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Process Optimization for Recovery of Zein (A Valuable Maize Protein) From Corn Meal and Corn Gluten Meal using Microbial Enzymatic Hydrolysis

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ABSTRACT: In present study Bajaura Makka a corn variety of H.P., India was used for preparation of corn meal (CM) and corn gluten meal (CGM). Both CM and CGM were analyzed for total protein, carbohydrate and fat in the initial substrate and used further for enzymatic treatment individually with protease (*Bacillus* sp.), amylase (*Aspergillus oryzae*) and lipase (*Aspergillus niger*). All enzyme treatments with CM and CGM (100mg/ml w/v) were carried out at pH6.0-10.0 and in all treatments the protein, sugar and zein release was optimum at pH 9.0. In protease treated CM and CGM, 0.174 mg and 0.132 mg of protein, 0.006mg and 0.010mg of reducing sugar was determined with spectrophotometric method. Whereas, in protease treated CM & CGM, 5.01% and 4.81% (w/w) zein respectively was detected with HPLC. Treatment of CM and CGM with amylase resulted in 0.780mg and 0.486mg of protein, 0.326 mg and 0.576mg of reducing sugar and 6.47% and 3.80% of zein release respectively. With lipase treatment 0.202mg and 0.240mg protein, 0.016mg and 0.012mg reducing sugar and trace amount of zein was released respectively from CM and CGM. However, treatment of CM and CGM with mixture of enzymes (protease, amylase and lipase) resulted in increase in yield of zein recovery to 7.14% and 6.20%.

Keywords: Bajaura Makka, corn meal (CM), corn gluten meal (CGM), protease, amylase, lipase, zein, HPLC

INTRODUCTION

A variety of maize crops are grown across the world. Corn gluten meal (CGM) is a valuable co-product of the wet milling process and is used primarily as poultry feed because of its high protein content. Zein proteins were first described by Gorham (1821) after extraction from Indian corn. According to Wilson (1987), the corn endosperm contains the prolamin zein which accounts for 60% of the total protein, glutelins account for 26% of total protein, and albumins and globulins account for 6% of total protein. Pure zein is clear, odourless, tasteless, hard, water-insoluble, and edible, making it valuable in processed foods and pharmaceuticals (Shukla, 1992). Presently the zein recovery from corn meal and corn gluten meal is done using organic solvents extraction using non-green technology. The recovery of organic solvents further add to the overall cost of the process and is the main barrier to the commercial success. Zeinis used in a variety of applications viz., plastics, coatings, inks, chewing gum, adhesives, and fibers etc. (Sturken, 1938; Coleman, 1939; Croston *et al.*, 1945; Lougovoy, 1949; Simonds *et al.*,1949). Most of the zein obtained from CGM is used for food andpharmaceutical coatings (Shukla, 1992).

In the present study a green process using microbial enzymes (protease, amylase and lipase) was used for the treatment of corn meal and corn gluten meal for the recovery of zein.

MATERIAL AND METHODS

A. Preparation and characterization of corn meal (CM) and corn gluten meal(CGM)

A common cultivated corn variety Bajaura Makka obtained from the Department of Crop Improvement, Choudhary Sarvan Kumar Himachal Pradesh Krishi Vishwavidyalaya (CSKHPKV), Palampur, H.P., India was used in the present study. The cornkernels were grinded in local dry millto keep the particle size of corn meal (CM)to 50-100 µm. Corn gluten meal (CGM) was prepared by fermentation of CM with yeast (*Saccharomyces cerevisiae*).

For this a 200g of CM was added to 850ml of distilled water to make total volume to 1000ml.This mixture is stirred gently till it gets solubilised and to this 20 ml of overnight grown 1%(v/v)seed culture of yeast was added and incubated at 30°C for 2 days for the fermentation. After two days the liquid part is separated and the wet corn gluten meal is separated out and dried in a tray dryer at 50°C and made in to a fine powder with grinder and stored for further use.

The CM and CGM prepared above were further evaluated for total proteins (Lowry *et al.,* 1951), total carbohydrates (Hedge and Hofreiter, 1962), total fats (Sadasivam and Manickam, 1992) and water activity using Rotronic Hygrolab Bench type Water activity meter before its further use for treatment with different enzymes.

