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Aqueous Enzymatic Extraction of Oil from Mandarin Peels

Dheeraj MISHRA[1], A.K. SHUKLA[2], A.K. DIXIT[3] and K. SINGH[1]*

[1] Department of Biochemical Engineering and Food Technology, Harcourt Butler Technological Institute (Kanpur-208002, INDIA)

[2] Department of Oil and Paint Technology, Harcourt Butler Technological Institute (Kanpur-208002, INDIA)

[3] Department of Chemistry, V.S.S.D. College (Kanpur-208002, INDIA)

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Abstract: The Mandarin (*Citrus reticulata*) peels were utilized for recovery of essential oil. The essential oil is one of the important ingredients widely used in various food products. The flavedo part of peels of mandarin fruit was utilized for extraction of essential oil by hydro-distillation method and cold pressing extraction method. The peels were pre-treated with enzyme by varying its concentration from 0.1% to 0.3%. Essential oil was recovered from these enzyme treated samples and yield obtained was compared with control samples. By varying the concentration of enzyme in pre-treatment the increase in the yield of essential oil as compared to control samples obtained were up to 15%. This increase in recovery may be due to rupture of oil sacs/glands by enzymatic action, resulting excess release of essential oil from oil sacs. The effect of concentration of enzyme at pH 4.5 and contact time 3 hrs on the yield obtained was studied during extraction.

Key words: essential oil, mandarin, peel oil, citrus reticulata

1 Introduction

Citrus fruits represent the third major fruit in India, next to Mango and Banana. Citrus fruits comprise about 10% of total fruits produced in India (4.57 million tones out of 44.04 million tones) (1). It occupies about thirteen percent of total area under fruit cultivation (0.48 million hectare out of 3.72 million hectare). India produces about five million tones of Citrus fruit cultivated over 0.5 million-hectare area. The production of Citrus fruit in India is about seventy four percent of the world citrus fruit production. India has a huge potential of producing Citrus peel oil i.e. about 2700 metric tones per annum (based on 0.6% recovery of oil from whole fruit), while very small amount (2-3 tones) is being produced presently.

Genus Citrus comprises sixteen species and eight varieties. Among these *Citrus reticulata* (Mandarin) is the most important commercial crop which occupies about 39% of the area under cultivation of Citrus fruit. Citrus flavours are among the most popular fruit flavours for beverages. Citrus fruit comprised of about 25-30% peel. The peel consists of two parts inner part i.e. albedo which is white in colour and outer part i.e. flevedo which is green in colour. Peel oil is confined in oblate to spherical shaped oil glands which is known as oil sacs. The oil sacs are intercellular cavities embedded irregularly at different depth in the flavedo beneath

*Correspondence to: K. SINGH, Department of Biochemical Engineering and Food Technology, Harcourt Butler Technological Institute, Kanpur-208002, INDIA

E-mail: dh_mishra@rediffmail.com

epicarp and hypoderm and above the albedo.

The peel consists mainly of cellulose, hemicellulose, lignin, pectin, pentosans, sugars, glycosides, bitter substances and mineral matter. The oil sacs are neither all located equally deep below the cuticle, nor of the same size. The yield of the oil in cold pressing process based upon pressure/force applied on the peel. The sacs themselves contain not only oil but also tissue fluid, rich in pectin. The product of expression is thus never a simple mixture of oil and water, but a very thin emulsion of all from which oil can be separated (2). As the oil sacs are buried deep inside the flavedo portion of the peel therefore they were digested with xylanases enzymes which facilitated the recovery of oil from oils sacs.

Occasionally essential oil are present in combination with sugar as glycosides and are insoluble in water but freely soluble in alcohol, ether, fatty oils and mineral oils. They are commonly liquid at ordinary temperature many of them are optically active, lighter than water and possess a high refractive index. They are composed of a number of chemical compounds e.g. hydrocarbons, alcohols (straight chain, terpene and phenol) esters, aldehydes, ketones, oxides and lactones and occasionally compounds of nitrogen and sulphur (3). Among the many essential oils one of the most important group is the Citrus oil, of which the peel oil of Mandarin, Orange and Lemon are most widely known and being used. The Citrus oils, which occur in small ductless glands scattered in the skin (flavedo or epicarp) of the Citrus peel are natural flavouring products made up of numerous constituents that give the characteristic odour and flavour associated with the respective fruits. The compounds, which give them their desirable flavour characteristics, may be classified chemically as aldehydes, ketones, esters, alcohols, terpenes and sesquiterpenes (4-6). The primary aroma carriers are oxygenated substances. The terpenes and sesquiterpenes are unsaturated compounds and frequently contribute to the whole odour and flavour spectrum of the oil source.

