Newer Chemical & Technological approaches for the preparation of flavourant in selected spices

Thesis

Submitted to
THE UNIVERSITY OF MYSORE
for the award of the degree of
DOCTOR OF PHILOSOPHY
In
Food Science

By
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NOVEMBER 2006

November 20th, 2006

CERTIFICATE

I hereby certify that the thesis entitled "Newer Chemical & Technological approaches for the preparation of flavourant in selected spices submitted by **Smt H.B. Sowbhagya** for the award of the degree of **Doctor of Philosophy** in **Food Science** to the University of Mysore, India is the result of the research work carried out by her in the Department of Lipid Science and Traditional Foods, **Central Food Technological Research Institute,** Mysore, under my guidance during the period 2002 - 2006.

(Dr. N. Krishnamurthy)
(Research Guide)

DECLARATION

I hereby declare that the thesis entitled, "**Newer Chemical & Technological approaches for the preparation of flavourant in selected spices**" submitted to the University of Mysore, India for the award of the Degree of Doctor of Philosophy in Food Science, is the result of the research work carried out by me in the Department of Lipid Science and Traditional Foods, Central Food Technological Research Institute, Mysore under the Guidance of **Dr. N. Krishnamurthy** during the period 1997– 2004.

I further declare that the results presented in this have not been submitted for the award of any other degree or fellowship.

November 20th 2006.

(**H.B. Sowbhagya**)

Signature of Doctoral candidate

Signed by me on……

Signature of Guide *Date:*

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Signature of Chairperson/Head of Department/
Institution with name and official seal.

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ACKNOWLEDGEMENT

My sincere thanks are due to Dr. N. Krishnamurthy for the valuable guidance for carrying out the work for doctoral thesis reported here.

I am thankful to our Director, Dr. V. Prakash for his encouragement to carry out my doctoral work.

I am thankful to Sri S.R. Sampathu, Retd. Scientist, CFTRI for his constant encouragement for his valuable discussions of the subject while carrying out the doctoral work

I also thank Mr. B. Raghavan for his constant encouragement during carrying out the doctoral work

My thanks are due to Mr. B.V.Satyendra Rao, and GST Dept. for their Help in carrying out the flaking of cumin and celery.

My thanks also are due to Mr. K.Venkatesh murthy and Mr. Jayaprakash, Food Engineering Dept. for their co operation in carrying out pilot plant work on steam distillation.

My thanks are due to Dr. Venkateswara Rao, Mrs. Jyotsna and Mrs. D. Indrani FMBCT Department for their co operation to carry out my investigation work on food application studies.

I am thankful to all my collegues of PPSFT Department for their co operation while carrying out investigation work.

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INTRODUCTION

Flavour is one of the most important factors which governs the consumer's selection of food. Creation and utilization of flavours of highest quality are the factors of major concern in the manufacture and sale of food products. There are several plant materials which can be good sources of flavour. Spices form a unique commodity due to the essential oil content rich in flavour compounds. Though the quantity of essential oil present in them is in small amounts, they are in the concentrated form and give a characteristic flavour depending on the composition of the oil. Spice oils and oleoresins play an important role as source of flavourants. Synthetic flavour compounds are preferred to naturals because of the following reasons (a)naturals are non uniform in composition and flavour strength, (b) mostly unstable and (c) more expensive than synthetics on an equal flavour strength basis .As a consequence of the expanding use of synthetic flavours and synergetic enhancers there is a renewed interest in the research on the composition of natural food flavour and their recovery and newer approach for extraction of flavourant of high quality with good yield to replace the synthetics. India is a major producer and exporter of spices and during the year 2003 – 2004 the production was 28 lakh tonnes and exported about 3.0 lakh tonnes valued at Rs. 170 crores (Spices Board statistics). Fifty two plant materials have been listed as spices under the purview of Spice Board. They are listed in Table 1(Spices Board).

Table 1. List of Spices under the purview of Spices Board

1. Cardamom (Small & Large)
2. Pepper
3. Chilly
4. Ginger
5. Turmeric
6. Coriander
7. Cumin
8. Fennel
9. Fenugreek
10. Celery
11. aniseed
12. bishop's weed
13. Caraway
14. Dill
15. cinnamon
16. Cassia
17. Garlic
18. Curry leaf
19. Kokam
20. Mint
21. Mustard
22. Parsely
23. Pomegranate
24. Saffron
25. Vanilla
26. Tejpat
27. Pepper long
28. Star anise
29. Sweet flag
30. Greater galangal
31. Horse radish
32. Caper
33. Clove
34. asafoetida
35. Cambodge
36. Hyssop
37. Juniperberry
38. Bay leaf
39. Lovange
40. Marjoram
41. Nutmeg
42. Mace
43. Basil
44. Poppyseed
45. All-spice
46. Rosemary
47. Sage
48. savory
49. Thyme
50. Oregano
51. Tarragon
52. Tamarind

Ref : Spices Board

Spices are used in all categories of food and food industry like meat, fish, fruit & vegetable products, bakery products, beverages and convenience foods. Spices in their native forms are not very convenient to be used in all food products. Traditionally, they are used in powdered form for uniform dispersion of flavour but they have the disadvantages of variability in flavour strength and quality, loss of flavour strength during storage, unhygienic and inconvenient to handle. As an answer to these problems, a number of spice products have come into use in the

processed food industry viz., spice oils, oleoresins, encapsulated spices, dispersed spices and emulsions. Spices are classified into (Lewis,1984)

(i) Major spices: pepper, ginger, turmeric, cardamom, capsicum

(ii)Tree spices: cinnamon, cassia, cloves, pimento, nutmeg, mace, star anise

(iii) Seed spices: coriander, cumin, dill, fennel, caraway, celery

(iv) Leafy spices: basil, marjoram, oregano, mint

Table 1. Export of spices from India

Spices	2001-02		2002-03		2003-04		2004-05		2005-06	
	Qty. (MT)	Value (Rs. lakhs)	Qty. (MT)	Value (Rs. Lakhs)	Qty. (MT)	Value (Rs. lakhs)	Qty. (MT)	Value (Rs. lakhs)	Qty. (MT)	Value (Rs. lakhs)
Cumin	17247	14818.03	10422	9326.33	7957	5883.79	13750	10190.00	12000	8800.00
Celery	4251	1236.59	3959	1225.43	4815	1520.33	4100	1300.50	3400	1265.00
Spice oils & oleoresins	4510	37311.10	4838	39094.23	5133	37991.76	5600	46375.00	6225	50000.00

SPICE OIL & OLEORESINS AS FLAVOURANT

Spice oils and oleoresins are value added items derived from spices and accounts for 15 % of total earnings from Indian spices ( ). As whole spices do not provide uniform flavour strength and colour, the spice oils and oleoresins are used in flavour formulations. Use of spice oils and oleoresins have advantage over whole spices in their flavour concentration, solubility, stability,

uniformity and hygienic quality and hence preferred in modern food industries. Spice oils are volatile substances which are mostly terpienic in nature and are obtained by steam or hydro-distillation methods (Pruthi,1984). Volatile oil represents the aroma of the spice devoid of the colour and pungency of the spice. Oleoresins are obtained by organic solvent extraction of the powdered spices and contain the essential oil fraction, bite principles, waxes, colour and resinous matter which act as fixatives for the volatiles. Some of the important uses of spice oils and oleoresins are:

(a) As a flavouring agent in food formulations, beverages, candy, sugar confectionery, cookies etc.,

(b) In cosmetics, toiletries and de-odourant industry,

(c) In medicines as internal and external antiseptics, carminaters and

(d) For the isolation of bio-active principles to use as neutraceuticals.

The volatile oil content of different spices varies accordingly, and it may be as low as 0.1% and as high as 18% (Lewis, 1984). The oil yields of some of the spices are given along with major component. (Table 4).

Spice oil

Spices contain generally 2-3% of essential oil except in the case of clove and nutmeg which are characteristic of the spice (Lewis, 1984). They are volatile and are mainly terpienic in nature with a low concentration of oxygenated compounds. Over the years, numerous procedures have been proposed for the isolation of aromatic compounds (as a source of flavour) from plant materials.

Table 2. Volatile oil (%) and constituents of some spices

Spice	% yield (V/W) of volatile oil	Terpenes & Sesquiterpenes (%)	Important oxygenated compounds present (%)
Cardamom	4 – 5	5 – 7	1, 8 cineole 25 - 45 Terpinyl acetate 35 - 50
Cinnamon	2 – 3	8 – 10	Cinnamaldehyde 60 - 65
Clove			Eugenol 10 – 15
	16 – 18	9 – 10	Eugenol 75 – 80 Acetyl Eugenol 7 – 10
Coriander	0.1 – 0.5	25 – 30	Linalool 60 – 70
Cumin	2 – 3	30 – 40	Cuminaldehyde 35 – 40
Celery	2 – 2.5	60 – 70	Limonene 70 – 80 Pthalides 1
Ginger	1.5 – 2.5	50 – 60	Sesquiterpene Alcohols 15 – 20
Nutmeg	8 – 10	40 – 60	Myristicin 10-12 Linalool 5 – 11
Pepper	2 – 4	90 - 95	Oxides of alcohol sesquiterpenes 5 – 6

Generally, the methods of extraction followed are solvent extraction, hydro distillation, steam distillation, super critical carbon dioxide extraction.

Hydro-distillation

The plant material is powdered and boiled along with water, volatile aroma compounds gets condensed along with the steam. Volatile compounds are separated from water and dried over anhydrous sodium sulphate. Generally, this method is followed at laboratory level to screen the quality of the raw material. Hydro-distillation is not practiced commercially because the distillation time is more and the resulting mass after hydro-distillation is not easily amenable for oleoresin extraction with solvents.

Steam distillation

Cleaned spice with moisture content of 10-12 per cent is ground in a plate mill, to get a coarse powder to pass through 20 mm mesh size (BIS sieve20) . The powdered spice is subjected to distillation as quickly as possible or stored in airtight containers till it is used. A commercial steam distillation unit consists of a stainless steel vessel of 500kg capacity provided with a perforated false bottom, on which powdered material is packed uniformly and loosely without applying any pressure, which helps in the prevention of channeling of steam (Ravindran, 2002). The powder is charged from the top of the vessel and leveled periodically not packed to a full capacity leaving a head space of 40-50cm. The lid at the top is secured and connected to a water cooled condenser of suitable capacity, which in turn is led to an oil water separator. From the bottom, steam is let in slowly which passes through the bed of material. By entrainment, the steam carries along with it the volatile principles of the spice and gets condensed when it passes through the condenser. The condensate enters the oil water separator and the oil floats at the top. When sufficient volume of oil is collected, it is taken out through a trap provided for this purpose draining the condensate water. It is not desirable to leave the oil in contact with water till the end of the distillation, since this could result in the loss of oil due to saturation with water and may cause compositional variation due to differential dissolution of the components. Distillation is continued for longer hours to recover the high boiling fractions which also contribute to flavour of the oil. Early fractions of oil will be rich in low boiling hydro carbons and subsequent fraction will contain oxygenated compounds like ketones, ester etc.

Spice oleoresin

Oleoresin is made up of two components viz.,(i)the volatile oil and (ii)resin. Total flavour of a spice is obtained by mixing both oil and resin. The resin is obtained by solvent extraction. The spice powder is loaded on to the extractor and extracted with a suitable solvent like acetone, alcohol, methanol, ethylene dichloride, hexane, isopropyl alcohol, methylene chloride. Solvent selection is based on criterias like extraction efficiency, toxicity, cost and availability. The resin is made up non-volatile matter like colour, fat, pungent constituents, waxes etc., The total flavour of a spice is obtained by mixing both oil and resin.

The main steps involved in the oleoresin process are:

i. Selection of right type of raw material
ii. Grinding the spice to the optimum particle size
iii. Extraction with a selected solvent
iv. Distillation of the miscella (extract)
v. Blending of finished product

For oleoresin extraction, either fresh ground spice or essential oil free (spice powder from which oil has been distilled off) is used. Spice is ground to coarse powder of particle size 500-700 microns which helps in the rupture of flavour cells and is amenable for ready extraction by solvents. Fine grinding should be avoided since it causes volatile oil loss and creates problems during extraction, like slow percolation of the solvent, channeling and engagement of the extractor for longer periods of time. The spice powder is loaded into extractor (percolator) and extracted with a suitable solvent like acetone, alcohol, methanol, ethylene dichloride, hexane, isopropyl alcohol, ethyl acetate, ethyl methyl ketone, methylene chloride or a mixture of solvents (Ravindran, 2002). Solvent selection is very crucial and should be done on a small scale at laboratory level before venturing into commercial production. Selection of solvent is based on certain

criteria viz., extraction efficiency, boiling point, inflammability, miscibility, with water, residual notes, toxicity cost and availability. The selected solvent is percolated through the bed of material by keeping the bottom drain valve open for the escape of air. When the entire material is soaked with solvent, the bottom drain is closed and sufficient contact time is given for the leaching of the solutes into the solvent. After the contact time, the extract called as 'miscella' is drained and collected.

The "miscella" is carefully distilled to remove the solvent. Most of the solvent (90-95%) is removed by normal atmospheric distillation while the remaining solvent is removed by distillation under reduced pressure. The trace amounts of solvent are removed by azeotropic distillation using innocuous solvent like ethyl alcohol. Alternatively bubbling nitrogen into the thick viscous material is carried out to drive away the residual solvent. The maximum permitted residual limits for some of the solvent in spice oleoresins are: 30 ppm for acetone and chlorinated solvents, 50 ppm, for methyl alcohol and 25 ppm for hexane(CFR, 1995)

After completion of the solvent stripping, the product, while hot, is discharged from the bottom of the still and stored in suitable containers viz., oleoresin is stored in aluminium or stainless steel containers, Epoxy –coated drums and food grade high molecular weight high density polyethylene containers are also being employed for storage and export.

The finished product (oleoresin) is a dark viscous liquid. The resin obtained is mixed with separately distilled essential oil to achieve a balance between pungency and aroma. Since oleoresins are too viscous and concentrated to be

used as such in flavour blends or food products, it is customary to disperse them in on solid or liquid media to dilute them for easy application to foods.

Standards for spice oleoresins have been given by the Essential Association of America. Other organizations like the Bureau of Indian Standards and the International Standards Organizations have also prescribed such standards for spice oil (physico-chemical properties) and for spice oleoresins including solvent residues.

Quality Evaluation

Ideally, the quality of spice oils should be assessed organoleptically by experienced, well-trained panels. But this method is slow and impractical when quick evaluations are needed by industry. Physico-chemical properties like specific gravity, solubility, refractive index and optical rotation are used to characterize essential oils, but they do not give complete information on the quality. Thin layer chromatography, gas-liquid chromatography, infrared spectroscopy, mass spectrometry, etc., are modern methods used in the objective assessment of quality of essential oils.

Super critical carbon dioxide extraction

This method involves using carbon di oxide gas above its critical temperature and pressure for extraction of flavour. In critical state carbon di oxide exhibits physiochemical properties intermediate between liquid and gas, At constant reduced pressure its solvent power increases and thus techniques is used for the extraction of various food materials, so that the resulting component is rich in top notes. Super critical Fluid Extraction (SCFE) is employed for difficult

separation processes based on high degree of relativity, low volume and high valued products.

The use of carbon di oxide for flavour extraction has several advantages over the traditional methods using other solvents like carbon di oxide is abundantly available, cheap, non-inflammable, non-toxic, non-corrosive and do not cause any environmental pollution and also solvent residues is absent in the product. It behaves either as a polar or non-Polar solvent depending on pressure and temperature employed. Carbon di oxide is a liquid below its critical point (31.2 °C,7.38 milli Pascal pressure) and above its critical point it exists as a Super Critical Fluid (SCF) under normal conditions density of carbon di oxide is less than 100 g/l. The diffusion coefficient and viscosity are higher in SCF and this helps in better and faster mass transfer rates from the matrix to the solvent. Hence carbon di oxide is preferred over liquid carbon di oxide for extraction.

Carbon di oxide has been widely accepted as a permitted safe solvent for flavour extraction. The technical advantages of using SCF extraction for spices like pepper, ginger, and cumin has been studied by(Udayashankar,1989). It is yet to be assessed as to how the products derived from SCF compete with the traditional solvent process quality-wise and price-wise in the international market. A one tonn capacity plant for SCFE will cost around Rs. 100 million. Attempts have been made to prepare oleoresin by using super critical carbon extraction technique (Udayashankar,2000). The process is yet to be commercialized. Though it is generally considered that SCF-derived products are superior in quality with no

residual solvents, cost wise they will be much higher and their acceptability by the food processing units is a point to be considered.

Ultra sound assisted extraction

Various non-thermal processes like the microwave heating, application of pulse electric heating, magnetic and electric fields are gaining importance in the food industry. Ultrasonic waves are elastic waves with a frequency higher than 20 kHz. Being elastic waves, they need a medium to propagate and the characteristics of the medium will influence their propagation. The waves can be longitudinal, shear, or surface according to how the particles move during the propagation of the wave. Like any other wave, sonic waves are characterized by their frequency, velocity and amplitude. According to their frequency, the waves show different penetration characteristics; the higher the frequency, the higher the attenuation and thus lower the penetration. Consequently, high-frequency ultrasonic waves are of no practical interest for many food applications.

The use of ultrasound in the food industry is classified according to the effect sought. Intensities for low-intensity ultrasounds (LIUs) are lower than 1 W/cm^2 and high-intensity ultrasounds(HIUs), intensities higher than 1W/cm^2. Since penetration characteristics change according to the frequency, this variable should also be considered for a particular application.

According to Mason,(Mason,) HIU are usually found at frequencies of 18-100kHz and LIU at 100kHz to 1 or more MHz. Further more, LIUs are operated in pulses, whereas in HIUs the operation is continuous.

Ultra sonics is being used for process control, particle sizing, drying, crystallisation, separation, food cutting, food preservation, quality control etc. in the food industry (Dharmendra,2000).The food and beverage industry has the potential of utilizing sonic energy for treating heat sensitive materials without loss of flavour, taste, or other damage. Ultra sonic extraction has been used for the extraction of oil from oil seeds and extraction of alkaloids from herbaceous and plant like materials and an enhancement in the extraction is reported (Ramachandra Rao , 2003).

Use of ultra sound water bath as a means of extraction of volatile and semi volatile compounds has become a promising technique which does not require heat. Studies on ultrasonic extraction of sugar from sugar beets show that while acoustic streaming enhances the extraction rate somewhat by reducing the external boundary layer, the mechanism believed to be primarily responsible for the larger increases in the cell disruption and dispersion of suspended solids coupled with enhanced mass-transfer rates due to acoustic streaming are believed to be responsible for the increased mass-transfer rates. The solid-liquid extraction processes in which the application of acoustic waves result in the increased extraction rates are:

- Extraction of soluble matter from cellular solids (ex.Sugar from sugar beets)
- Solvent extraction (oils from oil seeds)
- Extraction of alkaloids from herbaceous and plant –like materials

Ultrasonically-augmented extraction particularly suited to the small-scale, batch type extraction of drugs from plants where minutes of ultrasonic can replace the

soxhlet process requiring several hours for the extraction needs to be studied for the extraction of spice flavourants.

Solvent Mixtures

Restrictions on the use of chlorinated solvents have led to the use of alternative solvents for extraction a necessity for future. Solvents like acetone, Ethylene di chloride were conventionally used solvents for many years. . Solvents like ethyl acetate, hexane, and ethanol can be safe alternate solvents as a replacement for chlorinated solvents. Mixtures of above mentioned solvents viz. acetone-hexane, ethanol-hexane, ethyl acetate-hexane will be a new dimension in extraction of spice oleoresin and needs to be studied in terms of yield of resin and quality of the product.

Solvent mixtures of propanol: cyclohexane : water (8:10:11 v/v/v) has been used for rapid extraction of fat as a replacement for chloroform a chlorinated solvent. (Smedes,1999). The solvents viz., heptane. ethanol, propanol, isopropanol in single and in combinations have been studied as a replacement for hexane for the extraction of oil. It has been reported that n-heptane, n-propanol, iso -propyl alcohol and ethanol were all equally efficient in extraction of soybean oil with nutrient content of oil and meal similar to that obtained with n-hexane. Solvent mixtures of 4+1 v/v of ethanol+water, methanol+water, acetone+water and 7+7+6 v/v/v of ethanol+acetone+water have been studied for efficient extraction of phenols from barley flowers (Bonoli,2004).

On similar lines, study on extraction efficiency of different solvents in single and in combination at different ratios for the oleoresin extraction of spices needs to

be carried out. The information on this line will find direct application industries since in future all chlorinated solvents may be banned completely because of their toxic nature.

Enzymatic extraction

Considerable work has been reported on the application of enzymes for flavour extraction. It is interesting to note that enzymes have been used as a pre-treatment in combination with the conventional methods for the extraction of flavours and this has resulted in an increase in the yield of flavourant (volatile oil), colour and pungency in case of few spices like ginger, chilli, mace and vanilla(Freeze,1993; Santamaria, 2000). Enzymes mediated aqueous extraction of oil is an emerging technology in oil and fat industry. It has been reported that pre treatment of oil seeds with oil seeds with enzymes helps to overcome the low extraction efficiency of the conventional methods thus increasing the yield of oil and quality of the meal. Enzymes have been used for the extraction of carotenoids (Barzana, 2002) lycopenes from tomato (Boehm, 2003) and pigments from grapes (Munz, 2004).

One of the advantages of using enzymes would be low consumption of solvent and increase in the rate of extraction. Limitation of enzyme application in place of solvents would be its high cost, availability, immobilization of enzymes etc., Though newer fungal strains and enzymes from plant sources will help in availability of enzymes with reasonable cost. The important factors in enzyme extraction are pH, temperature, selection of appropriate enzyme, enzyme concentration etc

Though there are reports in literature on extraction of bio-active compounds from plant materials, application of the technique for the extraction of volatiles or resin in spices is not reported as an extraction tool in the preparation of spice flavourant. Applications of enzymes for spice flavourant extraction needs to be studied in detail.

In the present study, application of newer approaches/techniques such as ultra sonication, solvent mixtures and enzymes for the extraction of flavourant from two selected seed spices viz., celery and cumin have been studied under three major headings,

(1) physico-chemical and (2)enzymatic methods for the preparation of flavourant and the other aspect being the(3) food application of the flavourant prepared from the two spices and spent residue utilization for food application.

The physico-chemical characteristics of the two seed spices selected for the study are presented below:

I Celery (*Apium graveolens L.*)

Celery is a commercially important seed spice belonging to the family *Umbelliferrae.* Celery is cultivated for seeds extensively in India, France and United States. Celery seeds are exported to some of the continental countries like France , Italy, Netherlands, Germany as well as to Australia and New Zealand. Celery essential oil lends a floral like odour to oriental perfumes to which it imparts warm and clinging notes (Lewis, 1984). The ground seed is mixed with salt to give “celery salt” which is used in flavouring fish, salads, and eggs. Out of a world production of 6,000 tonnes, India produces 4000 tonnes and exports about 3,000

tonnes. For flavouring foods celery is used in various forms such as fresh herb, seeds, oil and oleoresin. The seeds contain around 2% essential oil which is used both in flavour and fragrance industries. The seeds contain on an average 7 % moisture, 3,5 % volatile oil, 15 % fixed oil , 18% protein, 9 % total ash. 6 % starch 12% crude fibre and 36% total carbohydrates (Lewis , 1984) (Table 4)

Celery plant

Celery stalk

Celery seeds

The celery seed oil has been widely studied by many workers and 3-n butyl phthalide, 3-n butyl-4,5-dihydropthalide (sedanenolide) and sedanolide have been reported as the major flavour components of the oil (Choudhary & Kaul,1992: Verghese,1990).The seeds contain 15-17% fixed oil and fatty acids in fixed oil are made up of petroselenic (64.3%), oleic(8.1%), linoleic(18%) ,linolenic(0.6%)and palmitic acids ( ).

Table 3. Proximate composition of celery seed

Component	Content (%)
Moisture	8.0
Volatile oil	2.0
Fixed oil	15.0
Protein	18.7
Total ash	8.0
Crude fiber	11.0
Carbohydrate	36.60
Starch	6.0

The specification prescribed by Essential Oil Association(E.O.A., 1978),U.S.A. for celery volatile oil and oleoresin are given below:

E.O.A. Specification for celery oil (E.O.A. 85)

Optical rotation +45 ° - +78° at 20°C

Refractive Index 1.4800 – 1.4900 at 20°C

Specific gravity 0.8720 - 0.9100 at 25 °C

The flavour compounds in celery volatile oil reported in literature are : limonene 80%,ά- pinene 0.2%,β-pinene- 0.7%,myrcene – 1%,ά-p-di methyl styrene – 0.9%, carryophyllene – 0.5%,ά-Selinene- 0.5%,n-butyl phthalide 1%, sedananenolide 0.5%,n-pentyl benzene 1%,traces of sabinene, linalool, Carvone and terpeneol (,1986)

II CUMIN (*Cuminum cyminum L.)*

Cumin is a commercial importance seed spice of export commodity belonging to the family umbelliferae. It is grown mainly in Punjab and Rajasthan in India. Indian production of cumin is about 1.1 lakh tons and export about 4250 tons. Seeds contain volatile oil in the range of 3-4% . Cumin seeds are valued for its aroma and medicinal and therapeutic properties. Volatile oil finds application in perfumery and as a flavourant in food industries especially in curries and oriental dishes. Cumin powder form an important ingredient of curry powders and used in bakery products In recent years there is an increase in the demand for cumin in export market. Cumin seeds contain 10% moisture, 3-4% volatile oil, fixed oil 15%, 12 % protein, 10% total ash, 11% crude fiber, 11 % starch and total carbohydrates 33% (Lewis 1984) (Table 6) . Major flavour component of the oil is cuminaldehyde (20-45%), p-cymene (11-14%), α-pinene(15-20%) and terpene (14%). It is reported that naturally occurring aldehyde in fresh cumin is 1,4-p-methadien-7-al and cuminaldehyde and other related compounds are only artifacts formed during storage of ground seeds and during distillation of oil (Borges & Pino ,1993)

Cumin plant

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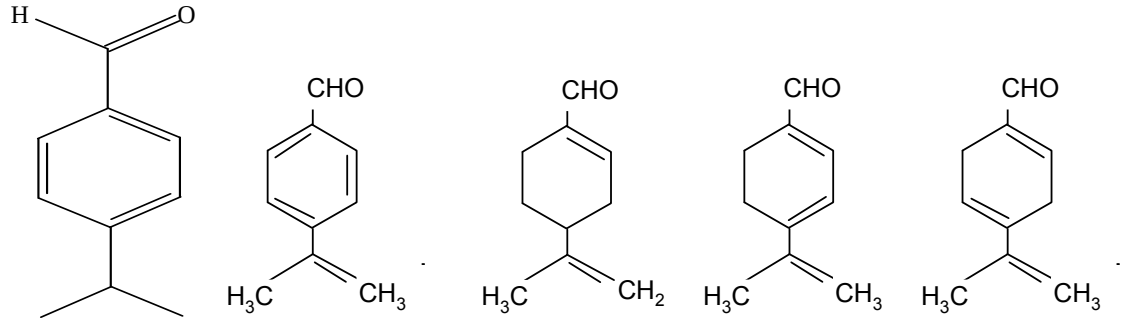
Cumin seeds

Table 4. Proximate composition of cumin seed

Component	Content (%)
Moisture	7.0
Volatile oil	3.5
Protein	12
Total ash	10
Fiber	11
Carbohydrate	33
Starch	11
Fat	15



Cuminaldehyde

E.O.A. Specification for cumin oil and oleoresin are as follows :-

Specific gravity : 0.8923- 0.9250 at 25°C

Optical rotation : + 3° - + 8° at 20°C

Refractive index : 1.5010 -1.5060 at 30°C

The problems of heat generation during grinding and long hours of distillation for oil recovery could be reduced by pretreatment of the spice and this would considerably help the spice processing industries.

