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Enzyme-assisted extraction for enhanced yields of turmeric oleoresin and its constituents

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ABSTRACT

In the present study, enzyme-assisted extraction of turmeric oleoresin was carried out using acetone as a solvent. The enzymes used for the pretreatment of turmeric prior to solvent extraction were α-amylase, glucoamylase, and a commercial mixture of enzymes containing cellulase and xylanase. The oleoresins were quantified for the content of curcumin and volatile oil after each extraction. Process parameters such as enzyme concentration, pH, incubation time and time of extraction were optimised to get maximum yield of the extractives. An increase of 13.7% and 22.5% oleoresin and 26.04% and 31.83% increase in curcumin yield were observed on enzymatic pretreatment of the turmeric powder by α-amylase and glucoamylase, respectively. The yield of volatile oil also increased significantly by 70% and 53.75% after α-amylase and glucoamylase pretreatment suggesting the feasibility of this process.

1. Introduction

Whole spices generally have flavouring material locked within the cellular structure provided by nature. This structure could be disrupted by numerous grinding techniques to release the flavour. Ground spices have a limited shelf life, and are also prone to microbial contamination as well as insect infestation. Hence oleoresins and/or essential oils are formulated as alternatives to the spices (Deline, 1985). Turmeric is primarily used in the form of powdered rhizomes for the colouring and flavouring of foods (Buescher & Yang, 1996). Turmeric oleoresin represents a concentrated form of the spice and possesses high replacement values. Generally, 3.86 kg of turmeric oleoresin is equivalent to 45.5 kg of freshly ground turmeric in colour enhancing value and other sensory attributes. Its odour is described variously as earthy, spicy, warm, irritating, orient like and heavy, while flavour is bitter, pungent, aromatic, spicy, metallic and earthy (Farrell, 1985).

The use of turmeric oleoresin is becoming increasingly important in developed countries where processed food industry has made phenomenal progress. Besides convenience in processing (such as mixing, uniformity of flavour and economy in use), freedom from microbial contamination is very significant in processed foods such as frozen-stored, ready to eat, and other convenience foods and food adjuncts. Turmeric is essentially used in ready-to-eat preparations for its colour. Although it is used in fewer foods compared to other spices, it is used exclusively and in large amounts in some formulations. Turmeric oleoresin chiefly functions as food colour, and in some products, it imparts a characteristic mild spicy aroma compatible with mustard, pickles, and relish formulas (Govindarajan, 1980).

Curing is an important post-harvest operation that affects the yield of active principles in the extractives from turmeric. It ensures even distribution of colour in the rhizomes. In commercial practice, different methods are adopted without

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proper control over the curing process. Trials have indicated that boiling of rhizomes in water for 1 h give a satisfactory product as compared to the commercial methods involving prolonged boiling. Boiling the turmeric rhizomes ensures an even distribution of colour, results in quicker drying, and hardens the rhizomes due to gelatinization of starch. This property makes the cured samples less susceptible to insect attack as observed in storage trials of cured and uncured samples. As opposed to mechanical drying, sun drying of slices shows surface bleaching with the core having a darker colour than the rind (Govindarajan, 1980).

Turmeric oleoresin commonly contains 30–45% curcuminoids pigments and 15–20% volatile oil. The amounts of individual curcuminoids vary considerably, although typically the amount of curcumin is more than demethoxycurcumin (Balakrishnan, 2007). In some oleoresins, demethoxycurcumin and bisdemethoxycucumin may account for as much as 50% of the total curcuminoid content. Sesquiterpenoids are the predominant components of the volatile oil extracted from turmeric rhizomes. While many components have not been identified, *ar*-turmerone, turmerone, and curlone are known to be the major components in turmeric volatile oil. Further refinement of the pigments through additional processing steps may be done to eliminate volatile oils that may influence flavour in some food products. The volatile oil derived from the tubers of *Curcuma longa* L. is an orange-yellow, occasionally slightly fluorescent liquid with an odour reminiscent of the tubers (Balakrishnan, 2007). It is rich in turmerones and zingebrene signifying its importance in flavouring and aromatic compounds and its use in confectionary and in aerated water industry. Turmeric oil with its pleasant aroma also exhibits antiseptic properties in toilet soaps (Rankhunde, Munjal, & Patil, 1998).