Major applications of citrus essential oils are found in beverages, bakery productes, ice-cream flavours, confectionery, condiments, cosmetics, soaps and detergents, perfume, paint, rubber, leather and textile industries. Now-a-days Citrus oils are being used as a solvent in pet shampoos. Use of waterless hand cleaners takes advantage of the solvent properties of terpenes in the oil. Other uses of Citrus peel oil are as antimicrobial agent, masquito-larvicide and as insecticide (7).

2 Experimental

The raw material taken for extraction of citrus essential oil was mandarin (*Citrus reticulata*). Fruits were purchased from local market of Kanpur (INDIA). Xylanase enzyme purchased from Biocon India Ltd., Banglore (INDIA) was used for pre-treatment of flavedo portion of peel. Buffer solution was prepared by L.R. grade lab chemical of S.D. Fine chemicals Pvt. Ltd. Boiser, Maharashtra (INDIA), Clevenger apparatus (**Fig.1**) was used for hydro-distillation of peel oil.

The flavedo portion of the peel was scrapped off and crushed into pulp form with the help of mixer. This pulp was filtered and the filtrate was centrifuged at 10,000 rpm with the help of high-speed C-30 cooling centrifuge (Remi Instrument, Bombay, INDIA). The oil was separated and quantity was measured by volume.

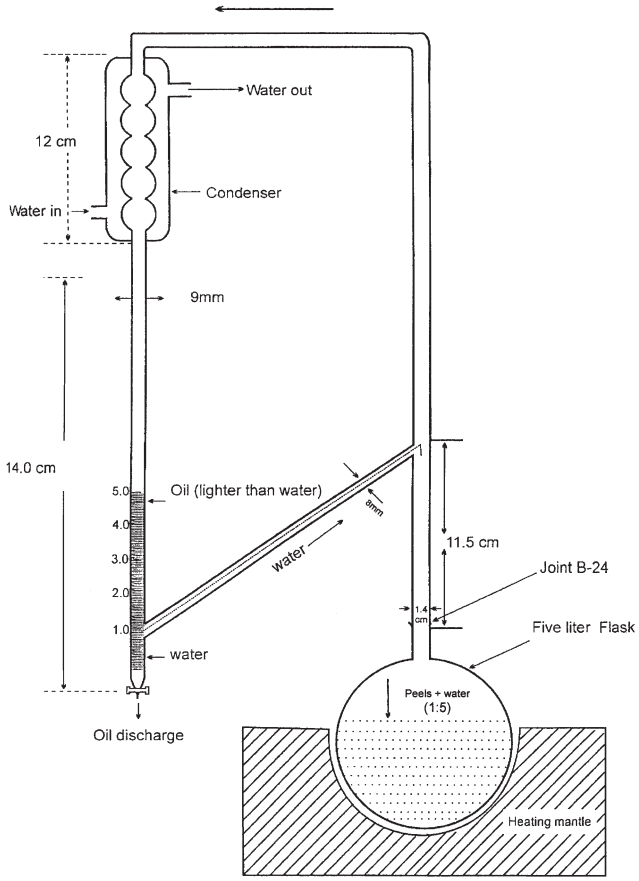

Fig. 1 Clevenger Apparatus.

Pre-treatment of Flavedo Portion with Enzyme

The flavedo portion was scraped off from the fruits and mix thoroughly for uniformity. The mix was divided into eight lots of 75g. each. Out of this four lots were used for oil recovery by hydro-distillation method only.

First lot was dipped in buffer solution of pH 4.5 at temp. 31-33℃ for 3.0 hours and then crushed in the form of pulp by use of mixer and the oil was recovered from the pulp (control sample) by hydro-distillation method with the help of Clevenger apparatus (**Fig. 1**). Remaining three lots were pre-treated with 0.1%, 0.2% and 0.3% Xylananses enzyme at same temperature and time period and oil was recovered by using same method i.e. hydro-distillation. The yield of oil obtained by using different concentrations of Xylanases enzyme were compared with the yield obtained in control sample and improvement in the recovery of oil by enzymatic pre-treatment were determined.

Oil from other remaining four lots was recovered by cold pressing of peel and hydro-distillation of residue. One lot of peel was pre-treated in buffer and converted to pulp as earlier, followed by filtration of pulp to two parts i.e. residue and filtrate. The residue was washed 3-4 times with distilled water and total filtrate was collected in a beaker. Oil was recovered from this with the help of high-speed centrifuge and quantity of oil obtained was measured by volume. Residue obtained by filtration of pulp was hydro-distillated for oil-recovery and total yield of oil from flavedo portion by both method i.e. cold pressing and hydro-distillation was calculated.

Similarly oil was recovered from other three lots after pre-treatment with 0.1%, 0.2% and 0.3% Xylanases enzyme and the total yield of oil (i.e. some of the two yield obtained) was estimated.

