced from *Bacilius sp* PKD-9 under solid state fermentation (SSF) and was partially purified. Mannanase producing *Bacillus sp*. CAM-21 was procured from laboratory culture collection. endo-mannanase was produced from from *Bacillus sp*. CAM-21 under solid state fermentation (SSF) and was partially purified.

1. Defatting of Niger seed meal

Niger seed meal was procured from local market of Mysore, India and was defatted (for 24 h) in percolator by solvent extraction using n-hexane in 1:1.5 ratio (meal residue: n-hexane). Thereafter, spent solvent was decanted and defatted meal residue obtained was dried for 24–48 h and used in further experiments.

2. Estimation of total phenolic content (TPC) by Folin-Ciocalteu method

The total phenolic content (TPC) was determined by the Folin-Ciocalteu's method, in which 3.75ml of water, 0.25ml of twofold diluted Folin-Ciocalteu reagent, 0.5ml of phenolic extract and 0.5ml of 10% sodium carbonate were mixed. After 1hour at room temperature, the sample absorbance was measured at 765 nm. A standard curve of gallic acid was prepared in range of 10-50 µg/ml using above mentioned conditions. The total phenolic content was expressed as mg Gallic acid equivalents/L extract (Laroze et al., 2010; Soto et al., 2008).

3. *Optimization of polyphenol release from Niger seed meal using endo-xylanase*:

Defatted niger seed meal (1.0 g) was incubated with either 500.0-5000.0 IU of endo-xylanase from *Bacillus* sp. PKD-9 in 9.0 ml of Tris-HCl buffer (100.0 mM, pH 8.0) at 50C under shaking conditions (100 rpm). Aliquots of 500.0 µl were withdrawn at various time intervals (up to 10.0 min and up to 60 minutes), centrifuged (10,000 rpm, 5.0 min., RT) and polyphenol released was estimated by Folin Ciocalteu method (Laroze et al., 2010) using gallic acid as standard.

4. Optimization of polyphenol release from Niger seed meal using endo-mannanase :

Defatted Niger seed meal (1.0 g) was incubated with either 50.0-90.0 IU endo-mannanase in 9.0 ml of phosphate buffer (100.0 mM, pH 7.0) at 50C under shaking conditions (100 rpm). Aliquots of 500.0 µl were withdrawn at various time intervals (up to 10.0 min and up to 60 minutes), centrifuged (10,000 rpm, 5.0 min., RT) and polyphenol released was estimated by Folin Ciocalteu method (Laroze et al., 2010) using gallic acid as standard.

5. Optimization of polyphenol release from Niger seed meal using synergistic interaction of endo-mannanase and endo-xylanase

Defatted niger seed meal (1.0 g) was incubated with a combination of enzyme (endo-xylanase: 4000.0-5000.0 IU, endo-mannanase: 80-90 IU: or endo-xylanase: 500 IU, endo-mannanase: 50 IU) at 500C under shaking conditions (100 rpm). Aliquots of 500.0 µl were withdrawn at various time intervals (up to 1 h), centrifuged (10,000 rpm, 5.0 min., RT) and polyphenol released was estimated by Folin Ciocalteu method (Laroze et al., 2010).

Results and Discussion

Preparation of gallic acid standard curve for estimation of total phenolic content (TPC) by Folin-Ciocalteu method:

A standard curve of gallic acid was prepared in range of 10-50 µg/ml for estimation of total phenolic content as mg Gallic acid equivalents/L extract. Results obtained indicated good linearity (R2=0.9986) between values tested (Figure 1).