The above data shows the importance the two seed spices selected for the study and the results of the proposed study will have a direct implication in the spice processing industries.

AIM AND SCOPE OF THE STUDY

India produces about 28 lakh tons of various spices out of which 7.28 lakh tons, is seed spices. The share of celery and cumin is 2.12 lakh tons which accounts for 29% of total seed spices. Seed spices such as coriander, fenugreek, fennel, cumin and celery either in whole form or as extractives are used as food flavourants. There is a steady increase in the export of celery oil and oleoresin as well as cumin oil and oleoresin (Mathew, 2000). During the year 2002-03 the export of celery oil and oleoresin was 18 tons and 271 tons respectively while the export of cumin oil was 0.6 tons and that of oleoresin was 7.6 tons. The export earning from celery flavourant was Rs. 961 lakh while that of cumin flavourant was 121 lakhs. With this trend of increasing demand for cumin and celery oil and oleoresin it will be appropriate to carry out studies for the preparations of spice flavourant by employing suitable newer techniques and the results would find direct application in spice industries. The collection of data on these lines will fill a gap in the literature on spice flavourant extraction also.

Considerable work has been reported in literature on flavour extraction with respect to coriander, fenugreek, and fennel. The flavour extractives from these spices have been obtained either by steam distillation or by using solvents like EDC, acetone, hexane. At present in industry only single solvents are used for flavour extraction. However, there is no information on the application of enzymes for flavour extraction with respect to these two seed spices. For the extraction of flavours newer approaches/ techniques such as physical, chemical and enzymatic methods have been studied for flavour extraction.

In the present study, two seed spices namely celery and cumin have been selected for the extraction of flavours which includes volatile oil as well as oleoresin. The problems encountered during grinding of spices are the heat generation which results in considerable loss of volatiles for which these spices are valued. An attempt has been made to reduce the heat generation during grinding by suitable techniques like flaking. The flavourants (oleoresins) have been extracted by using either single solvent or mixture of solvents such as acetone +hexane, ethyl acetate+ hexane and rectified spirit +hexane. The use of ethylene dichloride for extraction has not been studied though it is being industrially used by some flavour extractors. Use of EDC is banned in some western countries because of its carcinogenic property and is likely that the industries in India may also stop using it. Effect of enzyme in combination with solvent mixtures have been studied for the enhancement of flavour quality and yield.

Application of flavourant from celery and cumin in some food preparations have been carried out.

After flavour extraction the left out spent material has been examined for their (i) fiber quality and (ii) antioxidant properties.

Ultimate aim of the present investigation is

Optimization of processing parameters to develop suitable methods for improved extraction of flavourants from these two selected spices and a comparative evaluation of the flavourants so obtained with the flavourant obtained by conventional method.

STUDIES ON CELERY

CHAPTER 1

1a. Flaking studies on celery volatiles

The effect of grinding/flaking with and without pre cooling of celery seeds, employing selected laboratory size reduction equipment was evaluated. The yield of oil, as well as physical and chemical characteristics of the volatile oil obtained by hydro distillation and steam distillation were determined. Celery seed is the dried fruit of *Apium graveolens* Linn. belonging to the Umbelliferae family. The composition of celery volatile oil has been studied by many workers (Wilson,1970 ; Ramesh, Gupta & Basalas,1978 ; Lawerence,1998) and GC-MS analysis has shown that d-limonene and selinene form about 60% and 20% of the oil respectively. However the important flavour constituents of the oil responsible for the typical aroma are 3-n-butyl-4-5-dihydro phthalide (sedanenolide), 3-n butyl phthalide, sedanolide and sedanonic anhydride which are present in very low levels (Chowdhary & Kaul, 1992). Six major compounds viz.,piperitone, eugenol, ß-pinene, terpinolene 3-carene, myrcene and menthone have been reported in steam distilled celery seed oil (Guenther,1990). Distillation of fresh celery juice and identification of flavour compounds such as phthalides and hydro phthalides have been reported (Gold and Wilson,1963). Myrcene, Limonene, butyl phthalide, pentyl benzene and ß-carryophyllene have been reported in the oil of a selected Indian variety of celery seed. A review on celery with composition of oil has been published (Verghese,1998).

Celery seed oil is a valued product both in flavour and fragrance industries. Celery seed contains higher amount of fixed oil which poses certain problem during grinding operations by employing plate mill or hammer mill. Problems like over-heating, clogging of mill and loss of volatiles are usually encountered. Cryo-grinding is another method which can be employed but it is expensive. As an alternative method these methods, flaking of the seeds prior to distillation has been studied. The objective of the present study was to evaluate the effect of flaking on the yield and physico-chemical quality of volatile oil fractions obtained

by steam distillation and on the recovery of oil from condensate on which there are no reports in literature.

2. Materials and Methods

Materials

Celery seeds were procured from Punjab, India ,

Reference flavour standards were obtained from Sigma Aldrich Co.

solvents acetone and hexane were of analytical grade

Mini plate mill Laboratory model Buhler miag, Italy

Grinder – domestic model Sumeet , India

Flaker, Laboratory model, Pascal Engineering, Sussex , England

For bigger batch (10 kg) Hammer mill , Batliboi, India

Twin drum roller fabricated at CFTRI, Mysore, India

Precooling of the spice were carried out by keeping the spice overnight in a refrigerator at a temp. Of 5 °C

Laboratory scale experiment (200g batch)

Celery seeds were powdered in a plate mill as well as mixer and 200 g of powder was subjected to hydro-distillation by Clevenger method (ASTA, 1991) for 5 hours. In another batch, celery seeds were flaked in a flaker with a gap adjustment of 0.1 mm. between the rollers and 200g flakes were subjected to hydro distillation for 5 hours. The yields of volatile oils were expressed as per cent (v/w) on dry weight basis. In another set of experiments, the spice was pre cooled to 5 °C and subjected to size reduction as mentioned earlier.

Scale up experiment (10 kg batch)

Celery seeds were powdered in a hammer mill using a sieve (550 microns) and 10 kg of the powder was subjected to steam distillation for 5 hours. In another batch, celery seeds were flaked in a double roller flaker with a clearance of 0.1 mm between the rollers. Flakes (10 kg) were subjected to steam distillation for 5 hours. Volatile oil samples were collected and yield of oil obtained was expressed as per cent (V /W) on dry weight basis (dwb). The oil samples were collected at every 30 minutes interval and altogether four fractions F1, F2, F3 and F4 were collected. The fractions were analysed individually for their chemical composition by GC with FID detector. Confirmation of phthalides was carried out by GC-MS analysis. Oil samples obtained by pooling all the four fractions were analysed for their physical properties viz., specific gravity, refractive index and optical rotation (ISI,1980)

Analysis of volatile oil by Gas Chromatography

Shimazdu 15-A Gas Chromatograph with Column- SE-52 on Chromosorb B (10 ft length 1/8 " i.d.) with a temp. programme of 75/5/180/2/200°C with a Injector temp: 150 °C, Detector temp: 210 °C, Carrier gas flow : 30 ml/min. The oil (0.05 ml) was diluted in acetone (1 ml) and 1 µl was injected to GC

Recovery of oil from condensate

During steam distillation, the condensate was found to be turbid, indicating that certain amount of oil was getting dispersed in water. An attempt was made to recover the oil from condensate by (i) hydro distillation and (ii) hexane extraction. Well mixed condensate (five liters) obtained during 5 hours of steam distillation

was subjected to hydro distillation for 30 minutes. The well mixed condensate from steam distillation (2.0liters) was taken in a separating funnel and 200 ml of hexane was added ,shaken well and allowed for the separation of two layers. Hexane layer separated at the top was collected . The extraction was repeated with fresh 200 ml hexane and pooled extract was distilled to get the oil .

Kinetics

Kinetics of the extraction of celery volatile oil during steam distillation was calculated (Levenspiel,1972)

In the general equation for kinetic study

$$-\frac{dC_A}{dt} = k\ C_A^n \qquad (1)$$

C_A is the yield of oil at any time θ, K is the rate constant and n is the order of change. It is the common experience that the first order (n=1) changes are encountered in most of the cases. Assigning n=1 in equation (1) and rearranging, we get

$$-\frac{dC_A}{C_A} = k\ dt \qquad (2)$$

Integrating equation (2) at conditions C_A=C_A *at* $t=0$, and C_A at any time t, we get

$$-\ln\ \frac{C_A}{C_{A_0}} = k\ t \qquad (3)$$

The rate constant K was obtained by using equation (3)

Results & discussion

Grinding methods play a very important role on the yield and quality of a spice oil. Grinding of celery seeds by the conventional method in plate mill, or hammer mill resulted in the clogging of the mill and also a rise in the temperature of the powdered material which will lead to loss of oil. Loose Bulk Density (LBD) of powder was 500g/l while LBD of flakes was 320g/l Flaking as an alternate to grinding by conventional milling, has shown promising results.

Lab scale studies

In small batch size (200g) distillation, the yield of oil was almost equal both in case of powder and flakes, probably because difference between the material temperature during grinding (32°C) and flaking (27°C) was only 5°C. The yield of oil was 1.9% and 1.8% in the powders obtained in plate mill and grinder respectively which favourably compares with 1.98% oil yield from flakes (Table 1). Pre cooling of the spice resulted in higher oil yield viz., 2.2%, 2.05% and 2.2 % for powder obtained in plate mill, dry grinder and flaker respectively The higher yield of oil may be due to the low temperature attained by the product (20 °C) during grinding or flaking compared to normal grinding without pre cooling where the temperature went up to 32°C.

Scale Up studies

In case of 10 kg batch, flaking of celery seeds resulted in higher yield of oil by steam distillation (1.76%) as against (1.42%) by grinding in a hammer mill.(Table 2). Compared to laboratory hydro distillation, the recovery of oil from flakes was 89% and 79% from powder. The oil yield of celery powder by hydro distillation in lab was 1.5% and in case of flakes it was 1.8%. Reason for lower

yield of oil from powder could be due to the fact that the temperature of the powder went up to 60°C, whereas flaking did not appreciably increase the temperature .

Table 5. Effect of grinding methods on the yield of celery volatile oil (hydro distillaltion)

Methods of Grinding	Volatile oil * (% v/w dwb)	
	Without Pre cooling	With Pre cooling
Mini plate mill (powder)	1.9*± 0.08	2.20 ± .12
Laboratory grinder (powder)	1.80 ± 0.20	2.05 ± .19
Laboratory flaker (flakes)	1.98 ± 0.04	2.20 ± .05

** Average of triplicate values*
dwb = dry weight basis

Table 6. Effect of size reduction method on yield of celery seed oil (Steam distillation 10 kg batches)

Size reduction Method	Yield of oil (%) dwb	Percent oil recovery*
Powder (Hammer mill)	1.42 ±0.34	75
Flaking (flaker)	1.76 ±0.16	89

** In relation to celery seed analysis by hydro distillation of a sample ground using the mini plate mill (1.9%)*

Flaking

Flaking was done with different gap clearance between rollers (0.05mm-0.3mm) and clearance measured by thickness gauge. For each thickness, loose bulk density and the yield of oil by hydro distillation was determined. As the clearance

between rollers increased the flake thickness increased causing decrease in the yield of oil. An optimum clearance of 0.05 mm between the rollers resulted in maximum yield of oil (Table 3).

Scanning Electron Microscope Studies

To evaluate the effect of flaking or powdering on structural changes, both celery flakes and powder were subjected to scanning electron micrograph. The sample spread on a double sided conducting adhesive tape, placed on a metallic stub was coated (100u) with gold in a spatter coating unit for 2 minutes and observed in a LEO-435-VP electron microscope (LEO Electron microscopy Ltd., Cambridge, U.K.) at 20 kv.

Electron microscopy study reveal that there is a marked difference between the structures of powder and flakes (fig.1). In case of flakes, the cells have got ruptured and a total flattening observed which facilitated the release of higher amount of oil in a shorter duration. Lumps like structure is observed in case of powder and the particles would retain these spherical shape and cell rupture may be minimum which resulted in lower yield of oil and longer time of distillation.

Kinetics of Volatile oil extraction

The volume of oil fractions collected at different time intervals of distillation is shown in Table 4. In the first 30 minutes 73% of the oil is collected and by the end of 120 minutes most of the oil was recovered. Hence continuation of the distillation beyond 2 hours is unnecessary. Extraction of volatile oil from either powder or flakes followed first order kinetics as evidenced by low variance values of 0.04. The rate constants are 0.061 and 0.109 min^{-1} for the distillation of powder

and flakes respectively. These values show that the extraction of volatile oil was faster for flakes compared to the powder as evidenced by the values given in table The improved release of oil from flakes as compared to that from powder is further substantiated by the data on oil retention in the spent material. The oil yield from spent powder was 0.09% whereas it was 0.0166% from spent flakes. The spent after steam distillation was taken out form distillation still and a representative sample (200g) was subjected to hydro distillation in the lab. The volume collected was calculated as percentage (vol/wt). Similarly, in case of powder a s .Faster release of the oil in case of flakes may be attributed to the fact that flakes in the steam distillation still facilitates the passage of steam through flakes more easily compared to passage of steam through the bed of powder and surface area of the flakes is optimum for the release of the oil.

Table 7. Effect of roller gap on the yield of celery oil

Roller gap (mm)	Average flake thickness (mm)	Yield of oil (%)dwb Hydro distillation	Bulk density g/l
0.03	0.08	1.4	215
0.051	0.01	1.55	220
0.102	0.13	1.46	230
0.152	0.15	1.40	268
0.203	0.19	1.32	250
0.254	0.22	1.29	310
0.30	-	Bumped	

Table 8. Comparative evaluation on the yield of oil from flakes & Powder at different intervals of time

Time (min.)	Fractions	Powder (ml of oil)*	% recovery w.r.t. total oil collected	Flakes (ml of oil)*	% recovery w.r.t. total oil collected
0		0		0	
30	F_1	101.0	76.5	139.1	85.7
60	F_2	120.0	91	150.2	92.5
90	F_3	130.0	98	156.3	96.3
120	F_4	132.0		162.3	

***** cumulative values

Physico-chemical quality of volatile oil

The physical properties of the oil with respect to specific gravity, refractive index did not significantly change with flaking (Table 11) while a higher value of optical rotation(85°) in oil from flakes was observed which may be due to higher limonene content extracted compared to optical rotation in oil from powder (74°)

Table 9. Effect of flaking on physical properties of celery oil (steam distillation 10 kg batch)

Parameters	Oil from Powder	Oil from Flakes
Specific gravity (g) at 30°C	0.9307	0.9148
Optical rotation at 20°C	+ 73.99	+ 85.10
Refractive index 20 °C	1.4782	1.4792

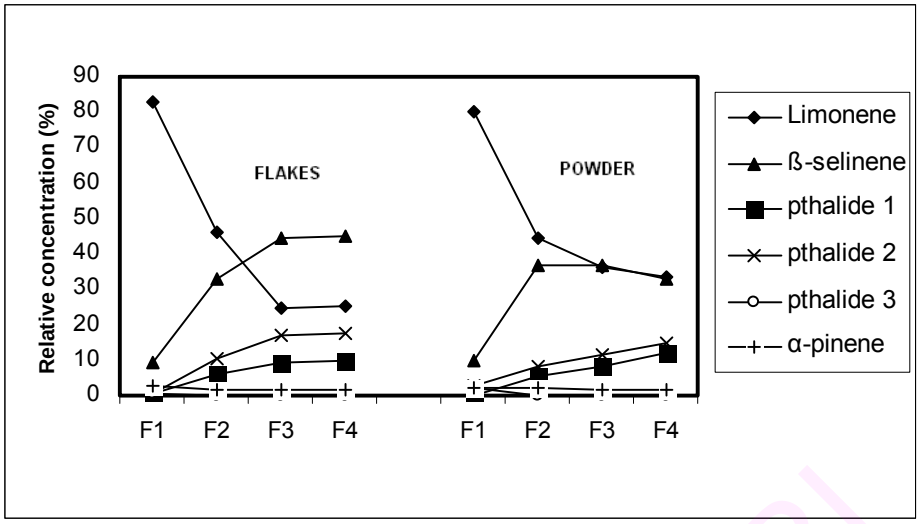
GC analysis of volatile oil

The relative concentrations of the flavour components of the volatile oil in different fractions were estimated by GC (fig. 2).Higher concentration of limonene (80%), the major flavour compound and linalool (1.46%) was observed in flakes as compared to that from powder which were 73% and 0.96% respectively. It was observed that mainly terpenes are extracted in first two fractions with a small amount of phthalides and the sesquiterpene β-selinene, the second major constituent gradually increased. Elution of the flavour impact compound phthalide was less in the first fraction. But from 3rd fraction onwards phthalide concentration gradually increased from 2.5-15% and concentration of terpenes decreased from 80 to 33%. By selective collection and pooling it is possible to obtain the specific

fractions of the oil having different flavour profile. The phthalides from celery are the most significant bio-active compounds exhibiting many health benefits like protection against cancer, high blood pressure and cholesterol. Sedanolide being the major flavour impact compound of celery volatile oil has been reported to be the most active of the phthalides in the reduction in tumours in laboratory animals (Momin & Nair,2002). By pooling the fractions 3,and 4 a phthalide rich fraction of oil could be obtained which may have greater significance from the point of view of health benefits An enriched fraction of sedanolide will be desirable for the treatment of aliments like hypertension and heart ailments. The total volume of oil collected was 132 ml and by pooling fractions 3 and 4, 12.0 ml of phthalide rich fraction was obtained.

The process of flaking and extraction resulted in higher yield of oil with no loss of flavour compounds. GC profile of the celery seed oil obtained from flakes did not significantly differ from oil obtained from powder (fig. 1). The technique of cryogrinding or freeze grinding of spices has been shown to improve the flavour quality of spice powders (Weistrich & Schafer,1968; Gopalkrishnan & Laxmivarma, Padmakumari, Symon, Umma &Narayanan 1990). It has also been reported that cryogenic grinding gives better quality of ground spices when compared to ambient grinding (Pesek, Wilson & Hammond, 1995). Head space analysis of the spice powders namely white pepper, nutmeg, cumin, oregano and cinnamon showed that significantly higher amounts of total volatiles, and lower molecular weight constituents with samples ground at ambient temperature.

Cryogenically ground spices retained more of the volatiles of the natural spices as assessed by sensory evaluation and headspace, but no significant increase in the yield of the oil was noticed. It has been reported that chilled water circulation during milling has resulted in better retention of flavour components in case of pepper compared to ambient grinding conditions (Murthy, Krishnamurthy, Girish & Srinivas Rao, 1996). In our study it was observed that flaking compared favourably with grinding of pre cooled spice with increase in yield of volatile oil in case of smaller batches.



Relative concentration of major flavour compounds of Celery volatile oil in different fractions.

In case of 10 kg batches, the temperature attained during flaking was 28°C while the temperature went as high as 70°C during powdering in the hammer mill. Oil yield of pre cooled celery seeds was almost same for mini plate mill and laboratory grinder. In the case of size reduction without pre cooling the oil yields were lower than with pre cooling, but within the grinding methods the yield range was similar (Table 1) Pre cooling resulted in higher yield of about 13% and this is due to the low temperature attained by the product during grinding .

Recovery of oil from condensate

Hydro distillation

By hydro distillation of the condensate, oil can be recovered within 30 Minutes. The recovery of oil was 1.4 ml in 5 liters of condensate (0.028%). In case of flakes oil in condensate was 0.028% while in case of powder it was 0.067%. In a 10 kg batch distillation, the total amount of condensate collected was 90 liters and 25 ml of oil was recovered from it.

Hexane extraction

The recovery of oil was 2.0 ml from 5 liters of condensate. By adopting either one of the above methods, 25-40 ml of oil can be recovered from the condensate in 10 kg batch steam distillation.

GC Analysis of volatile oil recovered by condensate

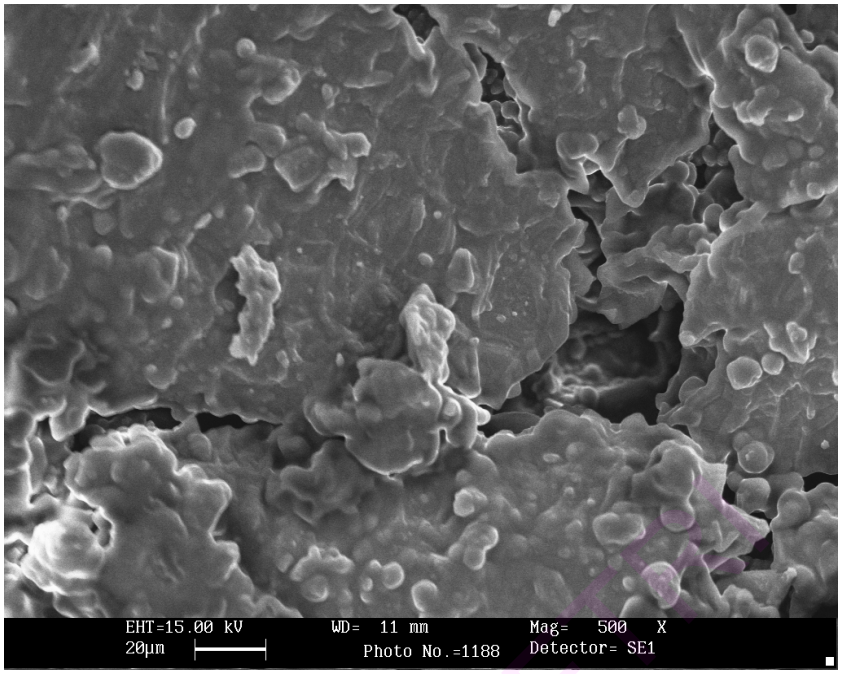
GC analysis of the recovered oil showed the presence of higher boiling compounds like phthalides in higher concentrations than the lower boiling compounds like terpenes. The oil obtained by hydro distillation contains both the major terpenes limonene and selinene and also the phthalides. The percentage of

terpenes was higher in oil recovered by hydro distillation compared to hexane extracted oil. The phthalides concentration in hexane extracted oil was 52%n-butyl phthalide, 18% sedanolide, 5.38% sedanenolide as against 13.9%, respectively in Hydro distilled oil.

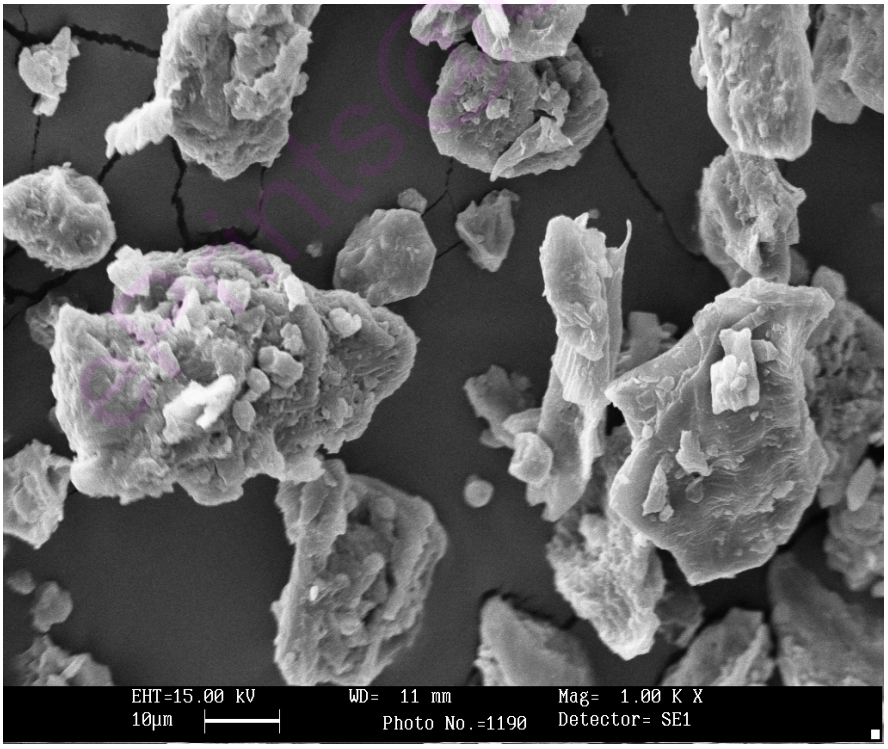
In terms of the volume of oil, it is possible to recover the oil from the condensate in the range of 25-40 ml per 10 kg batch distillation. The flavour of the oil recovered by from the condensate is not comparable to the neat oil, but it can be blended with the steam distilled neat oil to make it comparable with steam distilled oil or can be sold as a specialty oil rich in phthalides.

