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Development of a celery oil and extract industry

A report for the Rural Industries Research and Development Corporation

By Dr Linda Falzari and

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Development of a celery oil and extract industry in Tasmania

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Foreword

Celery seed oil is a widely traded product in international markets. It is traded as a flavour and fragrance product but also used in the therapeutic goods industry as an encapsulated oil of assistance in treating gout and arthritis. It has applications as a diuretic and has potential in the treatment of stroke and cancer. Additional celery products are leaf oil and seed and root extracts and oleoresins. The research reported here was designed to assess the feasibility of establishing a local celery oil and extract industry both as import replacement and for export.

In developing these potential products, this research project built upon experience in the development of a parsley oil industry. Previously, parsley seed oil was a commonly traded product, as was parsley leaf oil. Parsley herb oil, an oil produced by distillation of the immature umbels, was developed as a successful alternative product. In assessing the potential of celery products in the market place, research focussed on developing a celery herb oil in addition to testing varieties for their potential seed oil.

This report covers both an assessment of six celery varieties and the development of the protocols used to test them.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 1500 research publications, forms part of our Essential Oils and Plant Extracts R&D program, which aims to support the growth of a profitable and sustainable essential oils and natural plant extracts industry in Australia.

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Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

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We thank Dr Noel Davies for his contribution to the research in identifying the components of the oils and extracts.

We acknowledge Dr David Ratkowsky for providing assistance with experimental design and statistical analysis of the data.

We thank RIRDC, NPE and EOT for funding the research and trust that outcome of the project will be the future development of successful new industry.

Abbreviations

ANOVA	analysis of variance
DCM	dichloromethane
%DM	percentage dry matter in plant material (see methods for details)
DW	dry weight
EOT	Essential Oils of Tasmania Pty Ltd
FW	fresh weight
FID	flame ionisation detector
GC	gas chromatography
GC/MS	gas chromatography mass spectroscopy
HPLC	high performance liquid chromatography
hr(s)	hour(s)
ID	internal diameter
min(s)	minute(s)
mw	molecular weight
NPE	Natural Plant Extracts Cooperative
ppm	parts per million
®	registered brand name
RVE	rotary vacuum evaporator
SIM	single ion monitoring
TIC	total ion current

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Executive Summary

Celery, *Apium graveolens* L. is a member of the family Apiaceae (formerly known as Umbelliferae) along with carrots, parsley, fennel *etc*. The species under investigation in this research is *Apium graveolens* var *dulce* or stem celery.

Celery seed oil is traded with increasing demand on world markets. This oil is produced by steam distillation of the seeds and is used primarily for flavouring of foods. Trace amounts are used in fine perfumes. Both celery seed oil and celery seed extract have FEMA GRAS registration enabling trade as flavour products within the USA. Trade is established in international markets.

The aim of this project was to identify a celery variety, for use in local production of steam-distilled herb oil, for the flavour and fragrance market. Initially the product is aimed at the domestic market but later also for export. In addition, the same or a further variety could provide seed oil for use as a therapeutic good.

The phthalide components and β-selinene are the critical components imparting both the typical celery flavour and fragrance and the therapeutic value. An initial literature review showed that although many researchers have analysed the composition of celery products, there is still considerable confusion in the nomenclature and description of the phthalide compounds.

Study of two possible gas chromatography columns, HP1 and HP-INNOWax determined that the most suitable was the non-polar HP1 column. Two phthalides not frequently reported in celery oil were detected. The first of these appears to be 3-butyl-5,6-dihydro-4*H*-isobenzofuran-1-one. The second was not fully characterised, but has a molecular weight of 204 and the molecular formula $C_{12}H_{12}O_3$.

Data that were used to develop laboratory protocols for testing celery field samples are presented in the report. The nature of the duration of distillation curve is such that there is an initial phase of rapid release of oil components followed by an asymptotic release that continues for many hours. For celery herb, the initial phase is concluded within approximately one hour from breakthrough, though there are statistical differences between the increments to three hours. For celery seed, the nature of the distillation curve is influenced by grinding the seed. Grinding the seed greatly reduced the lag phase at the beginning of distillation but allowed volatilisation of the more volatile components while the charge was being packed into the distillation vats. This reduced the yield from ground seed by half and this preparation method was not recommended.

Change in oil composition with duration of distillation was also considered. The more volatile components distil over more rapidly and appear at higher concentrations in the early increments. For celery, the limonene concentration decreases with duration of distillation, while the concentration of β-selinene and the phthalides increases.

Based upon both the oil yield and composition data, arbitrary cut-off points for regular examination of celery field samples in the laboratory were set at 2.5 hours for herb and 6 hours for seed. Celery herb samples can be stored frozen at –18°C prior to distillation, without adverse affects on either oil yield or quality. If longer durations of distillation of celery seed are required, these can be conducted with a mid-distillation break, without substantial effect on the final oil yield and composition.

The criterion of primary importance in the selection of varieties for flavour and fragrance products is organoleptic assessment. The researchers made preliminary selections for oils that are reminiscent of fresh, crushed celery, but the final selection must be undertaken by the end-user. Two varieties, Salad Green and Tendercrisp, were selected as being the most promising of the six varieties tested. These were chosen for further analysis in a semi-commercial trial.

Both the absolute concentrations of the key aroma components and their relative proportions are potentially important in determining organoleptic character. Preliminary comparisons between the organoleptic assessments and the oil composition data suggest that the ratio of β-selinene to n-butyl phthalide may be important. Although sedanolide and sedanenolide are sensorially active compounds, they do not have particularly low odour thresholds. Insufficient data were available in this research project for a full assessment of this observation and it is unlikely that the ratio of two components alone will determine the acceptability of a fragrance or flavour product. However, this ratio may prove a useful reference point in later studies. The component n-butyl phthalide does not appear as part of the ISO standard but the concentration of this component should be included in quality assurance assessments. The absolute value of sedanenolide and the ratio of sedanolide to sedanenolide also appear to have a bearing on organoleptic quality.

Organoleptic properties are not relevant to selection of a variety for the therapeutic market, since the oil will be encapsulated prior to consumption. The criteria of importance here are oil yield per unit area and the concentration of physiologically active components *i.e.* β-selinene and the phthalides, particularly n-butyl phthalide.

Of the varieties studied, Salad Green was ultimately selected as the most promising variety for both the flavour and fragrance markets and as a therapeutic good.

A key point of interest to potential growers of celery, as an essential oil crop, is that such production is six months out of season with celery for the fresh vegetable market. This can pose some difficulties in the procurement of seed and potential growers for essential oil need to be aware that seed may not be readily available in autumn.

The physio-chemical properties of herb oils from plant material harvested in mid-February were found to fit reasonably well within the ISO standards for seed oil, although the β-selinene concentrations tended to be low. The physical properties of the two commercially distilled oils were inside the range specified by the ISO standard.

For flavour and fragrance oils, the optimum harvest time will be determined by the development of the appropriate organoleptic properties oil through the season. This will be determined by the concentration of β-selinene and the phthalide components. High levels of these components are also required in the production of celery as a therapeutic good. Serial harvest indicated that there is a marked change in oil composition as the plants begin to flower, after which, the oil composition becomes relatively stable. When the crop is harvested, even if it has mature seed, the seed should not be cleaned from the stem material before distillation. Analysis of the waste material from the variety trial indicated that the stem material contained high levels of phthalides, particularly sedanenolide. Removing this material may reduce distillation costs by decreasing the volume of material to be distilled but this must be weighed against reduced oil quality through lost phthalides.

Besides oil quality, oil yield is also important. Harvest must take place after the commencement of flowering to ensure sufficient yield for economic viability. Oil yields of between 30 and 60kg/ha are predicted when the herb is harvested from mid-to late February.

The oil composition resulting from the commercial distillation was very similar to that predicted by the 2.5 hour laboratory distillation of the herb collected from the commercial vat. Although there may be changes in yield not detected by a 2.5 hour distillation of serial harvest samples, the oil composition is a valid assessment tool.

The duration of commercial distillation will depend upon economic comparison of the cost of distillation per unit time against the value of the oil obtained. This must take into account the fact that oil yield decreases with increasing duration of distillation, but the value of the oil obtained increases due to the increasing concentration of the valuable components in each further increment. The

varieties in the semi-commercial trial were distilled to exhaustion, taking approximately three hours. Consideration must be given to breaking the seed coat prior to distillation to allow better penetration of the steam. Laboratory work in this project showed that the risk of loss to evaporation was significant and it is not recommended that the seed be ground. Crushing or rolling the seed are two possible solutions but any method which exposes the oil to the atmosphere will be subject to the risk of the loss of volatiles. The decision to grind the seed or to distil whole seed must be made based upon economic considerations of the cost of grinding or other preparation, the cost of a longer distillation and the value of the oil lost due to volatilisation of exposed oil before the charge can be sealed into the vat.

It is recommended that under commercial conditions, that the operator separate the first 30 minutes of oil from the remainder. This will improve the quality of the remaining fraction by decreasing the concentration of the monoterpenes and increasing the concentration of the valuable components. It may be possible to rectify the first oil fraction by fractionation-distillation and blend the valuable components back into the bulk oil.

It is recommended that distillation commence with the inner skin of the separator empty and that the separator temperature be maintained as high as possible to allow fast, clean separation of the oil from the water. It was shown in this project that a low separator temperature allowed the oil to cling to the sides of the separator, reducing oil recovery rates.

Oleoresin, produced by solvent extraction of the seed, is another potential product from celery. Guenther (1950) describes the oleoresin as having more of the body of celery than the oil, due to the presence of more of the less volatile components. This product would have potential as either a flavour or fragrance product but perhaps more particularly as a therapeutic good. The quality of the extract depends primarily on having a high concentration of β-selinene and the phthalides relative to the other components.

The important criteria for selection of the optimum protocol for extraction of the oleoresin are the yield and quality of the extract. Grinding dramatically increased the efficiency of release of solvent extractable components from the seed. Although the polar solvent increased the yield of the extract, the quality of the hexane extract was higher. The costs associated with extract clean-up and solvent recovery of a polar solvent may outweigh the economic value of the additional yield.

Further aspects of solvent extraction are the number and duration of the washes. Many short duration washes appear to be more effective than a few long duration ones.

Solvent extraction of the marc of steam distilled seed is a promising method, proposed to recover the remaining valuable, but less volatile sesquiterpenes (primarily β-selinene) and phthalides. The use of this method on laboratory distilled, dry seed produced 1.0-1.2% oil from a 6 hour distillation plus a further 0.3-0.4% of extract of valuable volatiles by solvent extraction.

Celery root extracts also have potential as a therapeutic good. There are substantial differences between the composition of extracts from above and below ground parts of celery and there are phthalides present in the roots of celery that are present only at trace levels in the tops of the plants. The primary phthalide component in the roots is *cis*-neocnidilide whereas its isomer, sedanolide, is the dominant form in the tops. The other major phthalide present is the mw 204 phthalide. The value of the root extracts as therapeutic agents depends upon the as yet unknown activity of the extracted components, particularly *cis*-neocnidilide and mw 204 phthalide. Market assessment of this product is necessary to establish its value.