These experiments were repeated six times for obtaining optimized results. The quantity of oil recovered was compared and improvement in oil recovery by enzymatic treatment were determined.

Pre-treatment of whole Fruit with Enzyme

Graded and cleaned *Citrus reticulata* fruits were washed with $KMnO_4$ solution and divided in to four equal lots. Each lot were pre-treated separately with Xylanases enzyme at different concentrations followed by scrapping off the flavedo portion and hydro-distillation of product as follows:

(a) Fruits were dipped in buffer solution of pH 4.5 at 31-33℃ for three hours and after this treatment flavedo portion was scraped and oil was recovered by hydro-distillation method.

(b) Fruits were dipped in 0.1% Xylanases enzyme solution at pH 4.5 and temperature 31-33℃ for three hours and after this treatment flavedo portion was scraped and oil was recovered by hydro-distillation method.

Similarly the concentration of enzyme solution was increased up to 0.2% and 0.3% at same pH and temperature for same time. The oil recovered from these lots were noted and compared. The effects of enzymatic treatment were determined.

3 Results and Discussion

Scraped peels were treated with enzyme by varying its concentration from 0.1% to 0.3%. Oil was recovered from these samples and the yield was compared with control samples. The effect of enzyme concentration on recovery of oil by hydro-distillation method are given in **Table 1**. It is apparent from the data obtained that yield of oil in control samples are in the range of 4.53-4.93% only. However, by using Enzyme at various concentration level as 0.1%, 0.2% and 0.3% the yield obtained in the range of 4.86-5.33%, 4.93-5.60% and 5.00-5.66%, respectively. Thus the increase in yield of oil in comparison to control samples are 6.84-9.58%, 8.82-15.06% and 8.69-13.69% at 0.1%, 0.2% and 0.3% concentration level, respectively. From the analysis of data it is clear that there is no remarkable increase in the yield of oil by increasing the enzyme concentration in pre-treatment from 0.2% to 0.3%. The increase in yield of oil is probably due to release of entrapped oil from sacs. It may be due to the action of enzyme on oil sacs resulting more release of oil from it.

The effect of enzyme concentration on yield of oil recovered from filtrate and residue by cold pressing and hydro-distillation method are given in **Table 2**. The recovery from both filtrate and residue was observed to be in the range of 4.46-4.86%, 4.66-5.13%, 4.73-5.46% and 4.86-5.46% for control, 0.1%, 0.2% and 0.3% concentrations of enzyme, respectively. The comparison of these results with the findings of **Table 1** indicates that hydro-distillation method of oil recovery is suitable with respect to cold pressing method for extraction of

Table 1 Effect of Enzyme Concentration on Recovery of Oil by Hydro-distillation Method.

S. no.	Wt. of peel(gm)	Control Sample*		Enzyme conc.* 0.1%			Enzyme conc.* 0.2%			Enzyme conc.* 0.3%		
		Recovery (mL)	%yield	Recovery (mL)	%yield	% increase in comparison to control sample	Recovery (mL)	%yield	% increase in comparison to control sample	Recovery (mL)	%yield	% increase in comparison to control sample
1	75.00	3.65	4.86	3.95	5.26	8.27	4.20	5.60	15.06	4.10	5.46	12.32
2	75.00	3.70	4.93	4.00	5.33	8.10	4.15	5.53	12.16	4.25	5.66	14.86
3	75.00	3.65	4.86	3.90	5.20	6.84	4.10	5.46	12.32	4.15	5.33	13.69
4	75.00	3.65	4.86	4.00	5.33	9.58	4.15	5.53	13.69	4.15	5.33	13.69
5	75.00	3.40	4.53	3.65	4.86	7.24	3.70	4.93	8.82	3.80	5.06	10.00
6	75.00	3.45	4.60	3.65	4.86	7.34	3.85	5.13	11.58	3.75	5.00	8.69

* pH 4.5, Temperature 31-33℃, Pre-treatment time 3hours

Table 2 Effect of Enzyme Concentration on Recovery of Oil by Cold Pressed and Hydro-distillation Method.