Conclusions

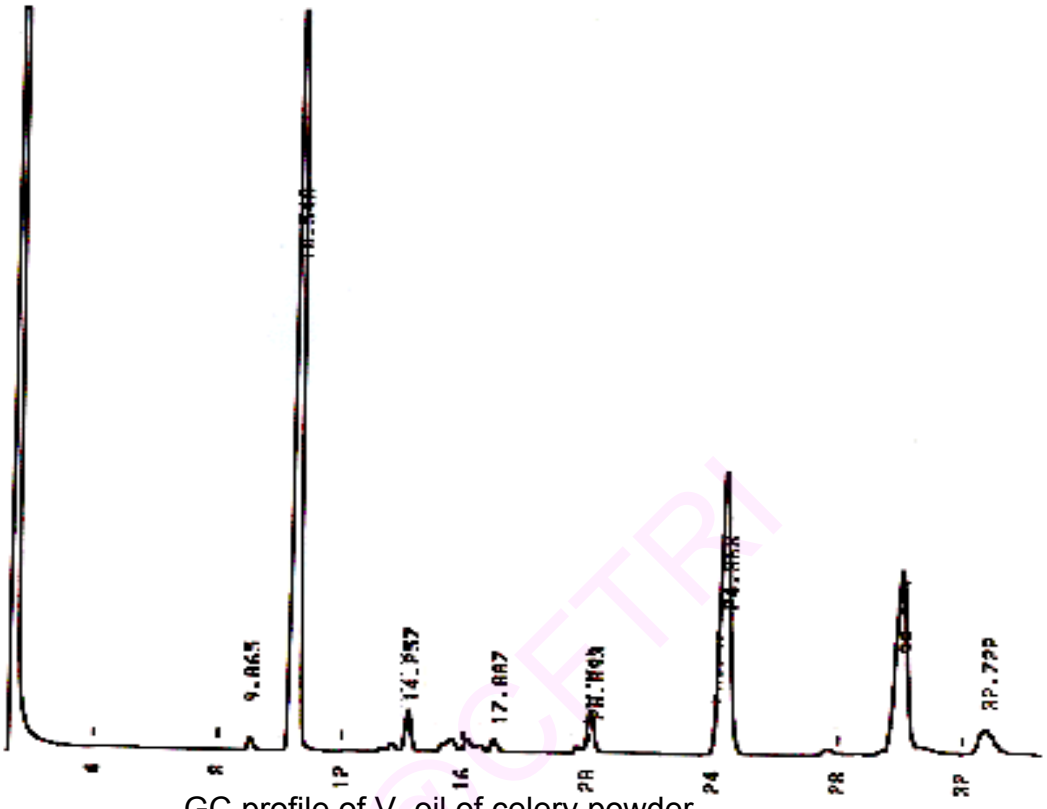
The study has clearly shown that by flaking the celery seeds prior to steam distillation, many drawbacks such as clogging of the mill, rise in the temperature of the ground material and loss of volatile oil associated with conventional method using hammer mill or plate mill were overcome. Flaking. and pre-cooling of celery seeds prior to flaking further improved the oil yield. and higher retention of the flavour components (terpenic compounds). Hence, for celery seed oil production by steam distillation, flaking is a promising replacement for the conventional size-reduction method of grinding. Selective collection of volatile oil at different intervals of time of distillation gives fractions of different flavour profiles. It is also possible to recover oil from the condensate and this oil can be added back to the total collected oil or can be used as a speciality oil having health benefits.



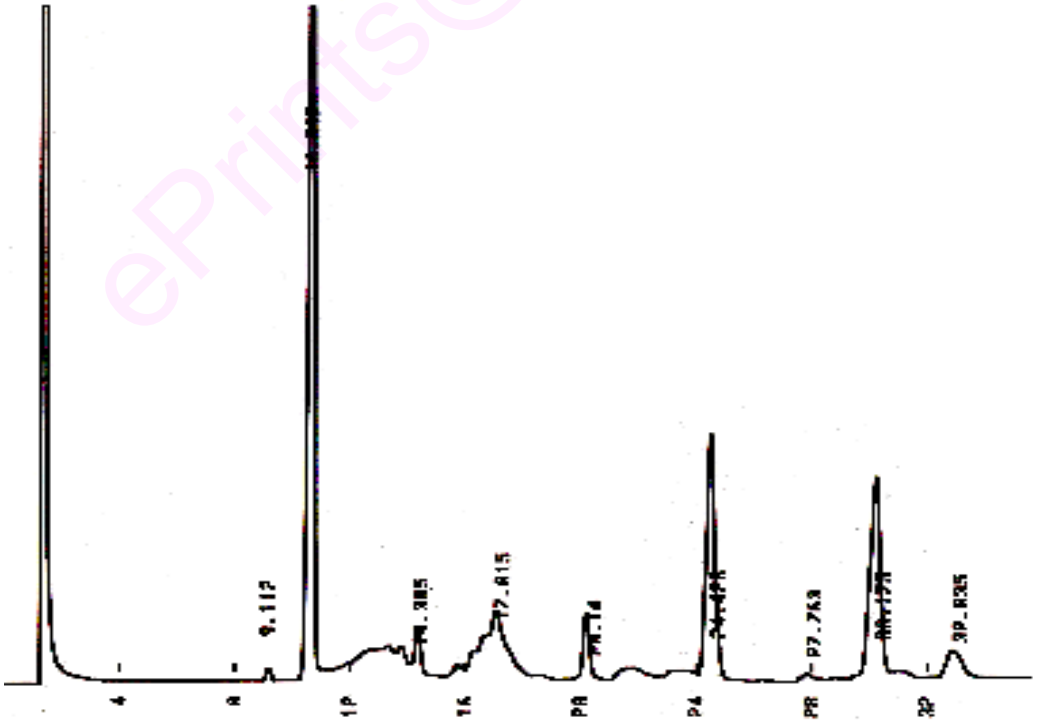
Celery flakes



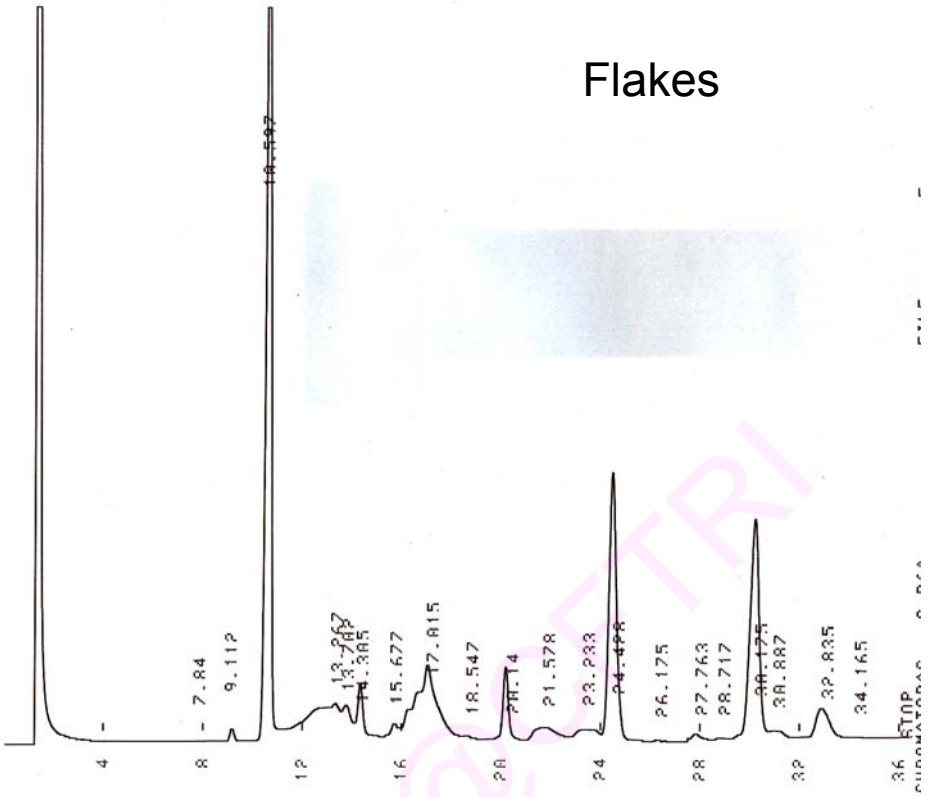
Celery powder



GC profile of V. oil of celery powder



GC profile of V. oil of celery flakes



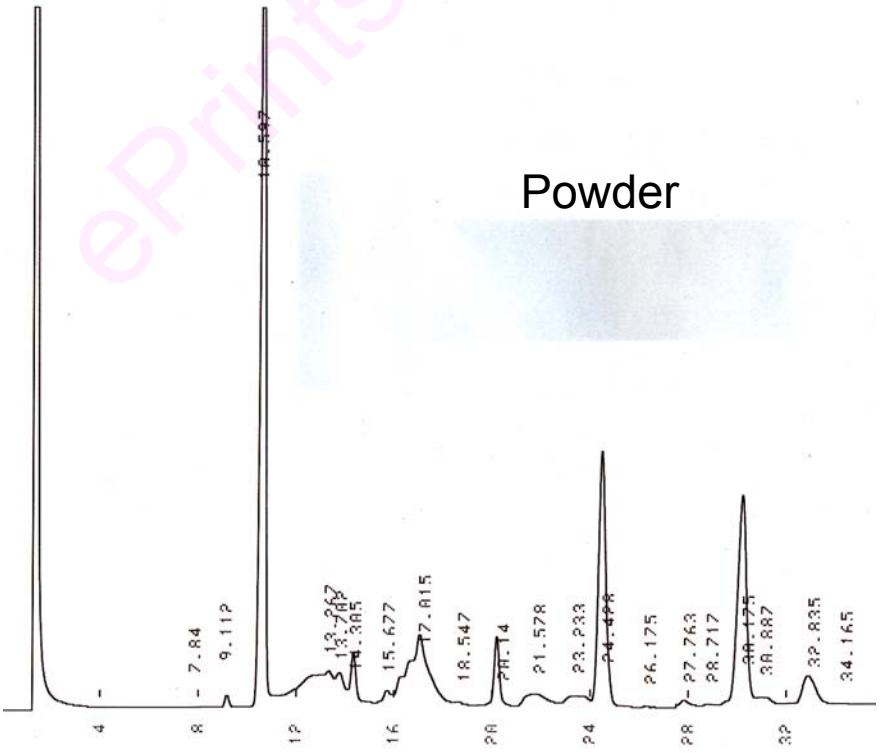


Table 3 : Compounds identified in Celery Seed Oil * by GC-MS

Sl. No.	Rt	Compounds	Relative Conc. (%)
01	8.85	mycrcene	1.3
02	9.54	limonene	67.8
03	12.47	Limonene oxide	0.2
04	12.99	Penthyl benezene	2.2
05	17.10	Carveol	0.1
06	20.62	β- caryophyllene	0.9
07	22.32	β- selinene	14.4
08	23.75	Caryophyllene oxide	1.2
09	25.62	3-n butyl pthalide	5.0
10	27.21	Sedanolide	1.8

* Oil obtained by hydrodistillation : laboratory mill grinding

1b. *Extraction of non-volatiles of Celery by solvent mixtures*

At present, single solvents like ethylene di chloride (EDC), acetone, methylene chloride and hexane are used for the extraction of spice oleoresins. EDC is being widely used in the industries because of its high extraction efficiency. Since the use of chlorinated solvents are likely to be banned because of their carcinogenic and toxic properties, use of alternative solvents as a replacement have been studied. Combination of a polar solvent and a non-polar solvent mixtures were tried for the extraction . In order to get a similar efficiency as EDC, use of solvent mixtures like 1) acetone + hexane ii) rectified spirit + hexane iii) ethyl acetate+ hexane have been evaluated at different proportions. For comparison individual solvents have also been studied. The quality of the product so obtained were assessed.

Materials & Methods

Resin extraction

The preliminary experiments were carried out on 5 g of Celery powder in a test tube with an overnight contact time with the solvents. The material to solvent ratio was maintained at 1:5 acetone + hexane, ethyl acetate +hexane and rectified spirit +hexane in two stages. In the first stage, 5g powder was treated with 25 ml of solvent/solvent mixtures in test tube and shaken well for 1 min. and left for overnight contact. Next day the clear supernatant was taken out (extract-1). The residue was again treated with 25 ml of the same solvent/solvent mixtures shaken thoroughly and left in contact for 8 hours. After 8 hours, the clear supernatant was taken out and mixed with the extract-1 .The pooled extract was taken in a 100 ml round bottom flask and distilled under vacuum and the flask was kept in a oven at 100± 2° C for 30 minutes and then the weight of the residue was determined. The weight of resin obtained was calculated for 100 g of Celery powder and expressed as % resin yield on moisture free basis. The same procedure was followed for the extraction of non volatile matter (resin) with the other two solvent mixtures.

Percent resin (mfb) – resin wt.x 100 /5(100-m)

m = moisture in celery powder

Fatty acid analysis

Fatty acid composition of the celery resin as their methyl resin was carried out by GC following the method of Christie,1984 . Celery resin 0.5g was taken in a wide mouth test tube and 1 ml of benzene added ml of sodium methoxide added and kept in water bath at 56 C. After 20 minutes test tubes were taken out from water bath and 5 ml chloroform added, shaken and left for layers separation. Chloroform layer collected, evaporated in a flash evaporator to 0.5 ml and 1 ul injected to GC.

Results and Discussion

Resin content

Yield of celery resin by employing different solvent mixtures are presented in Table. In case of acetone + hexane mixtures, the highest yield of resin(14.22%) was obtained with 90+10 proportions while the resin yield was lowest at 10+90 solvent proportion. Different proportions of acetone and hexane of 80+20,70+30 60+40 gave a resin yield of 14% ,13%and 14% respectively. Pure solvent acetone and hexane gave 13.4% and 12.1% of resin respectively. With respect to mixtures of ethyl acetate+ hexane mixtures, the highest yield of resin (13.5%) was obtained with 90+10 The resin yield was 13.4% and 12% with ethyl acetate and hexane respectively. In case of rectified spirit +hexane mixtures, highest yield of resin (12.5%) was obtained by extraction with 40+60 proportions and lowest yield of 7.1% was obtained with 90+10, 80+02 and 70+30 proportions of rectified spirit+hexane. The resin yield was 9.5 and 11.0% with rectified spirit and hexane respectively.

Yield wise A+H is good compared to other two solvent mixtures. At present, acetone and hexane individually are used for extracting various spice flavourants. These two solvents are commonly available at a much lower rate compared to ethyl acetate or rectified spirit, besides rectified spirit is a excise commodity and needs control on the stock of solvent by excise inspector which adds to the additional cost. Ethyl acetate though a good solvent for extraction, it leaves a characteristic note in the final product which is not desirable. In view of this,

acetone+hexane mixture has been found to be ideal for the extraction of flavourant from celery.

Fatty acid analysis

In case of celery non volatile components there is no marker compound reported in literature unlike in the cases of pepper and ginger where the non volatiles are characterized by the pungent compounds piperine and gingerol . The fatty acid profile by GC was studied as the quality parameter of the resin obtained by different solvent mixtures. Hence the fatty acid profile of the resin of celery which represents the non volatile were analysed. Fatty acid analysis was carried out by the preparation of methyl esters of the fixed oil followed by gas chromatography analysis by the method Christie,1984 . The

The fixed oil of celery contains the fatty acids petroselinic, oleic, linoleic, myristic, palmitic, palmitoleic, stearic, and myristoleic. In literature in celery isomers of oleic acid cis 9(18:1)-petroselenic acid , cis11(18:1)- vaccenic acid have been reported. It is not possible to separate them by baseline separation by the general method of FAME. A method of dibutyrate derivatization has been carried out for baseline separation of petroselenic acid and vaccenic acid (Destaillats,2002). In celery fixed oil a major amount of fatty acid 64-70% is petroselenic acid. In the present study by FAME method, it was not possible to separate petro selenic acid and oleic acid with the available facilities. Hence, oleoic acid content reported in the table of values is actually a mixture of petroselenic acid and oleic acid. The results reveal that with all the solvent mixtures studied, there was a similar pattern observed with respect to the fatty acids present and also the ratio of their distribution. Oleic acid was the found to be the major fatty acid in all samples (Table) an average of 65% of the total measured fatty acids. Linoleic acid was present on an average of 26% and palmitic around 4.5%. A similar trend was observed in all the samples extracted with respect to different solvent mixtures. Of the fatty acids present, 92% were unsaturated fatty acids while the saturated fatty acids formed only 8%.

Table 10. Effect of solvent mixtures on resin yield of celery powder

Solvent	Resin yield %	Solvent	Resin yield %	Solvent	Resin yield %
Acetone + hexane		Ethyl acetate+hexane		Alcohol+hexane	
Acetone 100%	13.4	Ethyl acetate 100%	13.4	100%	9.5
90+10	14.2	90+10	13.5	90+10	7.1
80+20	14.1	80+20	10.7	80+20	7.1
70+30	13.0	70+30	13.0	70+30	7.1
60+40	14.0	60+40	13.2	60+40	10.3
50+50	13.0	50+50	13.0	50+50	12.2
40+60		40+60	12.9	40+60	12.5
30+70	12.9	30+70	11.5	30+70	9.3
20+80	12.5	20+80	11.5	20+80	11.0
10+90	12.2	10+90		10+90	10.2
Hexane 100%	12.1	Hexane 100%		Hexane 100%	11.0

Table 11. Fatty acid composition (area %) of celery resin (Acetone + Hexane)

Solvent	C14 Myristic	C16 Palmitic	$C_{18:1}$ Petroselenic+Oleic	$C_{18:2}$ Linoleic	Others
E.A. 100%	1.2	4.7	64.8	25.0	4.4
90+10	1.0	4.8	65.4	24.8	
80+20		4.5	65.9	26.7	
70+30	-	4.5	67.0	24.7	
60+40		4.5	67.1	24.6	
50+50	-	4.7	66.2	26.0	
40+60	-	4.5	65.9	26.8	
30+70	-	4.4	64.7	27.9	
20+80		0.3	5.1	68.0	
10+90	-	0.4	5.1	68.5	
Hexane 100%			4.2	68.3	

Table 12. Fatty acid composition (area%) of celery resin(Ethyl acetate:Hexane)

'Solvent	C14 Myristic	C16 Palmitic	$C_{18:1}$ Petroselenic+Oleic	$C_{18:2}$ Linoleic	Others
E.A. 100%	1.2	4.7	64.8	25.0	4.4
90+10	1.0	4.8	65.4	24.8	
80+20		4.5	65.9	26.7	
70+30	-	4.5	67.0	24.7	
60+40		4.5	67.1	24.6	
50+50	-	4.7	66.2	26.0	
40+60	-	4.5	65.9	26.8	
30+70	-	4.4	64.7	27.9	
20+80		0.3	5.1	68.0	
10+90	-	0.4	5.1	68.5	
Hexane 100%			4.2	68.3	

COMPARATIVE EVALUATION OF FLAKES AND POWDER FOR THE RECOVERY OF RESIN AND SOLVENT FROM SPENT MEAL

After the extraction of the volatile oil by steam distillation, the deoiled powder was extracted with acetone with a material to solvent ratio of 1:7 for resin recovery. After the extraction, the spent meal, still contains solvent equivalent to 60-70% of the material weight. This solvent has to be recovered for the process to

be economical. A comparative study has been made to recover the solvent held up in the meal with respect to flakes and powder.

Materials & methods

Celery flakes and powder 1 kg each was packed individually in a jacketed column of dimension of ht. 45 cms, i.d. 10 cms and extracted with acetone as solvent which is commercially used. A solvent ration of 1:7 was used in case of powder and the total quantity of solvent (7000 ml) was added in 7 installments of 100 ml each and in case of flakes, the total amount of solvent (10,000 ml) was added in 10 installments of 1000 ml each. A solvent to material ratio of1:10 was employed in case of flakes. In each installment of solvent addition one hour contact time was given . After the contact time, the extracts were withdrawn and pooled, the solvent distilled off to recover the resin. Resin yield was recorded.

The solvent adhered to the spent meal was recovered from the column in situ by circulating hot water at 60 °C through the condenser and condensate collected with vacuum application at the end of the distillation. The distillation was continued for 100 minutes. The volume of solvent collected was measured and quantity of the solvent assessed by GC.

GC conditions

Column -SPB capillary column
Injector temp.- 150 °C
Detector-200 °C
Column – 50(2)/5/180/2/250
FID detector, Nitrogen flow- 30 ml min.

RESULTS

The experiment was conducted to study the effect of flaking on solvent flow rate, resin yield, solvent recovery from spent meal in comparison to powder. The flow rate of the solvent in case of flakes was 1.5 min/lt , while it was 30 min/lt in case of powder. The total quantity of solvent used for extraction of powder was 7 lts. and the total extract collected was 6.3 lts in 190 minutes , while in case of

flakes total quantity of extract collected was 9.4 lts. in 140 minutes. The yield of resin in case of flakes was 17% and by powder it was 21.1%. In order to get a resin yield comparable to powder extraction was continued with further addition of 3 llts of solvent which resulted in resin yield (21%) similar to that of powder. In spite of using additional quantities of solvent in case of flakes the total time taken was 140 minutes while it was 190 min. in case of powder. Flaking has an advantage of saving 50 min. in extraction time in spite of using additional amount of solvent.

Solvent recovery

Weight of wet spent meal in case of powder was 1.5 kg wherein the solvent wt. was 710 g (797 ml). When the wet spent meal was subjected to solvent recovery and 696 g which represents 98% of solvent recovery. Weight of wet meal in case of flakes was 1.4 kg . The per cent recovery of the solvent was 98% from flakes and powder (Table). The time taken to recover 98% of solvent was 100 minutes both in case of powder and flakes. The GC analysis of recovered solvent has a similar GC profile (fig.) compared to solvent used for extraction . Thus the solvent so recovered can be reused.

Conclusion

1. Flaking has the advantage of no heat generation normally associated with large scale powdering

2. Flaking helps in faster solvent flow rate

3. Flaking results in higher yield of volatile oil 24% compared to powdering without affecting the physical and chemical quality of the oil.

4. Recovery of solvent held up in bed of material in case of flakes is 98% similar to that of powder

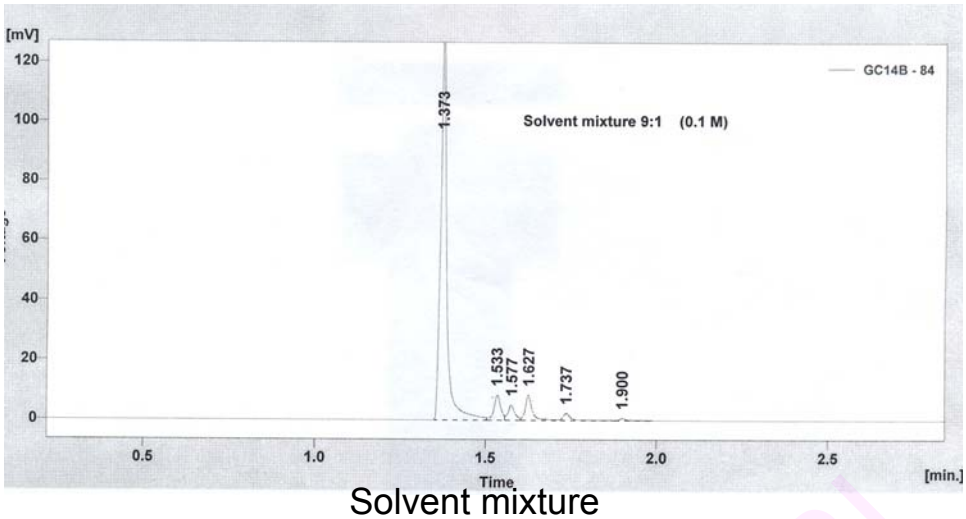
5. The recovered solvent has the same GC profile as the solvent used for extraction both in case of both powder and flakes which can be reused

Table 15 Recovery of resin & solvent from Celery flakes& powder

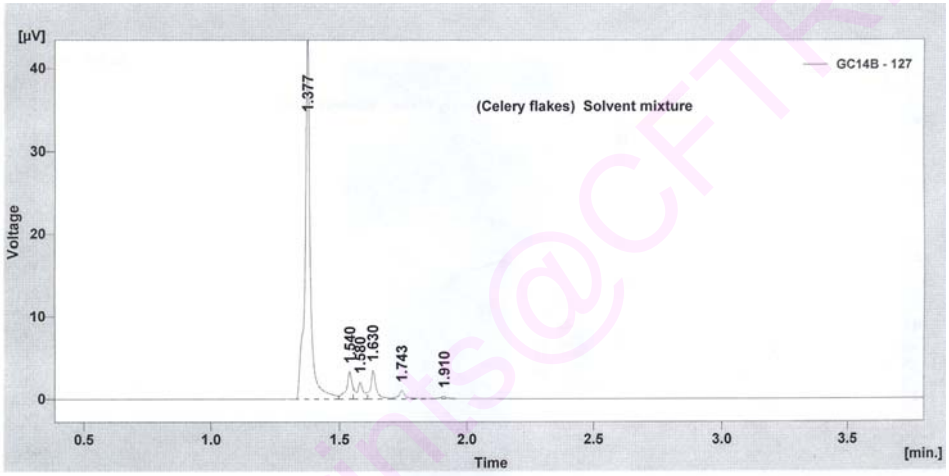
Parameters	**Celery flakes**	**Celery powder**
Weight of sample taken (g)	1 kg	1k g
Bulk density g/l	320	500
Solvent used	acetone	acetone
Column dimension ht x i.d. (cm)	45x10	45x10
Bed height (cm)	88	74
Volume of solvent for wetting (ml)	81	84
Wetting time (min.)	15	30
Flow rate (ml/min.) lt/hr	2	4
Total volume of extract collected (lt)	9.4	6.3
Solids in extract (g)	21 (17+4)	21.0
Wt. of solvent held up in the spent (kg)	1.4	1.5
Wt. of solvent recovered (g)	770	696

Recovery of solvent(%)	97.5	98.0

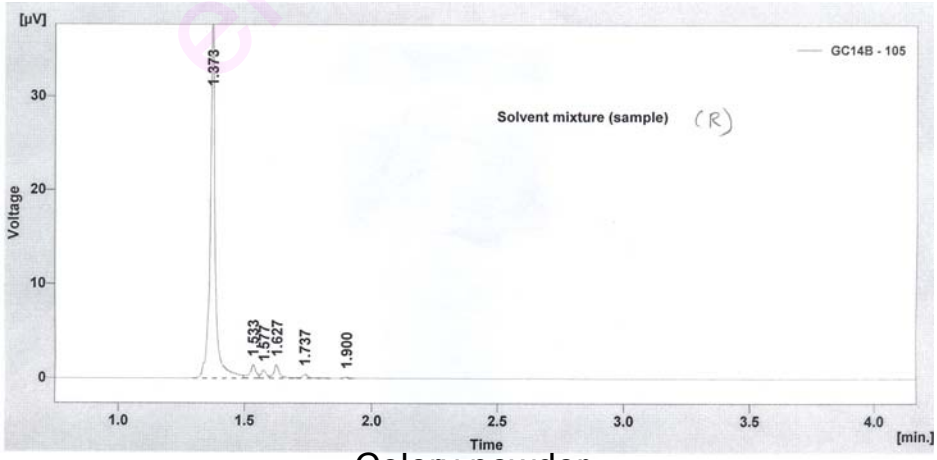
GC Profile of solvent recovered from celery spent (Flakes & Powder)



Solvent mixture



Celery flakes



Celery powder

ENRICHMENT OF BIO-ACTIVE COMPOUNDS FROM CELERY SEED OIL

Celery (*Apium graveolens L.*) a herb, grown as a biennial or as an annual, is cultivated as a popular vegetable, for the green and blanched leaf stalks and to a limited extent for the edible thickened roots and crowns. The pungent seed is used in salads, soups, stews, vegetable dishes, meat dishes, and celery salt (a mixture of table salt and ground celery seed). Celery seeds contain about 2% volatile oil and 15% fixed oil (0. The essential oil of celery seed includes d-limonene (> 60%), selinene (10-20%) and pthalides (1-4%). The pthalides are reported to be 3-n-butyl phthalide, sedanenolide and sedanolide and the phthalides are responsible for the characteristic odor of celery. The phthalides, 3-n-butyl phthalide, sedanenolide and sedanolide are separable by GC analysis. The compounds are identified by their characteristic molecular ions (M+) at m/z 190, 192 and 194 respectively (Uhlig , 1987). The fragmentation pattern for n-butyl phthalide is 133(100 %), 105 (38 %), 77 (20 %), 134 (10 %) and 190 (4 %). Sedanenolide is characterized by its mass spectrum consisting of peaks at 107 (100 %), 108 (22 %), 79 (19 %), 192 (17 %), 77 (16 %), 133 (9 %), and 135 (5 %). The mass spectrum of sedanolide consists of peaks at 108(100 %), 79 (15 %), 80 (15 %), 109 (12 %), 137 (4 %), and 194 (1.7 %) (.

