Biocatalysis and Agricultural Biotechnology 7 (2016) 95–101

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Effect of enzyme pretreatment on yield and quality of fresh green chilli (*Capsicum annuum* L) oleoresin and its major capsaicinoids

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ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form
17 May 2016
Accepted 20 May 2016
Available online 21 May 2016

Keywords:
Capsicum annuum
Capsaicinoids
Enzyme pretreatment
High Performance Liquid Chromatography (HPLC)
Scanning Electron Microscopy (SEM)
Green chilli

ABSTRACT

The effect of enzyme pretreatment on extraction yield and quality of oleoresin from fresh green chilli (*Capsicum annuum* L) samples and its major capsaicinoids were evaluated using various enzymes viz., Celluclast 1.5L, Pectinex Ultra SP.L, ViscozymeL, Protease and an equal combination of Celluclast 1.5L, Pectinex Ultra SP.L and ViscozymeL. Optimization of the enzyme activity was carried out by varying the enzyme concentration, reaction temperature, pH, and incubation time. The quality of the oleoresin was further evaluated using High Performance Liquid Chromatography (HPLC) coupled with Photo Diode Array (PDA) detector. Scanning Electron Microscopic (SEM) studies were conducted to visualize the effect of these enzymes on green chilli cell walls. It was observed that the capsaicinoids recovery was higher in ViscozymeL pretreated green chilli (22%) followed by chilli pretreated with enzymes Celluclast (20%) Pectinex (17.5%) and Protease (14%) with respect to the control sample. The HPLC finger printing of capsaicinoids showed no alterations in their profile as compared with the control sample. The SEM analysis revealed that the enzyme pretreatment, especially with enzymes ViscozymeL and Celluclast was potentially effective in rupturing the cell walls and making them more susceptible to extraction medium. In economic point of view, the results of the present study could be used effectively in the spice industry to increase the extraction yield and quality of oleoresin from green chilli.

1. Introduction

Chillies belong to the genus *Capsicum* and family solanaceae. The genus capsicum consists of about 22 species in which five are domesticated viz. *Capsicum annuum, Capsicum chinense, Capsicum frutescence, Capsicum pubescence,* and *Capsicum baccatum* (Dias et al., 2013; Wahyuni et al., 2013). India is considered as one among the ten top chilli producing countries in the world and the major chilli producing states in India are Andhra Pradesh (49%), Karnataka (15%), Maharashtra (6%), and Tamil Nadu (3%) which constitute nearly 75 per cent of the total area of chilli cultivation and remaining quantities are contributing by Orissa, Bengal and Madhya Pradesh (Jagtap et al., 2012). As per the latest statistics, India produced 800,100 t of dry chilli from an area of 930,000 ha. India, the largest producer of chillies in the world, accounts for 11 lakh tons of annual production followed by China (around 4 lakh tones) and Mexico and Pakistan (around 3 lakh tons each) (dedicated website http://ffymag.com/admin/issuepdf/Chilli_Mar09.pdf). India exported green chilli oleoresin worth USD 365,633 with total quantity of 2780. United States is the largest buyer of green chilli oleoresin accounting for exports worth USD 185,423 followed by Malaysia and South Korea which imported green chilli oleoresin worth USD 49,476 and USD 46,909 respectively (dedicated website https://www.zauba.com/exportanalysis-green+chilli+oleoresin-report.html). Chilli contains many chemicals of which the two most important groups are the carotenoids and capsaicinoids (Bosland and Votava, 2012). There are studies which showed that *Capsicum* species are the best source of phenolic compounds, flavonoids, capsaicinoids and other bio accessible carotenoids such as β-Carotene, β-cryptoxanthin, capsanthin, antheraxanthin, zeaxanthin, violaxanthin, neoxanthin and lutein (Pugliese et al., 2013; Pugliese et al., 2014; Loizzo et al., 2015). Carotenoids contribute the color and capsaicinoids provide the pungency to the food matrix. Chilli can provide color, pungency, taste and flavour to many cuisines and dishes around the globe, thus it becomes the favorite of the chefs (Zhuang et al., 2012). The pungent component, capsaicinoids of chilli are not only used in food as a flavouring, but also used as an ingredient in pharmaceutical products (Sanatombi et al., 2008).

Modern food industries are equipped with lots of new technologies to manufacture newly demanded products which force them to find new forms of raw materials including natural, nature

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http://dx.doi.org/10.1016/j.bcab.2016.05.010
1878-8181/

identical and artificial flavours. Oleoresins are the natural flavours essentially the concentrated liquid form obtained from spices. The flavours of many spices are due to the presence of phenols, alkaloids, terpenes and other aromatic compounds. These bio ingredients are isolated from plant materials either by solvent extraction, super critical fluid extraction (SCFE), ultra sonication, membrane adsorption, molecular imprinting, or by enzyme assisted extraction.