As with the seed extracts, hexane appears to be the best solvent for the production of root extracts. The use of slightly more polar solvents such as isohexane, pentane or light petroleum should be considered. Roots should be harvested immediately after harvest of the tops for distillation. Care must be taken that volatiles are not lost during air-drying or wilting of the roots. Rough chopping and

extraction of the fresh roots should result in yields of extract approximating 10-15mg/g of root extracted or 100ppm total phthalide.

The researchers are confident that this industry is economically viable. Yields of 50kg oil per hectare should be achievable under commercial conditions and at a farm-gate price of $50/kg for the oil; the gross margin for this crop makes it comparable to poppy and pyrethrum production. The inclusion of additional products, in the form of either marc or root extracts or both, should increase this gross margin further.

Chapter 1 Introduction

Celery seed oil is traded with increasing demand on world markets. It has a pervasive, spicy, warm and very persistent odour, produced primarily by two lactones, 3-n-butylphthalide and 3-n-butyl-4,5-dihydrophthalide (sedanenolide). It has a very low odour threshold and therefore is used in very small amounts. This oil is produced by steam distillation of the seeds and is used primarily for flavouring of foods. Trace amounts are used in fine perfumes. Leaf oil, extracts of seed and roots and seed oleoresin are additional products from celery. Celery leaf oil contains more monoterpenoids and less sesquiterpenoids than the seed oil (Dorland and Rogers 1977; Bauer and Garbe 1985).

1.1 Taxonomy

Celery, *Apium graveolens* L. is a member of the family Apiaceae (formerly known as Umbelliferae) along with carrots, parsley, fennel *etc*.

The species is divided into two varieties: *Apium graveolens* var *dulce* and *Apium graveolens* var *rapaceum*. The former of these is commonly known as “celery” or “stem celery” and is grown for its stems and leaves which are used as a vegetable, particularly in salads and soups. The latter, known as celeriac, forms a large root tuber, resembling a bulb, which is cooked and eaten. The plants which form the basis of the current research are of the variety *dulce* (Husain et al. 1988; Heaton 1997).
In this report the term ‘variety’ is used to refer to named seedlines belonging to the taxonomic variety *dulce* since this is the context in which the commercial producers of seed use the term.

1.2 Agronomic issues

Celery is well suited to a cool temperate climate in areas with well-drained, fertile soils (Husain et al. 1988). Poorly drained soils lead to root disease through the winter months.

Celery has a very fine seed size and the crop can be difficult to establish. The seed should be sown 1-2mm deep and requires consistent irrigation for good germination. In this research project, seed was sown and germinated under glasshouse conditions and trials planted using transplants to avoid establishment problems. In order to reduce costs under commercial conditions a seed to seed technique must be established.

Local growers of celery seed crops recommended a planting density of four plants/m^2 and this was used in this research. However, future research is recommended to test higher planting densities which may improve the uniformity of the crop and perhaps also produce individual plants of smaller stature that are less prone to lodging.

Celery is very susceptible to nutrient-deficiency related diseases, in particular black heart caused by calcium deficiency and hollow stem caused by boron deficiency. Regular foliar sprays of calcium and boron during the growing season can be used to overcome soil deficiencies (Rubatzky et al. 1999). High levels of nitrogen fertiliser should be avoided as van Wassenhove et al. (1990) showed that high nitrogen levels reduce the production of volatiles in celery.

Celery is also susceptible to fungal diseases including *Septoria* (leaf spot), *Erysiphe* (powdery mildew) and foliage and root blights such as *Alternaria*, *Cercospora* and *Phoma* (Dixon, 1981; (Rubatzky et al. 1999). In field trials of the current research program, these diseases were well controlled using regular applications of fungicide (section 3.3.3).

Careful monitoring of water use and application of irrigation is needed to produce a sturdy crop that is not prone to lodging.

1.3 Markets and marketing issues

Celery herb oil has potential as a flavour and fragrance product. Both celery seed oil and celery seed oleoresin have FEMA GRAS registration enabling trade as flavour products within the USA. Trade is established in international markets.

In the past, perfumers in Europe showed great interest in Tasmanian-grown celery herb oil. In 1987, this laboratory produced small quantities of celery herb oil for market evaluation in Europe and the USA. The product had the characteristics of freshly crushed celery and had immediate appeal. The 1992 preliminary market research on celery herb oil met with very favourable results and showed a potentially strong international market for Tasmanian-grown celery oil. The local industry was not in a position to develop the market at that time but it is now appropriate to extend the range of oils for import replacement and export. The authors believe that there will be strong demand for celery herb oil, in the same way that parsley herb oil was accepted on the international market. Oil from the variety Avon Pearl was of particular interest. Unfortunately, this variety is no longer available and new varieties with desirable oil must be identified.

Celery seed oil is also sold as a therapeutic good. There is mounting evidence in the literature to substantiate the pharmaceutical activity of the phytochemicals in many “herbal remedies”, including celery. Research such as that reviewed by Craig (1999) has shown that natural components, such as the phthalides present in the Apiaceae (Umbelliferae) have a biochemical basis for their alleged ability to treat or prevent disease and the market for medicinal herbs and related commodities is growing. According to Craig, the 1999 market for such in the US exceeded $2 billion.

Capsules of celery seed oil are used to treat arthritis and gout. Celery is claimed to be particularly good in this respect because it has low gastrointestinal side-effects when compared with current synthetic drugs. The component n-butyl phthalide has also been shown to be of benefit as a diuretic, useful in the treatment of high blood pressure, high cholesterol and in brain damage *e.g.* as a result of stroke. Both limonene and the phthalides have potential as anti-cancer compounds. This project did not investigate the physiological activity of celery components or their uses as therapeutic agents.

The 1984 review, by Lawrence, of the international essential oil industry revealed an annual celery seed oil production of 25 tonnes and a celery herb oil production of 0.5 tonnes. Current data are difficult to obtain but one current estimate of the annual consumption of celery seed oil is 40 tonnes/annum, with some 50% of this produced in India. Other producers are USA, Southern France, Holland, Hungary and China. Imports to Australia, in the year 2000, were approximately 1.2tonnes of seed oil. Local production could replace this import, which is used in flavours and in soft gel capsules.

1.4 Oil composition

Quantifying the volatile composition of the oils and extracts is vital in achieving objective comparisons between the potential products for the flavour and fragrance markets. The phthalide components and β-selinene are the critical components imparting the typical celery flavour and fragrance. The phthalide components are also critical to imparting therapeutic value. Assessment of the quality of the various oils and extracts requires comparison with the composition of other commercial products, ISO standards and other published data.

An initial literature review showed that although many researchers have analysed the composition of celery products, there is still considerable confusion in the nomenclature and description of the phthalide compounds. Since these compounds impart the characteristic celery aromas, a summary of the literature will provide a useful basis for the experimental work to follow.

Most early work was on the constituents of celery seed oil, although Guenther (1950) makes mention of celery herb oil in addition to seed oil when he summarised the knowledge to that time. The principal component of celery oil is *d*-limonene. Other terpenes were considered to be absent.

Sesquiterpene components present in significant quantities are α- and β-selinene and α- and β-eudesmol. At this time the odour of celery was attributed to two lactones, sedanolide and sedanonic anhydride (Guenther 1950).

Later studies elucidated a suite of monoterpene, sesquiterpene and alcohol components in celery oil that are common to many other essential oils, including β-pinene, sabinene and myrcene (Ikeda et al. 1962), (Gold and Wilson 1963), (Wilson 1969). These components, of which limonene was determined to be the major component, are easily separated, identified and quantified by a combination of GC/MS and FID. Lawrence, (1979-1980) and Lawrence (1981-87) provide reviews of this work.

In the 1980's Gijbels and associates did much to clarify the identification and nomenclature of phthalides, as did the 1994 review of Vernin (Gijbels et al. 1980; Gijbels et al. 1982a; Gijbels et al. 1982b; Gijbels and Svendsen 1982; Gijbels et al. 1983; Gijbels et al. 1984; Gijbels et al. 1985; Vernin et al. 1994). Sedanonic anhydride is not a component of celery oil and the presence of this component was in fact a misidentification of sedanenolide (Bjeldanes and Kim 1977). The important phthalides are n-butyl phthalide, sedanolide and sedanenolide. Other phthalides (often synonyms) reported in the literature include cnidilide, neocnidilide (sedanolide) and senkyunolide (sedanenolide). A brief review of the literature on the nomenclature of sedanolide is provided in Appendix A.

The ISO standard for celery seed oil defines the levels of the following five components:

β-pinene 0.5 - 2%, myrcene 0.3 - 1.4%, limonene 58 - 79%, β-selinene 5 - 20% and sedanenolide (3-butyl-4,5-dihydrophthalide) 1.5 - 11%.

Chapter 2 Objectives

This research was designed to establish celery as a new addition to the suite of crops grown in Tasmania for essential oils. The primary product to be developed is a herb oil which could have application as a flavour or fragrance product. Alternatively, celery seed oil would provide import replacement for the industry partner. In addition, extracts of roots and seeds may find a niche in the pharmaceutical market.

Chapter 3 Materials and Methods

The overall approach to this research was an initial germplasm screening, from which six varieties were selected for field-testing. These were grown in a field trial. Serial sampling and distillation of these crops was used to examine changes in oil composition through the growing season and to determine the optimum harvest time for oil of the desired quality. Further material was taken for laboratory assessment of the distillation and extraction procedures, in order to maximise extraction of the desirable oil components. In the final growing season, two of the varieties were selected and planted in a semi-commercial trial. The crop was processed by commercial distillation and an analysis of the commercial distillation process was made. Investigation included assessment of a selection of oil and extract products from herb, seed and roots.

Local growers of fresh vegetable celery and celery for the seed market provided advice on agronomic issues.

3.1 Plant material

Seed of seven celery varieties was obtained from three commercial seed companies (Table 3.1). Following initial laboratory screening, (section 3.3.2) seed of six varieties was germinated by a commercial nursery (Hills Transplants Pty Ltd, Devonport, Tasmania) and seedlings transplanted to a field trial (section 3.3.3). Additional samples of plant material from this trial were taken for testing laboratory protocols.

Table 3.1 Commercial sources of seed

	Variety	**Supplier**
1	Triumph	Bejo Seeds, inc
2	Salad Green	Royston Petrie Seeds Pty Ltd
3	Green Crunch	Arthur Yates and Co Ltd
4	USA Green Stringless	Royston Petrie Seeds Pty Ltd
5	Darklet	South Pacific Seeds Pty Ltd
6	Tendercrisp	South Pacific Seeds Pty Ltd
7	Excelsior	South Pacific Seeds Pty Ltd

Seed was collected from primary umbels of plants in the first trial to provide planting material for a semi-commercial planting in the final year of the project. Further details will be supplied in the descriptions of the methods for each trial.

3.2 General analytical procedures

Several general techniques were used on a regular basis throughout the experimental period. These are detailed here. They and any modifications are then referred to under the description of each individual experimental description.

3.2.1 Laboratory steam distillation

Laboratory samples were distilled in stainless-steel cohobation stills. These were comprised of stainless steel vats and glass, water-cooled condenser units. The oil was collected over water in a glass separator.

For distillation of herb material, the entire sample was weighed and then chopped into approximately 10cm lengths and mixed well. Duplicate samples were taken for determination of dry matter (section 3.2.5). Sufficient of the remaining material was used to fill the stainless steel vat, containing 3L of warm water, and the vat sealed. The vat was heated using an electric hotplate. Timing of the distillation commenced at breakthrough and the distillation was continued as appropriate for each experiment, in general for 2.5 hours.

