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Analytical Methods

Enzyme-assisted extraction of volatiles from cumin (*Cuminum cyminum* L.) seeds

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ABSTRACT

The effect of various enzymes on the extraction of the volatile oil of cumin (*Cuminum cyminum* L.) is reported in the present study. The oil yield, after pre-treatment of cumin seeds with cellulase, pectinase, protease and Viscozyme, was in the range 3.2–3.3% compared to 2.7% in a control sample. Profiling of the cumin oil by GC–MS showed that the total hydrocarbon content was 63.7%, 66.1% and 70.1% in control, cellulase and Viscozyme treated samples, respectively. However, there was no change in the content of cuminaldehyde, the principal flavour-impact constituent, in any of the volatile oils. The study demonstrated that enzymes facilitated the extraction of cumin oil with increase in oil yield, with little change in either flavour profile or physicochemical properties of the oil.

1. Introduction

Cumin (*Cuminum cyminum* L.), an important commercial seed spice, belongs to the family Apiaceae and is widely used as a spice and a seasoning in food. Cumin seed is used as a flavouring agent, either as a whole seed or as the ground powder, often mixed with other spices in curry powders. India is the world's largest producer and consumer of cumin. Turkey, Syria, China, USA, Indonesia and Iran are the other producers of cumin. India's production was estimated around 120,000 tons during 2008–2009, of which 31,000 tons was exported (Spices Board Statistics, 2008). Cumin contains 3–4% of volatile oil, which is extracted by steam distillation or hydro-distillation Borges & Pino, (1993). The main constituents of cumin seed oil are cuminaldehyde, β-pinene, γ-terpinene, *p*-mentha-1,3-dien-7-al and *p*-cymene Varo & Heinz (1970a, 1970b). Cumin seed oil from China was found to contain cuminaldehyde (36.3%) and cumin alcohol (16.9%), as principal components along with γ-terpinene (11%), *p*-cymene (9.8%) and β-pinene (7.7%), with a relatively high concentration of safranal (10.8%) (Li & Jiang, 2004). Flavour compounds present in the volatile oil of cumin extracted by super critical fluid extraction of cumin seeds and effect of feed rate and temperature on grinding of cumin on the flavour compounds is reported (David, Brendan & Nick 2001; Singh & Goswami, 1999). Volatile oil obtained from seeds is used in the perfume and pharmaceutical industries (Lewis, 1984). Cumin seeds extract is reported to exhibit antioxidant activity (Krishnakantha & Lokesh, 1993), anticancer effect, a protective effect against induced colon cancer in mice (Gagandeep, Dhanalakshmi, Mendiz, Rao, & Kale, 2003), hypolipidaemic effect (Dhandapani, Subramaniam, Rajagopal, & Namasivayam, 2002) and antimicrobial activity (Iacobellis, Cantore, Capasso, & Senatore, 2005).

Volatile oil recovery from plant materials is generally carried out by solvent extraction, hydro-distillation or steam distillation. Sangani, Patel, & Golakia (2005) have underlined the importance of particle size of ground cumin on the yield of volatile oil by hydro-distillation. Of late, the use of enzymes for flavour extraction from a few spices, e.g., fenugreek, pepper, mustard, chilli and citrus peels, has been reported. Application of enzymes, in the case of fenugreek seeds, to get a flavorant in liquid, as well as powder, form has been patented (Blank, Jaeger, & Zurbriggen, 2000). Enzymatic treatment of pepper with a combination of cellulase and pectinase results in pepper extracts with good sensory compositional properties (Freese & Binning, 1993). Treatment of mustard seeds with cellulolytic enzymes results in an increase (20–30%) in the yield of oil (Dobozi, Halasz, Kovacs, & Szacks, 1988). Enzyme preparations of cellulase, hemicellulase and pectinase have been used for the treatment of chilli and an increase in the yield of carotenoids (11%) and capsaicinoids (7%) has been reported (Santamaria

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et al., 2000). Enzymatic treatment of chilli fruits has been shown to result in improved extraction yields of carotenoids and capsaicinoids (Salgado-Roman et al., 2008). Application of enzyme preparation containing hemicellulolytic, pectolytic and polygalacturonase activities to recover oil from citrus peel has been reported (Coll et al., 1995). Pre-treatment of garlic with cellulolytic enzymes has been reported to increase the yield of volatile oil by twofold with little change either in flavour profile or physicochemical properties of the oil (Sowbhagya, Purnima, Florence, Appu Rao, & Srinivas, 2009). Enzymatic pre-treatment of celery seeds also results in 22–27% increase in the yield of volatile oil (Sowbhagya, Srinivas, & Krishnamurthy, 2010). Application of enzymes to ginger and garlic for the extraction of volatile oil results in increase in the yield of oil by 50% (Shamala et al., 2009). In recent years, there has been an increase in demand for these oils and oleoresins in export market.