B. Optimization of treatment of CM and CGM with free enzymes

Three enzymes viz., a protease produced by *Bacillus* sp. APR-4 (Kumar *et al.,* 2002), an amylase produced by *Aspergillus oryzae* (Hi Media) and lipase produced by *Aspergillus niger* (HiMedia) were used for the hydrolysis of CM and CGM for the recovery of zein. The protease activity was determined by Manachini *et al.* (1988), amylase by Miller (1959) and lipase activity by Winkler and Stuckmann (1979) methods. One unit of enzyme activity is defined as an amount of enzyme required to liberate one microgram of respective substrate/ml/min under assay conditions. In this experimental setup, the treatment of CM and CGM were done individually with protease, amylase and lipase enzymes and release of total protein, sugar content and zein was analysed. All enzyme treatments with CM and CGM (100mg/ml w/v) were carried out at pH6.0-10.0. The effect of use of mixture of these three enzymes on treatment of CM and CGM was also evaluated to check the recovery of zein.

C. HPLC analysis of enzyme treated samples for the recovery of zein

The different samples of CM and CGM after enzymatic treatments were analysed for the recovery of zein using Agilent 1200 series HPLC system with chem-station software, version 6.0 containing TSK 250-Biogel (Phenyl-5PW) column with 7.5 X 50mm dimensions. Two mobile phases were used i.e. mobile phase A contained 0.1M potassium sulphate methanolic solution, pH~6(0.1M HCl) and mobile phase B contained 30-50% ammonium sulphate solution with ~5%methanol. The runtime for the analysis was adjusted to 55 min with a flow rate of 0.5 mL/min and detection was done at 280nm wavelength using Diode Array (DAD) UV-VIS detector.

RESULTS AND DISCUSSION

A. Preparation and characterization of CM and CGM

Corn meal (CM) prepared by grinding and corn gluten meal (CGM) prepared after fermentation with *Sacchromyces cerevisiae* is shown in Fig. 1.

(a) (b)

Fig. 1. a). Corn meal (CM) prepared from Bajaura Makka variety corn kernels of H.P. and b) corn gluten meal (CGM) prepared after fermentation with *Saccharomyces cerevisiae.*

The CM and CGM obtained from Bajaura Makka were analysed for its characteristics before its further use for recovery of zein. The initial amount of total proteins, total carbohydrates, total fats and total water activity present in CM and CGM before hydrolysis with different enzymes were determined as discussed in methodology and the results are shown in Table 1.

Table 1: Characteristics of Corn meal (CM) and Corn gluten meal (CGM) prepared from Bajaura Makka variety of corn from H.P., India.

Parameters	Corn Meal (CM)	Corn Gluten Meal (CGM)
Total Protein	0.9%	1.95%
Total Carbohydrates	29%	23%
Reducing sugars	0.08%	0.10%
Total fats	7%	5%
Total Humidity (Equilibrium Relative Humidity)	59.87 % rh	62.3% rh
Water Activity	0.598 aw	0.623 aw

It has been reported that starch forms 62 % dry weight of the corn followed by protein (9-11%), fat (2.5%) and crude fibre (2.0%) (Earle, 1977). Wu *et al.* (1997) reported effect of various factors on yield and composition of zein extracted from commercial corn gluten meal.