Turmeric oleoresin is extracted by conventional methods such as solvent extraction, hydro-distillation (Braga, Leal, Carvalho, & Meireles, 2003), steam distillation (Manzan, Toniolo, Bredow, & Povh, 2003), and also by using supercritical carbon dioxide (Gopalan, Goto, Kodama, & Hirose, 2000). The application of enzymes have been found to be advantageous for the extraction of oil from corn, soybeans (Jung, Maurer, & Johnson, 2009), coconut, and peanut (Sowbhagya & Chitra, 2010; Sharma, Khare, & Gupta, 2002) over traditional methods. Various enzyme combinations are used to loosen the structural integrity of botanical material and enhance the extraction of the desired flavour and colour components. Liu, Du, Yuan, and Zhu (2009) successfully extracted silybin (a standardized extract of flavanolignans) with the help of enzymes. Sharma, Khare, and Gupta (2001) used mixtures of protizyme (protease, α-amylase and cellulase) for the extraction of rice bran oil, while Zu et al. (2009) used this technique for the extraction of paclitaxel and related taxanes from needles of *Taxus chinensis*.

It was envisaged that enzymatic pretreatment of the spice matrix could release the trapped oleoresin that is not extracted by conventional solvent extraction. In this study, enzyme-assisted extraction of turmeric oleoresin was done using acetone as a solvent after different enzymatic pretreatments. The oleoresins so obtained were quantified for curcumin and volatile oil content after each extraction. Various process parameters such as enzyme concentration, pH, incubation time and time of extraction were optimised for maximising the yield of extractives.

2. Materials and methods

2.1. Materials

Turmeric fingers of 'Salem' variety were procured from APMC, Vashi, Mumbai, India. Standard curcumin was provided by Samilabs Limited, Bangalore, India as a gift sample. Methanol for spectroscopy was procured from Merck, India. Acetone (AR grade) was procured from S. D. Fine Chemicals Limited, Mumbai, India. All the enzymes used in this work were provided as gift samples by Lumis Biotech, Mumbai, India.

α-Amylase activity was expressed in terms of dextrinizing unit (DU). One unit of enzyme activity was defined as the quantity of α-amylase that dextrinized soluble starch at a rate of one gram per hour at 30 °C. The α-amylase used in present study had an activity of 3×10^5 DU/ml.

One international unit (IU) of enzyme activity was defined as the amount of enzyme that released 1 μmol of product per min (glucose equivalents for glucoamylase, and xylose+glucose equivalents for xylanase+cellulase mixture). The assay for glucoamylase was based on liberation of p-nitrophenol from p-nitrophenol-alpha-glucopyromoside at pH 4.3 and 50 °C. Glucoamylase and xylanase+cellulase mixture used in the present study had an activity of 1×10^5 IU/ml. The major fraction of dried turmeric is carbohydrate which consists of starch (44.15% w/w), fibres (8.85% w/w) and free sugars (10.05% w/w) (Leonel, Sarmento, & Cereda, 2003). The enzymes amylase and glucoamylase were selected for starch hydrolysis while xylanase+cellulase mixture was used for fibre breakdown.

2.2. Methods

2.2.1. Extraction of turmeric oleoresin

Turmeric fingers procured from the local market were powdered, sieved through 40 mesh sieve to get particle size <425 μm and stored in air-tight plastic containers in a freezer (−10 °C) to avoid loss of volatiles and uptake of moisture. Particle size <500 μm is desirable for good extraction efficiency. Aqueous enzyme pretreatment was given to turmeric powder before extraction as follows.

Accurately weighed 50 g of turmeric powder was taken in a 250 ml conical flask. Fifty millilitres of McIlvaine's buffer pH 4.5 was then added to the conical flasks. An enzyme solution of different concentrations (0–5% w/w of turmeric powder) was then added along with 125 ml of distilled water. Water is a poor solvent but is required for the enzymatic treatment prior to extraction. The flask was sealed with non-adsorbent cotton and kept on a shaker at 180 rpm at room temperature (32 ± 2 °C) for different incubation times (2.5–10 h). The paste obtained after enzyme treatment was dried at 60 °C for 12 h to get a powder having a moisture content of about 8%. After drying, the enzyme treated turmeric powder was again ground to get uniform particle size (<425 μm). This powder was then extracted with acetone in a Soxhlet apparatus (70 °C, 400 ml) for varying time periods. The extract was cooled and then concentrated by rotary vacuum evaporator (250 mbar, 40 °C). Further, concentration of the extract was done in an evaporating dish on a water bath at 60 °C. A