As a medicinal plant, celery has been used as an aphrodisiac, anthelmintic, antispasmodic, carminative, diuretic, laxative, sedative, stimulant, and tonic. Preparations of celery are also used for blood purification, for regulating elimination of the bowels, for glandular stimulation, and as cure in case of gall and kidney

stones. Celery seed extracts have been shown to possess anti-inflammatory properties. The phthalides from celery are the most significant bio-active compounds exhibiting many health benefits like protection against cancer, high blood pressure and cholesterol. Sedanolide has been reported to be the most active of the pthalides in the reduction of tumours in laboratory animals. The sedanolide being the major flavour-impact compound of celery volatile oil, possessing the above mentioned health benefits, an enriched sedanolide fraction will be useful for the treatment of ailments like hypertension and heart ailments. Antioxidant, cyclo-oxygenase and topo isomerase inhibitory activities have been shown to be associated with compounds including sedanolide isolated from Apium graveolens Linn. Seeds (Momin,2002).

3-n-Butyl phthalide, and sedanolide, isolated from celery seed oil exhibited high activities to induce the detoxifying enzyme glutathione S-transferase (GST) in the target tissues of female A/J mice (Zheng ,1993). After treatment with 3-n-butyl phthalide and sedanolide, the tumor incidence was reduced from 68% to 30% and 11%, respectively. About 67% and 83% reduction in tumor multiplicity was also observed with 3-n-butyl phthalide and sedanolide, indicating that 3-n-butyl phthalide and sedanolide were both active in tumor inhibition and GST assays, suggesting a correlation between the inhibitory activity and the GST-inducing ability. The results suggest that phthalides, as a class of bioactive natural products occurring in edible umbelliferous plants, may be effective chemopreventive agents.

Specific herbal preparation containing phytochemicals from ginger, cayene, turmeric, yucca, devil's claw, nettle leaf, black cohosh, alfalfa and celery seeds have been used to treat prophylaxis and the therapy of joint and connective tissue disorders in vertebrates (US patent No. 5916565, 1999). Extracts of celery seed have been evaluated for the treatment and prevention of inflammation and

gastrointestinal irritation(US patent NO. 6352728, 2002 ; US patent No. 6576274, 2003).

Materials and methods

Two approaches were tried for the enrichment of phthalides in celery seed oil (i)solvent-solvent partition , (ii) column chromatograpy

Solvent –solvent partition

Celery seed volatile oil obtained by steam distillation, containing limonene (70-80%) , β-selinene(15%) and phthalides was subjected to solvent partition using aqueous ethanol 65% in a separating funnel when two layers were obtained, the upper layer containing limonene and selinene, and the lower layer containing phthalides. The phthalide layer was subjected to repeated extraction(3 times) using ethyl acetate as solvent. The ethyl acetate layer was separated and distilled under reduced pressure (10 mm) to get a phthalide rich fraction (80-85%). This fraction was subjected fractional distillation under reduced pressure in the range of 1-0.1mm Hg to obtain a highly enriched phthalide preparation (90-95%), which finds application as a neutraceutical and a flavourant.

Column chromatography

Another approach was tried for the enrichment of phthalides from celery seed volatile oil. The oil was loaded on to a silica gel (60-120 mesh) column and eluted with the solvent hexane and mixture of ethyl acetate and hexane. Silica gel was activated in oven at 110°C before extraction. The bed height of silica gel packed was 8 cm. First two fractions were eluted with hexane and 3to 7^{th} fractions were eluted with increasing strength of ethyl acetate in hexane. Finally 8^{th} fraction was collected with acetone. Each fraction was analysed by GC and yield calculated.

Results and Discussion

Column Chromatography

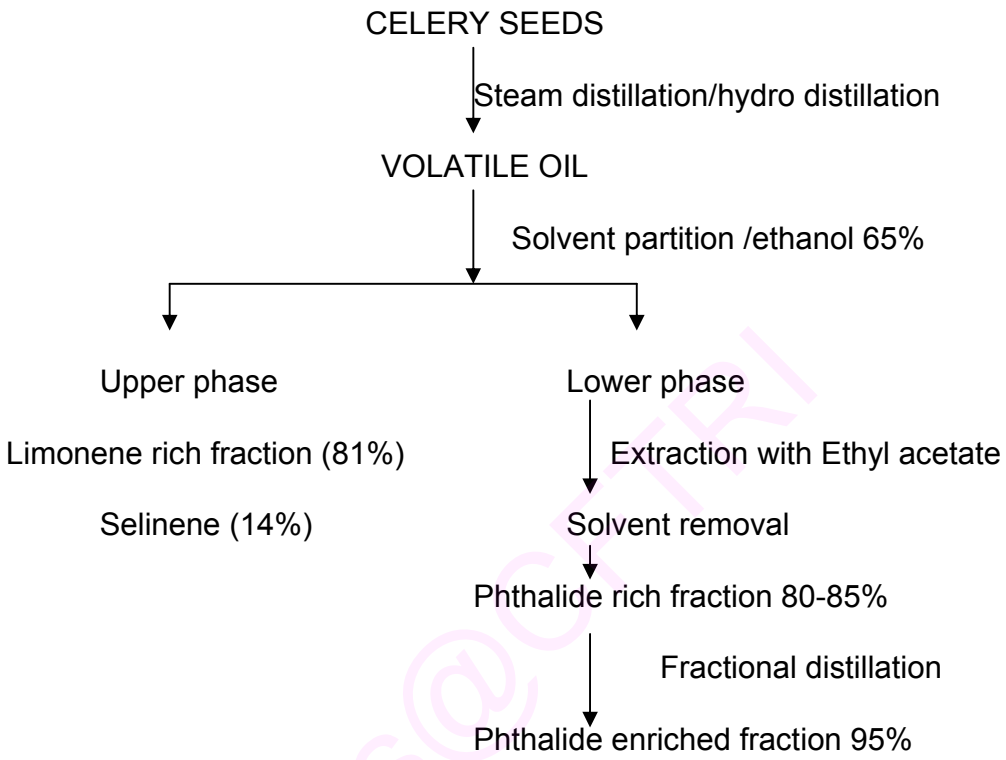
By column fractionation of celery seed oil a phthalide rich fraction can be obtained. The purity of the phthalides starts from 6^{th} and 7^{th} fraction. The yields of the fraction is given in table

Table 16

Fraction No.	Eluting solvent	Wt. of the fraction(g)	Purity of phthalides
1	Hexane	1.54	
2	Hexane	1.54	
3	2% E.A. in hexane	0.074	
4	10%E.A.i n hexane	0.3081	
5	10% " "	0.142	
6	20% " "		
7	20%		
8	acetone		

Though by column fractionation of oil , a phthalide rich fraction could be obtained, the method is not viable industrially. So an alternate method was sought and by simple solvent-solvent partition with aqeous alcohol 90% of the hydrocarbon fraction of the oil the limonene being the major one could be separated from the oil to get a phthalide enriched fraction (0%) which on further extraction with ethyl acetate and by subjecting to fractional distillation at high vacuum 0.1 mm Hg , an enriched fraction of celery (95%) could be obtained. The method has the advantage of simple two steps of operation without any sophisticated equipment involved.

Fig. Flow chart for the preparation of phthalide enriched fraction



III ULTRASONICATION STUDIES ON CELERY

Sonication technique has been used to get higher yield of active components from plant materials and also to reduce the extraction time drastically compared to conventional methods of extraction. Sonication technique was applied to celery seeds for the extraction of volatile oil. The probe type of sonicator was used for the sonication. In the first set of experiments ,sonication was carried out to whole seed spice without powdering. The samples (15g in each case with 30 ml water) were taken in a beaker and the probe was dipped into the sample and irradiated with sonication at 60 % amplitude at 0.5 cycles for different time intervals viz.,1 , 5 , 10 and 30 minutes. After the sonication , the samples were subjected to hydro distillation for 4 hours.

In another study, celery powder was subjected to sonication under above mentioned conditions. After the sonication, the sample was subjected to hydro distillation for 4 hours.

Results

In case of whole seeds sonication resulted in increased oil yield (20%) irrespective of sample to solvent ratio and times of sonication. Material to solvent ratio 1:2 was kept as optimum and the sonicaion was carried out with powdered samples. In case of powdered samples there was an increase in the yield of oil with sonication (30%). The release of oil with sonication was higher when compared to control samples without sonication. The effect of sonication on quality of the oil obtained is yet to be studied.

Cumin

Results and Discussion

Whole seeds with sonication resulted in increase in oil yield (%) irrespective of sample to solvent ratio and times of sonication. Whole seeds Yield of oil with different times of sonication. Material to solvent ratio 1:2 was kept as optimum and the sonicaion was carried out with powdered samples. Even incase of powdered samples there was an increase in the yield of oil with sonication. It was decided to observe the release of oil with sonication with respect to control

samples without sonication. The yield of oil at different periods of time was noted in both the cases. In case of sample with sonication for 15 minutes, the release of oil was faster in sonicated samples

Table 17 Yield of oil at different times of sonication (Whole seeds)

Sample	Time of sonication	Yield of oil(%) mfb
Whole seeds control		1.28
Whole celery seeds+30 ml water	5 minutes	1.5
Whole celery seeds+30 ml water	1 minute	1.5
Whole celery seeds+30 ml water	2 minutes	1.5
Whole celery seeds+60 ml water	3minute	1.5
Whole celery seeds+30 ml water	5 minutes	1.13
Whole celery seeds+30 ml water	30 minutes	1.13

Effect of sonication of whole celery seeds on yield of oil

Time of distillation	Control	sonicated sample
30	0.05	0.05
60	1.0	1.0
90	1.05	1.05
120	1.05	2.0

Sonicated powdered sample Yield of oil(%v/w) at different times of hydro distillation

Time of distillation	Control sample	Sonicated sample
1 hr	1.13	1.5
2 hr	1.13	2.06
3 hr	1.13	2.25
4 hr	1.13	2.25

CHAPTER 2

ENZYMATIC EXTRACTION OF CELERY FLAVOURANT

General methods of extraction followed for aroma recovery from plant materials are solvent extraction, hydro-distillation, steam distillation and solvent extraction. Of late the use of enzyme for extraction of flavour from plant materials has been initiated. Work has been carried out with this aspect in a few spices like ginger, pepper ,mace and garlic. Information is not available with respect to seed spices.

Celery oil is a highly value added product in terms of export market. In recent years there is an increase in the demand for cumin oil and oleoresin in export market. The application of enzymes in single and also in combination (mixture of enzymes) has been studied for the extraction of volatiles in cumin and quality and quantity of the flavourant (volatile oil) extracted was studied in comparison with the conventional method.

General methods of extraction followed for aroma recovery from plant materials are solvent extraction, hydro-distillation, steam distillation and solvent extraction. Of late the use of enzyme for extraction of flavour from plant materials has been initiated. Application of enzymes for the extraction of volatiles from spices is a new area. Work has been carried out with this aspect in a few spices like ginger, pepper ,mace and garlic ( ) . Information is not available with respect to seed spices.

Celery oil is a highly value added product in terms of export market. In recent years there is an increase in the demand for cumin oil and oleoresin in export market. The application of enzymes individually and also in combination (mixture of enzymes) has been studied for the extraction of volatiles in cumin and quality and quantity of the flavourant (volatile oil) extracted was studied in comparison with the conventional method.

Application of enzymes to whole celery seeds were carried out to enhance oil yield and reduce distillation time (hydro-distillation). Two variations were tried for oil distillation viz.,

(i) enzyme application, incubation, subjecting the whole seeds to distillation

(ii) enzyme application, incubation, drying the seeds at low temperature, powdering and subjecting the powder to distillation

Material and Methods

Enzymes used in the study were Commercial enzymes procured from Biocon, Bangalore Following enzymes were used : Cellulase , Hemicellulase, protease, pectinase, Combination of above enzymes and Enzyme mixture

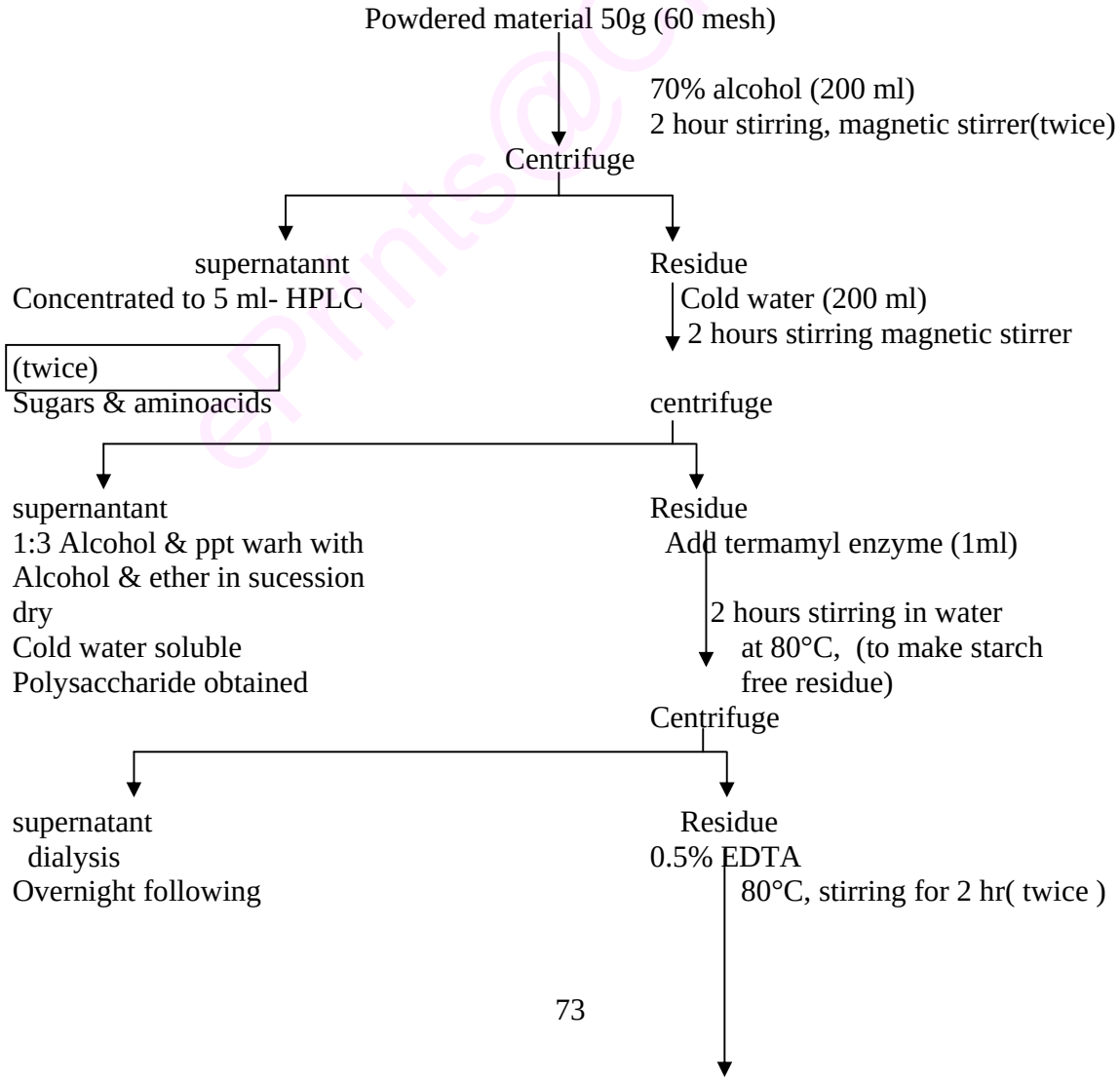
Celery seeds 200 g were treated with enzyme in aqueous solution (30 ml water) and mixed with citric acid (0.2 g in 10 ml water) mixed well and incubated for different period of time. After incubation, the enzyme treated seeds were dried in the oven. For one hour at 55 °C . The seeds were powdered in a mixer to pass through a sieve of mesh size 20-25 size microns and subjected to steam distillation for 3 hours. The volume of oil collected was measured and expressed

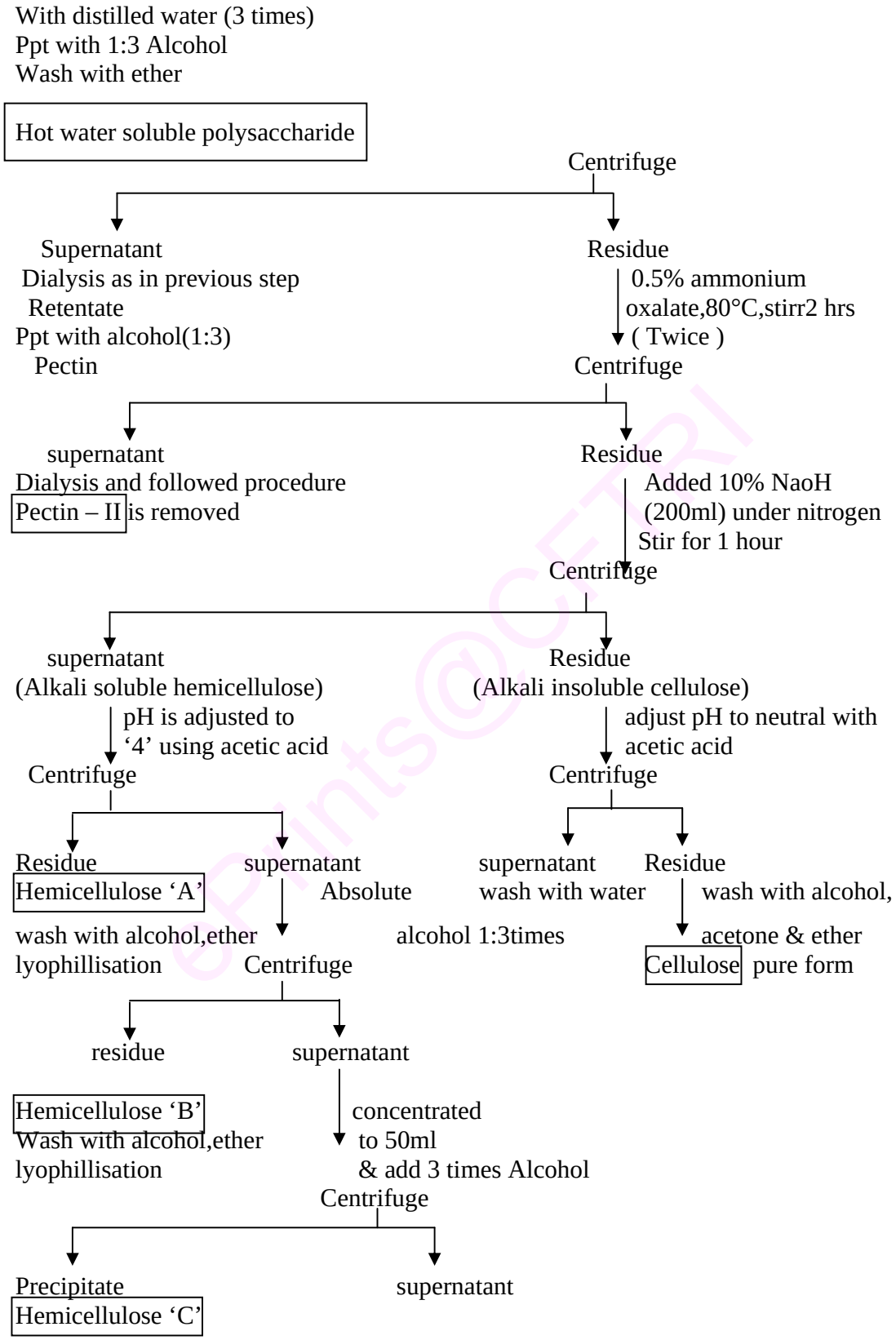
as per cent ml/100g . The oil collected was dried over anhydrous sodium sulphate and stored in refrigerator until further analysis by GC .

Carbohydrate Composition

To under stand the action of enzymes on cumin seeds and to get a clue on which point the enzyme is acting and also to decide on selection of enzymes to loosen the cell wall, it was decided to study the carbohydrate composition of cumin seeds. The carbohydrate composition of cumin seeds in terms of cellulose, hemicellulose, pectins, was carried out by the following the procedure of (Tharanathan, 2000) . The method followed in given in fig. .

Fig. Carbohydrate compositions in Cumin





Wash with alcohol and ether)

Physico-chemical quality

The volatile oil obtained by the enzyme pretreatment was analysed for the physical quality viz.,specific gravity, refractive index and optical rotation in comparison with the control sample (without enzyme treatment). Chemical quality i.e. effect of enzyme pretreatment on the flavour compounds was studied by GC analysis. The conditions followed for GC analysis are as given under chapter 1 for celery oil analysis.

Resin extraction

The cumin powder after the volatile extraction by seam distillation was extracted with solvent acetone in a material to solvent ratio of 1:10 and desolventised to get resin. The resin obtained was blended with volatile oil to get oleoresin.

Results and Discussion

Enzyme application was carried out to whole celery seeds since enzyme application to celery powdered resulted in decrease in yield of oil the reason may be oil escapes during incubation time

Application of enzymes to whole enzyme , incubation and distilling without powdering results in 15% increase in the yield of oil (Table 1,) while drying the enzyme treated seeds, powdering the seeds and stem distilling results in 21% increase in the yield of oil (Table 2). The reason may be powdering helps in easy release of oil because the surface area increases. After enzyme application and incubation, drying the seeds is required for powdering .