B. Optimization of treatment of CM and CGM with free enzymes for recovery of zein

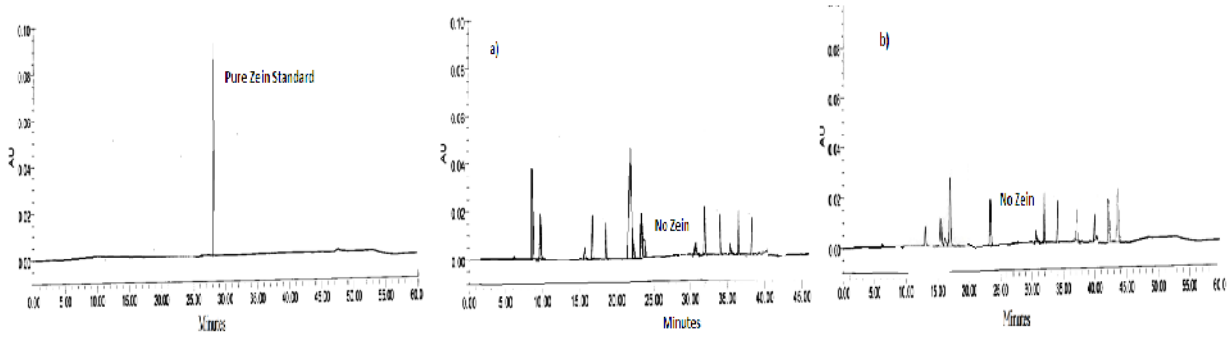
Initial activity of all enzymes used in the hydrolysis of CM and CGM was determined as discussed in Material and Methods. The results of various enzymatic treatments of CM and CGM are shown in Table 2. The initial activity of protease enzyme produced by *Bacillus* sp. APR-4 was 955.2U/ml. After pre-treatment of CM and CGM with free protease enzyme under optimized conditions, 0.174mg and 0.132mg of protein and 0.006mg and 0.010mg of sugar was released respectively. The initial activity in amylase was 1:2000 I.P Units/ml. After treatment of CM and CGM with amylase enzyme under optimized conditions 0.780mg and 0.486mg of protein and 0.326mg and 0.576mg of sugar respectively was released. On the other hand in lipase an initial activity of 16 U/mg was present before use in treatment and under optimized conditions 0.202mg and 0.240mg of protein and 0.016mg and 0.012mg of sugar was released respectively from CM and CGM samples.

However, on treatment of CM and CGM with mixture of all three enzymes under optimized conditions a 0.536mgand 0.062mg of proteins and 0.590mg and 0.122mg of sugar respectively were released.

The pure CM and CGM samples were also analysed for the presence of zein in untreated samples and no zein was detected in pure CM and CGM and the results are shown in Fig. 2. The recovery of zein was estimated with HPLC in all enzyme treated samples and 5.01% and 4.81% zein was determined in CM and CGM sample after treatment with protease enzyme (Fig. 3a). After treatment with amylase enzyme, 6.47% and 3.8% of zein respectively was reported in CM and CGM (Fig. 3b). During treatment with lipase enzyme, a very less amount of zein i.e. 1.05% and 1.27% respectively in CM and CGM was recovered (Fig. 3c). Whereas, on treatment of CM and CGM with mixture of all three enzymes the zein recovery increased to 7.14% and6.2% respectively (Fig.4).

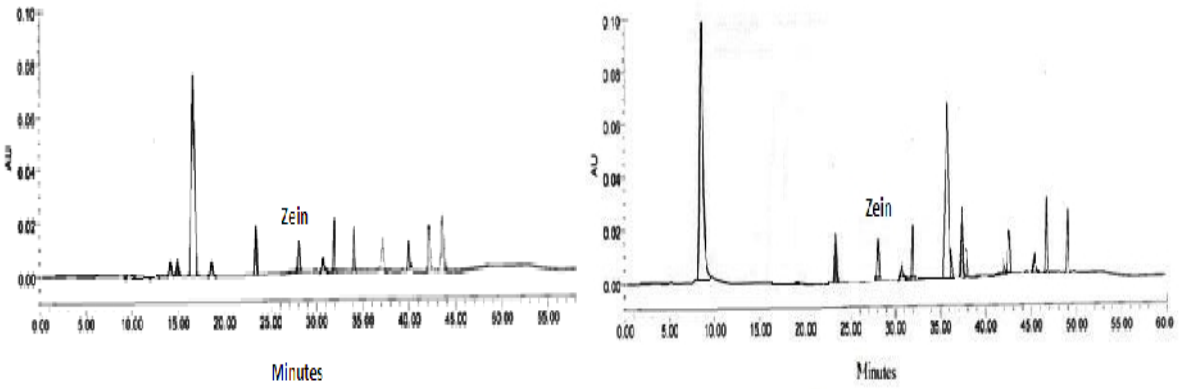
Table 2: Optimization of treatment of corn meal (CM) and corn gluten meal (CGM) with different microbial enzymes for the recovery of zein.