3.2.2 Solvent extraction

Solvent extractions were generally carried out in duplicate. Samples were weighed into 200ml conical flasks and extracted using two washes of approximately 100ml of solvent, for an appropriate length of time, as detailed in individual methods. Extractions were carried out on an orbital shaker, generally in a fume hood at room temperature (approximately 22°C). The marc was removed from the solvent by filtering, through either cottonwool or grade 4 Whatman® filter paper in a Büchner funnel. Oil was recovered by drying down at 30°C, using an RVE.

3.2.3 Oil yield determination

Oil yield was determined by weight and presented as a w/w percentage either of fresh or dry plant weight, as appropriate.

3.2.4 Oil composition

Oil composition was determined by gas chromatography. Oil samples were dried using anhydrous Na_2SO_4, then a sample of 10µL of oil was dissolved in 1ml of HPLC grade hexane. Oil composition was determined using a Hewlett Packard 5890 Series II gas chromatograph, fitted with a flame ionisation detector. Owing to the difficulties associated with the detection of phthalides in the oils and extracts two different GC columns and associated methods were used. The two columns used were a polar HP-INNOWax column and a non-polar HP1 column. The appropriate column and method are given in each individual experimental description.

HP-INNOWax column and method
The column was a 30m HP-INNOWax column (phase ratio 160), 0.32 mm ID, 0.5 µm film thickness. The carrier gas was high purity nitrogen with a split vent flow of 100mL/min, a column flow of 2mL/min (split ratio 50:1), a purge flow of 3mL/min and a head pressure of 10psi. The GC injector temperature was 250°C and the detector temperature 280°C. The oven temperature was programmed from 50°C (1 minute) rising at 8.0°C/min to 250°C, remaining at the final temperature of 250°C for 14mins.

Peak identification was initially based on GC/MS (section 3.4.1) and subsequently determined by retention time.

HP-1 column and method
The column was a 30m HP-1 (cross-linked methyl siloxane) column (phase ratio 320), 0.32 mm ID, 0.25 µm film thickness. The carrier gas was high purity nitrogen with a split vent flow of 100mL/min, a column flow of 2mL/min (split ratio 50:1), a purge flow of 3mL/min and a head pressure of 10psi. The GC injector temperature was 260°C and the detector temperature 290°C. The oven temperature was programmed from 50°C (1 minute) rising at 8.0°C/min to 285°C and remaining at the final temperature of 285°C for 16.6 minutes.
Peak identification was initially based on GC/MS (section 3.4.1) and subsequently determined by retention time.

3.2.5 Dry matter determination

Samples for dry matter determination were weighed into tared paper bags and dried for a period of one week at 50°C. Samples were then re-weighed and dry matter expressed as a percentage of the total fresh weight.

3.2.6 Specific gravity

The weight of samples was measured in duplicate against distilled water at 20.0°C, using a 10ml, 25ml or 50ml specific gravity flask depending on the volume of the oil available.

3.2.7 Optical rotation

Measured using an Atago polarimeter with either a 1 dm or 2 dm path-length, depending on the volume of oil available.

3.2.8 Refractive index

Measured using an Abbe Refractometer, with a sodium D-line light source (589nm). Measurements were made at 20°C to a precision of 2 parts in 10 000.

3.2.9 Statistical analysis

All statistical analyses were carried out using procedures of the SAS statistical package, version 6.12, 1989-1996,SAS Institute Inc., Cary, N. C. USA.

3.3 Production and sampling of plant material

As detailed earlier, apart from the preliminary samples supplied by local growers, plant material for laboratory testing of protocols was obtained as extra samples from the field trials.

In this report herb material refers to whole plants harvested approximately 10cm above ground level, when the plants are in full bloom. This covers a wide range of plant maturity and further details of maturity are presented in the relevant sections. Seed refers to dry, mature seed which has been cleaned of most other plant debris. Root material refers to the underground portion of the plant but also includes a short portion of stem (10cm above the ground) *i.e.* it refers to the plant material remaining after harvest of herb material.

3.3.1 Preliminary assessment of Triumph

In the initial stages of the project, herb material of the variety Triumph was obtained from a commercial seed crop, grown by Bejo Pty Ltd. This material was distilled by the method set out in section 3.2.1 to provide oil for establishment of chromatography techniques. Two pairs of duplicate samples were distilled, with one pair incorporating a hexane trap in the condenser, in order to collect any water-soluble fractions which are lost during standard distillation. Oil yield and composition were determined by the methods set out in sections 3.2.3 and 3.2.4. Chromatography was undertaken using the HP1 column.

3.3.2 Preliminary screening of seed samples

Each of the six varieties was extracted using a modified method based on the solvent extraction technique detailed in section 3.2.2. For most varieties approximately 10g of dry seed of each variety was weighed into 125ml conical flasks. The exceptions were Green Crunch and Darklet. For Green Crunch, only 2g seed was available and one extraction was made on the entire sample. For Darklet, one extraction was based on a 10g sample with the duplicate being an extraction of a 2g sample.

Redistilled n-hexane (50ml) was added and the samples ground with an Ultraturrax (IKA Labortechnik) for 10 seconds. These samples were left to extract for 2 hours and then filtered through cottonwool and dried down at 30°C on the RVE. They were weighed to obtain the yield of extract and analysed for composition by GC using the HP1 column. The extracts were then compared using organoleptic assessment. No seed of the variety Triumph was available at this time. The distilled herb oil obtained from the variety Triumph (section 3.3.1) was included in the organoleptic assessment.

3.3.3 Screening six varieties

Six varieties, Triumph, Salad Green, Green Crunch, USA Green Stringless, Tendercrisp and Excelsior were assessed in a field trial. Seed of each was germinated by a commercial nursery (Hills Transplants, Devonport, Tasmania) and the plants transplanted as small rosettes on 7th June 2002. Plants established well under the following management regimen.

Experimental layout

The trial was a completely random design laid out on an area 50m x 30m of good loam soil. Each plot was 15m long by two beds wide, with 0.8m between beds. Each bed contained 80 plants in alternate double rows 50cm apart, equating to approximately 3plants/m^2 over the trial area. The trial contained the six varieties; each replicated three times to give a total of 18 datum plots. Four guard plants at

either end of each datum plot were not sampled. Plants were sampled from the two inner rows of each pair of beds.

Management regimen
The site was prepared using a mouldboard plough followed by offset discs. Beds were formed and approximately 1 tonne/ha dolomite applied before the beds were rotary hoed and rolled.

Three applications, 125kg/ha, 250kg/ha and 200kg/ha, of CM Supreme fertiliser (N:P:K 12:5:14 plus micronutrients) were made over the growing season. One application, 250kg/ha, of Nitram (34% nitrogen) was made just prior to the plants bolting. Boron and calcium were applied as a foliar spray at fortnightly intervals, in conjunction with the fungicides.

Fungicides and herbicides were applied in accordance with accepted practice in the industry.

Serial harvest
Serial harvesting began on 18 November 2002, when the first plants were beginning to flower. Harvesting took place on a fortnightly basis. Harvesting was conducted by randomly selecting single plants from the inner datum rows of each plot. Plants were cut at ground level, bagged into plastic bags and stored at -18°C before being distilled (section 3.2.1) for 2.5 hours in the laboratory cohobation stills. Dry matter and oil yield were measured (sections 3.2.5 and 3.2.3) and oil composition determined using the INNOWax column (section 3.2.4).

Organoleptic assessment
Organoleptic assessment of herb oils from each variety was made on oil from plants harvested on 4/2/03. Oil was sampled using paper tapers. Freshly dipped oil was assessed first, followed by a second assessment on dry out.

Herb oil production
On 17/2/03, 15 plants were harvested from each plot and the plants of each variety pooled. Plants were harvested at ground level but most of the stem material was then removed and the material stored at –18°C before distillation. The material was distilled in a semi-commercial distillation plant as a preliminary assessment of each variety for harvestability and commercial oil production. Material of each variety was distilled in batches, with each batch distilled for approximately 3 hours. All batches of each variety were distilled in series before the oil for that variety was collected. Oil was collected in a stainless steel separator. Oil was recovered from the separator using hexane. The hexane layer was dried using anhydrous sodium sulphate and the oil recovered on an RVE at 30°C. Oil yield was measured (sections 3.2.3) and oil composition determined using the HP1 column (section 3.2.4).

Seed oil production
At the completion of the growing season plants were allowed to dry in the field. They were harvested by hand and seed threshed by mulching entire plants in a garden mulcher from which the cutting blades had been removed. Seed was separated from the trash material by sieving. A sample of the trash material was taken for laboratory distillation (section 3.2.1), to give an indication of the quantity and quality of the oil not recovered (section 3.2.3 and section 3.2.4, using the INNOWax column).

The yield of seed per plot was determined by weight and approximately 1kg of dried seed of each plot was distilled (section 3.2.1). The first fraction of oil produced after one hour was collected and distillation continued for a total of six hours. The fractions between one and six hours were maintained separately. Oil yield and composition of the fractions were determined and expressed on a dry weight basis (section 3.2.1, 3.2.3, 3.2.4 (HP1 column) and 3.2.5).

The marc remaining after distillation of the seed from each plot was extracted using re-distilled n-hexane to estimate the quantity of oil components not recovered by steam distillation (section 3.2.2). Samples of 300-400g of marc were extracted with 700ml solvent for 3 hours followed by two further washes of 400ml each. The total extraction time was 8hrs. The yield of extract from the marc was

expressed on a dry weight basis (section 3.2.3 and 3.2.5). The composition of the extract was determined (section 3.2.4) using the HP1 column.

Harvest for root extraction
Following the final harvest, root material of fifteen plants from each plot was collected. Roots of each variety were pooled. Roots were roughly cleaned by hosing and air dried for one week before extraction with solvent. The detailed method of this extraction is presented later, under the heading of laboratory protocols (section 3.4.6).

3.3.4 Semi-commercial trial

Two varieties, Tendercrisp and Salad Green, were selected for semi-commercial testing. Seed was collected from the primary umbels of plants growing in the inner rows of the first field trial. This was germinated by the commercial nursery (Hills Transplants Pty Ltd) to produce 12,500 plants of each. These were planted as transplants using a tractor driven seedling planter.

Experimental design
Each variety was planted on a 0.5ha block, in double rows 0.5m apart with 0.8m between beds. Each variety was maintained under commercial management conditions. The trial was not laid out for a statistical comparison of the two varieties.

Management regimen
The crop was managed as a commercial crop with a regimen based upon that of the first field trial and detailed in section 3.3.3.

Serial harvest
Four plants of each variety were harvested at random on a fortnightly basis. These were distilled in pairs (section 3.2.1) and oil yield expressed on a dry weight basis (sections 3.2.3 and 3.2.5). Oil composition was determined using the HP1 column (section 3.2.4).

The roots of these plants were washed, air dried and frozen. At the end of the season, all roots were chopped and solvent extracted using n-hexane to determine extract yield and composition (sections 3.2.3, 3.2.4 and 3.2.5).

Commercial harvest of herb
Commercial harvest of both varieties took place on 10/2/04. The crop was slashed just above the ground and left to wilt in the paddock for three days. Each variety was harvested to a separate vat.