Optimization of time course of endo-xylanase assisted polyphenol release from Niger seed meal:

Figure-1. Standard curve of gallic acid using Folin-Ciocalteu method

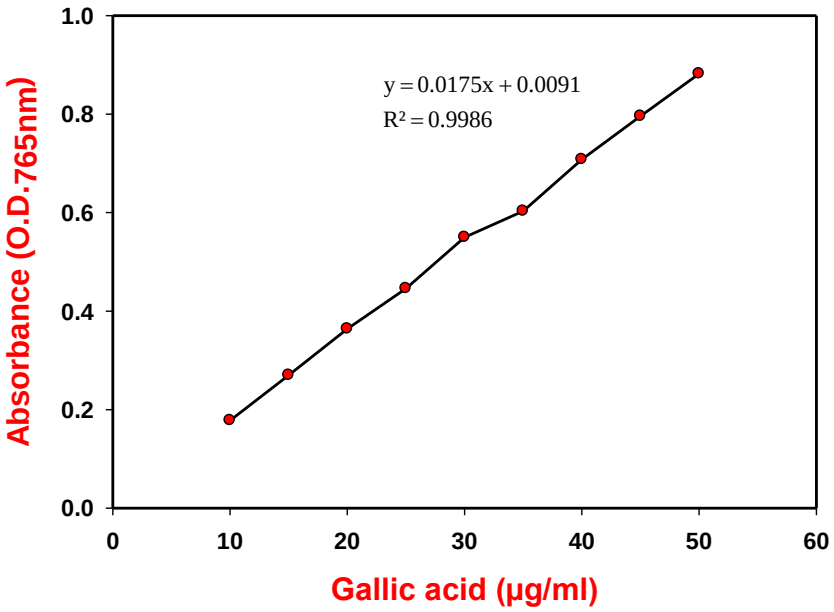
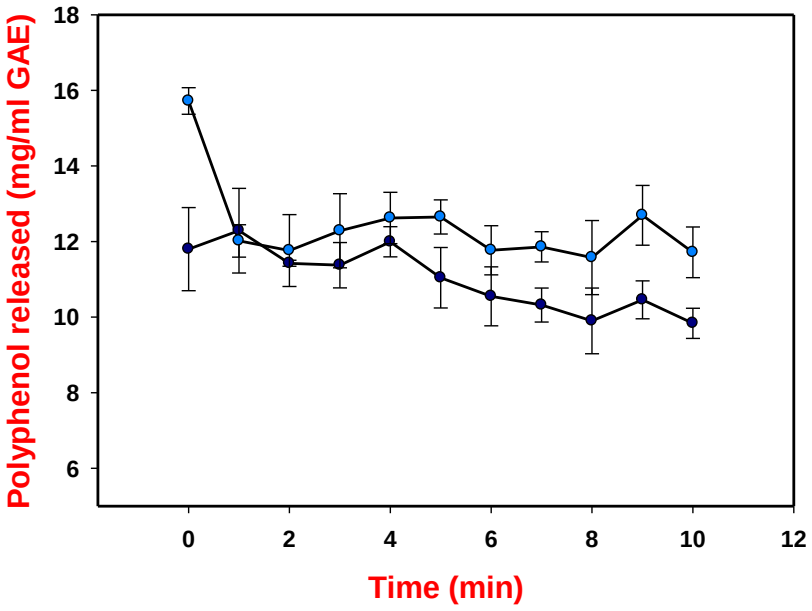


Figure-2. Release of polyphenols from 2 gram of Niger seed meal using *Bacillus* sp. PKD-9 endo-xylanase treatment for up to 10 min.



Defatted Niger seed meal (2.0 g) when incubated with 4000.0 IU of endo-xylanase in 9.0 ml of Tris-HCl buffer (100.0 mM, pH 8.0) at 500C under shaking conditions (100 rpm) for 10.0 min showed good release of polyphenol (15.81 mg/2g GAE) from Niger seed meal (Figure-2). Xylanase treatment increased release of polyphenol when compared to control by 24.89% at the start of reaction itself. However, there was no major difference in polyphenol release between enzyme and control thereafter.

