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Enhancing anti-diabetic potential of bitter gourd juice using pectinase: A response surface methodology approach

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ABSTRACT

Bitter gourd exhibits significant anti-diabetic activity. In the present study, pectinase concentration (4.04 −15.92 ml kg^{-1}), incubation time (48−191 min) and temperature (31−55 °C) were optimized using response surface methodology (RSM) for obtaining increased juice yield with higher anti-diabetic (α-amylase and α-glucosidase inhibition) activity, total phenolics and antioxidant activity. The optimum conditions i.e. pectinase concentration (10.2 ml kg^{-1}), incubation time (140 min) and temperature (48.8 °C) resulted in juice yield of 82%, α−amylase and α-glucosidase inhibition activity of 23 and 59%, respectively, with total phenolic and antioxidant content of 710 and 198 μg GAE ml^{-1}, respectively. In comparison, control (prepared without enzyme treatment) had significantly ($p < 0.05$) lower juice yield, α−amylase and α-glucosidase inhibition activity of 59, 8 and 17%, respectively. Control samples also demonstrated significantly ($p < 0.05$) lower total phenolic and antioxidant content of 464 and 162 μg GAE ml^{-1}, respectively. RSM optimized juice also demonstrated significantly ($p > 0.05$) higher contents of phenolic acids as compared to control juice.

1. Introduction

Occurrence and prevalence of obesity and associated metabolic disorders such as cardiovascular diseases and type 2 diabetes mellitus are on the rise worldwide. Based on a collective study from different parts of the world, the World Health Organization (WHO) has projected that maximum increase in incidence of diabetes could occur in India, China and US (King, Aubert, & Herman, 1998).

Bitter gourd (*Momordica charantia* L.), an annual climbing plant belonging to the Cucurbitaceae family, is an extensively investigated tropical medicinal plant. Its fruit, also known as balsam pear, bitter melon or *karela* has a distinct bitter taste which enhances as the fruit ripens. Bitter gourd has been popular as a natural remedy in Ayurvedic systems owing to its anti-diabetic, anti-malarial, anti-inflammatory, antiviral, contraceptive and insecticidal properties (Grover & Yadav, 2004). Bitter gourd juice (BGJ) has a rich array of bioactive phytochemicals including vitamins, minerals and phenolic compounds (Horax, Hettiarachchy, & Islam, 2005). Phenolic compounds have been widely reported to have both high antioxidant potential (Wu & Ng, 2008) and anti-diabetic activity (Vinayagam, Jayachandran, & Xu, 2016). Other bioactive molecules in bitter gourd include steroidal saponins, charantin, insulin like peptides, glycoalkaloids, vicine, momorcharin, oleanolic acids, triterpenes, trehalose and momordin (Grover & Yadav, 2004).

There is a need to develop protocols for juice preparation with enhanced extraction of bioactives which is simple, cost effective as well as rapid. Microbial pectinases have been widely used in preparation of vegetable and fruit juices for higher yield, higher clarity, lower viscosity and cloudiness, reduced bitterness and improved nutrient profile (Kashyap, Vohra, Chopra, & Tewari, 2001). It may be hypothesized that the use of pectinase for treating bitter gourd pulp would enhance juice yield and facilitate the release of bioactive compounds.

Response surface methodology is a statistically designed tool which uses minimum number of experiments to provide an optimized response outcome by comparing net interactions of various parameters. Taking into consideration the immense potential of bitter gourd to become a dietary beverage for diabetic and pre-diabetic patients, this study aimed at optimization of parameters (incubation time, incubation temperature and enzyme

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concentration) for extraction of BGJ with desirable responses (yield, total phenols, total antioxidant potential, α-glucosidase inhibition and α-amylase inhibition) using enzymatic pretreatment of pulp. It also attempts to identify and quantify the phenolic content in native and enzyme pretreated BGJ.

2. Materials and methods

2.1. Plant material

Bitter gourd fruits were purchased from a local grower on the outskirts of Mumbai (India). Fruits were brought to the laboratory within 12–14 h after harvesting and immediately stored at refrigerated temperature (10 °C). Selection of fruits was based on maturity (14–16 days after fruit set), free from visual defects, dark green color, 20–25 cm long and pendulous in shape.