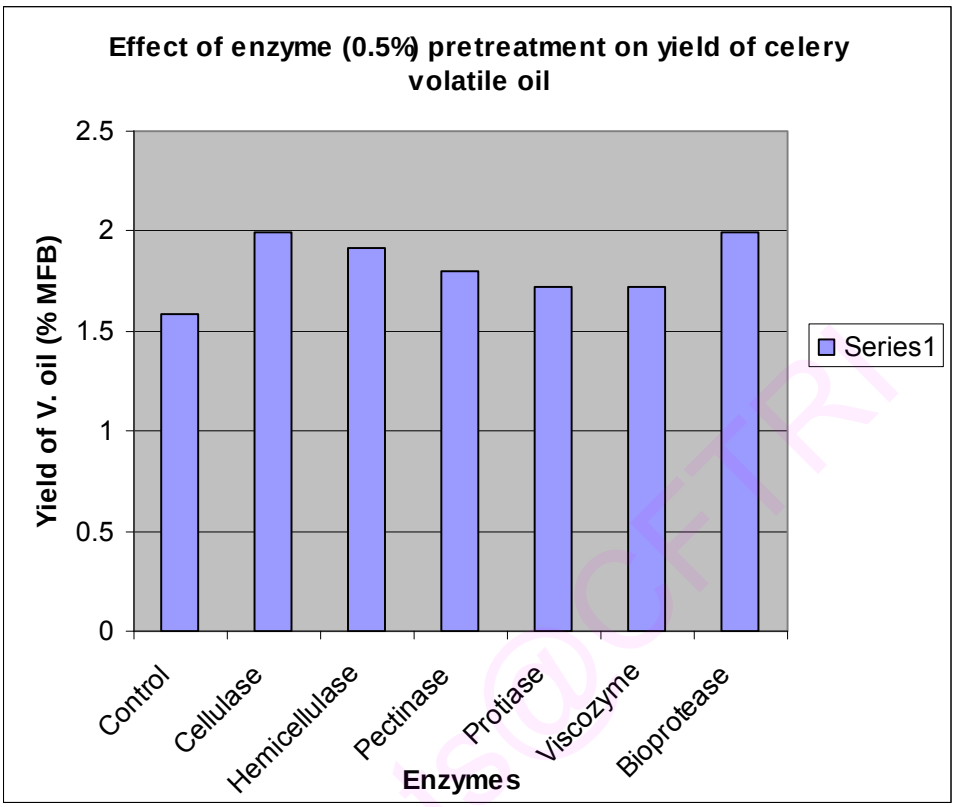
Enzyme concentration

Among all the enzyme pretreatments, cellulase treatment gave the best result in highest yield of oil even at lowest concentration of 0.5% (Table) .Next best treatment is with hemicellulase at 0.5 and 1.0% conc. The reason may be due to the effect of these enzymes on the cell wall enhancing the release of volatile compounds and at this same concentration, protease and pectinase being less active. At 1% level, both cellulase and hemicellulase have found to have the equal effect on the yield of oil (12% increase in oil) Cellulase at 1% level has shown same effect of protease at 2% addition (8% increase) A combination of cellulase and hemicellulase at 0.5 and 1.0%level have given equal yield of oil (12% increase). There was no synergistic effect of cellulase and hemicellulase observed

Incubation time

In case of each of the enzyme incubation time of 30 minutes, 60 minutes,90 minutes and 120 minutes were evaluated in terms of yield of oil. In case of cellulose 120 minutes of incubation was found to be optimum(3.3% oil) compared to 30,60 90 minutes(3.2% oil). In case of hemicellulase 60 minutes of incubation was found to be optimum (2.5% oil) compared to 30 minutes (2.9% oil) 90 minutes (3.2%), 120 minutes (3.15%). In case of Protease 60 minutes incubation was found to be optimum (3.25%) compared to 30 minutes (3%), 90 minutes (3.2%), 120 minutes (3.2%). In case of viscozyme mixture of all enzyme, 90 minutes

incubation was found to be optimum (3.25%) compared to 30 minutes (3.1%), 60 minutes (3.2%) and 120 minutes (3.2%)



Effect of enzyme pretreatment on Physico-chemical quality of celery oil

Physical Quality

As seen from Table specific gravity, refractive index and optical rotation of the volatile oil obtained by enzyme pre-treatment does not differ too much from the control sample (without enzyme In case of optical rotation, the values were higher in all the cases of enzyme treated samples. This may be due to the higher quantities of the active components of volatile oil extracted.

Table 18 Effect of enzymes on whole celery seeds (steam distillation without powdering)

enzymes	Enzyme concentration(%)	v.oil % vwb	% increase

Control		2.1	
Cellulase			
Hemi cellulose			
Pectinase			
Protease			
Viscozyme			
Cellulase+Hemicellulase			

Table 19 Effect Of Enzymes on yield of celery seed oil
(steam distillation after powdering)

Enzymes	Enzyme concentration	v.oil % mfb	% increase
Control		2.7	
Cellulase	0.25	3.1	14.8
	0.5	3.3	22.2
	1.0	3.3	22.2
	2.0	3.3	22.2
Hemi cellulose	0.25	3.1	14.8
Hemi cellulose	0.5	3.2	18.7
	1.0	3.2	18.7

	2.0	3.2	18.7
Pectinase	0.25	3.1	14.8
Pectinase	0.5	3.2	18.7
	1.0	3.2	18.7
	2.0	3.2	18.7
Protease	0.25	3.0	11.1
Protease	0.5	3.0	11.1
	1.0	3.2	20.3
	2.0	3.2	18.7
Viscozyme	0.25	3.1	14.8
	0.5	3.3	22.2
	1.0	3.3	22.2
	2.0	3.3	22.2
Cellulase+Hemicellulase	0.25+0.25	3.2	18.7
Cellulase+Hemicellulase	0.5+0.5	3.2	18.7
	1.0+1.0	3.2	18.7
Cellulase+Pectinase	0.25+0.25	3.17	17.4
Cellulase+Pectinase	0.5+0.5	3.17	17.4
	1.0+1.0	3.17	17.4
Cellulase+Protease	0.25+0.25	3.2	18.5
Cellulase+Protease	0.5+0.5	3.2	18.5
	1.0+1.0	3.2	18.5

Chemical Quality

The effect of enzyme pre-treatment on chemical quality of oil was studied by Gas chromatography (GC). The concentration of major flavour compounds viz., β-pinene, p-cymene and cuminaldehyde were selected as marker compounds. The peak area has been taken into consideration for comparison. The GC profile of cumin with and without enzyme treatment are given fig 1 & 2. It can be seen from the fig that there is no change in the GC pattern of the oil with enzyme treatment. The concentration of the three major flavour compounds cuminaldehyde, p-

cymene and β-pinene were highest in powdered cumin oil compared to whole seed oil (oil obtained by distillation of seed) and a slight increase in the concentration of these compounds was observed with enzyme treatment of whole cumin seed . The oil obtained by pretreatment of enzyme to whole cumin seeds , powdered and steam distilled had higher amounts of marker compounds .

Application of enzymes to whole celery seeds were carried out to enhance oil yield and reduce distillation time (hydro-distillation). Two variations were tried for oil distillation viz.,

(i) enzyme application, incubation, subjecting the whole seeds to distillation

(ii) enzyme application, incubation, drying the seeds at low temperature, powdering and subjecting the powder to distillation

Table 20 Effect of enzyme pretreatment on the flavour compounds of celery oil

Sl No.	Compound	Relative area (%) Retention time (min)	control	Cellulase treated	Viscozyme treated
1.	α-pinene	12.76	0.7	0.8	1.1
2.	β-pinene	15.05	0.6	0.44	0.8
3	Myrcene	16.3	0.45	0.47	1.2
3.	Limonene	19.5	63.8	67.9	82.2
4.	3-carene	20.7	0.66	1.10	0.92
5.	Pentyl benzene	27.1	1.13	1.4	1.4
6.	Carvone	31.9	0.26	0.18	0.53
7.	carryophylllene	44.2	0.14	**0.17**	**0.41**
8.	Carryophyllene oxide	53.1	0.6	0.6	0.2
9	n-butyl phthalide	56.0	5.3	3.8	1.1
10	sedanolide	59.6	5.7	4.4	1.1
11.	sedananolide	32.92	11.7	10.0	9.6

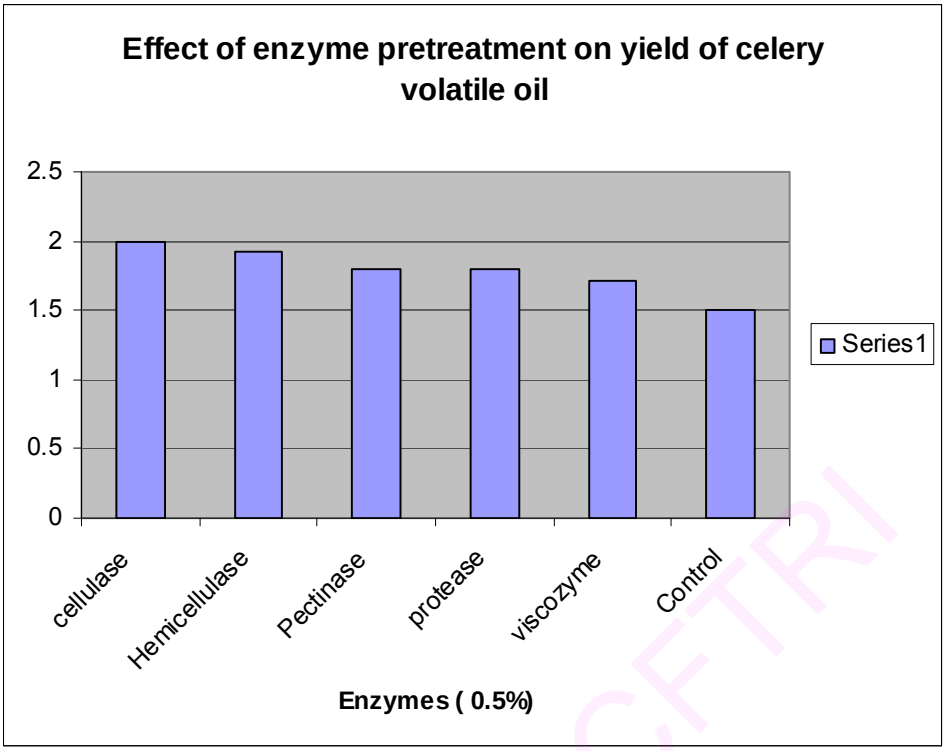
Table 21 Effect of enzyme treatments on physical quality of celery volatile oil

Sample	*Specificgravity@ 25°C	*Optical rotation @ 25°C	Refractive Index @30°C
Control	0.8967	3.6	1.4912
Enzyme treated-Cellulase	0.8926	4.0	1.4902
" Hemicellulase	0.8912	4.3	1.4960
" Pectinase	0.8915	4.3	1.4970
" Protease	0.8904	4.2	1.4923
" Viscozyme	0.8939	4.3	1.4922
" Cellulase+hemicellulase	0.8924	4.0	1.4908
" Cellulase+pectinase	0.8939	4.3	1.4948
" Cellulase+protease	0.8897	4.3	1.4934
" - Cellulase+hemicellulase+pectinase	0.8964	4.0	1.4910

Effect of enzyme pretreatment on yield of celery seed volatile oil (hydro-distillation)

Sl. No.	Sample	Enzyme conc. (%)	Incubation temp.(° C)	Incubation time (hrs)	Yield of volatile oil (%)
1	Celery seeds control	1	40	--	1.60
2	Celery seeds+ Cellulase	1	50	2½	1.80

3	Celeryseeds+ Hemicellulase	1	45	2½	1.77
4	Celery seeds + energex	1	45	2½	1.77
5	Celery powder control	--	--	--	2.00



CHAPTER 3

FOOD APPLICATIONS OF CELERY FLAVOURANT AND SPENT UTILIZATION

Food application of Celery flavourant

Some bland foods which are nutritious in nature are not acceptable if they are not suitable flavoured. Flavourants from cardamom, ginger, cinnamon, pepper are being used in various bakery products and beverages. Different forms of celery flavourant viz., celery oil and oleoresin and celery powder were used to impart flavour in crackers. The flavourants were incorporated at the dough stage in the range of 0.05-1% level. The dosage of the flavourants were optimized in each case. Effect of addition of flavourant on physical and chemical characteristics of celery crackers were analysed.

Materials and methods

Celery oil : obtained by steam distillation of celery powder in the laboratory

Celery oleoresin: obtained by solvent (acetone) extraction of celery powder in the laboratory

Texture analyzer: Model Tahdi, Stable micro system, U.K.

Total ash, dry gluten, zeleny sedimentation value and protein content of the wheat flour used were determined by AACC method (AACC, 2000)

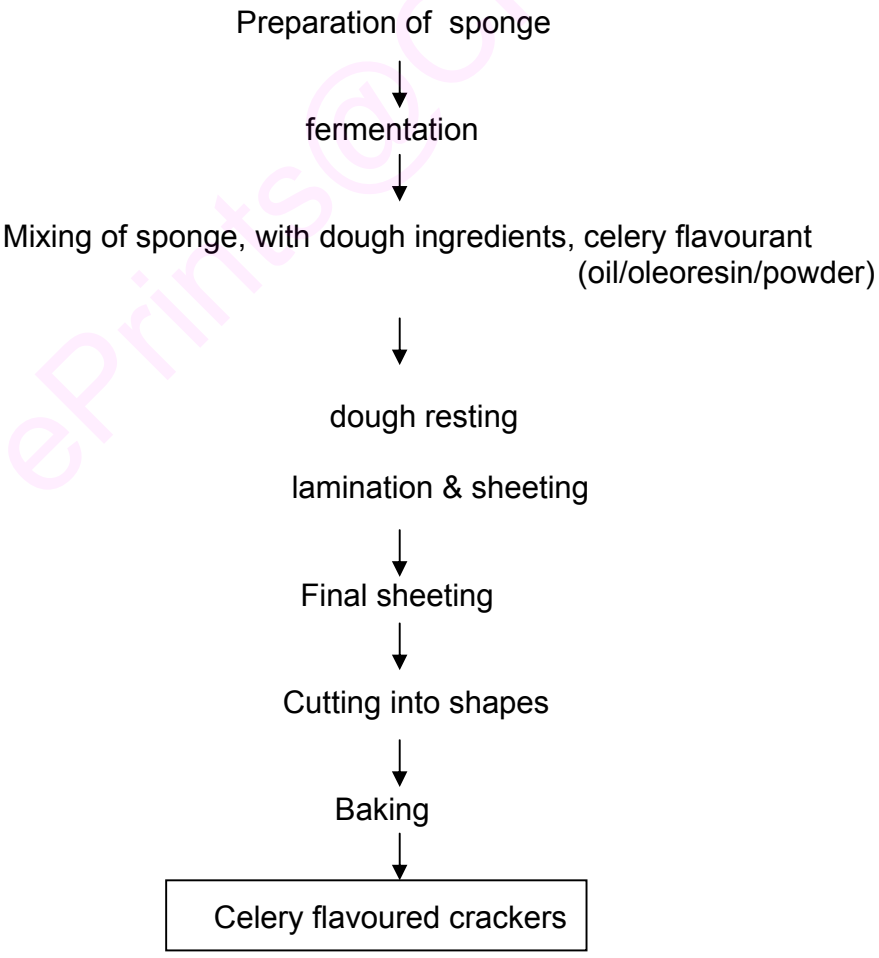
Breaking strength of crackers was measured by Triple Beam Snap Technique of Gaines (using Texture Analyser The samples were rested on two supporting beams spaced at a distance of 2.5cm another beam connected to Texture Analyser was brought down to break the crackers at cross head speed of 10mm

per minute and load cell of 10kgs. The peak force (in g) at break representing breaking strength was recorded and average values were calculated.

Preparation of crackers

The crackers were prepared using the sponge and dough method. The sponge was prepared by mixing 30 g flour, 0.5g yeast,130g water and left for fermentation for 18 hours at 30 C. and 75% RH. Sponge was mixed with 70g flour,1g salt and 20 g fat and 0.2g sodium bi carbonate and celery flavourant (oil/oleoresin/powder). The cracker dough was then sheeted, laminated and sheeted finally cut into shapes and baked (fig.1).

PREPARATION OF CELERY CRACKERS



Effect of celery flavourant addition on quality characteristics of crackers

The wheat flour used in the study had

The evaluation of physical and sensory characteristics of celery crackers were carried out. The statistical analyses of data were carried out on 8 experimental groups with 4 replicates each using Duncan's New Multiple Range Test, as described by Steel & Torrie ,1960 and were tested at 5% probability level.

Results

Celery flavoured crackers possessed golden brown colour, crisp and flaky nature in all cases of celery flavourant incorporation comparable to control samples.

The data on physical characteristics of celery crackers is presented in Table 1. The result showed that the control cracker had a spread ratio of 9.33 whereas for crackers with different forms of celery it ranged from 8.5-10.12. The breaking strength of crackers with Oleoresin varied between 1200 – 1250g as against the control value of 1440g indicating a lighter texture when compared to texture of crackers containing celery oil, seed powder and spent. The data on sensory characteristics of celery crackers (Table 2) indicated that crackers with oleoresin at 0.3% had golden brown colour and smooth surface. Whereas the texture was fragile and crackers had a prominent pungent taste. Hence crackers containing 0.2% Oleoresin were considered optimum. The crust colour of the crackers was affected at 0.4% level of seed powder. The crackers had a dark colour. There was an adverse effect on the layers and mouth feel of crackers also. The crackers had

an unacceptable bitter taste. Hence celery seed powder at 0.3% was optimum. The overall quality of crackers with 1% celery spent was 45 out of 60 and produced acceptable crackers. Hence it was considered that addition of 0.2% celery oleoresin, 0.4% celery oil, 0.3% seed powder and 1% spent was considered optimum. Celery residue (residue after volatile oil and oleoresin extraction) at preset not having food application has been successfully used in crackers and incorporation at 1% level does not produce any adverse effect on the quality characteristics of crackers.

Table 22 Physical characteristics of celery crackers

	Weight (g)	Diameter (D)(cm)	Thickness (T) (cm)	Spread ratio (D/T)	Texture** (g, force)
Control	5.7a	4.95a	0.53b	9.33c	1440a
Oleoresin					
0.2%	4.8c	4.88b	0.50b	9.76b	1250c
0.3%	4.8c	4.86b	0.48c	10.1a	1200c
Oil					
0.3%	5.8a	5.00a	0.53b	9.43c	1420a
0.4%	5.8a	4.83b	0.53b	9.11d	1350b
Powder					
0.3%	5.2b	5.06a	0.50b	10.12a	1400a
0.4%	5.4b	5.10a	0.60a	8.50d	1380b
Spent					
1%	5.4b	5.10a	0.55b	9.27c	1380b
SEM*(±)**	0.1	0.05	0.09	0.11	15

** Breaking strength measured using Texture Analyser

***Means in the same column followed by different letters differ significantly (P≤0.05) Standard error of the mean at 24 degrees of freedom

Table 23 Sensory characteristics of celery crackers

Sample	Crust Colour (10)	Crust Surface (10)	Crumb Colour (10)	Layers (10)	Mouthfeel (10)	Flavour (10)	Overall quality (60)
Control	9.0a	9.0a	9.5a	9.5a	9.0a	9.0a	55a
Oleoresin							
0.2%	8.5ab	9.0a	9.0ab	8.5ab	8.0b	8.0ab	51ab
0.3%	8.5ab	8.5ab	8.5b	7.5c	7.0c	7.0c	47bc
Oil							
0.3%	9.0a	8.5ab	8.5b	8.0ab	8.5ab	8.0ab	50.5bc
0.4%	9.0a	9.0a	9.0ab	8.5ab	8.5ab	8.5ab	52.5ab
Powder							
0.3%	8.5ab	8.5ab	8.0bc	8.0ab	7.5b	7.5ab	48.0bc
0.4%	7.5c	8.0c	7.0d	7.0d	6.5d	6.5d	42.5d
Spent			0				
1%	8.5ab	8.0c	7.5c	7.0d	7.0c	7.0c	45.0c
SEM(±)**	0.25	0.30	0.25	0.35	0.35	0.25	2.0

**Means in the same column followed by different letters differ significantly (P≤0.05).
Standard error of the mean at 24 degrees of freedom.

Conclusions

It was found that celery flavourant was feasable to use in crackers. Addition of 0.4% celery oil,0.2% oleoresin,0.3% celery powder were found to be the optimum dosage for incorporation as flavourant in crackers which was arrived at after sensory evaluation of the product by trained panelists

Addition of celery oil and oleoresin both were found to have same effect with respect to flavour perception and acceptance in celery flavoured crackeres.

Addition of celery flavourants as a new flavour to crackers has neutraceuticals benefits as celery possess anti hypertensive, anticardiac and anti-inflammatory properties.

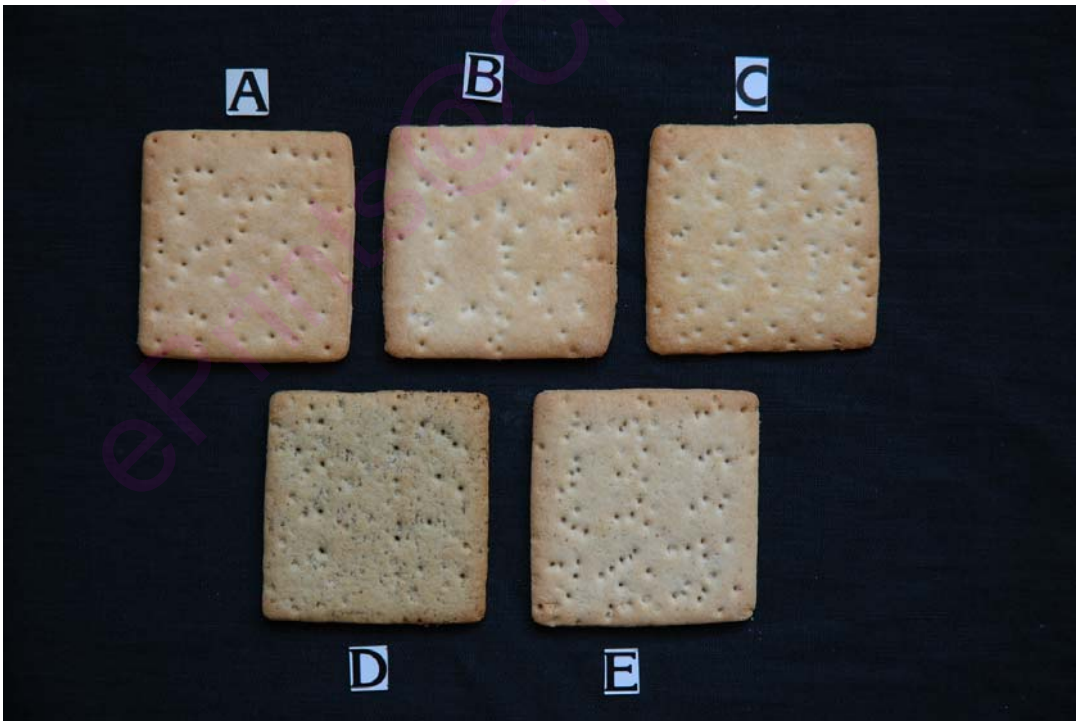


FIG 6: CELERY FLAVOURED CRACKERS

A = Control
B = Celery powder
C = Celery oil
D = Celery oleoresin
E = Celery spent

3. b STUDIES ON SPENT FROM CELERY

Celery powder after oil and oleoresin extraction does not have any commercial utility at present for food applications. Information on the usage or on value addition to the spent material is not available. It is highly desirable to find a use for the huge residues left behind after extraction of essential oils and oleoresins by the spice industry in India from the point of view of environmental protection by the effective utilization of industrial wastes. Possibility of utilizing spent residue from celery as a source of dietary fiber was explored. Soluble and insoluble dietary fibers, considered as important elements in human diet, are the storage and cell wall polysaccharides of plants that cannot be hydrolyzed by human digestive enzymes. Consumption of dietary fiber consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in plants, has received much attention due to its role in preventing certain diseases like cardiovascular disease, diabetes ,colon cancer and obesity (Chau,2004). A diet that provides adequate fiber is usually less energy dense and larger in volume and thus may bring a feeling of satiety sooner (saris,2003). National Advisory Committee in Great Britain has recommended a fiber intake of 25-30g/day /person (Daskti,2003)].

Spices are reported to contain 15-55 % crude fiber and except for a few, very little information is available on dietary fiber content in spices. Cereal brans are used as a source of dietary fiber but alternative source of dietary fiber and data on nutritional input is also required. Production and export of celery oleoresin is around 271 metric tons for the year 2002-2003 (Mathew,2000). Around 1234 tons

of celery spent is obtained which is going as a boiler feed. Only about 1% of the spent which is going as a veterinary feed, the spent which is not having any commercial value has been evaluated as a source of dietary fiber. The aim of this study was to explore the spent residue from cumin as a new source of dietary fiber for its quality, physicochemical characteristics and application potential.

Materials and Methods

Celery spent

The cumin powder after the solvent extraction is taken out from the column, air dried, and designated as spent residue which is used in the present studies.

Proximate composition

The spent was analysed for proximate composition viz.,moisture, fat, protein and ash content by A.O.A.C. methods and total dietary fiber (soluble and insoluble fiber) by the method described by (Asp,1993)

Hydration properties

Water holding capacity, is the quantity of water that is bound to the fiber without the application of any external force (except for gravity and atmospheric pressure),was determined by accurately weighing sample (1 g) into a graduated test tube, and added 30 ml of water and was allowed to hydrate for 18 hr at ambient temperature. The supernatant was removed by passing through a sintered glass crucible under vacuum. The hydrated residue weight was recorded and dried at 105^0C for 2 hr to obtain the residue dry weight

$$\text{Water holding capacity (g/g)} = \frac{\text{Residue hydrated weight - residue dry weight}}{\text{Residue dry weight}}$$

Water retention capacity, is the quantity of water that remains bound to the hydrated fiber following the application of an external force (pressure of centrifugation) was determined by accurately weighing sample (1 g) into a graduated centrifuge tube, added 30 ml of water and was hydrated for 18 hr,

centrifuged (3000 x g, 20 min) and the supernatant solution was removed by passing through a sintered glass crucible under applied vacuum. The hydrated residue weight was recorded and then sample was dried at 105^0C for 2 hr to obtain its dry weight.

$$\text{Water retention capacity (g/g)} = \frac{\text{Residue hydrated weight after centrifugation - residue dry weight}}{\text{Residue dry weight}}$$

Swelling capacity, is the ratio of the volume occupied when the sample is immersed in excess of water after equilibration to the actual weight. Accurately weighed dry sample (0.2 g) was placed in a graduated test tube, 10 ml of water was added and was hydrated for 18 h, and the final volume attained by the sample was measured.

$$\text{Swelling capacity (ml/g)} = \frac{\text{Volume occupied by sample}}{\text{Original sample weight}}$$

Results and discussion

Proximate composition of the celery spent residue revealed 5.0% residual fat, and 23% protein, 8.0 % starch ,49.1% insoluble dietary fiber and 8.5% soluble fiber. The fiber fractions from the spent residue were isolated as per the scheme shown in Fig.1. It was found to contain TDF of 57.6%, of which IDF was the major constituent (49.1). It is known that spices are generally rich in TDF, spices like pepper, coriander,cumin, fenugreek, fennel contain TDF in the range of 23-45%with chilli as high as 43.3% (potty,2005),

Effect of particle size on hydration properties

The hydration properties viz., water holding, water retention, and swelling capacity of the fiber actually determine its optimal usage levels in various processed foods for a desirable texture as well as beneficial physiological-functional characteristics [15]. The hydration properties of the spent residue increased with decrease in particle size (Fig. 2). The particle sizes studied were

from –850 - +699 microns to –500 microns. The increase in the hydration properties with decrease in particle size was due to the higher packing density by the smaller fiber particles, which enhances the surface area for better water absorption and higher swelling capacity.