Optimization of polyphenol release from Niger seed meal using endo-xylanase:

Defatted Niger seed meal (1.0 g) when incubated with endo-xylanase (500.0-5000.0 IU) from *Bacillus* sp. PKD-9 in 9.0 ml of Tris-HCl buffer (100.0 mM, pH 8.0) showed higher release of polyphenols when treated with 4000.0 IU/g of xylanase at the start of enzyme treatment. Increasing the incubation time lead to no further increase in release of polyphenols (Figure 3)

Optimization of polyphenol release from Niger seed meal using endo-mannanase:

Defatted Niger seed meal (1.0 g) when incubated with endo-mannanase (50.0-90.0 IU) from *Bacillus* sp. CAM-21 9.0 ml of phosphate buffer (100.0 mM, pH 7.0) showed higher release of polyphenols (9.4 mg/g GAE) when treated with 60.0 IU/g of endo-mannanase for 40 min. at the start of enzyme treatment. Increase in the incubation time lead to no further increase in release of polyphenols (Figure-4). Endo-mannanase treated Niger seed meal showed up to 68.0% higher release of polyphenols when compared to control.

Optimization of polyphenol release from Niger seed meal using synergistic interaction of endo-mannanase and endo-xylanase:

Defatted Niger seed meal (1.0 g) when treated with a combination of enzyme (endo-xylanase:

Figure-3. Release of polyphenols from Niger seed meal by treating with endo-xylanase from Bacillus sp PKD-9 at various levels.

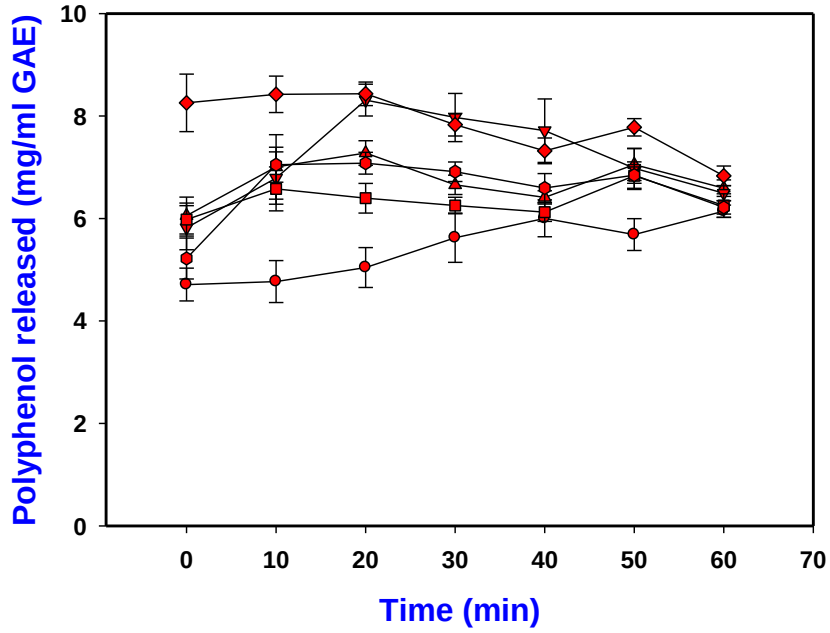
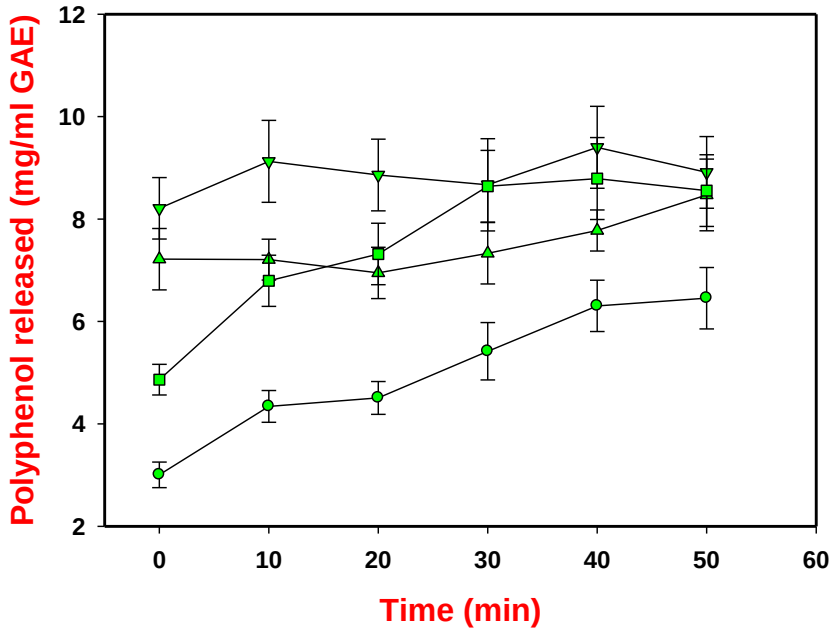
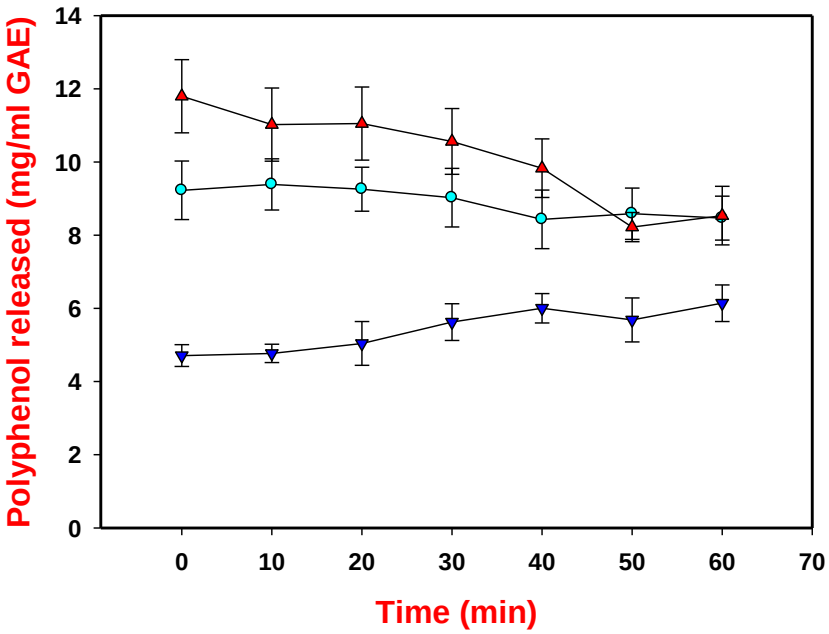