Harvest of roots
Two weeks after the commercial harvest, bulk samples of the root material remaining were collected for each variety. These were air dried and chopped with a garden mulcher. The material was then frozen at –18°C.

Sub-samples of 70-100g of each variety were extracted in triplicate using isohexane (2-methylpentane). Yield and composition were determined (sections 3.2.3, 3.2.4 and 3.2.5).

Commercial distillation of herb
The two vats were connected to a commercial, wood-fired steam generator and distilled concurrently, each into a separate separator. The capacity of each stainless steel vat is approximately 30m^3 and is such that full vats hold approximately 5-6 tonnes of wilted plant material. The vats have a false floor to allow steam to be directed at atmospheric pressure, from the boiler, through the plant material and to the inlet of the condenser, situated at the top of the vat. The stainless steel separators each had a capacity of approximately1000L in total divided between an inner and outer skin. The inlet to the separator allows the oil and water condensate to enter the vat at the base of the inner skin, under a flange that reduces turbulence and improves separation of the oil droplets from the water. Wastewater passes out of the vat through an outlet at the base of the outer skin.

Distillation began with the inner skin of the separators empty and the outer skins filled with hot water (~60°C). Some fluctuations in condenser temperatures occurred because of changes in the rate of steam production. This caused some changes in the rate of flow from the condensers, but these fluctuations were minimised by continual adjustment of the flow rate of cooling water to the condensers. Distillation continued until the volume of oil in the distillate was negligible (see next section).

Assessment of commercial distillation
Samples of the herb prior to distillation and marc following distillation were collected for distillation in the laboratory.

Samples of the distillate from each vat were collected at the inlet to the separator at regular intervals during distillation. The volume of oil per 3L of distillate was estimated and its composition determined. Each commercial distillation began with the inner skin of the separator empty. Once the inner skin of the separator was filled, water from the separators began to flow to waste. Samples of this first waste water were collected at the commencement of flow to waste and further samples collected one hour later. Additional samples were collected at the completion of distillation. Distillation continued until the volume of oil in the distillate was negligible. Oil was recovered from the separators and weighed. Waste water was sampled from the inner skin of the separator after collection of the oil.

The yield of oil per litre of waste water for all waste water samples was assessed by back extraction of the waste water with hexane. All oil samples were assessed for composition using the HP1 column (section 3.2.4).

3.4 Testing laboratory protocols

The following methods were used to develop protocols for laboratory assessment of the potential varieties.

3.4.1 Identification of oil components

Identification of the oil components was based upon GC/MS analysis of oils and extracts of the variety USA Green Stringless. Samples of oils or extracts from other varieties were included to identify further compounds as necessary.

Components of oils and extracts were analysed by GC/MS using a Varian 3800GC connected to a 1200 triple quadrupole ms. A Varian 1177 injector was used in split mode (20:1 split). The initial analyses of the oils and extracts were carried out using a Varian, 30m VF5-ms column, 0.25mm ID, 0.25µm film thickness. The carrier gas was helium, in constant flow mode, at a flow rate of 1.2mL per minute. The injector temperature was 220°C. The oven temperature was programmed from 60°C (1 minute) rising at 12.0°C/min to 260°C. The ion source temperature was 200°C and the transfer line 280°C. The range 35 to 350mz was scanned 3 times per second. Additional analyses of the oils and extracts were carried out using the HP1 and HP-INNOWax columns described in section 3.2.4.

Identification was based on comparing GC/MS spectra and retention times with library GC/MS data and published lists of components of celery oils and extracts. The library data available included both the NIST MS database and a comprehensive in-house database of volatile compound spectra. Most of the phthalide components were identified from spectra published in the relevant literature.

As with most essential oils, a large number of components were present at trace levels. Most components present at greater than 0.1% of the total FID area were identified. Coelution of components caused some peaks to be identified on one column but not on the other. Where two or more compounds coelute (elute with the same retention time) and they are present at similar concentrations, the peaks are identified as mixed peaks. Where peaks are composed primarily of only

one component but have trace amounts of other components, the peaks are labelled by the primary component and not labelled as mixed.

Subsequently, identification was based upon GC retention time and quantification of the oil components was based upon FID peak areas (section 3.2.4).

3.4.2 Duration of distillation

In order to determine an appropriate length of time for distillation under laboratory conditions the rate of release of oil during distillation was measured on both herb and seed material. Time zero was defined as breakthrough and oil fractions were collected at intervals described in the method for each experiment. Each fraction was held separately and oil yield determined by weight and expressed on a dry weight basis. The composition of each oil fraction was determined by GC.

Further experiments were then conducted in an attempt to obtain the oil more quickly and/or to improve the extraction of the desirable oil components.

Distillation of herb oil
Duplicate samples of herb material of the varieties Excelsior and Triumph were collected from the field trial (section 3.3.3) and the distillation method tested across the two varieties. The material was distilled fresh by the method described in section 3.2.1. This method was modified so that distillation continued for six hours from breakthrough, with the oil collected at hourly intervals. Oil yield was calculated on a dry weight basis (sections 3.2.3 and 3.2.5) and oil composition assessed using the INNOWax column (section 3.2.4).

Distillation of seed
Two experiments were conducted.

In the first experiment, dry seed of the varieties Salad Green and Excelsior was distilled by a modification of the method described in section 3.2.1. Two preparation treatments, whole seed and ground seed were compared. The material for the whole seed treatment was distilled in the raw state as harvested from the variety trial (section 3.3.3). Seed from the same batches was prepared for the ground seed treatment by grinding in an AEG sk1 mill using a 1.5mm screen. Both the whole and ground seed were sub-sampled for analysis of dry matter content (section 3.2.5). Treatments were distilled in duplicate.

The whole seed was stored at -18°C prior to distillation. The ground seed was also stored at -18°C for 3 days between grinding and distillation.

Distillation was continued for 43 hours with fractions collected at 1, 2, 3, 4, 5, 7, 9, 12, 16, 20, 24, 30, 36 and 43 hours.

The experiment was then repeated using the variety Green Crunch, with the material prepared in the same manner but the distillation was carried out immediately upon grinding the seed *i.e.* the seed was not stored between grinding and distillation. In this experiment, the duration of distillation was 12 hours.

3.4.3 Assessment of modifications to standard distillation procedure

Two further experiments were conducted to assess the rate of oil release during distillation. In the first of these experiments a comparison was made between herb distilled fresh and herb distilled frozen. This experiment included an examination of the oil remaining in the marc and in the waste cohobation waters at the completion of distillation.

A second experiment was conducted to examine the effect on yield of breaking the distillation of dry seed. Commercial experience has shown in other crops (principally fennel) that a break during distillation can lead to a large flush of oil being released when distillation is resumed.

Comparison of fresh and frozen material
Paired samples of herb material of variety Triumph were from the variety field trial (section 3.3.3). One pair of each sample was frozen overnight and the other stored at 5°C and distilled fresh. The duration of distillation was 2.5hours. Samples were sub-sampled for dry matter content (section 3.2.5). The yield of oil was determined (section 3.2.3) and its composition assessed using the INNOWax column (section 3.2.4).

At the completion of distillation, the marc was collected and weighed. Two duplicate subsamples were weighed for dry matter determination and a further two duplicate subsamples extracted with DCM for 2 hours (section 3.2.2). The DCM extract was weighed to determine yield and composition assessed on the INNOWax column (section 3.2.4).

The cohobation waters were collected and stored at 5°C before processing. The waters were partitioned with chloroform, the oil/extract obtained weighed and its composition assessed on the INNOWax column (section 3.2.4).

Comparison of distillations conducted with and without a mid-distillation break
Whole seed of the variety Salad Green was distilled by a modification of the method described in section 3.2.1. The two treatments compared were distillation with and without a mid-distillation break.

The treatments were carried out in duplicate. All stills were packed using 5L warm water and 1kg whole seed. Heat was applied to two of the stills and distillation continued for two hours from breakthrough. The heat supply was then turned off, and the stills left to cool. The oil was collected and the stills left overnight. Distillation was commenced on all stills the following morning and continued for 7.5 hours, *i.e.* the total duration of distillation for those stills distilled without a break was 7.5hours while for those distilled with a break the total duration of distillation was 9.5 hours. Oil was collected at 2 hours, 4 hours and 6 hours from breakthrough, for all stills. It was also collected at 7.5hours for those stills with no mid-distillation break and at 9.5 hours for those stills that had a mid distillation break. Oil yield was determined by weight (section 3.2.3 and oil composition was determined using the INNOWax column (section3.2.4). The results of oil yield and composition are presented for each fraction separately.

3.4.4 Optimisation of separator temperature

A preliminary assessment of the optimum temperature for separation of the oil from water was made by adding 5ml of oil to water in two 100ml measuring cylinders. The first cylinder was at 30°C and the second at 60°C. The cylinders were held in water baths to ensure maintenance of the desired temperatures. The cylinders were covered and each inverted ten times to mix the oil and water. The time required for the majority of the oil droplets to rise to the surface was recorded. In addition, the clarity of the resultant oil layer was assessed.

3.4.5 Solvents extraction of seed

Dry seed samples (400g) were taken from the three replicates of all six varieties from the first field trial, pooled and stored at room temperature. This experiment tested extraction protocols across varieties but cannot be used to assess differences between varieties.

Two preparation treatments were compared; one being whole seed and the other seed prepared by grinding with an AEG sk1 mill fitted with a 1.5mm screen. All samples of both whole and ground seed were assessed for dry matter content.

Two extracting solvents were compared; chloroform and hexane. The experimental design was a 2 x 2 factorial with twelve replicates of each treatment, arising from duplicate samples within each variety.

A modification of the extraction method detailed in section 3.2.2 was used. Samples (~50 g) of material of each sample were weighed into each of 2 x 150ml Erlenmeyer flasks. One of each pair of flasks was extracted with hexane and the other with chloroform. Each wash consisted of 50ml of the appropriate solvent. There were a total of four washes, of the following durations: 0-2 hours, 2-4 hours, 4-6 hours and 6-24 hours. Upon removal of the final wash a quick rinse of 50 ml solvent was used to improve recovery of the final wash. This rinse was added to the final wash.

During extraction, flasks were covered with alfoil and placed on an orbital shaker at 100 revs per minute, in a fumehood.

At the completion of each wash, the solvent wash filtered off using cottonwool. The solvent was immediately replaced with the next wash. The filter cottonwool was added to the extracting flask to ensure that any plant material was not discarded. Where necessary, further fine debris was removed by filtering the organic layer through Whatman grade 4 filterpaper over a glass funnel.

Washes were maintained separately and stored at 5°C before being dried down at 30°C on a RVE. Dry down continued until the solvent was removed and continued for a further 5 mins. Yield of extract was determined by weight. Composition was determined on the HP1 column using the method set out in section 3.2.4, with C18 as an internal standard.

The results are presented to enable a comparison of extracts after each wash with the standard error calculated from the pooled variance of the sum of the extracts obtained in the full extraction period of 24 hours.

3.4.6 Solvent extraction of roots

Root samples were collected from all six varieties of the field trial. Eight roots were collected from each plot and then the material from three field plots of each variety to be pooled. Roots were cleaned, air dried for two days and stored at 5°C before processing.

The experimental design is similar to the design of the seed extraction (section 3.4.5) but using three preparation treatments with two solvents to give a 3 x 2 factorial design.