Table 24 Proximate composition of celery spent

Parameter	G /100g
Moisture	6
Crude fat	5.0
Total ash	7.3
Starch	11.0
Protein	20.0
Insoluble Dietary fiber	49.1
Soluble dietary fiber	8.5
Total dietary fiber	57.6

Conclusions

Interest on health foods and focus on the health benefits of dietary fiber in the human diet invites the speculation that the spent residue from celery could provide a new source of inexpensive dietary fiber in selected food products. The dietary fiber content of the spent celery residue (57.6%) was much higher compared to many spices. Compared to other spices celery spent residue has a high TDF which can b a good potential for use as a dietary fiber.

3c. ANTIOXIDANT ACTIVITY OF CELERY SPENT REISDUE

Since celery is reported to posses medicinal properties and antioxidant property, it was decided to investigate the antioxidant activity of the spent after solvent extraction. 2,2 diphenyl picryl hydrazyl (DPPH) method was employed for evaluation of inhibition of radical scavenging activity. The methanolic and aqueous extracts of the cumin spent residue were examined for the radical scavenging activity. The radical scavenging activity of purple coloured solution of DPPH, following the methodology of . Basically, 0.1mM methanolic solution of DPPH was prepared freshly and 1ml of this solution was added to 3 ml sample solution in methanol at different concentrations. After 30 minutes, the aabsorbance was measured at 577 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

MATERIALS AND METHODS

Celery spent obtained as mentioned under the dietary fiber studies

2,2 diphenyl picryl hydrazine procured from Sigma

Methanol AR grade from Glaxo

Spectrophotometer Shimadzu

Sample solution

Celery spent (40g) was extracted with 70% methanol and water in separate columns with a material solvent ratio of 1:10. The solvent was evapourated and

resin obtained was used for the study. Resin (0.1g) was dissolved in 10 ml of methanol.

Method

Resin solution 20,40 and 80ul equivalent to 50,100 & 200 ppm of the sample was pipetted into different test tubes and 0.2 ml of the DPPH reagent in methanol was added , total volume made up to 5 ml and incubated at 30 °C for 1 hour and absorbance was measured in a spectrophotometer at 515nm against reagent blank (methanol and reagent). Similar experiment was carried out with Butylated hydroxyl anisole(BHA) a synthetic antioxidant.The percent inhibition of the radical scavenging activity was calculated using the following equation :-

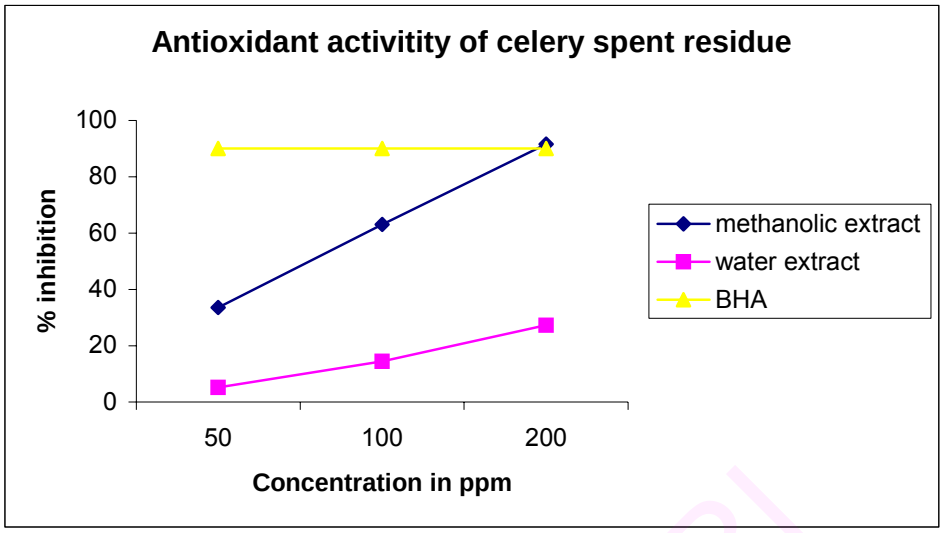
$$\text{Inhibition}(\%) = \frac{100 \times (A_0 - As)}{A_0}$$

A_0 = absorbance of blank

As = absorbance of the sample at 515 nm

Results and Discussion

Aqueous methanol extract at 50 ppm showed 33% radical scavenging activity while 100 and 200 ppm exhibited 63 and 92% radical scavenging activity respectively. The aqueous extract exhibited 5,14 and 27% inhibition of radical scavenging activity at 50,100 and 200 ppm respectively.



Conclusions

The methanolic extracts exhibited higher antioxidant activity compared to the aqueous extract. The antioxidant activity of 200 ppm of the methanolic extract was equivalent to antioxidant activity of 50 ppm BHA the commonly used synthetic antioxidant. Thus the spent residue from celery has a good potency as an antioxidant for use in food applications

STUDIES ON CUMIN

CHAPTER 4

FLAKING STUDIES ON CUMIN

Cumin seeds one of the commercially important seed spice contains about 3% volatile oil and 15% fixed oil. Owing to its high content of fixed oil, grinding of cumin seeds on large scale causes problems like heat generation leading to volatile loss, clogging of mill and dust in grinding area. Extraction of essential oil from cumin at different particle sizes has been reported (Sangani,2005) and it is reported that particle size of -35+48 gives higher recovery of essential oil by hydro distillation. Effect of feed rate and temperature on grinding of cumin and studies on cryognenic grinding of cumin seeds have also been reported (Singh,1998). Identification of flavour compounds in volatile oil extracted by super critical fluid extraction of volatile oil from ground cumin has been carried out(David ,2001). Mohammed,1991 and Vero ,1970 have standardized the extraction of volatile oil from ground cumin and have reported the major flavour components of cumin seed volatile oil as β-pinene, p-cymene, γ-terpene, cuminaldehyde, phellandral, cuminyl alcohol, p-mentha-1-4dien-7al and perilladehyde. The objective of the present study is to evaluate flaking as an alternative to powdering to avoid the heat generation during grinding and to study the effect of flaking on the yield and physico-chemical quality of volatile oil obtained n comparison with conventional grinding methods. Flaking studies were carried out in smaller batches (200g) and in larger batches (10 kg).

MATERIALS AND METHODS

(a) Cumin seeds procured from local market, India

(b) Equipments used for size reduction on laboratory scale (200g) :-

Laboratory model mini plate mill Buhler miag, Italy

Laboratory model grinder – Sumeet , India

Laboratory model flaker - Pascal Engineering, Sussex , England

(c) Equipment used for size reduction for scaled –up batches (10 kg)

Hammer mill , Batliboi, India

Large scale flaker - Twin drum roller fabricated at CFTRI, Mysore, India

Laboratory scale experiment (200g batch)

Cumin seeds were powdered in a dry grinder at laboratory level and 200 g of powder was subjected to hydro-distillation by Clevenger method (9) for 5 hours. In another batch, celery seeds were flaked in a laboratory model flaker with a gap adjustment of 0.1 m.m. between the rollers. Flakes (200g) were subjected to hydro distillation for 5 hours. The yields of volatile oil were expressed as percentage (wt./vol.)

Scale up experiment (10 kg batch)

Cumin seeds were powdered in a hammer mill using a sieve (550 microns) and 10 kg of the powder was subjected to steam distillation for 5 hours. In another batch, celery seeds were flaked in a double roller flaker with a clearance of 0.1 mm between the rollers. Flakes (10 kg) were subjected to steam distillation for 5 hours. Loose Bulk Density (LBD) of powder was 500g/l while LBD of flakes was 350g/l

Volatile oil samples were collected and yield of oil obtained was expressed as percentage (wt /vol).

Volume of oil collected for every 30 minutes was measured and altogether four fractions F1, F2, F3 and F4 were collected. The fractions were analysed for their physical properties viz., specific gravity, refractive index and optical rotation and chemical composition by GC with FID detector. Confirmation of flavour compounds was carried out by GC-MS analysis.

Gas Chromatography : Following experimental conditions were employed

Instrument: Shimazdu 15-A

Column- SE-52 on Chromosorb B (10 ft length 1/8 " i.d.)

Temp.programme: 75/5/180/2/200°C

Injector temp: 150 °C

Detector temp: 210 °C

Carrier gas flow : 30 ml/min.

The oil (0.05 ml) was diluted in acetone (1 ml) and 1 µl was injected to GC

Recovery of oil from condensate

During steam distillation, the condensate was found to be turbid, indicating that certain amount of oil was getting dispersed in water. An attempt was made to recover the oil from condensate by (i) hydro distillation and (ii) hexane extraction

(i) Well mixed condensate (five liters) obtained during 5 hours of steam distillation was subjected to hydro distillation for 30 minutes.

(ii) The well mixed condensate from steam distillation (2.0l iters) was taken in a separating funnel and 200 ml of hexane was added ,shaken well and allowed for the separation of two layers. Hexane layer separated at the top was collected . The extraction was repeated with fresh 200 ml hexane and pooled extract was distilled to get the oil .

Kinetics

Kinetics of the extraction of celery volatile oil during steam distillation was calculated (11)

In the general equation for kinetic study

$$-\frac{dC_A}{dt} = k\ C_A^n \qquad (1)$$

C_A is the yield of oil at any time θ, K is the rate constant and n is the order of change. It is the common experience that the first order (n=1) changes are encountered in most of the cases. Assigning n=1 in equation (1) and rearranging, we get

$$-\frac{dC_A}{C_A} = k\ dt \qquad (2)$$

Integrating equation (2) at conditions $C_A=C_A$ at $t=0$, and C_A at any time t, we get

$$-\ln \frac{C_A}{C_{A_0}} = k\ t \qquad (3)$$

The rate constant K was obtained by using equation (3)

Results and Discussion

Grinding methods play a very important role in the yield and quality of a spice oil. Grinding of celery seeds by the conventional method in plate mill, or hammer mill resulted in the clogging of the mill and also a rise in the temperature of the powdered material which may lead to loss of oil. Flaking as an alternate to grinding by conventional milling, has shown promising results.

Lab scale studies

In case of small batch size (200g), the yield of oil was almost equal both in case of powder and flakes, probably because difference between the material temperature during grinding (32°C) and flaking (27°C) was only 5°C. The yield of oil was 1.9% and 1.8% n the powders obtained in plate mill and dry grinder respectively which favourably compares with 1.98% oil yield from flakes (Table 1). Pre cooling of celery seeds to 5-8°C by keeping the celery seeds in freezing compartment n refrigerator, resulted in higher oil yields 2.2%, 2.05% and 2.2 % for powder obtained in plate mill, dry grinder and flaker respectively The higher yield of oil may be due to the fact that temperature attained by the product during grinding or flaking was lower

Pilot scale trials

In case of 10 kg batch, flaking of cumin seeds resulted in higher yield of oil by steam distillation (1.74%) as against (1.28 %) by grinding in a hammer mill.(Table 2). Compared to laboratory hydro distillation, the recovery of oil from flakes was 96% and 85% from powder. Reason for lower yield of oil from power

could be due to the fact that the temperature of the powder went up to 60°C, whereas flaking did not appreciably increase the temperature .

Flaking

Flaking was done with different clearance between rollers (0.05mm-0.3mm), clearance measured by thickness gauge. In each case thickness, loose bulk density and the yield of oil by hydro distillation was evaluated. As the clearance between rollers increased the flake thickness increased causing decrease in the yield of oil. The optimum clearance between the rollers to get maximum yield of oil was 0.05 mm (Table 5). Not much difference in yield of oil was seen when the clearance was 0.10 and 0.15 mm. The optimum roller gap in case of cumin is 0.05 mm to get maximum yield of oil and the next best is 0.1 and 0.15 mm. Flakes made with both 0.1 and 0.15 mm roller gap gave same yield of oil. If the roller gap cannot be adjusted to 0.5mm, then the next gap of 0.15 mm can be adjusted to get good yield of oil. The bulk density of the flakes increased with increase in the roller gap.

Table 25 Effect of roller gap on yield of cumin oil

Roller gap Mm	Average flake thickness mm	Yield of oil % mfb Hydro distillation	Bulk density G/l
0.05	0.29	2.33	200
0.10	0.38	2.27	200
0.15	0.45	2.28	225
0.208	0.48	2.2	280
0.254	0.42	2.2	260
0305	0.46	2.0	300

FIG 2: CUMIN SEEDS, FLAKES, POWDER

Kinetics of Volatile oil extraction

Table 26 Effect of grinding methods on the yield of cumin volatile oil

Method of grinding	Volatile oil % (v/w mfb)
Laboratory mixer	3.4
Laboratory flaker	3.4
Chilling & flaking	3.8
Hammer mill(10 kg)	2.8
Flaker (10 kg)	3.3

Table 27 Effect of flaking on the yield of oil with time

Fraction No.	Time min.	vol. of oil (ml) (Powder)	vol.of oil (ml) (Flakes)
F_1	30	175	147
F_2	60	15	33
F3	90	2	30
F4	120		219
Total time & volume	210	192	219
Yield of oil (%) Mfb		2.8	3.3

Physico-chemical quality of the oil

The physical properties of the oil viz., specific gravity, refractive index and optical rotation did not significantly change with flaking (Table 3). A slight higher value of optical rotation of oil from flakes was observed which may be due to higher limonene content extracted..

Table 28 Physical properties of cumin oil

Parameters	Oil from powder	Oil from Flakes
Specific gravity (g) at 25°C	0.9050	0.9039
Optical rotation at 25°C	+ 3.5	+ 4.0
Refractive Index at 30°C	1.4884	1.4907

The relative concentrations of the flavour components of the volatile oil was estimated by GC (fig. 1).Higher concentration of limonene (80%), the major flavour compound and linalool (1.46%) was observed in flakes as compared to that from powder (73% and 0.96% respectively). It was observed that mainly terpenes are extracted in first two fractions with a small amount of phthalides and the sesquiterpene □-selinene, the second major constituent gradually increased. Elution of the flavour impact compound phthalide was less in the first fraction. But from 3rd fraction phthalide concentration starts increasing 2.5-15% and concentration of terpenes decreased from 80 to 33%. By selective collection and pooling it is possible to obtain the specific fractions of the oil having different flavour profile. The phthalides from celery are the most significant bio-active compounds exhibiting many health benefits like protection against cancer, high

blood pressure and cholestrol. Sedanolide being the major flavour impact compound of celery volatile oil has been reported to be the most active of the phthalides in the reduction in tumours in laboratory animals. By pooling the fractions 3,and 4 a phthalide rich fraction of oil could be obtained which may have greater significance from the point of view of health benefits An enriched fraction of sedanolide will be desirable for the treatment of aliments like hypertension and heart ailments.(12). The total volume of oil collected was 132 ml and by pooling fractions.

Recovery of oil from condensate

Hydro distillation

By hydro distillation of the condensate, oil can be recovered within 30 minutes. The recovery of oil was 1.4 ml in 5 liters of condensate (0.028%). In a 10 kg batch distillation, the total amount of condensate collected was 90 liters and from it 25 ml of oil was recovered

Hexane extraction

The recovery of oil was 2.0 ml from 5 liters of condensate.

By adopting either one of the above methods, 25-40 ml of oil can be recovered from the condensate in 10 kg batch steam distillation.

GC Analysis

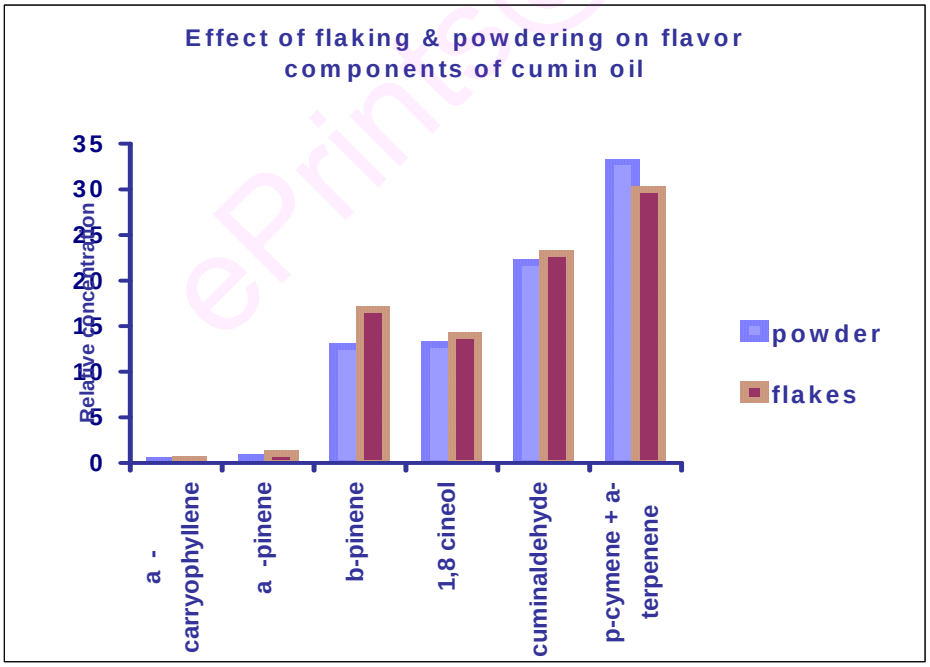
GC analysis of the recovered oil showed the presence of higher boiling compounds like phthalides in higher concentrations than the lower boiling compounds like terpenes. The oil contained by hydro distillation contains both the major terpenes

limonene and selinene and also the phthalides. The percentage of terpenes was higher in oil recovered by hydro distillation.

CONCLUSION

The study has clearly shown that by flaking the celery seeds prior to steam distillation, many drawbacks such as clogging of the mill, rise in the temperature of the ground material and loss of volatile oil associated with conventional method using hammer mill or plate mill were overcome. Flaking. and pre- cooling of celery seeds prior to flaking further improved the oil yield. and higher retention of the flavour components (terpenic compounds). Hence, for celery seed oil production by steam distillation, flaking is a promising replacement for the conventional size-reduction method of grinding. Selective collection of volatile oil at different intervals of time of distillation gives fractions of different flavour profiles. It is also possible to recover oil from the condensate and this oil can be added back to the total collected oil or can be used as a speciality oil having health benefits.

FIG 3: CUMIN SEEDS, VOLATILE OIL, OLEORESIN



Extraction of non-volatiles of Cumin by by solvent mixtures

At present , single solvents like ethylene di chloride(EDC), acetone, methylene chloride , hexane are used for the extraction of spice oleoresins. Since the use of chlorinated solvents are likely to be banned because of their carcinogenic and toxic properties. Use of alternative solvents as a replacement have been studied. Currently, EDC is being widely used in the industries because of its high extraction efficiency. In order to get a similar efficiency as EDC, use of solvent mixtures like 1) acetone and hexane ii) ethanol and hexane iii) ethyl acetate and hexane have been evaluated. For comparison individual solvents have been studied. The quality of the product so obtained is assessed in terms of resin and fatty acid content. The extraction of resin was carried out as described under chapter 1.

Fatty acid analysis

To assess the quality of cumin volatile i.e. resin there are no marker compounds reported either in literature or in the standards and specifications. Regulatory agency like Essential oil Association (E.O.A.) has not fixed no limits for any non-volatile component in oleoresin though it specifies 16-20% volatile oil in cumin oleoresin . In view of this an attempt was made to monitor the component of cumin resin i.e. fatty acid composition as a quality marker in resin extracted by different solvent mixtures. Fatty acid analysis was carried out by analysis of fatty acid methyl esters by GC(Christie,1985) as described under celery in chapter1

Table 29 Effect of solvent mixtures on resin yield of cumin powder

Solvent	Resin yield %	Solvent	Resin yield %	Solvent	Resin yield %
Acetone + hexane		Ethyl acetate+hexane		Alcohol+hexane	
Acetone 100%	14.7	Ethyl acetate 100%	14.6	100%	8.0
90+10	16.8	90+10	14.5	90+10	8.5
80+20	13.9	80+20	13.1	80+20	8.6
70+30	17.2	70+30	16.1	70+30	9.4
60+40	20.7	60+40	16.8	60+40	
50+50	18.5	50+50	16.2	50+50	12.7
40+60	20.6	40+60	15.3	40+60	13.8
30+70	16.4	30+70	14.9	30+70	11.2
20+80	16.7	20+80	14.8	20+80	12.0
10+90	16.4	10+90	12.4	10+90	12.9
Hexane 100%	14.5	Hexane 100%	14.2	Hexane 100%	14.2

RECOVERY OF SOLVENT FROM CUMIN SPENT

After the extraction of the volatile oil by steam distillation ,the deoiled powder was extracted with a solvent in a column with a material to solvent ratio of 1:6 for resin recovery. After the extraction, the spent meal , still contains solvent

equivalent to 50-60% of the material weight. The spent was sparged with steam to recover the solvent held in the material. A comparative study has been made to recover solvent held up in the meal with respect to flakes and powder. Effect of flaking is studied with respect to solvent recovery in comparison with conventional powdering in case of cumin.

Experimental details

Cumin 100 g was packed in to a jacketed glass column of dimensions15 length and 7 i.d. and extracted with a mixture of solvent mixture acetone:hexane (9:1) , the most effective solvent mixture as found by the initial experiments with solvent mixtures in small batches of extraction 5g batch,) After the extraction was complete, hot water (60-70°C) was circulated through the jacket . Top of the jacketed column was connected to the condenser and the condensate collected in a receiver. Vacuum was applied at the end. The extraction was carried out for 100 minutes in all the cases. The volume of the solvent collected was measured and quality of the solvent collected was assessed by GC.

Results

Table 30 Extraction of non -volatiles from Cumin

Parameters	Cumin Powder	Cumin flakes
Weight of sample taken (g)	100 g	100 g
Solvent used	A+H (9:1)	A+H (9:1)
Moisture in the material (g)		
Column dimension (cm)	169.3	172.0
Bed height (cm)	82	104
Volume of solvent for wetting (ml)	86.7	83
Wetting time (min.)		6.5
Flow rate (ml/min.)		
Total volume of extract collected **(**ml**)**		
Solids in extract (g)		

Table Solvent recovery from Cumin spent meal

Parameters	Cumin Powder	Cumin flakes
Weight of sample taken (g)	100 g	100 g
Solvent used	A+H (9:1)	A+H (9:1)
Weight of wet meal after extraction (g)		
Weight of solvent recovered (g)	82.0	104
Weight of spent meal recovered (g)	86.7	83
Solvent held up in spent meal (g)		6.5
Percent recovery of solvent (%)		
Loss of solvent (%)		

Table 31 Fatty acid composition of cumin resin (Acetone:Hexane)

Solvent	C14 Myristic	C14	C16 Palmitic	$C_{18:1}$ Oleic	$C_{18:2}$ Linoleic	others
Acetone 100%		0.5	4.1	63.3	29.7	2.4
90 +10		0.4	4.0	64.7	29.7	1.7
80+20	0.20	0.5	4.3	64.1	24.8	
70+30	-	-	4.3	64.5	29.6	1.7
60+40		0.3	4.1	64.3	30.4	1.0
50+50	-	0.5	4.3	60.3	34.6	0.3
40+60	-	0.5	4.3	61.2	33.0	1.0
30+70	-	0.4	4.5	63.2	31.0	0.9
20+80		0.3	4.2	63.7	30.1	1.4
10+90	-	0.4	4.1	63.6	30.5	1.4
Hexane 100%			4.5	64.2	29.8	1.5

Oleic and linoleic acid are the prominent fatty acids present in all the resin samples extracted by acetone and hexane solvent mixture in different proportions. Linoleic acid is in the range of 24 to 35%. Oleic acid in the range of 60-65%.

Table 32 Fatty acid composition of cumin resin (Ethyl acetate:Hexane)

Solvent	C_{14} Myristic	C_{16} Palmitic	$C_{18:1}$ oleic	$C_{18:2}$ Linoleic	Others
E.A. 100%	0.49	4.1	68.2	26.0	1.2
90 +10	0.41	4.6	67.8	26.8	0.4
80+20	0.49	5.6	69.2	24.5	0.02
70+30	-	6.1	71.0	22.9	0.02
60+40	0.29	5.0	67.8	27.0	0.09
50+50	0.50	-	68.2	26.0	5.33
40+60	0.50	5..0	68.2	27.0	0.57
30+70	0.43	5.6	70.8	23.2	
20+80	0.29	5.1	68.00	26.6	0.02
10+90	0.37	5.1	68.5	25.9	0.29
		4.2	68.3	26.6	0.96

E.A.: Ethyl acetate

CHAPTER 5

ENZYMATIC EXTRACTION OF CUMIN FLAVOURANT

Aroma recovery from plant materials are generally carried out by solvent extraction, hydro-distillation or steam distillation. Of late the use of enzyme for extraction of flavour from plant materials like fenugreek, ginger, pepper(Freese,1993),mustard(Dobozi,1995)Chilli(Santamaria,2000),citrus peel (Coll,2000) have been initiated. Application of enzymes for the extraction of volatiles from spices is a new area and no work has been reported with respect to cumin.

Cumin oil is a highly value added product in terms of export market and in recent years there is an increased demand for cumin oil and oleoresin in export market. The application of enzymes individually and in combination (mixture of enzymes) have been studied for the enhanced extraction of volatiles from cumin The effect of enzyme application on quality and quantity of the flavourant (volatile oil) extracted has been studied in comparison with the conventional method. The economics has been worked out.

Materials and Methods

Materials

Cumin seeds – procured from local market

Enzymes used in the study were

Cellulase - activity of 1460 units/ml.

Hemicellulase -

Protease ----1100 units/g

Commercial enzymes procured from Biocon, Bangalore

pectinase -- 9.29 units/ml

Viscozyme – commercially available mixture of enzymes containing cellulase, hemicellulase pectinase, amylase, arabinase, beta-glucanase and xylanase with an activity of 5000-12,000 pectin solubilising units/g and fungal beta glucanase activity of 50-120 units/g

procured from Novozyme, Netherland.