Enzyme supported isolation is the recent method for recovering bio ingredients from the plant materials. Enzymes pretreatment with the plant materials prior to the traditional method of extraction will help to isolate high yield of bio ingredients. Enzymes such as cellulase, hemicellulase, pectinase, protease or a proportionate blend of these enzymes have been used for the pretreatment of the different raw materials. The action of cellulase and hemi-cellulase make a significant impact on the cell walls of the plant materials. They react with the chemical molecules found in the cell wall and hydrolyse them and eventually the permeability of the cell wall increases and it leads to a better recovery of volatile oil and resin. Some of the reports available in this regard include that of Sowbhagya et al. (2009, 2010a, 2010b, 2011) and Freeze and Binning (1993). Many researchers had identified the effect of enzyme pretreatment on capsaicinoids and carotenoids recovery from the chilli powder. A study conducted by Santamaria et al. (2000) reported that the chilli powder pre-treated with the enzyme Viscozyme L (prepared from *Aspergillus*) followed by repeated ethanol extraction lead in a higher extraction yield of capsaicinoids (7%) than the conventional method. Roman et al. (2008) also reported that the enzymatic extract from chilli powders obtained as a result of *Rhizopus nigricans* metabolism yielded a higher percentage of capsaicinoids (11%). Another study conducted by Sampathu et al. (2006) reported that the pretreatment of chilli with multi enzyme preparation consisting of pectinase, beta glucanase and hemicellulose followed by solvent extraction resulted in a higher yield of capsaicinoids.

Accounting the industrial and agricultural viewpoints, it is of substance to improve the quality and yield of spice oleoresins especially the one like green chilli which is of high demand in food industries due to its high flavour profile. More over compared to the raw green chilli, due to the absence of moisture content in the green chilli oleoresin it can be stored up to three years without quality reduction. Hence it is imperative to develop an alternate method which enhance the extraction yield as solvent extraction alone is inadequate to extract the capsaicinoids completely. Hence, in the present study, enzyme pretreatment of green chilli followed by solvent extraction as an alternate method for the enhanced extraction of oleoresin was studied. Also the effect of enzyme pretreatment on the total capsaicinoids recovery and the distribution of its chemical constituents has been investigated. Scanning electron microscopic (SEM) studies were conducted to scientifically explain the structural difference between green chilli with and without pre enzyme treatment. Variations in the major components of the oleoresin obtained by the enzyme pretreatment of green chilli with control were also taken into account.

2. Materials and methods

2.1. Materials and reagents

Pre mature green chilli was procured from the local market in Kerala, India. Commercial enzymes used in this study, celluclast 1.5L (Cellulase) declared activity 700EGU/g (Endo-Glucanase Units/g). Pectinex Ultra SP-L (Polygalacturonase) declared activity 3800PGNU/g (Polygalacturonase unit) Alcalase 2.4LFG (Protease 2.4 AU-A/g) and ViscozymeL (Beta- Glucanase (endo-1, 3(4)-) declared activity 100FBG/g (Beta-glucanase unit) were procured from Novozymes, Bangalore, India. All the solvents including the HPLC water used for our study were purchased from Merck Life Science Private Limited, Mumbai, India. The reference standards of capsaicinoids, capsaicin (95%) and dihydrocapsaicin (90%) were obtained from Sigma (St. Louis, MO, USA).

2.2. Enzyme pretreatment method

Green chilli from a selected batch was used for all the experiments. Different trials have been conducted for optimizing the concentration, pH, temperature, and incubation time for various enzymes like Celluclast1.5 L, Pectinex Ultra SP.L, ViscozymeL, Protease and their combinations (Mix of first three enzymes in 1:1:1 ratio). In each case 1000 g of green chilli with 65–70% moisture was used for all the experiments. A measured amount of the enzymes (different concentrations of 0.5%, to 2.5% of enzymes were used for optimization) was dissolved in 10 mL of water and sprayed uniformly over the green chilli paste after wet grinding and adjusted the pH 4–6 by adding citric acid. Followed by the incubation for 30–150 min at a temperature between 45–65 °C the wet paste was squeezed for removing the water. The capsaicinoids were recovered by solvent extraction of these squeezed green chilli sample (with moisture content 25–30%). The experiments were repeated in triplicate. Green chilli (1000 g), without enzymatic pretreatment, were wet ground, squeezed and subjected to solvent extraction and the oleoresin obtained was designated as control samples.

2.3. Recovery of capsaicinoids

For solvent extraction, whole green chilli (1000 g) was cleaned and ground to get a uniform paste. Then the wet paste was uniformly mixed with the different concentrations of enzymes 0.5–2.5%) in different trials and loaded in a round bottom flask. Then the mixture was incubated for 30–150 min at 45–50 °C. After incubation, the moisture was removed from the wet paste by applying mechanical force and loaded in an extractor for solvent extraction. Based on the quantity of raw material, 5 times acetone and hexane blend in 1:1 ratio was used for the first wash. After 1 h contact time the miscella was collected through a 20 μm filter cloth. Five more washings were done with the same solvent ratio giving each of 20 min of contact time. After filtration all six washes were combined together and concentrated under reduced pressure. After removing the solvents and moisture the extracts were collected, weighed, and analyzed for getting the yield and capsaicinoids recovery.

2.4. Qualitative evaluation of green chilli oleoresin

2.4.1. Spectrophotometric determination of the unit cap

The unit cap was determined by the already established method (BPC, 1968). Briefly weigh accurately about 5 g of green chilli oleoresin in a 300 mL ground joint flask. After addition of 100 mL of 70% methanol, shake for 30 min. Let the solution settle for 5 min and filter. The first 25 mL of the filtrate is discarded and the rest of the filtrate mixed well. Afterwards, solutions are prepared in 100 mL volumetric flasks as described by Table 1. Mix the solutions well and fill the flasks to 100 mL with methanol. Measure the absorbances A_1 to A_4 of the four solutions at 248 nm and 296 nm. Calculate the concentration of capsaicin from:

Absorbance at 248 nm,

$$\%\ \text{Of capsaicin (a)} = \frac{\left[(A_2 - A_1) - (A_4 - A_3)\right] \times 2500}{314 \times \text{Weight of sample(g)}}$$