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Effect of enzymes on extraction of volatiles from celery seeds

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ABSTRACT

Celery (*Apium graveolens* L.), belonging to the family Apiaceae, is widely used as a spice, in perfumery and pharmaceutical applications. It is reported to possess several nutraceutical attributes, such as anticoagulation activity of blood plasma and prevention of cardiovascular diseases. Effects of various enzymes on the extraction of volatile oil of celery are reported in the present study. The oil yield, after cellulase, pectinase, protease and viscozyme pretreatment, was in the range 2.2–2.3% as against 1.8% in a control sample, by steam distillation. Profiling of the celery oil thus obtained by GC–MS showed that limonene, the major terpene, increased from 63% to 82% with enzyme treatment. The other major flavour compounds identified were β-selinene (16–17%), butyl phthalide and sedanolide. The study demonstrated that enzymes facilitated the extraction of celery oil with increase in oil yield with little change in either flavour profile or physicochemical properties of the oil.

1. Introduction

Celery (*Apium graveolens* L.), an important commercial seed spice, belongs to the family Apiaceae and is widely used as a spice and a seasoning in food. India produces 40,000 tons of celery and exports 29,250 tons (Spices Board, 2008). It is grown commercially in the USA, France and other parts of Europe. In India, it is grown in Punjab. Celery contains 1.5–2.0% of volatile oil, which is extracted by steam distillation or hydro-distillation. Volatile oil contains mainly about 60–70% of (+)-limonene and 1–2% of β-selinene. The characteristic celery odour is due to oxygenated compounds present in the oil, namely sedanolide and sedanonic acid anhydride (Bjeldanes & Kim, 1977). Celery seed is used as a flavouring agent, either as a whole seed or as celery salt; the ground powder mixed with salt. Volatile oil obtained from seeds is used in the perfume and pharmaceutical industries (Lewis, 1984). Volatile oil recovery from plant materials is generally carried out by solvent extraction, hydro-distillation or steam 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 resulted in increase (20–30%) in the yield of oil (Dobozi, Halasz, Kovacs, & Szacks, 1988). Enzyme preparations of cellulase, hemicelluase 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 et al., 2000). Recently, enzymatic treatment of chilli fruits has been shown to result in improved extraction yields of carotenoids and capsaicinoids (Salgado-Roman et al., 2008). Pretreatment 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). Also, application of enzyme preparation containing hemicellulolytic, pectolytic and polygalactouranase activities to recover oil from citrus peel has been reported (Coll et al., 1995). Application of enzymes to ginger and garlic for the extraction of volatile oil resulted in increase in the yield of oil to an extent of 50% (Shamala et al., 2003). 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 celery for flavour extraction.

Celery oil is a highly value-added product in terms of the export market. In recent years, there has been an increased demand for these oils and oleoresins in the export market. It has been generally observed that, when spices are subjected to hydro-distillation, the yield of oil is higher than with steam distillation. However, steam distillation is commonly employed in industries where the residual

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material is subsequently used for resin extraction by solvents. To obtain a higher yield of oil and to overcome the problem of powdering of celery seeds, flaking of celery seeds has been adopted (Sowbhagya, Sampathu, & Krishnamurthy, 2007). In order to obtain an oil yield, by steam distillation, similar to that of hydro-distillation, the role of enzymes has been explored in the present investigation. Different enzymes at various concentrations have been evaluated for the pretreatment of celery for the extraction of volatiles from celery.

In the present study, cellulolytic enzymes, single or as a mixture of enzymes, were used for the pretreatment of celery which was then subjected to steam distillation and hydro-distillation. Also the effect of enzyme pretreatment 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

Celery was purchased from the local market in Gujarat, India and solvents used were of Analytical grade from Merck. 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 pretreatment

Celery seeds (200 g) were sprayed with the selected four enzymes, individually, as well mixed aqueous solution and pH of the material was adjusted to 4.5–5.0 with citric acid in water. The material was thoroughly mixed and incubated in an incubator at 45 ± 2 °C for a period of 30–120 min. The spice was dried in an oven at 55 ± 2 °C for 1 h. This dried material was powdered to pass through a 20 British standard sieve (BSS) mesh size of and subjected to steam distillation (Nambudiri, Lewis, Rajagopalan, & Natarajan, 1968). Celery seeds (200 g), without enzyme pretreatment, were powdered and subjected to steam distillation (control sample). In both cases, distillation was carried out for 3 h.

2.3. Volatile oil extraction

Celery 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 celery oils obtained from control and enzyme-pre-treated celery 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

Celery 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 ft length, 1/8 in internal diameter) with a temperature programme of 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

The celery oil obtained by enzyme pretreatment and the 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 celery 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. Optimization of incubation time

The effect of different times of incubation on the yield of celery oil is given in Fig. 1. It can be seen from the graph that, for enzymes cellulase and viscozyme, 60 and 90 min of incubation were optimum (Fig. 1a and b), resulting in a higher yield of oil (2.0%) compared to the control (1.8%). For pectinase (Fig. 1c), 2 h of incubation time was found to be optimum, resulting in higher oil yield (2.0%). In the case of viscozyme (Fig. 1b), the yield of oil was marginally different and 60 min was taken as the optimum incubation time, since there was no difference between 90 (1.9%) and 120 min (2.0%) of incubation.

3.2. Optimization of enzyme concentration

Enzyme pretreatment resulted in 22–27% increase in the yield of oil compared to the control. Cellulase (0.5%), viscozyme (0.5%), protease (1%) and pectinase (1%) gave higher yields of oil. At equal concentrations (0.5%) of different enzymes, cellulase gave the highest yield of oil, followed by viscozyme, protease and pectinase (Fig. 2). Similar results have been reported earlier in the case of garlic with the above enzymes with a twofold increase in yield of volatile oil (Sowbhagya et al., 2009).

3.3. Volatile oil yield

By enzyme pretreatment, an increase in the yield of oil was obtained in the case of single enzyme treatments with cellulase, pectinase and protease and also with the viscozyme mixture of enzymes (Table 1). The oil yield in the case of cellulase, pectinase, protease and viscozyme pretreatment was in the range of 2.2–2.3% as against 1.8% in the control sample by steam distillation. A single enzyme, cellulase at 0.5%, resulted in higher yield of oil, which is better than the combination of enzymes.

3.4. Physicochemical quality of oil

The physical properties of the oil, namely specific gravity, refractive index and optical rotation, of the oil obtained from enzyme pretreatment did not significantly change compared to control samples. The refractive index of the control oil sample was 1.4816 as against 1.4816–1.4820 in different enzyme-treated