The three preparation treatments were: (a) fresh, chopped; (b) chopped, dried; (c) dried, ground. The fresh samples, treatment (a) were processed immediately. The remainder of the roots were dried at 30°C for seven days. Material for treatment (b) was extracted at this stage while material for treatment (c) was ground in a coffee grinder.

Two extraction solvents, hexane and chloroform, were used on each preparation treatment. The experimental design was a factorial with six varieties by three preparation treatments by two extracting solvents.

Each preparation treatment was assessed for dry matter content (section 3.2.5).

For each extraction, ~100 ml samples of material of each treatment were weighed into 125ml Erlenmeyer flasks. A constant volume of material was used since the different levels of drying of the original material would result in quite different densities of material. Weight was determined from the estimate of dry matter content.

The extraction procedure was the same as that used for the solvent extraction of seeds (section 3.4.5) above.

The yields of extracts were determined by weight (section 3.2.3) and the composition by GC analysis (section3.2.4) using the INNOWax column.

3.5 Assessment of potential commercial oils

Certificates of analyses were produced for each of the herb and seed oils resulting from the variety trial. These oils were only available in small quantities, 10-50ml. Herb oils were produced by the distillation of the herb from the variety field trial in the semi-commercial still (section 3.3.3). Seed oils were produced by pooling the 1-6 hour fraction of the seed distilled in the laboratory (section 3.3.3).

Further Certificates of Analyses were produced for the two herb oils produced by commercial production and distillation in the final season of the project (section 3.3.4).

For certificates of analyses the composition of the oils was assessed by GC (section 3.2.4) using both columns. The physical parameters of specific gravity, optical rotation and refractive index were measured (sections 3.2.6, 3.2.7 and 3.2.8).

3.6 Gross margin analysis

A gross margin analysis was prepared, based upon the variable costs associated with the semi-commercial trial.

Chapter 4 Results

The identification of the oil components and a suitable chromatography method for routine quantification by FID is fundamental to the rest of the project. The components identified are presented first, followed by an assessment of the laboratory protocols used in analysing the field trials. The final section of the results presents the assessment of the potential varieties, the commercial distillation process and the commercial implications of the project.

4.1 Identification of oil components

Tables 4.1 and 4.2 present the components identified eluting from the HP1 and INNOWax columns respectively. Components were first identified in seed and herb oils by GC/MS using the VF5 and HP1 columns. The component peaks 1 to 25 were first numbered in order of elution from these columns. Further components, peaks 26 to 28 were detected and numbered in extracts of seeds and roots. The process was then repeated on the INNOWax column and further components, peaks 29 to 41 were numbered accordingly.

When presenting the results the integration of the chromatograms was adjusted so that approximately 40-50 peaks per chromatogram were quantified for seed and herb oils *i.e.* generally most components forming greater than 0.03% of the oil were identified.

Table 4.1 Components identified as eluting on HP1 column

Peak Number	Component
1	α-pinene
2	β-pinene
3	myrcene
4	p-cymene
5	limonene
6	*cis*–β-ocimene
7	γ-terpinene
8	fenchone
9	n-pentylbenzene
10	a n-pentylcyclohexadiene
11	*trans*-anethole
12	*trans*-pinocarveyl acetate
13	carveyl acetate
14	caryophyllene
15	humulene
16	β-selinene
17	α-selinene
18	elemol
19	caryophyllene oxide
20	n-butyl phthalide
21	β-eudesmol
22	α-eudesmol
28	unknown phthalide (mw 192)
26	unknown phthalide (mw 204)
23	sedanenolide
24	sedanolide
27	*cis*-neocnidilide
25	neophytadiene

Identification of the monoterpenes and sesquiterpenes was relatively straight forward as most are common to many essential oils. Identification was achieved by comparing GC/MS data with library data. Identification of the phthalides required analysis of the literature on celery oil composition and comparison of published GC/MS spectra, retention times and Kovat Indicies with those obtained for analysis of the oils and extracts produced during this research.

Table 4.2 Components identified as eluting on INNOWax column

Peak Number	Component
2	β-pinene
3	myrcene
5	limonene
6	*cis*-β-ocimene
4	p-cymene
10	a n-pentylcyclohexadiene
9	n-pentylbenzene
34	*cis*-limonene oxide
35	*trans*-limonene oxide
40	pinocarvone
29	β-elemene
14	caryophyllene
38*	*cis*-p-mentha-2,8-dien-1-ol
12	*trans*-pinocarveyl acetate
30	β-farnescene
39*	*trans*-p-mentha-2,8-dien-1-ol
15	humulene
31	β-himachalene
16	β-selinene
17	α-selinene
33	carvone
32	*ar*-curcumene (mixed peak)
37	*trans*-carveol
36	*cis*-carveol
25	neophytadiene
41	limonene diepoxide (mixed peak)
19	caryophyllene oxide
18	elemol
22	α-eudesmol
21	β-eudesmol
28	unknown phthalide (mw192)
20	n-butyl phthalide
24	sedanolide
27	*cis*-neocnidilide
23	sedanenolide
26	unknown phthalide (mw 204)

**N.B.* There are inconsistencies in literature as to order of elution of these two components. The identification here is drawn from Wilson (1969) using a packed carbowax column, supported by data obtained from McLeod and Ames (1989) and Hunter and Moshonas (1966).

Two peaks coeluted on the HP1 column. These were peaks 22 and 28, or α-eudesmol and the unknown phthalide mw 192. The phthalide in question was not detected in more than trace quantities in herb and seed oils and therefore did not present a problem in these oils. It was, however, a problem in some extracts, particularly extracts of the marc from steam-distilled seed. It was not possible to

identify this component on the INNOWax column, as it was unstable on that column, tending to isomerise and dehydrogenate into sedanenolide and n-butyl phthalide respectively.

The components listed in table 4.3 were selected as representative of the spectrum of components across the chromatograms and are reported as indications of the composition of oil samples. Peak number refers to order of elution on HP1 column. These components include all those listed on the ISO standard. They are selected so that the three main regions across the chromatograms *i.e.* the monoterpenes, sesquiterpenes and phthalides are represented. Additional components are reported in certain sections *e.g. cis*-neocnidilide is a major component of root extracts and is reported where the composition of roots is discussed.

Table 4.3 List of peaks, selected as representative of celery herb and seed oils, to be reported in comparisons between oils

Peak Number	Component
2	β-pinene
3	myrcene
5	limonene
10	a n-pentylcyclohexadiene
12	*trans*-pinocarveyl acetate
16	β-selinene
20	n-butyl phthalide
23	sedanenolide
24	sedanolide

4.2 Assessment of laboratory protocols

Steam distillation is the primary commercial means of extracting essential oil from celery. The more volatile components will be distilled over first, followed by the less volatile components. The duration of distillation can be adjusted to optimise the balance between components. The optimum duration of distillation may not be the same for different types of plant material. Pre-distillation treatment such as freezing or grinding may interact with the duration of distillation to modify the rate of oil release and composition of the oil.

The protocols developed for solvent extraction of seeds and roots are presented next and followed by the results of the assessment of the optimum temperature for separation of celery oil from water.

4.2.1 Preliminary distillation of Triumph with and without hexane trap

Herb distilled without the use of a hexane trap produced a yield of 0.48%±0.15% on a dry weight basis. With a hexane trap, the oil yield was 0.67%±0.05%. Comparison of the relative compositions indicates that both limonene and sedanenolide are extracted more fully by the use of a solvent trap.

Table 4.4 Composition of essential oil from steam distilled Triumph based on relative percentage peak areas of FID response

Component	With hexane trap	Without hexane trap
β-pinene	4.3%	4.8%
myrcene	1.4%	1.1%
limonene	63.9%	42.3%
a n-pentylcyclohexadiene	4.2%	4.1%
trans-pinocarveyl acetate	0.3%	0.1%
β-selinene	2.7%	2.2%
n-butyl phthalide	0.6%	0.4%
sedanenolide	10.4%	4.2%
sedanolide	4.0%	3.7%

4.2.2 Duration of distillation of herb

Changes in oil yield with duration of distillation are presented in figures 4.1, 4.2, and 4.3. These figures show both the incremental release of oil over time and the effect of each additional increment on the overall oil accumulation with increasing duration of distillation. In figures 4.1 and 4.2, the results are expressed as weight of oil per unit weight of dry matter distilled. Figure 4.3 expresses the yield at each collection time as a percentage of the total oil obtained after six hours of distillation.

Figure 4.1 shows that the increment of oil obtained in the first hour about three times the amount of oil released in the following hour. The flush of oil released in the first hour is followed by a steady release that continues throughout the six hours at a slowly decreasing rate. Figure 4.2, illustrating cumulative oil yield, also shows this rapid release of oil in the first hour followed by a linear increase in oil yield after the first 2.5 hours. Figure 4.3, showing the yield as a percentage of the total obtained after six hours, indicates that 70-80% of the oil obtained after 6 hours is obtained in the first 2.5 hours.

Figure 4.1 Incremental essential oil yield of celery herb, with increasing duration of distillation

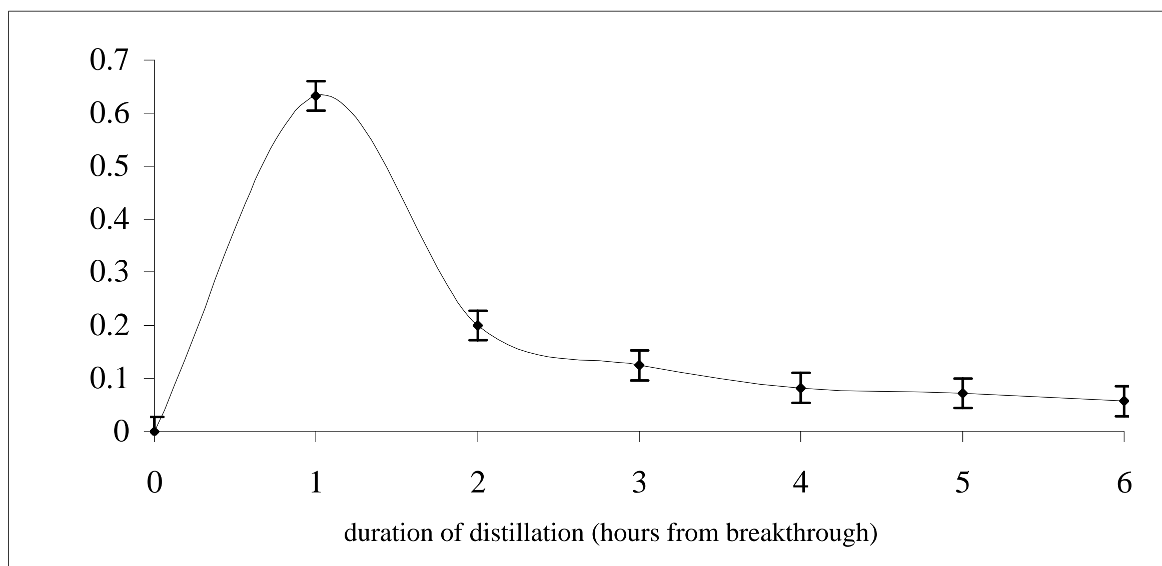
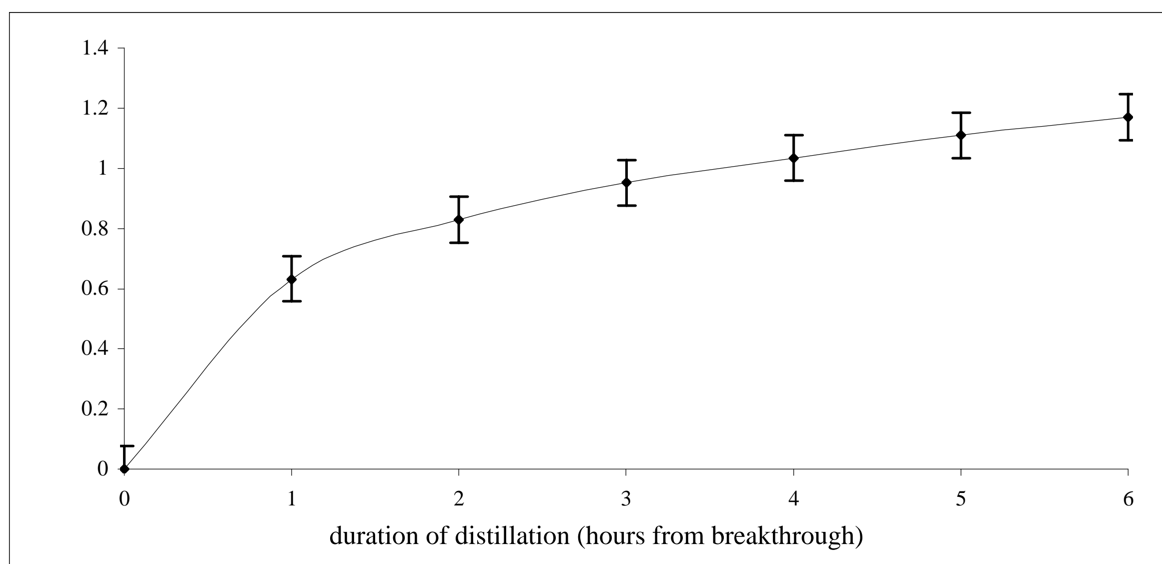