Figure-4. Release of polyphenols from Niger seed meal by treating with endo-mannanase from Bacillus sp CAM-21 at various levels.



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Figure-5. Release of polyphenols from Niger seed meal by synergistic interaction of endo-xylanase and endo-mannanase (Triangle down: Control, Circle: 4000.0 IU of endo-xylanase + 80.0 IU of endo-mannanase, Triangle up: 5000.0 IU of endo-xylanase + 90.0 IU of endo-mannanase.



4000.0-5000.0 IU, endo-mannanase: 80-90 IU: or endo-xylanase: 500 IU, endo-mannanase: 50 IU) at 500C under shaking conditions (100 rpm) resulted in higher release of polyphenols (11.05 mg/g GAE) up to 45.62% when compared to control at the start of incubation period itself. Prolonging of incubation period did not lead to any further increase in polyphenol release (Figure-5).

In the processing of pectic polysaccharide from mangosteen for enhancing extraction of an antioxidant, utilization of pectinase has been reported (Gan et al., 2010). The enzyme at 0.1% w/w increased extraction from 1.7 to 7.4 g/kg of raw material in dry weight.

Conclusion

The results of the present study showed that enzyme-assisted extraction was effective in enhancing the recovery of Polyphenols from defatted Niger seed meal with endo-xylanase and endo-mannanase. Both enzyme preparations proved to be effective in extracting polyphenols. Endo-mannanase was found to be more efficient than endo-xylanase. A higher effect was obtained when both enzymes were combined. In general enzyme assisted extraction is not exploited to its maximum potential within the food industry. Also, a more in-depth knowledge and understanding of the polysaccharide structure of the plant substrate and the use of certain specific enzymes for improved hydrolysis would help the enzyme to reach the active site.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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