Optimization of conditions for zein recovery using different microbial enzyme treatments							
Microbial Enzymes	**Corn Meal (CM)**				**Corn Gluten Meal (CGM)**		
	pH of treatment	**Protein released (mg/100mg)**	**Sugar released (mg/100mg)**	**Zein released at optimized pH 9.0 (%)**	**Protein released (mg/100mg)**	**Sugar released (mg/100mg)**	**Zein released at optimized pH 9.0 (%)**
Protease	6.0	0.132±0.01	0.002±0.01	5.01	0.086±0.02	0.006±0.01	4.81
	7.0	0.144±0.02	0.004±0.02		0.092±0.01	0.002±0.01	
	8.0	0.114±0.01	0.002±0.01		0.086±0.01	0.004±0.01	
	9.0	0.174±0.03	0.006±0.03		0.132±0.03	0.010±0.02	
	10.0	0.134±0.01	0.004±0.01		0.084±0.01	0.002±0.01	
Amylase	6.0	0.678±0.05	0.320±0.02	6.47	0.444±0.02	0.524±0.01	3.80
	7.0	0.684±0.03	0.246±0.04		0.480±0.01	0.484±0.02	
	8.0	0.756±0.01	0.286±0.01		0.384±0.04	0.418±0.02	
	9.0	0.780±0.04	0.326±0.03		0.486±0.02	0.576±0.06	
	10.0	0.762±0.02	0.264±0.01		0.420±0.05	0.440±0.04	
Lipase	6.0	0.198±0.01	0.016±0.03	1.27	0.198±0.01	0.012±0.01	1.05
	7.0	0.192±0.01	0.010±0.02		0.198±0.03	0.012±0.02	
	8.0	0.198±0.03	0.012±0.01		0.198±0.01	0.008±0.01	
	9.0	0.202±0.01	0.016±0.03		0.240±0.01	0.012±0.03	
	10.0	0.180±0.02	0.008±0.04		0.230±0.02	0.004±0.01	
Enzyme Mixture	9.0	0.536±0.06	0.062±0.03	7.14	0.590±0.05	0.122±0.02	6.20



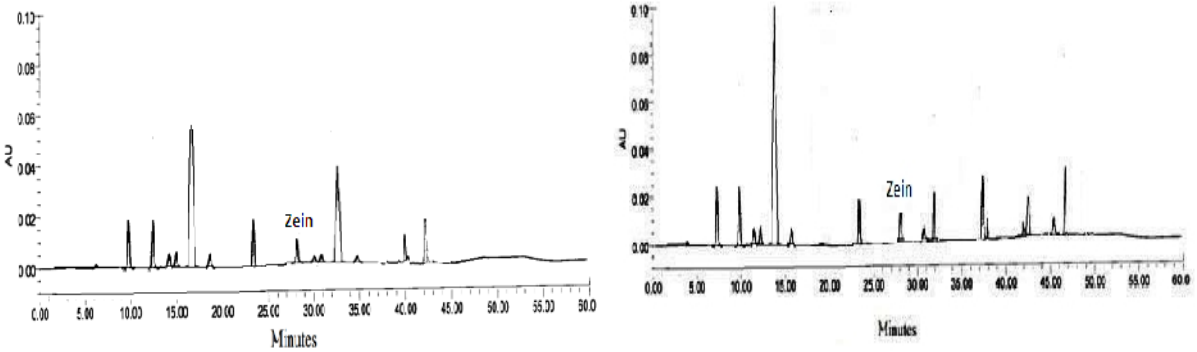
(a) **(b)** **(c)**

Fig. 2. HPLC analysis of pure corn meal (CM) and corn gluten meal (CGM). a) Chromatogram showing pure zein standard and b) and c) detection of zein in pure CM and CGM.