Figure 4.2 Cumulative essential oil yield of celery herb, with increasing duration of distillation



Analysis of individual means shows that the incremental change in oil yield is not significantly different after 3 hours. Oil continues to be released at the same rate between 2.5 and six hours.

The bar graph, Figure 4.4, illustrates the changes in composition of each increment of oil. Although the relative concentration of the components in the oil increments changes as the distillation progresses, all the oil components of interest are present in the first increment of oil. No further components were detected in the later increments. Figure 4.4 shows that β-pinene, myrcene and limonene are released from the herb at beginning of distillation and the levels of these components fall in each subsequent increment.

The release of the n-pentyl cyclohexadiene isomer component is greater in the second increment and then falls, however this apparent trend is less than the limits of the standard error of these means.

The concentration of β-selinene and the phthalides increases in each subsequent increment as distillation continues.

Figure 4.3 Cumulative essential oil yield from celery herb, as a percentage of the total yield after six hours distillation

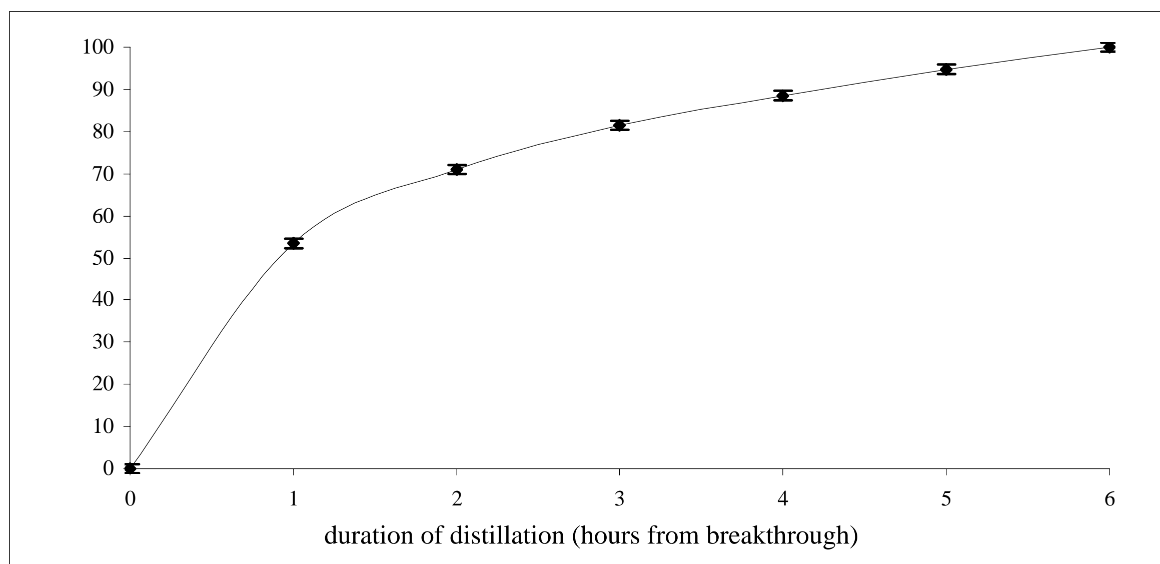
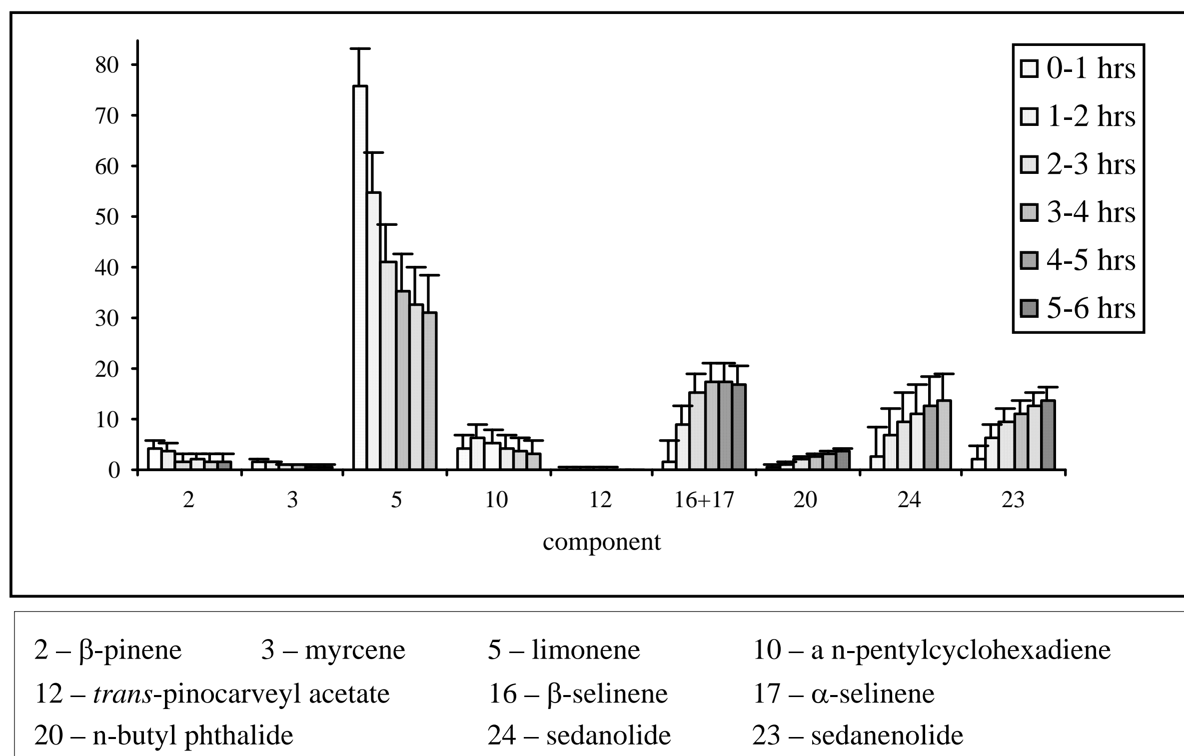


Figure 4.4 Changes in essential oil composition of celery herb, with increasing duration of distillation



2 – β-pinene	3 – myrcene	5 – limonene	10 – a n-pentylcyclohexadiene
12 – *trans*-pinocarveyl acetate		16 – β-selinene	17 – α-selinene
20 – n-butyl phthalide		24 – sedanolide	23 – sedanenolide

4.2.3 Duration of distillation of seed

The results of these experiments are presented in the same manner as those of the distillation of herb (section 4.2.2). Oil yield is expressed on both an incremental (figure 4.5 and figure 4.10) and a cumulative (figure 4.6 and 4.11) basis. Standard errors are derived from the estimate of the pooled variance.

In the first experiment (figure 4.6) the yield of oil from whole seed shows an increase with each increment for first three hours, followed by a rapid fall until about 6 hours at which time each further increment is of a similar size. The pattern of oil release from ground seed tends to be more like the pattern of oil release from the herb. There is a rapid release in the first hour followed by a steady decrease as distillation progresses. For both curves 50% of the oil obtained after 43 hours is obtained after 7 hours (figure 4.7). Although grinding removed the lag phase in oil release during the initial stages of distillation, it also greatly reduced the cumulative yield of oil, with the difference increasing with duration of distillation (figure 4.6).

The changes in composition of the oil with increasing duration of distillation (figure 4.8 and 4.9) follow the same trend as that shown by the herb. The monoterpenes, β-pinene, myrcene and limonene decrease in concentration with each increment as the distillation progresses. The n-pentyl cyclohexadiene component peaks in concentration at around six hours of distillation for both the ground and whole seed. The concentration of the desirable components, β-selinene and the phthalides increases as the distillation progresses.

The most important difference between the ground and whole seed with respect to oil composition is the rate of release of the phthalides. For whole seed the rate of release in each increment increases while the rate of release from ground seed is consistent and the overall level of phthalides is higher.

The experiment was repeated to ensure that the storage time between grinding and distillation did not bias the results. The results of the second experiment (figures 4.10 to 4.14) were in agreement with the results of the first experiment (figures 4.5 to 4.9). The second experiment highlighted the fact that the oil was more quickly extracted from the ground seed but that the yield was reduced by grinding the seed (figure 4.12).

The components lost by grinding are the monoterpenes, therefore the concentration of β-selinene and the phthalides is higher in the oil from ground seed (figures 4.13 and 4.14).