Acetone, hexane, ethyl acetate AR grade

Rectified spirit 95%

Incubator JEIO, korea

Mixer- Sumeet domestic model

GC- Shimadzu 15-A

GC-MS Perkin Elmer

Method

Carbohydrate Composition

To under stand the cell wall composition of cumin and to select the proper enzymes to loosen the cell wall, a study was carried out on the carbohydrate composition of cumin seeds. The carbohydrate composition of cumin seeds in terms of cellulose, hemicellulose, pectins was estimated by the following the procedure of Tharanathan, 2000 . The flow sheet of the method is given in fig.1

.*Enzyme pretreatment*

Cumin seeds 400 g was sprayed with cellulase enzyme (2.0 g cellulase in 30 ml water) and further sprayed with citric acid solution(0.2 g in 10 ml water),

thoroughly mixed and incubated in an incubator maintained at 50±2°C for a period of 30 min.The material after the incubation period was dried in an oven at 55±2°C for one hour. This dried material was divided into 2 portions of 200 g each. One batch of 200g cumin as such (whole) was subjected to steam distillation (Nambudiri, 1988) and another batch of 200 g was powdered to pass through mesh size of 20 BIS and steam distilled. In both cases distillation time was 3 hours.

(i) different enzymes namely 0.5,1.0,1.5,2.0%

(ii) different periods of incubation 30,60,90,120 minutes

The volume of oil collected was measured and expressed as per cent (v/w). The oil collected was dried over anhydrous sodium sulphate and stored in refrigerator until further analysis by GC .

Steam distillation

Steam generation was done by boiling water in a 2lt. round bottom(r.b.) flak on a mantle and the material to be distilled was filled onto a glass column and placed on the r.b. flask and connected to a condenser and a receiver a separationg funnel. After 3hrs.of distillation, the oil collected was separated and dried over anhydrous sodium sulphate, volume recorded and yield expressed as per cent (v/w). The yield of oil and as well as quality have been evaluated in comparison with the control sample without enzyme treatment.

Resin extraction

The cumin after the volatile oil recovery was air dried , ground to a mesh size of 25-30 mesh mesh (BIS) and 20 g were taken in two separate glass columns. The first column was extracted with acetone(commercially used solvent) and second column extracted with optimized solvent mixture(acetone+hexane,9+1)

as mentioned in chapter 1 (P.no.). The total quantity of solvent used for the extraction in each case was 140 ml (1:7) material to solvent ratio. This quantity of solvent was added in installments of 20 ml each .After the addition of the each installment 1hr.contact time was given. The extracts obtained were pooled(125 ml), desolventised in a flash evaporator to get the resin and the yields were recorded.

Physico-chemical quality of cumin volatile oil

The volatile oil obtained by the enzyme pretreatment was analysed for the physical quality viz.,specific gravity, refractive index and optical rotation in comparison with the control sample (without enzyme treatment). Chemical quality i.e. effect of enzyme pretreatment on the flavour compounds was studied by GC analysis. The conditions followed for GC analysis are as given under chapter 1 for celery oil analysis.

GC-MS Analysis

GC-MS analysis of volatile oil from cumin control and enzyme treated was carried out to study the effect of enzyme treatment on flavour profile of the oil. (identification of flavour compounds). The conditions of GC-MS were as follows:

Column SPB capillary i.d.

Injector: 150

Detector : 250 °C

Column: 40º (4)/2/180/4/210º C

Results and Discussion

Cell wall analysis

Cumin was found to be rich in cellulose with 27%, hemicellulose 5.1% and pectins 4.7%.

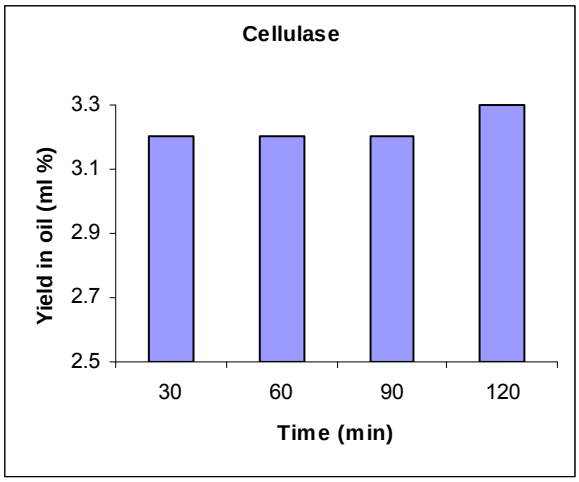
Incubation time

After the enzyme application to cumin seeds, Different times of incubation period viz., 30, 60, 90 and 120 minutes were evaluated in terms of yield of oil in case of each enzyme. In case of cellulase 120 min. of incubation was found to be optimum (3.3% oil) compared to 30, 60, 90 min. (3.2% oil) as against control (2.7%) (fig.1a). In case of hemicellulase 60 min. of incubation was found to be optimum (3.2% oil) compared to 30 min. (2.95% oil) 90 min. (3.2%) and 120 min. (3.15%) (fig.1b). In case of Protease 60 minutes incubation was found to be optimum (3.25%) compared to 30 min. (3%), 90 min. (3.2%) and 20 min. (3.2%) (fig.1c). In case of pectinase,60 min. was found to be optimum (3.23% oil),compared to 30 min. (3.1% oil) and 120 min. (3.17%) (fig.1d). In case of viscozyme mixture of enzymes, 90 min. incubation was found to be optimum (3.25%) compared to 30 min. (3.1%), 60 min. (3.2%) and 120 min. (3.2%) (fig.1e). From the above results it is observed that optimum incubation period for all the enzyme treated samples is 60 min. except in case of cellulase where the yield of oil is 3.3% at 120 min. while at 60 min. it is 3.2% which is only marginal. In view of this for cellulase treated cumin sample, 60 min. incubation period can be considered satisfactorily.

Volatile oil recovery

Application of enzymes to whole cumin seeds ,incubation and distilling without powdering results in 19% increase in the yield of volatile oil compared to control

with 0.5% viscozyme pretreatment) (Table 1,). Yield of oil in control sample is 2.1%, while with cellulase enzyme (0.5%) yield was 2.5%. Cellulase at 0.5% gave the highest yield of oil compared to all the enzymes studied. Hemicellulase at 1.0% resulted in 2.4% yield of oil. Similarly, Pectinase at 0.5% and protease at 1.0% gave a yield of 2.4% and 2.3% respectively.



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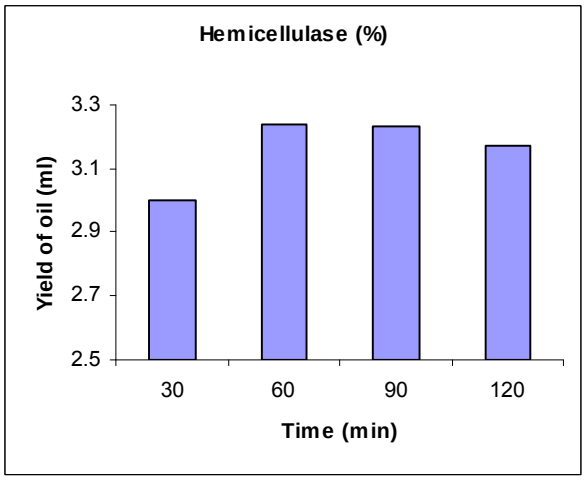

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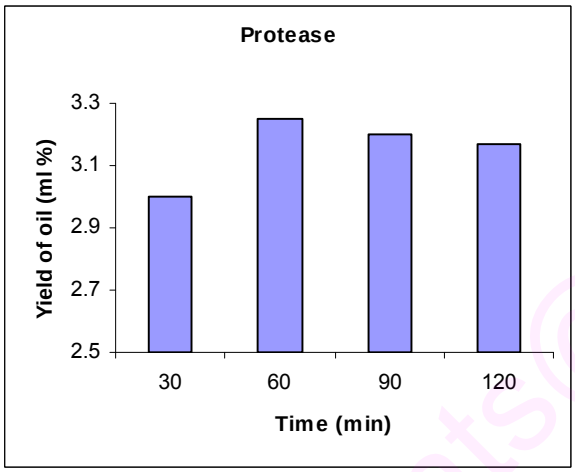

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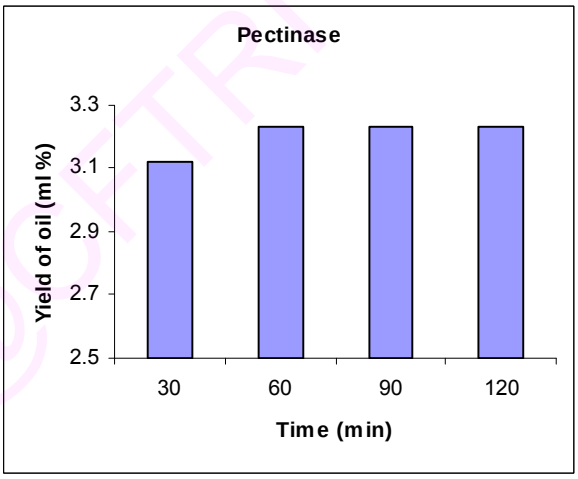

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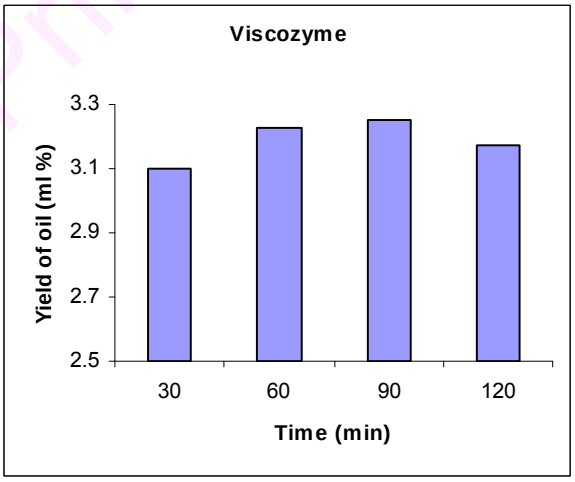


2e

Fig. 2: Effect of incubation time on the yield of cumin volatile oil (temperature 50°C, pH 4.5)

Table 33 Effect of enzyme pretreatment on whole cumin seeds (steam distillation without powdering)

enzymes	Enzyme concentration (%)	v.oil % vwb	% increase
Control		2.1	
Cellulase	0.25	2.3	9.5
	0.5	2.5	19.0
	1.0	2.3	9.5
	2.0	2.2	4.8
Hemi cellulose	0.25	2.1	-
	0.5	2.2	4.8
	1.0	2.4	14.3
	2.0	2.4	14.3
Pectinase	0.25	2.3	9.5
	0.5	2.4	14.3
	1.0	2.1	-
	2.0	2.1	-
Protease	0.25		
	0.5	2.3	9.5
	1.0	2.3	9.5
	2.0	2.1	-
Viscozyme	0.25	2.4	14.3
	0.5	2.4	"
	1.0	2.4	"
	2.0	2.4	"

Enzyme concentration

In case of cumin powder, cellulase and viscozyme treatment gave best result in highest yield of oil (3.3%) at a concentration of 0.5% compared to control sample (2.7%) (Table). Other enzymes namely hemicellulase, pectinase at 0.5% level gave 3.2% which is less and 1.0% conc. The reason may be due to lightly lower than the cellulose and viscozyme . Protease at 1% level and cellulase +hemicellulase and cellulase+protease and cellulase+pectinase at 0.25+0.25 gave 3.2% volatile oil. Cellulase treatment at 0.5% gave an oil yield of 3.3% which has resulted in an increase 22%. Since cumin oil is highly value added product, this

higher yield can be considered significant and profitable for the industry. A similar trend was noticed with viscozyme also. However cost of cellulase enzyme (600 Rs/kg) is cheaper compared to viscozyme Rs 1000/kg. There was no synergistic effect of cellulase and hemicellulase observed

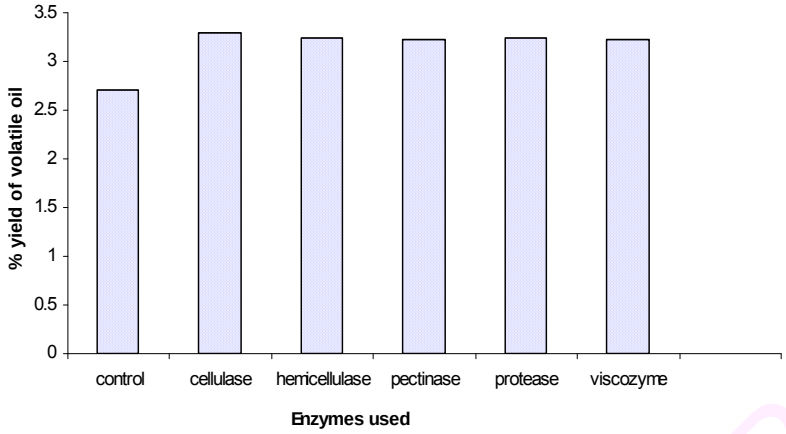
If the oil yield of cumin powder by hydro distillation (3.2%) is taken as 100% then distillation of whole cumin seeds without enzyme treatment and with enzyme treatment results in 66% and 84% recovery of oil. Pretreatment of seeds with enzymes, powdering and steam distillation resulted in 100 % recovery of oil. In some cases slightly more than the oil obtained by hydro distillation is also achieved.

The effect of different enzymes at a uniform concentration of 0.5% on the yield of oil was studied by steam distillation. It was observed that cellulase was best resulting in highest yield of oil (fig.) compared to all the enzymes studied.

Table 34 Effect of enzyme pretreatment on yield of cumin seed oil (steam distillation after powdering)

Enzymes	Enzyme concentration	v.oil % mfb	% increase
Control		2.7	
Cellulase	0.25	3.1	14.8
	0.5	3.3	22.2
	1.0	3.3	22.2
	2.0	3.3	22.2
Hemi cellulose	0.25	3.1	14.8
Hemi cellulose	0.5	3.2	18.7
	1.0	3.2	18.7
	2.0	3.2	18.7
Pectinase	0.25	3.1	14.8
Pectinase	0.5	3.2	18.7
	1.0	3.2	18.7
	2.0	3.2	18.7
Protease	0.25	3.0	11.1
Protease	0.5	3.0	11.1
	1.0	3.2	20.3
	2.0	3.2	18.7
Viscozyme	0.25	3.1	14.8
	0.5	3.3	22.2
	1.0	3.3	22.2
	2.0	3.3	22.2
Cellulase+Hemicellulase	0.25+0.25	3.2	18.7
Cellulase+Hemicellulase	0.5+0.5	3.2	18.7
	1.0+1.0	3.2	18.7
Cellulase+Pectinase	0.25+0.25	3.17	17.4
Cellulase+Pectinase	0.5+0.5	3.17	17.4
	1.0+1.0	3.17	17.4
Cellulase+Protease	0.25+0.25	3.2	18.5
Cellulase+Protease	0.5+0.5	3.2	18.5
	1.0+1.0	3.2	18.5

Effect of enzymes (0.5% level) on yield of oil



Effect of enzyme treatment on rate of extraction of volatile oil

The enzyme treated samples release oil at a much faster rate compared to the control (Table). The volatile oil collected in case of enzyme treated samples were 5.5 to 5.6 ml while it was 5 ml in control samples at 30 min. of diatillation. After 2 hrs of distillation, the release of oil is almost complete in the control sample while 0.1 ml of oil was still being extracted in case of enzyme treated samples. At the end of 180 min. of distillation, the total recovery of oil from control sample was 5.3 ml while in enzyme treated samples it was 5.3 ml which is a significantly higher amount i.e. 30% increase over the control sample.

Table 35 Effect of enzyme pretreatment on rate of volatile oil extraction

Time (min)	Volume of oil collected (ml)					
	control	Cellulase	H.cellulase	pectinase	protease	Viscozyme
30	5.0	5.5	5.6	5.5	5.5	5.5
60	0.2	0.2	0.2	0.2	0.2	0.2
90	0.1	0.1	0.1	0.1	0.1	0.1
120	0.05	0.1	0.1	0.1	0.1	0.1
150	0.0	0.1	O.05	0.1	0.1	0.1
180	0.0	0.0	0.0	0.0	0.0	0.0
Total vol. of oil collected(ml)	5.35	6.0	6.05	6.0	6.0	6.0

Table 36 Effect of enzyme treatments on physical quality of cumin Volatile oil

Sample	*Specific gravity 25°C	Refractive Index 30°C	*Optical rotation 25°C
Control	0.8967	1.4912	3.6
Enzyme treated-Cellulase	0.8926	1.4902	4.0
" Hemicellulase	0.8912	1.4960	4.3
" Pectinase	0.8915	1.4970	4.3
" Protease	0.8904	1.4923	4.2
" Viscozyme	0.8939	1.4922	4.3
" Cellulase+hemicellulase	0.8924	1.4908	4.0
Cellulase+pectinase	0.8939	1.4948	4.3
Cellulase+protease	0.8897	1.4934	4.3
" - Cellulase+hemicellulase + pectinase	0.8964	1.4910	4.0

Physical Quality

As seen from Table specific gravity, refractive index and optical rotation of the volatile oil obtained by enzyme pre-treatment does not differ too much from the control sample (without enzyme treatment). In case of optical rotation, the values were higher in all the cases of enzyme treated samples. This may be due to the higher quantities of the active components of the volatile oil extracted by enzyme pretreatment.

Chemical quality

Volatile oil obtained from control and enzyme pre-treatment were subjected to Gas chromatography (GC) analysis.The concentration of major flavour compounds viz., β-pinene, p-cymene, r-terpenene and cuminaldehyde were selected as marker compounds. The peak area(%) has been considered for comparison. The GC profile of cumin with and without enzyme treatment are given fig 2. It can be seen from the fig that there is no change in the GC pattern of the oil with enzyme treatment. The concentration of the three major flavour compounds cuminaldehyde, p-cymene and β-pinene were highest in powdered cumin oil compared to whole seed oil (oil obtained by distillation of seed) and a slight increase in the concentration of p-cymene, r-terpenene and β-pinene was observed while cumnaldehyde conc. remained same in cumin seed enzyme treated , powdered and steam distilled.

From the compilation of data from GC-MS (fig. 4) concluded that enzyme pretreatment does not have any drastic change in either of the total hydrocarbon content or aldehyde content. Two samples viz., cellulase treated as a case of single enzyme treatment and viscozyme treated as a mixture of enzymes was selected were selected to study the effect of enzyme on flavour compounds. Control samples without enzyme treatment contained 19.5% cuminaldehyde and 61.9% of total hydrocarbons out of which p-cymene and r-terpenene were major accounting for 23.7 and 22.1% respectively , whereas cellulose and viscozyme treated samples had 66.6% and 70.6% terpene content.

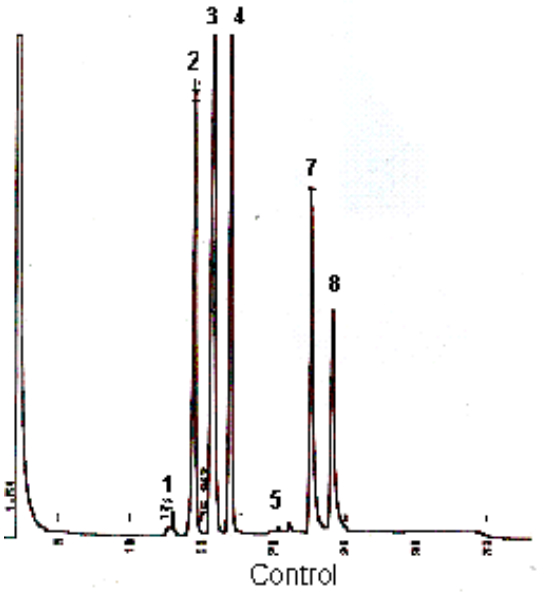
With cellulase and viscozyme treatment there was no drastic change in total aldehyde content which remained same 35%. The total aldehydes (cuminaldehyde+ perialldehyde +p-mentha 3-dien al + p-mentha 4-dien al) content were 35.32and 30% in cellulase, viscozyme treated samples respectively. The total hydrocarbon content was 62% in control and 61.8 and 68.0% in cellulase and viscozyme treated samples respectively . Total hydrocarbon and aldehyde content were higher in viscozyme treated samples compared to cellulase treated sample. In case of cellulase treatment, along with flavour compounds presence of additional compounds were observed identified as C_{14} , C_{16} , C_{18} and $C_{18:1}$ fatty acids accounting for 1.0, 3.6,1.0 and 2.0% respectively.

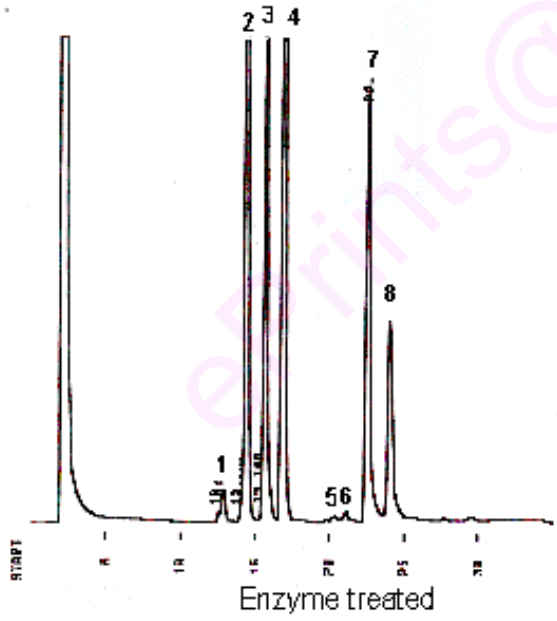
There are patents reported in literature on enzymatic extraction of volatiles from spices like ginger, cloves, garlic and tarragon which resulted in increase in yield of volatiles(WO2005063953, 2005) (XP 002287412, 1991) (11222410 ,1999). Santamaria have reported higher yield of carotenoids and capsaicinoids by treating chilli with cellulolytic enzymes(Santamaria,2000).

In the present study, a similar trend is observed with the enzyme pretreatment of cumin seeds with cellulolytic enzymes resulting in higher yield of oil of 15-20%.

Steam distillation of whole cumin seeds without powdering is 2.1%, while after enzyme treatment with cellulase and steam distillation resulted in 2.5% of oil and seeds treated with cellulase enzyme, incubation, drying powdering resulted in an yield of oil of 3.2%. By enzyme treatment 15-21% of oil could be recovered compared to control sample. Drying the enzyme treated seeds, powdering and

stem distilling results in 21% increase in the yield of oil (Table 2) compared to yield of oil by conventional method. The reason may be powdering helps in easy release of oil because the surface area increases. After enzyme application and incubation, drying the seeds is required for powdering.





1. α-pinine
2. β-pinine
3. P-cymene
4. r-terpinene
5. Sabinene hydrate
6. Limonene
7. Cuminaldehyde
8. p-metha 4-dien 7-al

Fig. 4 GC Profile of control & enzyme treated cumin volatile oil

Resin extraction

Resin yield in control sample with acetone extraction was 17% in case of control and enzyme pretreated sample respectively which indicates that there is no effect of enzyme pretreatment resin yield. However, the control sample and enzyme treated sample when extracted with acetone + hexane mixture (9:1) the yields were 23 and 28% respectively. The increase in the resin yield was 21% which is significant. The increase in yield of resin with respect to enzyme treated sample may be due to the enzyme action on cell wall facilitating leaching out of cell constituents to an higher extent compared to control.

Table 37 Effect of enzyme treatment on the flavour compounds of cumin oil

Sl No.	Compound	Retention time (min)	Relative area (%)		
			control	Cellulase treated	Viscozyme treated
1.	α-pinene	12.76	0.7	0.8	1.1
2.	β-pinene	15.48	14.3	13.8	16.6
3.	myrcene	16.5	1.0	0.9	0.9
4.	p-cymene	18.74	22.7	23.7	24.0
5.	r-terpenene	21.3		26.9	27.5
6.	Sabinene hydrate	23.39	0.59	0.53	0.53
7.	limonene	27.13			
8.	Cuminaldehyde	30.83	19.5	19.6	19.4
9.	Perilaldehyde	31.94	0.1	2.3	0.1
10	p-mentha-3-dien-7al	32.45	4.0	2.7	1.4
11.	p-mentha-4-dien-7-al	32.92	11.7	10.0	9.6

Economics for 100 kg batch

100 kg cumin ---------- 2700 ml oil control

100 kg cumin ----------3300 ml oil cellulase treated

Increase in vol.oil yield ---------- 600 ml

Enzyme used for 200 kg cumin cellulase(0.5%) ----------500 ml

Cost of enzyme @ 600 rs/lt ---------- 300

Cost of cumin oil /kg----------4000 Rs.

Increase in oil ---------- 2400 Rs.

Profit ----------

CONCLUSIONS

1. Cumin was found to contain 27% cellulose 5.3% hemicellulose and 5.5% pectin.
2. Optimization of enzyme conc. and incubation time with respect to cumin was carried out for the extraction of volatile oil.
3. Enzyme pretreatment of cumin with cellulolytic enzymes resulted in 17-22% increase in the yield of volatile oil which is a new line of work with promising results.
4. Enzyme treatment did not have drastic effect either on physical quality or chemical quality of the volatile oil and there was a marginal increase in the quantity of major flavour compounds viz., α-pinene, β-pinene, p–cymene and cuminaldehyde of the oil. Enzyme treatment had a higher amount of p-cymene and γ-terpenene
5. In case of resin extraction, higher yield of resin was obtained with extraction of enzyme treated sample with solvent mixture.
6. The optimized enzyme pretreatment were:
    - Cellulase at 0.5% and hemicellulase at 0.25% were effective for enhanced recovery of volatile oil.
    - A mixture of cellulase and hemicellulase (1:1) at 0.25% level was effective.
    - Viscozyme a commercial mixture of enzyme (mixture of cellulase, hemicellulase, pectinase, arabinase & xylanase) was effective at 0.5% level.

Since cumin oil is a highly value added product, the increase in the yield of oil 17-22% is a significant result achieved.

CHAPTER 6

FOOD APPLICATIONS OF CUMIN AND SPENT UTILIZATION

Application of cumin flavourant

Feasability of application of cumin flavourants viz., cumin oil, cumin oleoresin and cumin powder as a flavourant in beverage was studied. A formulation was prepared with each form of cumin flavourant in optimum dosage. The optimum level of dosage of flavourant was finalized after the sensory evaluation by trained panelists.