It has been generally observed that when spices are subjected to hydro-distillation, the yield of oil is higher compared to steam distillation. However, steam distillation is commonly employed in industries where the de-oiled material is subsequently used for resin extraction by solvent. In order to get an oil yield by steam distillation similar to that of hydro-distillation, the role of enzymes has been explored in the present investigation. While enzyme application to the plant materials mentioned above has resulted in enhanced yield of volatiles and major active components, little work has been carried out on enzyme application to cumin for flavour extraction. An Indian patent (285/Del/2009) has been filed on enzyme application to a cumin and oleoresin preparation (Sowbhagya et al., 2009). Cumin oil is a highly value-added product in terms of the export market. To obtain a higher yield of oil and to overcome the problem of powdering of cumin seeds, flaking of cumin seeds has been adopted (Sowbhagya, Satyendra Rao, & Krishnamurthy, 2008). In the present study, cellulolytic enzymes, single or as a mixture of enzymes, were used for the pre-treatment of cumin, which was then subjected to steam distillation and hydro-distillation. Also the effect of enzyme pre-treatment on the yield and quality of the oil, in terms of flavour profile, by GC–MS, was studied in comparison to the oil obtained by the conventional method.

2. Materials and methods

2.1. Materials

Cumin was purchased from a local market in Gujarat, India and solvents used were of Analytical grade from Merck India. Commercial enzymes used in the study, cellulase (activity of 4000 U/ml), protease (activity of 1100 U/g) and pectinase (activity of 300 U/ml), were procured from Biocon, Bangalore, India. Viscozyme, with a pectin-solubilising activity of 5000–12,000 U/g and fungal β-glucanase activity of 50–120 U/g was procured from Novozymes, Bagsvaerd, Denmark.

2.2. Enzyme pre-treatment

For all the experiments, the same batch of cumin seeds with a moisture content of 9% was used. Cumin seeds (200 g) were sprayed with aqueous solutions of five enzymes in different concentrations, viz., 0.1, 0.2, 0.5, 1.0 and 2.0% (w/w) individually. Enzymes were dispersed in 20 ml of water in each case and sprayed uniformly on cumin seeds followed by adjustment of pH of the material to 4.5–5.0 with 0.1% citric acid solution (~10 ml). The material was thoroughly mixed and incubated at 45 ± 2 °C for a period of 30–120 min. The spice was dried in an oven at 45 ± 2 °C for 1 h. This dried material was powdered to pass through a 20 British standard sieve (BSS) mesh size and subjected to steam distillation (Nambudiri, Lewis, Rajagopalan, & Natarajan, 1968). In a control experiment, cumin seeds (200 g), without enzyme pre-treatment, were powdered and subjected to steam distillation, a normal procedure followed by spice oil industries. Also in another experiment, cumin seeds were treated with only water and citric acid followed by incubation, drying, powdering and steam distillation.

2.3. Volatile oil extraction

Cumin seeds (200 g) were powdered in a domestic mixer and packed into a glass column and steam was passed, from the bottom, through the bed. The column was attached to Clevenger trap and subjected to distillation for 3 h. The volatile oil collected in the trap was collected and yield of oil obtained expressed as a percentage (v/w).

2.4. Physicochemical quality of the oil

The refractive index values of cumin oils obtained from control and enzyme-pre-treated cumin seeds obtained by steam distillation were measured using an Abbey refractometer; optical rotation was measured using a polarimeter (Perkin Elmer 243, Waltham, MA) and specific gravity using a pycnometer.

2.5. Gas chromatography

Cumin oils obtained by stream distillation of control and enzyme pre-treated samples were analysed by GC. GC analysis was carried out using a Shimadzu 015-A gas chromatograph (Osaka, Japan) with a packed column (SE-52 on Chromosorb B; 10′ length, 1/8′ i.d.,) with a temperature program: initial temperature 75 °C, raised to 180 °C at the rate of 5 °C/min and raised to 200 °C at the rate of 2 °C/min, with injector at 150 °C, detector at 210 °C and nitrogen gas flow of 30 ml/min. The oil (0.05 ml) was diluted in acetone (1 ml) and 1 μl was injected into the GC.

2.6. GC–MS analysis

Cumin oil obtained by enzyme pre-treatment and control were analysed by GC–MS in a Shimadzu GC-17 QP GC–MS system, with an SPB-1 column of 30 m length, 0.25 mm i.d., with 0.25 μm film thickness. Injector temperature was 150 °C, oven temperature was 40 °C (4 min) raised to 180 °C at the rate of 2 °C/min, and raised to 210 °C at the rate of 4 °C/min, with detector temperature at 250 °C. Ion source temperature was at 200 °C. The flavour compounds in the cumin oil were identified by GC–MS by mass fragmentation pattern and spectral comparison with standards in the Wiley Library.

3. Results and discussion

3.1. Optimisation of incubation time

Enzyme-treated cumin seeds were subjected to different periods (30–120 min) of incubation. The effect of different times of incubation on the yield of cumin oil is given in Fig. 1. The treatment of cumin with cellulase, resulted in higher yield of oil (3.2%) for incubation over periods of 30, 60 and 90 min with only a slight increase in oil yield (3.3%) for incubation over 120 min (Fig. 1a), as compared to control (2.7%) without the enzyme treatment, which is not significant statistically. Using Viscozyme, 90 min of incubation was found to afford optimum oil yield of 3.2% (Fig. 1e). Optimum incubation period was 60 min for experiments with hemicellulase, protease and pectinase (Fig. 1b–d). These results demonstrated that an incubation period of 60 min was ideal for all the enzyme treatments (Fig. 1a–e).