Figure 4.5 Incremental essential oil yield from celery seed, with increasing duration of distillation. Experiment 1

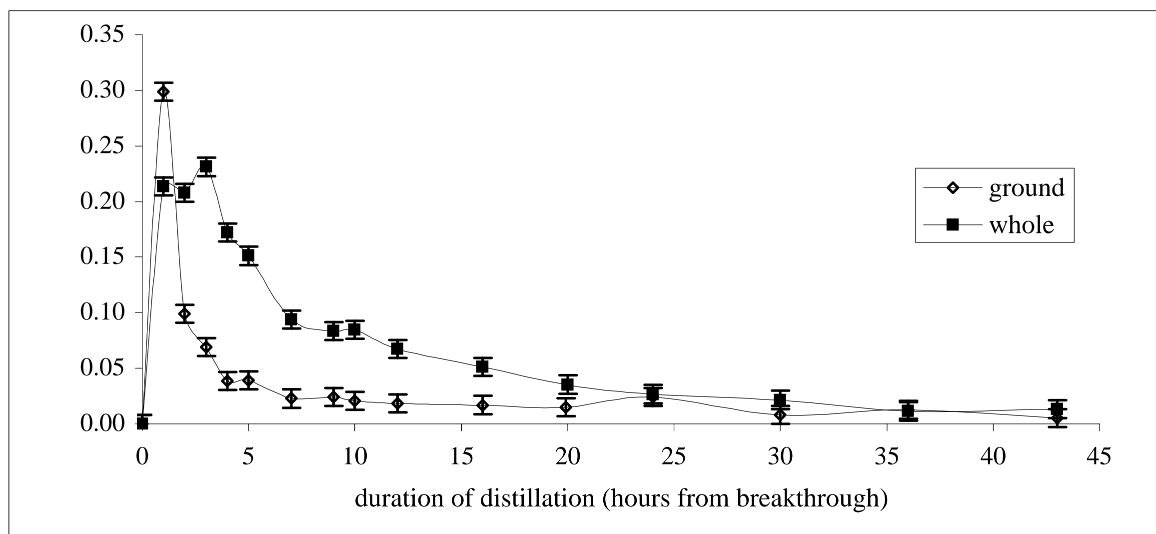


Figure 4.6 Cumulative essential oil yield from celery seed, with increasing duration of distillation. Experiment 1

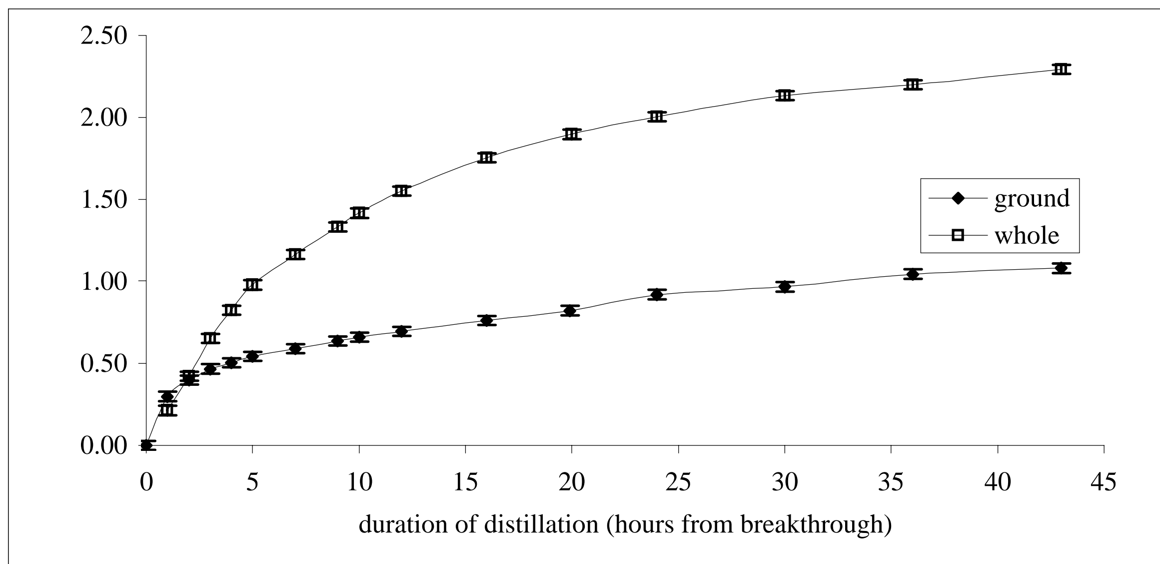


Figure 4.7 Cumulative essential oil yield from celery seed, as a percentage of the total after 43 hours distillation. Experiment 1

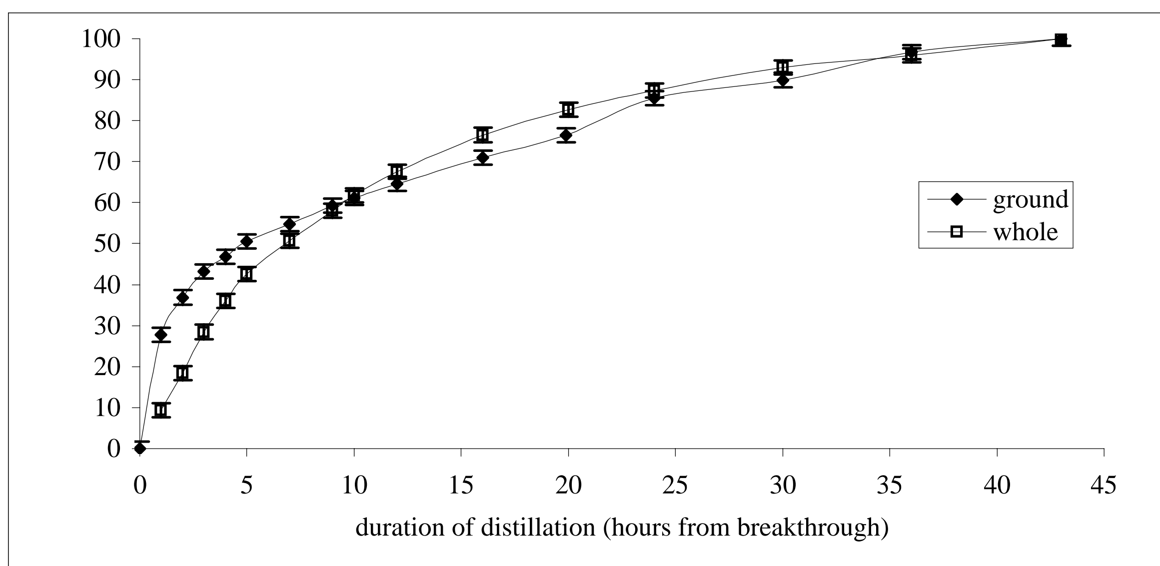
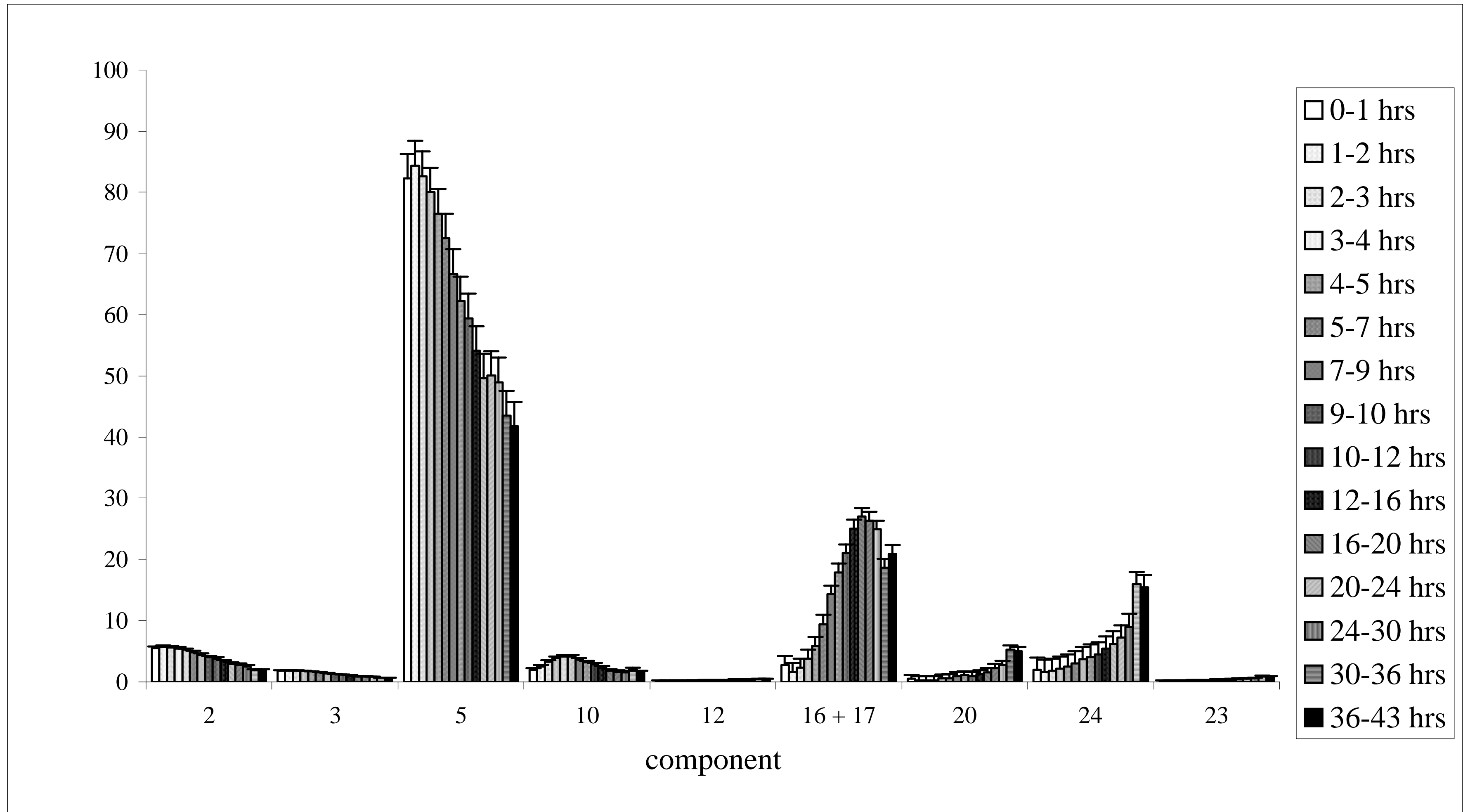


Figure 4.8 Changes in composition of essential oil from whole seed, with increasing duration of distillation. Experiment 1.