Materials

Cumin oil : obtained by steam distillation in the laboratory

Cumin oleoresin : obtained by solvent (acetone) extraction of cumin deoiled powder

Cumin powder: obtained by grounding cumin to pass through mesh size of 25 BIS sieve

Sugar

Salt

Citric acid

Method

Taking into consideration of the opinion of sensory evaluation of trained panelists 3 formulations were arrived at with different forms of cumin flavourant which are as given below :

Formulation 1

Sugar 70g

Cumin oil 1.2g

Citric acid 1.3g

Salt 0.75 g

Formulation 2

Sugar 45g

Oleoresin 0.7g

Citric acid 1.0 g

Salt 0.5 g

Formulation 3

Sugar 55g

Cumin powder 3.0g

Citric acid 1.0g

Salt 0.5g

The formulations were stored in air tight containers and 1.0 g of the formulation was dissolved in 10 ml of chilled water to obtain the beverage. Similar dilutions were made in case of each formulation to obtain ready to drink beverage. The beverage thus prepared was evaluated for sensory attributes by method. The results were subjected to statistical analysis by duncan multiple range method.

Results

The beverage prepared with all the three formulations were found to be acceptable.

The beverage prepared had a natural cloudiness which is a highly desirable factor.

STUDIES ON SPENT RESIDUE FROM CUMIN

Cumin powder after oil and oleoresin extraction does not have any commercial utility at present for food applications. Information on the usage or on value addition to the spent material is not available. Possibility of utilizing spent residue from cumin as a source of dietary fiber was explored. Dietary fiber plays an important role in health by providing a protection against cardiovascular disease, diabetes, and also the advantage of easier bowel movement.

Dietary fiber consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in plants, has received much attention due to its role in preventing certain diseases in human beings including lowering of blood cholesterol levels [1]. Consumption of dietary fiber reduces the risk of civilization diseases such as cardiovascular disease, colon cancer and obesity [2]. Soluble and insoluble dietary fibers, considered as important elements in human diet, are the storage and cell wall polysaccharides of plants that cannot be hydrolyzed by human digestive enzymes. Soluble fiber lowers serum cholesterol and helps to reduce the risk of heart attack and colon cancer. It dissolves in the gut to form a viscous gel that slows the release of glucose into blood stream. Soluble fiber also reduces total and low-density lipoprotein (LDL) cholesterol. Cellulose, hemicellulose and lignin are the main components of insoluble fibre, which

prevent or relieve constipation due to absorption of water from the digestive tract. Total dietary fiber reduces the risk of obesity, blood pressure, appendicitis and many other diseases [3]. A fiber rich meal is metabolized more slowly and nutrient absorption occurs over a longer period [4]**.** Further, a diet that provides adequate fiber is usually less energy dense and larger in volume and thus may bring a feeling of satiety sooner [5]. National Advisory Committee in Great Britain has recommended a fiber intake of 25-30g/day /person [6].

The total dietary fiber content of infant foods plays a central role in meeting the recommendations [19g/d] as well as stabilizing the intestinal population by stimulating the proliferation of bacteria capable of digesting dietary fiber and lowering the colonic pH [7]. Spices are reported to contain 15-55 % crude fiber and except for a few, very little information is available on dietary fiber of spices. Cereal brans are used as a source of dietary fiber but alternative source of dietary fiber is also needed and data on nutritional input is also required. Dearomatised cumin and coriander as a source of dietary fiber and minerals and their incorporation in bread for the nutritional input has been reported [8].

Cumin (*Cuminum cyminum*) is commercially an important seed spice valued for its aroma, medicinal and therapeutic properties. Oil and oleoresin from cumin are value added products, which have export value. Spent residue from cumin, obtained after volatile oil extraction by steam distillation and resin extraction by solvents, is not commercially exploited for food application at present except in veterinary feeds to a small percentage. Currently around 400 tones of cumin spent residue from cumin are disposed off as waste every year. The aim of this

study is to explore the spent residue from cumin as a new source of dietary fiber, for its quality, physiochemical characteristics and application potential. No information is reported in literature on the nature of dietary fiber and its characterization and also on the nature of starch in cumin. A systematic study has been carried out on the effect of residual fat and particle size on the hydration properties of the cumin fiber.

Materials and methods

Spent residue from cumin

Cumin seeds procured from local market were ground to 30-mesh size powder and the volatile oils were extracted by steam distillation. The resulting deoiled cumin powder (100 g) was taken in a glass column and extracted with acetone (200 ml) for 1 hr contact time. The extraction was repeated five times and the pooled extracts were flash evaporated under vacuum. The desolventised material is the resin to which volatile oil is added to get oleoresin. The cumin powder after the solvent extraction is taken out from the column and air dried, and designated as spent residue.

Proximate Composition

AOAC methods [9] were followed for determining the proximate composition viz., moisture, protein, fat, and ash of spent residue.

Moisture

Homogenized spent residue from cumin was accurately weighed in a tarred aluminum dish and dried overnight (about 16 h) in an oven. It was covered and cooled in a desiccator and then weighed. Sample was dried again for a further 2 hr and reweighed until a constant weight was obtained.

Protein

The total nitrogen content in spent cumin sample was determined by micro-Kjeldhal method. A factor of 6.25 was multiplied by % N_2 to get % protein value.

Crude fat

The crude fat was determined by extracting the sample in a Soxhlet apparatus for 16 hr using petroleum ether (40-60^{o}C), evaporated and the residue was weighed to get fat content.

Total ash: This was determined by igniting the spent residue from cumin until white ash was obtained followed by further ashing in a muffle furnace at 550^{o}C. The weight was recorded after cooling. The difference in weight was expressed as total ash content

Carbohydrate profile

The fiber fractions were acid hydrolysed followed by alditol acetate derivatization and GC on 3 % OV-225 (Chromosorb W,100-120 mesh) in a Shimadzu gas liquid chromatograph equipped with FID detector at 200^{0} C [10]

Starch Content

To the spent residue (0.5-1.0 g), dispersed in 50 ml water, was added heat stable alpha amylase (0.1 ml), then kept in a boiling water bath for 10 min and acetate buffer (pH 4.6) was subsequently added to 0.05 M concentration and equilibrated at 60^{0}C. To this glucoamylase (50 mg) was added and incubated in a shaking water bath at 60^{0}C for 2 hr. The solution was filtered and made up to a suitable volume and the liberated glucose was determined by the TGO (Tris Glucose Oxidase) method. The glucose value multiplied by a factor 0.9 gave the starch content [10].

Dietary fiber

The TDF, a measure of the sum of insoluble and soluble dietary fibers, based on digestion of food samples (1 g) with enzymes, was determined as described by Asp et al. [11].

Determination of hydration properties [12]

Water holding capacity, defined by the quantity of water that is bound to the fiber without the application of any external force (except for gravity and atmospheric pressure),was determined by accurately weighing dry sample (1 g) into a graduated test tube, and adding around 30 ml of water and it was allowed to hydrate for 18 hr at ambient temperature. The supernatant was removed by passing through a sintered glass crucible (G4) under vacuum. The hydrated residue weight was recorded and it was dried at 105^0C for 2 hr to obtain the residue dry weight

$$\text{Water holding capacity (g/g)} = \frac{\text{Residue hydrated weight - residue dry weight}}{\text{Residue dry weight}}$$

Water retention capacity, defined as the quantity of water that remains bound to the hydrated fiber following the application of an external force (pressure of centrifugation) was determined by accurately weighing dry sample (1 g) into a graduated centrifuge tube, adding 30 ml of water and it was hydrated for 18 hr, centrifuged (3000 x g, 20 min) and the supernatant solution was removed by passing through a sintered glass crucible (G4) under applied vacuum. The

hydrated residue weight was recorded and then sample was dried at 105^0C for 2 hr to obtain its dry weight.

$$\text{Water retention capacity (g/g)} = \frac{\text{Residue hydrated weight after centrifugation - residue dry weight}}{\text{Residue dry weight}}$$

Swelling capacity, defined as the ratio of the volume occupied when the sample is immersed in excess of water after equilibration to the actual weight. Accurately weighed dry sample (0.2 g) was placed in a graduated test tube, around 10 ml of water was added and it was hydrated for 18 h, and the final volume attained by the sample was measured.

$$\text{Swelling capacity (ml/g)} = \frac{\text{Volume occupied by sample}}{\text{Original sample weight}}$$

Isolation of starch

Starch from raw cumin powder (before steam distillation) was isolated by steeping in water followed by centrifugation (13). The crude starch isolate was purified by repeated washings with sodium chloride (0.1M)-toluene (10:1, v/v) and later by differential sedimentation in water.

Fourier transform infrared spectroscopy

FTIR spectra of native cumin starch was measured in a Nicolet 5700 FTIR spectrophotometer (from 400 to 4000 $cm^{-1)}$ under dry air at room temperature using KBr pellets. Sample (4 mg) was mixed thoroughly with 200 mg solid KBr, from

which 40 mg were taken for pellatization. Reproducibility of the data was verified on two preparations.

Scanning Electron Microscope

The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated (100 µ) with gold in a sputter coating unit for 2 min and observed in a LEO-435-VP electron microscope (LEO electron microscopy Ltd, Cambridge,UK) at 20 kV.

Results and discussion

Proximate composition of the cumin spent residue revealed 5.0% residual fat and higher protein content (23.0 %) compared to whole cumin (18%). The fiber fractions from the spent residue were isolated as per the scheme shown in Fig.1. It was found to contain TDF of 64.2%, of which IDF was the major constituent (53.8). The SDF value (10.4%) of spent cumin was comparable to that of the whole cumin (10.5%). The spent residue had a starch content of 8.0%**.** It is known that spices are generally rich in TDF, Viz. pepper (27.8%), coriander (36.4%), cumin (23.1%), fennel (28.7%), fenugreek (33.5%), and red chili (43.3%), and that the dearomatised cumin and coriander residues contain 44.28% and 31.73% fiber respectively. It has been recently reported that the defatted coconut residue, after the extraction of milk, also has a high amount of total dietary fiber (61-63%) with beneficial hydration properties[14].

Effect of particle size on hydration properties

The hydration properties viz., water holding, water retention, and swelling capacity of the fiber actually determine its optimal usage levels in various processed foods for a desirable texture as well as beneficial physiological-functional characteristics [15]. The hydration properties of the spent residue increased with decrease in particle size (Fig. 2). The particle sizes studied were from –850 - +699 microns to –500 microns. The increase in the hydration properties with decrease in particle size was due to the higher packing density by the smaller fiber particles, which enhances the surface area for better water absorption and higher swelling capacity.

Effect of fat on the hydration properties

The results (Fig. 3) show that the hydration properties of defatted spent were much better compared to spent residue with fat. This may be due to the residual oil getting trapped inside the fiber matrix, thus restricting the entry of water molecules and resulting in decreased hydration properties (Fig. 3)

Analysis of sugars in fiber fractions

By GC analysis, it was found that all six sugars viz., rhamnose, arabinose, xylose, mannose, galactose and glucose were present in both SDF and IDF fractions of spent cumin (Table 2). Arabinose followed by mannose were the major sugars in both the fiber fractions. It appears that the cumn fiber fractions essentially contain arabinannan and a mannan rich polysaccharides, the former probably accounting for its beneficial hydration characteristics. Glucose content was almost same in SDF and IDF before and after defatting the value ranging from 1.04 to 1.6%.

For starch isolation, the solvent defatted spent residue was not found to be suitable, as the isolated 'so called starch granules' were found to be partially swollen and highly covered (embedded) with fat,cell wall material and fiber matrix. This was probably attributed to the effect of steam distillation, allowing the starch granule hydration with better adhering to the fiber matrix. On the contrary, starch isolation from the raw cumin powder with successive sedimentations from the aqueous medium furnished spherically shaped starch granules (5.8 μm) in large numbers, attribute the presence of some cell wall debris. Starch content in the cumin spent residue was 8% .

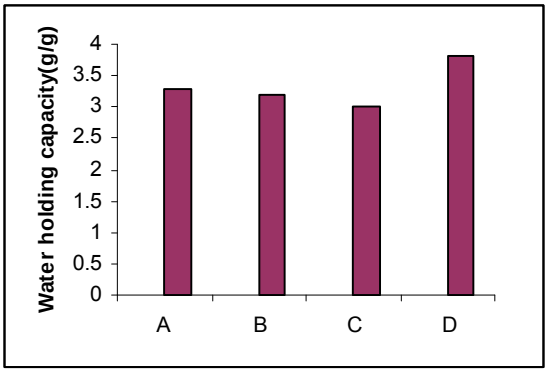
Scanning Electron Microscopy Studies

From the SEM studies, it was clear that the native cumin showed very few starch granules embedded well inside the fiber matrix (Fig. 3A), which showed distinct spherical starch granules (Fig. 3B) upon defatting by steam distillation. Upon further defatting by treatment with solvent the fiber matrix appeared to have a typical 'honey comb' structure, almost devoid of starch granules (Fig. 3C). Nevertheless, upon further purification by repeated differential sedimentation of spent residue, well separated spherically shaped starch granules of various sizes (5.8μm) were clearly visible (Fig 3D). The starch granules appeared to have smooth surface without any roughening , as seen in some legume starch granules [17]. Black pepper starch was shown to be unusually small sized (2-2.5 μ) and having polygonal shape [18].

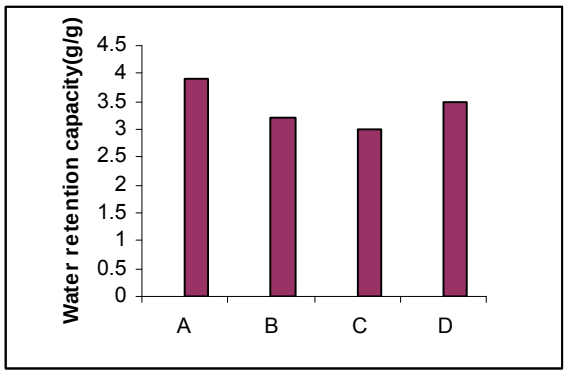
Conclusions

Interest on health foods and focus on the health benefits of dietary fiber in the human diet invites the speculation that the spent residue from cumin could provide a new source of inexpensive dietary fiber in selected food products. The dietary fiber content of the spent cumin residue (64.2%) was much higher than that of many fruits and vegetables, the fiber content of which varies from 6 to 17%, viz., grapes (1%), raspberries (4.4%), raisins (5.1%), barley (12.5%), oats (16.9%) and carrot (58%) . This study has revealed that the spent residue from cumin is a rich source of useful dietary fiber. It was also found to be rich in protein (23%). Utilization of the spent cumin residue in various functional food formulations augments sufficient value addition for an otherwise waste byproduct from the spice oleoresin industry.

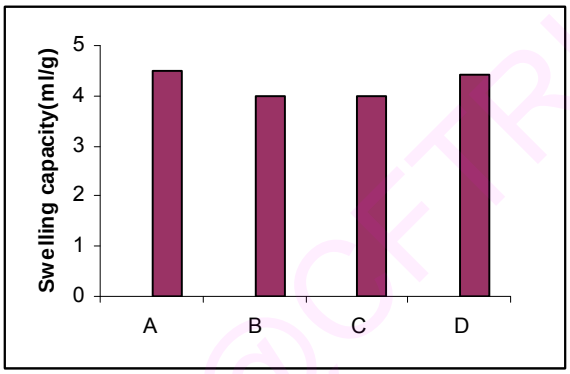
Fig. 2 Effect of particle size on the hydration properties of spent residue from cumin



(2a)



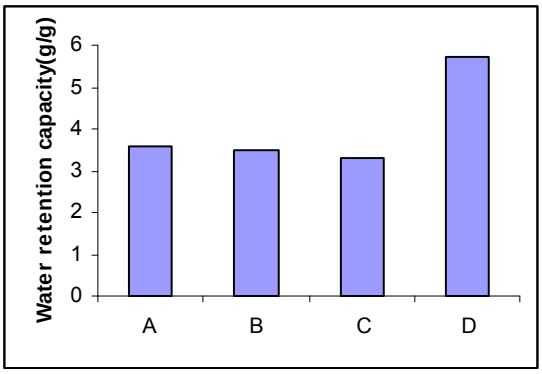
(2b)



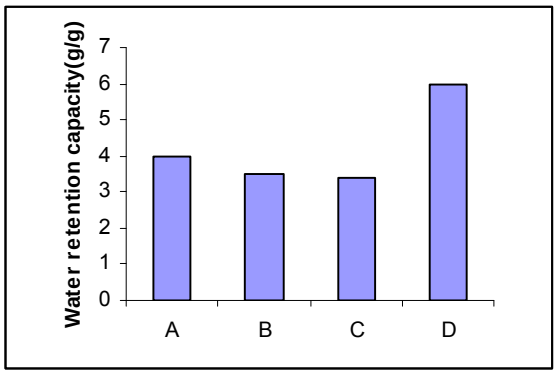
(2c)

A= -850+699 microns
B= -699+599 microns
C= -599+500microns
D= -500 microns

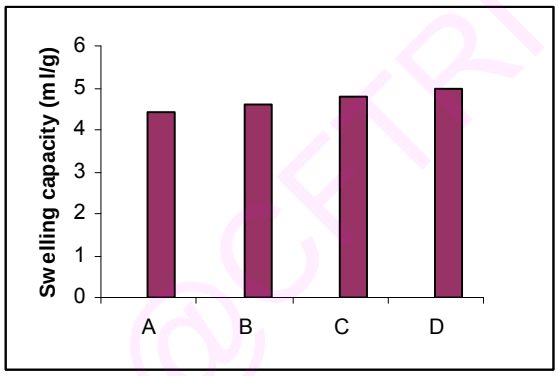
Fig.3 Hydration properties of defatted spent residue from cumin



(3a)



(3b)



(3c)

A= -850+699 microns
B= -699+599 microns
C= -599+500microns
D= -500 microns

Fig.1 Isolation of starch and dietary fiber from cumin

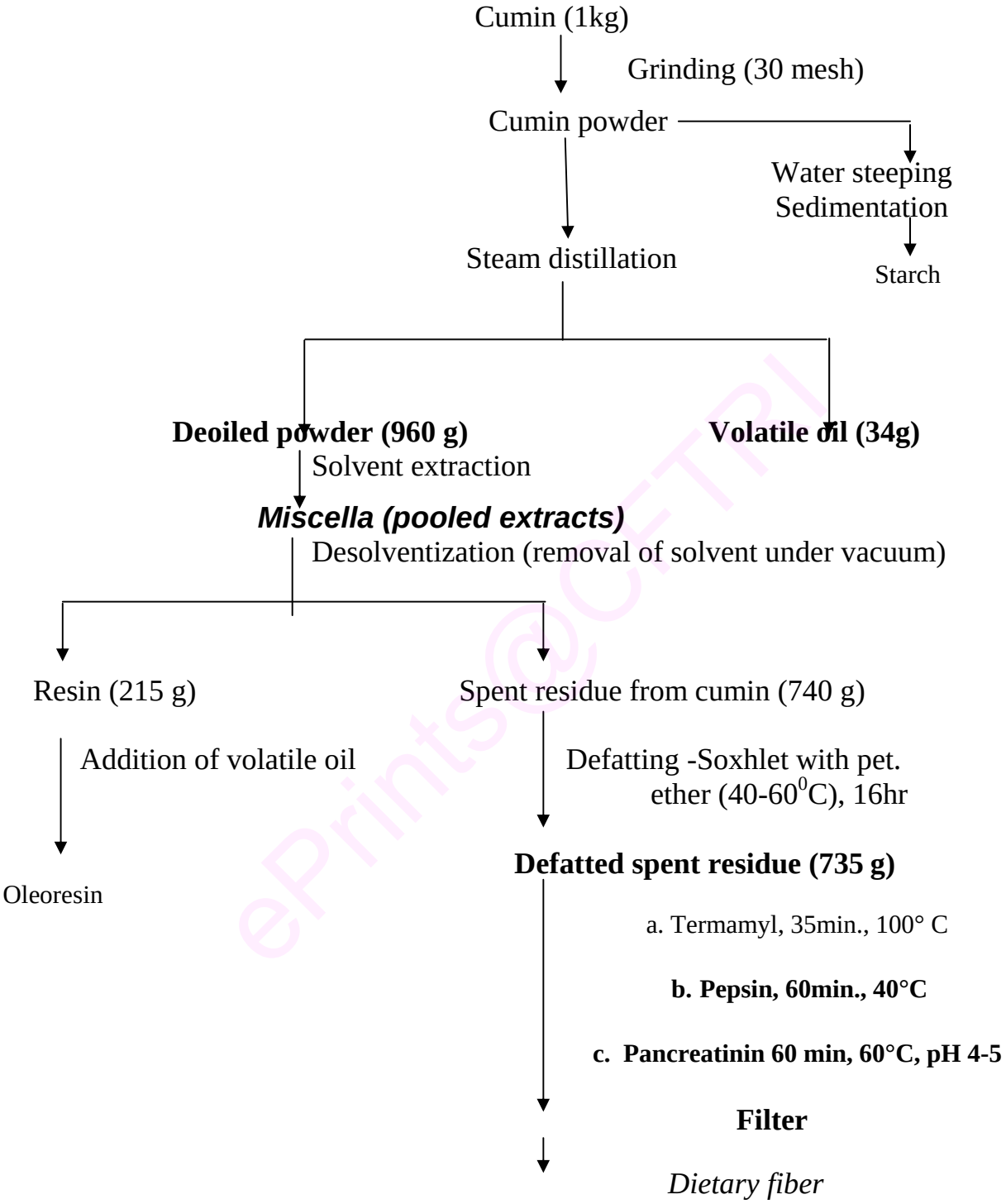


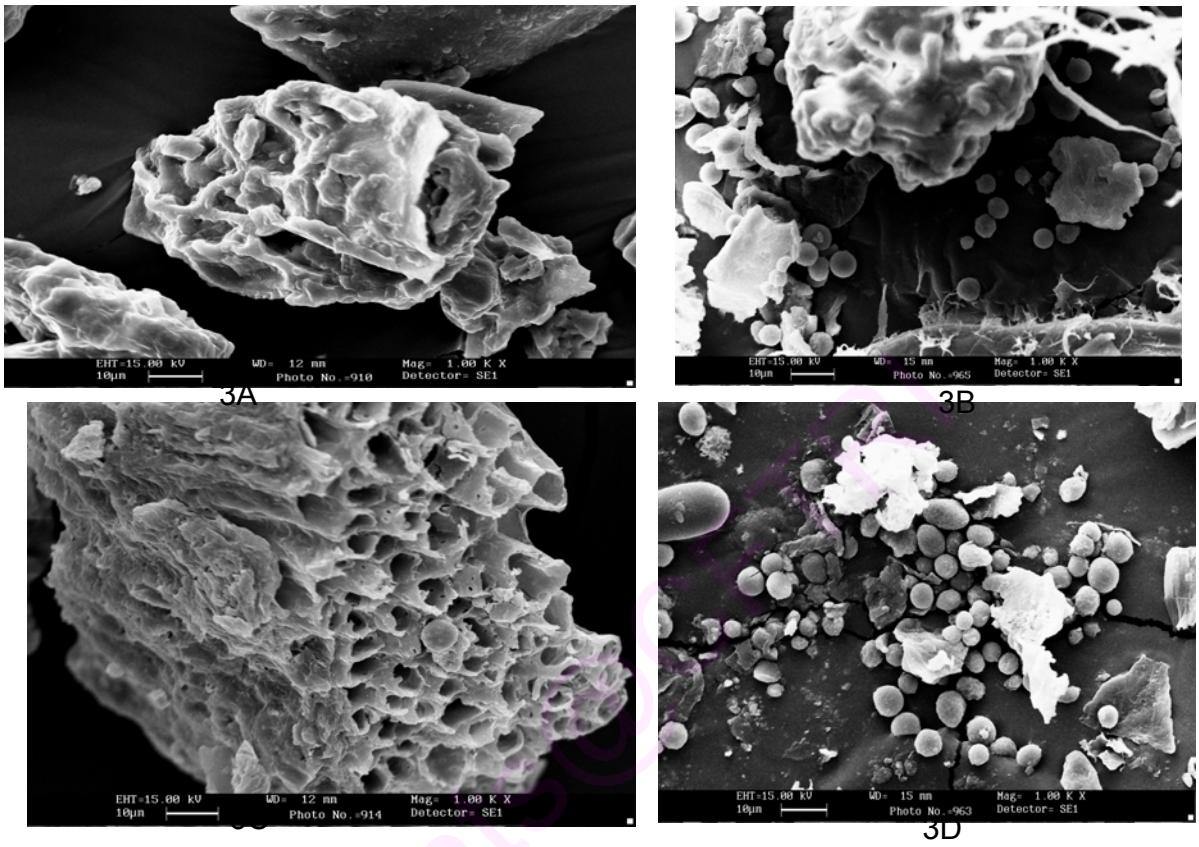
Table 1. Proximate composition (%) of spent residue from cumin

Components	Content (%)
Moisture	6.0
Crude fat	5.0
Proteins	18.0
Ash	11.0
Starch	7.9
Insoluble dietary fiber	53.8
Soluble dietary fiber	10.4
Total dietary fiber	64.2

Table 2 Sugar profile in fiber fractions of spent cumin

Sample		Sugars (g/100g)					
		Rahmnose	Arabinose	Xylose	Mannose	Galactose	Glucose
Cumin spent	SDF	6.5	52.3	4.8	31.1	3.8	1.4
	IDF	0.3	44.1	6.7	47.0	1.0	1.0
Defatted cumin spent	SDF	2.0	78.8	4.6	6.1	6.9	1.6
	IDF	3.1	37.8	11.4	46.1	0.5	1.2

Fig. 3 Scanning electron micrograph of cumin and isolated starch



A=cumin (native), B=cumin spent, C=cumin spent (defatted), D=isolated starch from cumin (native)

Appendix

Publication:

1. *Evaluation of grinding methods on the yield and quality of celery seed oil*

H.B.Sowbhagya, S.R.Sampathu, N. Krishnamurthy

Journal of Food Engineering (in press)

Papers presented in Symposia/Conferences

1. *Effect of grinding on the yield and quality of Cumin(cuminum Cyminum)*

Presented at Conference of Food Scientists &Technologists ICFOST 2004 at DFRL, Mysore on 3rd Dec. 2004

Poster was awarded III Prize

2. *Celery- A natural flavlourant for crackers*

H.B.Sowbhagya*, Jyostna Rajiv, D. Indrani ,N. Krishnamurthy*

Presented in ICFOST 2005at Bangalore on Dec. 9th, 2005