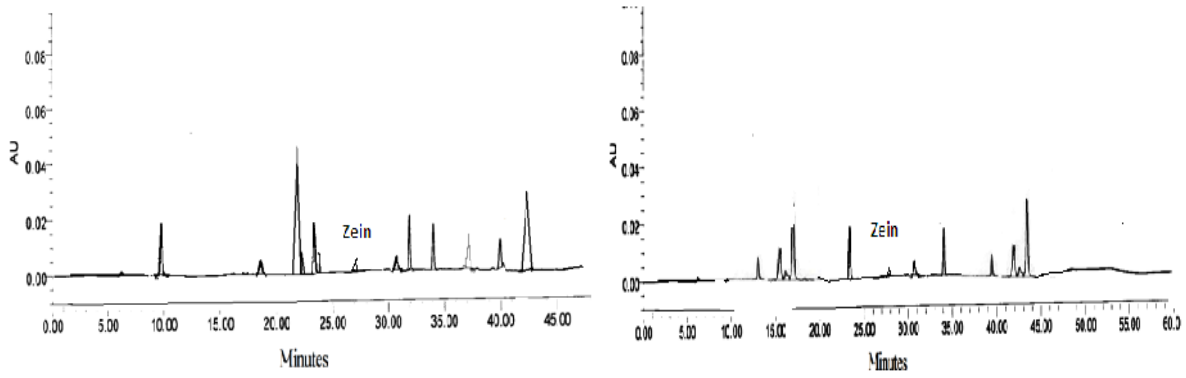
(i) **(ii)**

(A) Protease Treatment



(i) **(ii)**

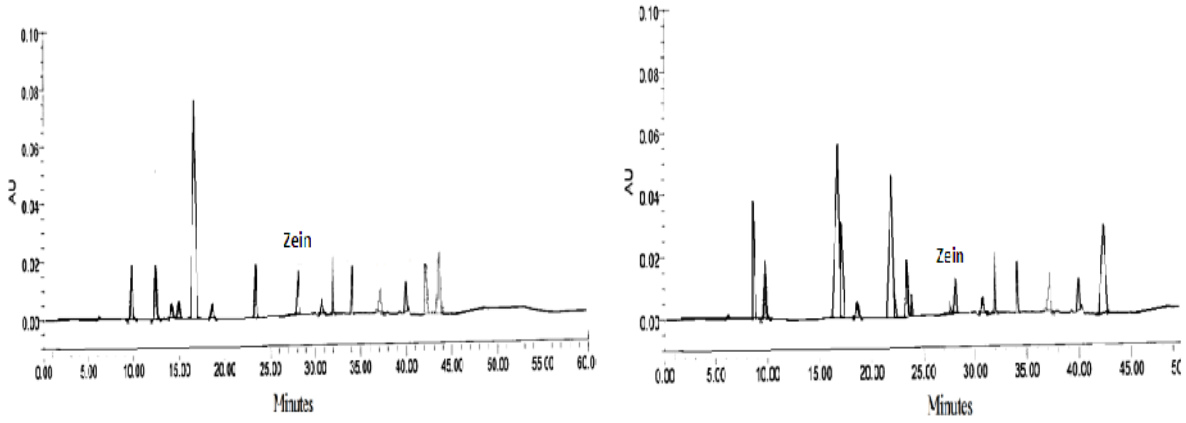
(B) Amylase Treatment



(i) **(ii)**

(C) Lipase Treatment

Fig. 3. Recovery of zein from corn meal (CM) and corn gluten meal (CGM) after treatment with protease (A), amylase (B) and lipase (C) enzymes. Chromatogram showing zein recovery from i) CM and ii) CGM.



(a) **(b)**

Fig. 4. Recovery of zein from corn meal (CM) and corn gluten meal (CGM) after treatment with mixture of enzymes (protease, amylase and lipase). Chromatogram showing zein recovery from a) CM (7.14%) and b) CGM (6.20%).

Dickey *et al.*(1998) described a process to extract zein from maize with 70% ethanol, followed by dilution of the extract to 40% ethanol and centrifugation to separate a solid containing 70-80% protein. At present, zeins are isolated from the corn ethanol co-product distiller's dried grains (Paraman and Lamsal, 2011; Parris and Dickey, 2001). Extraction of zeinis generally done using organic solvents involving non-green chemistry. This classical isolation method still needs a long process which further add to the final cost of the zein and associated pigments recovery. Now, aqueous ethanol is used as the solvent for the entire process of zein and xanthophylls recovery and extraction (Kale and Cheryan, 2009). To overcome these barriers, the commercial enzymes may help in removing the zein proteins from various types of cornstarch (Belles *et al*., 2000).

CONCLUSION

The recovery of zein from CM and CGM was done by treatment with three microbial enzymes (protease, amylase and lipase) individually and the yield of zein recovery increased when treatment was done with mixture of enzymes.

The whole optimized process used in the present study is unique and innovative green process and there is no reports available on use of enzymes for zein recovery in the literature. The reported process completely avoids use of organic solvents for the extraction of zein thus making it a completely green extraction process. Further the work is under process to use immobilized enzyme to improve the reusability of enzyme and reduce the cost of treatment process.

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