S. no.	Wt of peel (gm)	Control*				Enzyme conc.* 0.1%				Enzyme conc.* 0.2%				Enzyme conc.* 0.3%			
		Recovery of oil from filtrate (mL)	Recovery from residue by hydro-distillation (mL)	Total Yield (mL)	Total % Yield	Recovery of oil from filtrate (mL)	Recovery from residue by hydro-distillation (mL)	Total Yield (mL)	Total % Yield	Recovery of oil from filtrate (mL)	Recovery from residue by hydro-distillation (mL)	Total Yield (mL)	Total % Yield	Recovery of oil from filtrate (mL)	Recovery from residue by hydro-distillation (mL)	Total Yield (mL)	Total % Yield
1	75.00	3.25	0.30	3.55	4.73	3.40	0.40	3.80	5.06	3.85	0.25	4.10	5.46	3.80	0.30	4.10	5.46
2	75.00	3.35	0.30	3.65	4.86	3.45	0.35	3.80	5.06	3.30	0.85	4.15	5.53	3.40	0.65	4.05	5.40
3	75.00	3.25	0.40	3.65	4.86	3.45	0.40	3.85	5.13	3.50	0.50	4.00	5.33	3.55	0.40	3.95	5.26
4	75.00	3.30	0.30	3.60	4.80	3.40	0.35	3.75	5.00	3.70	0.35	4.05	5.40	3.75	0.35	4.10	5.46
5	75.00	3.00	0.35	3.35	4.46	3.15	0.35	3.50	4.66	3.25	0.30	3.55	4.73	3.25	0.40	3.65	4.86
6	75.00	3.00	0.35	3.35	4.46	3.25	0.45	3.70	4.93	3.20	0.60	3.80	5.06	3.10	0.75	3.80	5.06

* pH 4.5, Temperature 31-33℃, Pre-treatment time 3hours

Table 3 Recovery of Oil by Hydro-distillation Method (Enzyme Treated whole Fruit).

S. no.	Control Sample*				Enzyme Treated Sample* Conc. 0.1%					Enzyme Treated Sample* Conc. 0.2%					Enzyme Treated Sample* Conc. 0.3%				
	Wt of fruit (gm)	Wt of peel (gm)	Oil yield (mL)	% Oil yield	Wt of fruit (gm)	Wt of peel (gm)	Oil yield (mL)	% Oil yield	% Increase in comparison to control	Wt of fruit (gm)	Wt of peel (gm)	Oil yield (mL)	% Oil yield	% Increase in comparison to control	Wt of fruit (gm)	Wt of peel (gm)	Oil yield (mL)	% Oil yield	% Increase in comparison to control
1	143.05	19.27	0.85	4.41	174.20	17.29	0.80	4.62	4.91	147.71	20.87	1.00	4.80	8.80	176.17	20.05	1.0	4.98	13.09
2	104.75	12.91	0.55	4.26	114.51	11.81	0.55	4.65	9.32	101.01	13.42	0.65	4.84	13.69	115.74	14.24	0.70	4.91	15.39
3	118.49	18.87	0.75	3.97	94.56	11.79	0.50	4.24	6.82	117.56	16.72	0.70	4.18	8.57	89.80	14.90	0.65	4.36	9.88
4	120.42	18.72	0.70	3.73	109.46	13.72	0.55	4.00	7.47	121.32	19.72	0.80	4.05	11.57	101.41	13.17	0.60	4.17	11.96
5	108.72	13.42	0.55	4.09	157.23	14.42	0.65	4.50	10.21	110.72	13.74	0.65	4.73	11.03	170.20	15.02	0.70	4.66	13.94

* pH 4.5, Temperature 31-33℃, Pre-treatment time 3hours

oil, as recovery in this method is higher in comparison to cold pressing method.

Whole fruit was dipped in buffer solution only at pH 4.5 for 3.0 hrs (control) and enzyme solution of different concentration i.e.0.1%, 0.2% and 0.3% for same pH, temperature and time. Oil from flavedo part of peel was recovered by hydro-distillation method. The recovery of oil by hydro-distillation method (enzyme treated whole fruit) are given in **Table 3**. The yield of oil in control sample, 0.1%, 0.2% and 0.3% enzyme pre-treated whole fruit samples were observed in the range of 3.73-4.41%, 4.0-4.65%, 4.05-4.84% and 4.17-4.98%, respectively. The increase in recovery of essential oil ranges from 4.91-10.21%, 8.80-13.69% and 9.88-15.39% by varying the enzyme concentration from 0.1%, 0.2% and 0.3% respectively.

4 Conclusion

From the present study it is concluded that recovery of oil improves after enzymatic pre-treatment upto 0.2% conc. to peels and/or whole fruits. There is no remarkable increase in the % oil yield by varying the enzyme conc. from 0.2% to 0.3%. The yield obtained was slightly higher by using hydro-distillation in comparision to cold-pressing method.

Although the objective set out for this research have been fulfilled, the scale-up studies are required to understand the mechanism(s) of enzyme action on oil sacs for more yield of oil. Research is also required to observe the effect of application of Citrus oil and other vegetable oil for medicinal as well as insecticidal properties. Comparison of Citrus oil with other essential as well as vegetable oils of Cucurbitace family is also required for their insecticidal properties on vegetable crops and food grains storage. This may be useful in replacing use of harmful chemicals to bio-base natural eco-friendly insecticide.

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