2.2. Chemicals and enzymes

Pectinase from *Aspergillus niger* (specific activity 70.4 μKat ml^{-1}, protein concentration 10.6 mg ml^{-1}), α-amylase from porcine pancreas (Type IV-B), α-glucosidase from *Saccharomyces cerevisiae* and *p*-nitrophenyl-α-D-glucopyranoside were procured from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteau's phenol reagent (FC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), standard phenolic acids (gallic acid, protocatechuic acid, gentisic acid, (+) catechin, vanillic acid, caffeic acid, tannic acid, (−) epicatechin, ferulic acid, and *o*-coumaric acid) and HPLC grade solvents (acetonitrile, *o*-phosphoric acid and glacial acetic acid) were also procured from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals and reagents were of analytical grade and obtained from Merck (Mumbai, India).

2.3. Methods

2.3.1. Preparation of bitter gourd juice

Bitter gourd fruits were washed with running tap water to remove all adhering dust. Whole fruit including the rind were diced into small pieces (approximately 3 cm × 3 cm). Pulp was then prepared using a high speed homogenizer (BUCHI Mixer B-400, Switzerland). For each trial, 50 g of pulp was subjected to different treatments according to experimental design (section 2.3.2). At the end of each experimental trial, pectinase was inactivated by heating the pulp in a water bath at 90 °C for 5 min. After cooling to room temperature (24 ± 2 °C) using ice cold water bath, the pulp was immediately centrifuged at 1699×g at 25 °C for 20 min to obtain a clear supernatant. Juice samples were stored at −20 °C till all further analysis to prevent any deterioration or spoilage by microbial contamination.

2.3.2. Experimental design

Experiments were designed and analysis was performed using Design expert 8.0 software (Stat-Ease Inc. USA). A central composite rotatable design was used to study the combined effect of selected independent variables i.e. incubation temperature (X_1), enzyme concentration (X_2) and incubation time (X_3) coded at five levels (-α, −1, 0, +1 and +α). Based on preliminary experiments, the range of variables selected was as follows: incubation temperature: 25–50 °C, enzyme concentration: 0–20 ml kg^{-1} and time: 0–240 min. The natural pH of the juice was maintained at 4.1 which falls in the optimal pH range for acidic pectinases. The complete design consisted of 19 experimental points including 5 replications of centre point (all variables coded as zero) (Table 1). The experiments were carried out in triplicates to ensure repeatability of experiments.

2.4. Determination of juice yield

Juice yield was determined in terms of percentage.

Yield percent = [Juice weight/Total pulp weight] x 100

2.5. Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventós, 1999) with slight modification. In brief, 100 μl of aqueous dilution of juice (20 ml l^{-1}) was added to 650 μl of 1:10 diluted FC reagent (2 mol l^{-1}). The mixture was incubated for 5 min and then 650 μl of

Table 1
Experimental design indicating coded and actual values of independent variables for enzymatic treatment of bitter gourd juice.

Factors				
Standard	Run	X_1 (Incubation Temperature)°C	X_2 (Enzyme concentration) ml kg^{-1}	X_3 (Incubation Time) min
1	8	31.08 (−1)	4.05 (−1)	48.65 (−1)
2	10	48.92 (+1)	4.05 (−1)	48.65 (−1)
3	1	31.08 (−1)	15.92 (+1)	48.65 (−1)
4	14	48.92 (+1)	15.92 (+1)	48.65 (−1)
5	5	31.08 (−1)	4.05 (−1)	191.35 (+1)
6	19	48.92 (+1)	4.05 (−1)	191.35 (+1)
7	12	31.08 (−1)	15.92 (+1)	191.35 (+1)
8	16	48.92 (+1)	15.92 (+1)	191.35 (+1)
9	6	25 (-α)[a]	10 (0)	120 (0)
10	17	55 (+α)[a]	10 (0)	120 (0)
11	15	40 (0)	0 (-α)[a]	120 (0)
12	18	40 (0)	20 (+α)[a]	120 (0)
13	13	40 (0)	10 (0)	0 (-α)[a]
14	7	40 (0)	10 (0)	240 (+α)[a]
15	4	40 (0)	10 (0)	120 (0)
16	3	40 (0)	10 (0)	120 (0)
17	2	40 (0)	10 (0)	120 (0)
18	9	40 (0)	10 (0)	120 (0)
19	11	40 (0)	10 (0)	120 (0)

Actual values for various factors are shown along with their coded form (in parenthesis).
[a] α = 1.682.