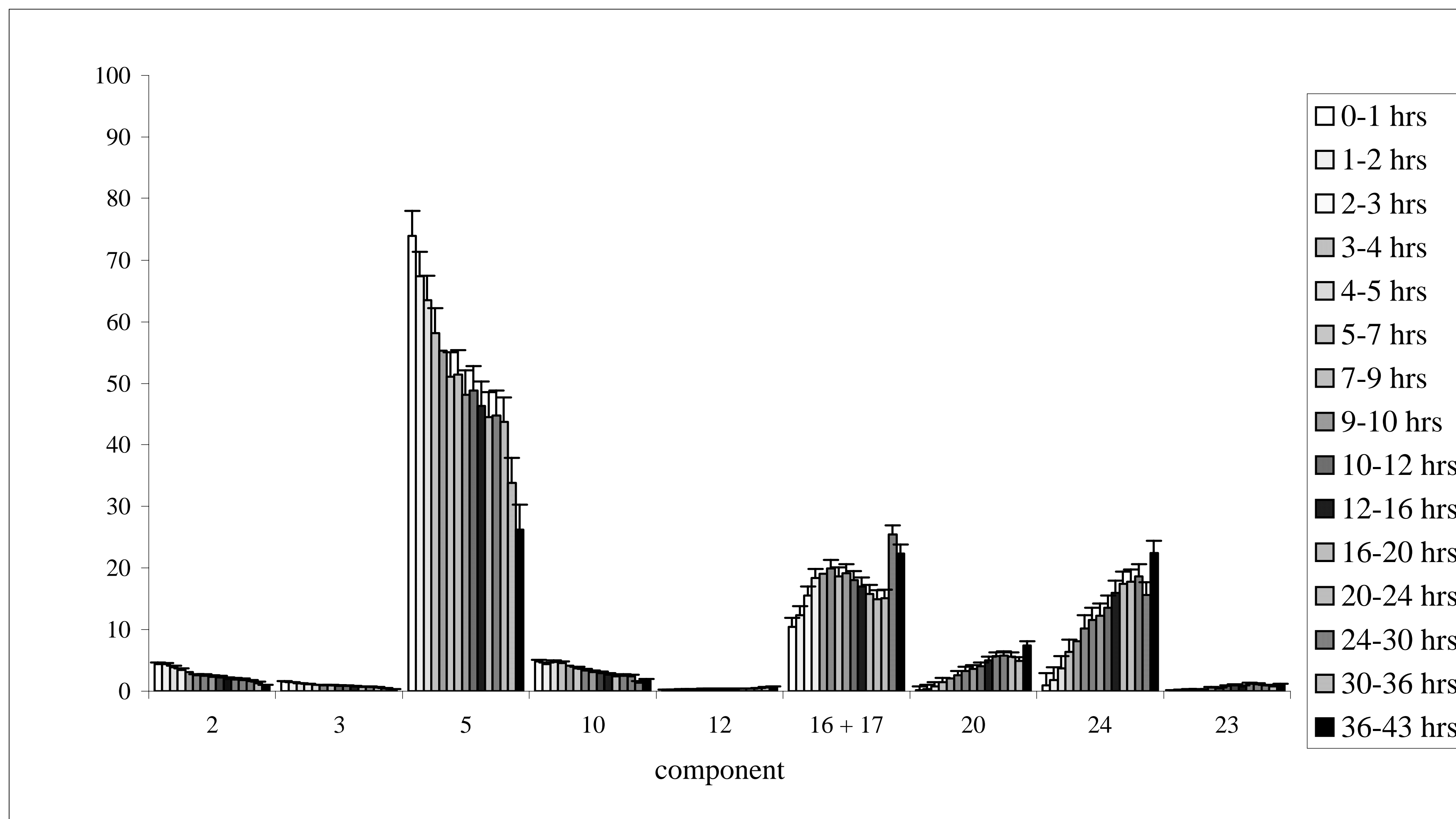


Key to components

2 - β-pinene | 3 - myrcene | 5 - limonene | 10 - n-pentylcyclohexadiene | 12 - *trans*-pinocarveyl acetate

16 - β-selinene | 17 - α-selinene | 20 - n-butyl phthalide | 24 - sedanolide | 23 - sedanenolide

Figure 4.9 Changes in composition of essential oil from ground seed, with increasing duration of distillation. Experiment 1.



Key to components

2 - β-pinene | 3 - myrcene | 5 - limonene | 10 - n-pentylcyclohexadiene | 12 - *trans*-pinocarveyl acetate

16 - β-selinene | 17 - α-selinene | 20 - n-butyl phthalide | 24 - sedanolide | 23 - sedanenolide

Figure 4.10 Incremental essential oil yield from celery seed, with increasing duration of distillation. Experiment 2

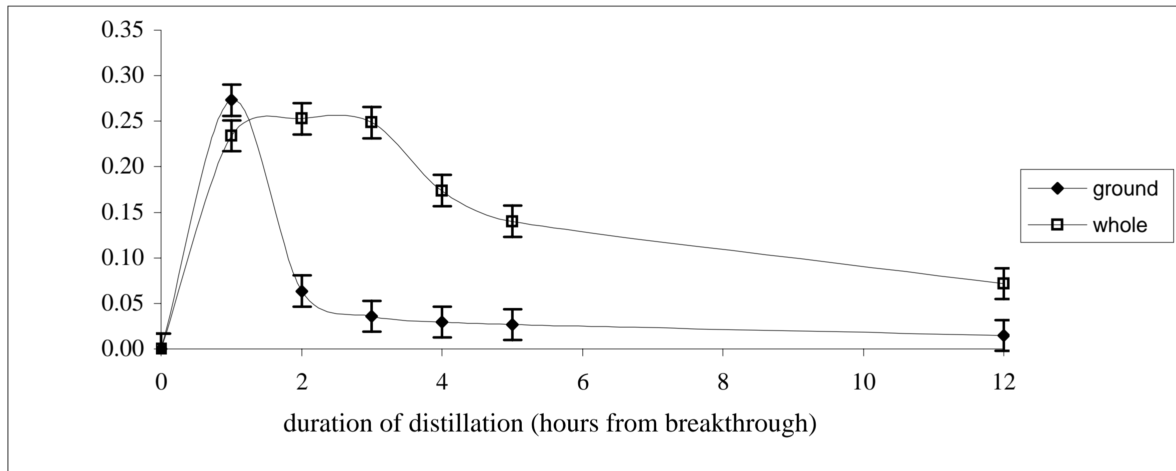


Figure 4.11 Cumulative essential oil yield from celery seed, with increasing duration of distillation. Experiment 2

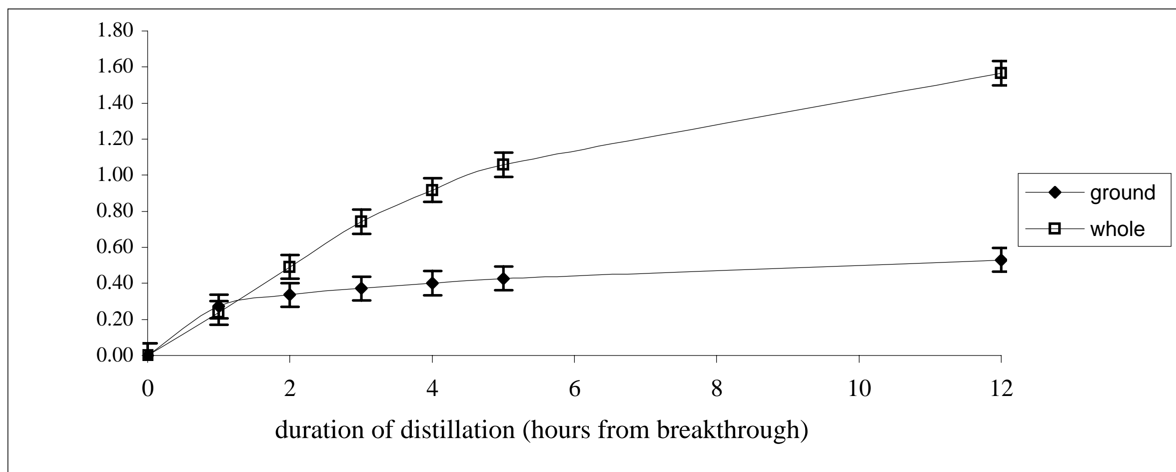


Figure 4.12 Cumulative essential oil yield from celery seed, as a percentage of the total yield after 12 hours distillation. Experiment 2

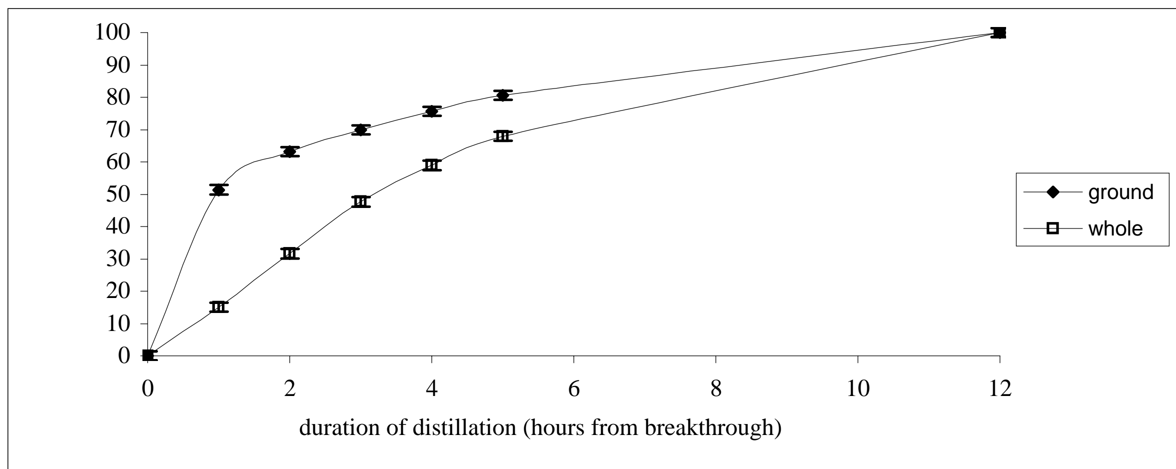


Figure 4.13 Changes in composition of essential oil from whole celery seed, with increasing duration of distillation. Experiment 2

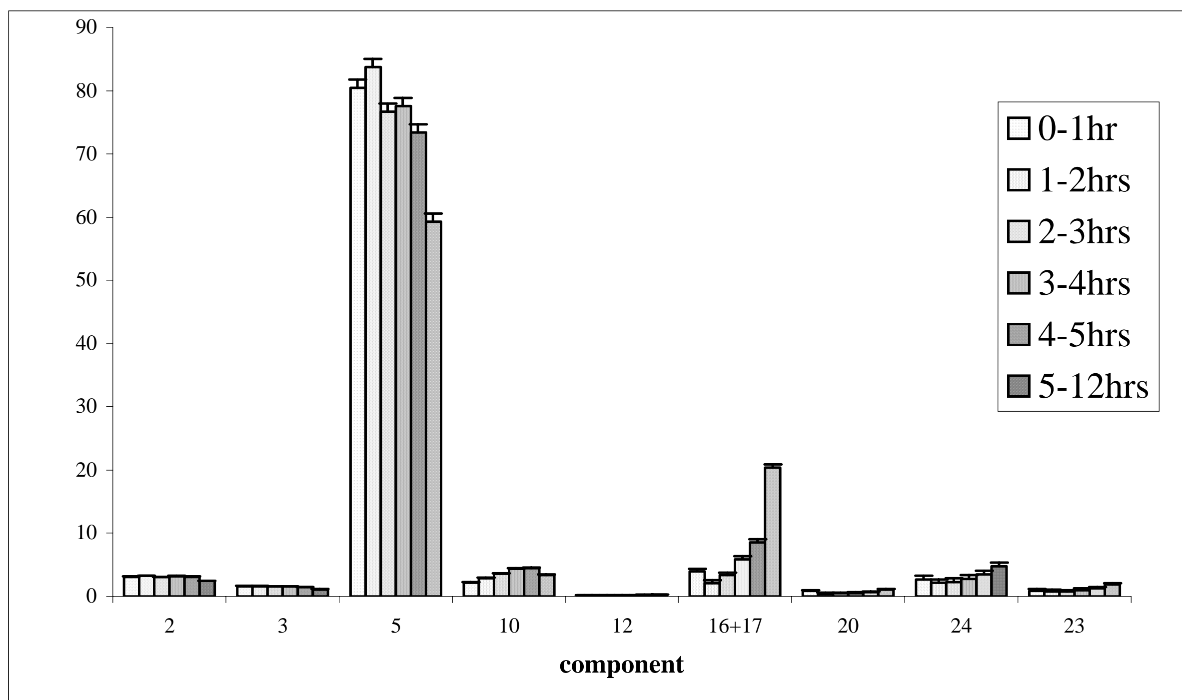
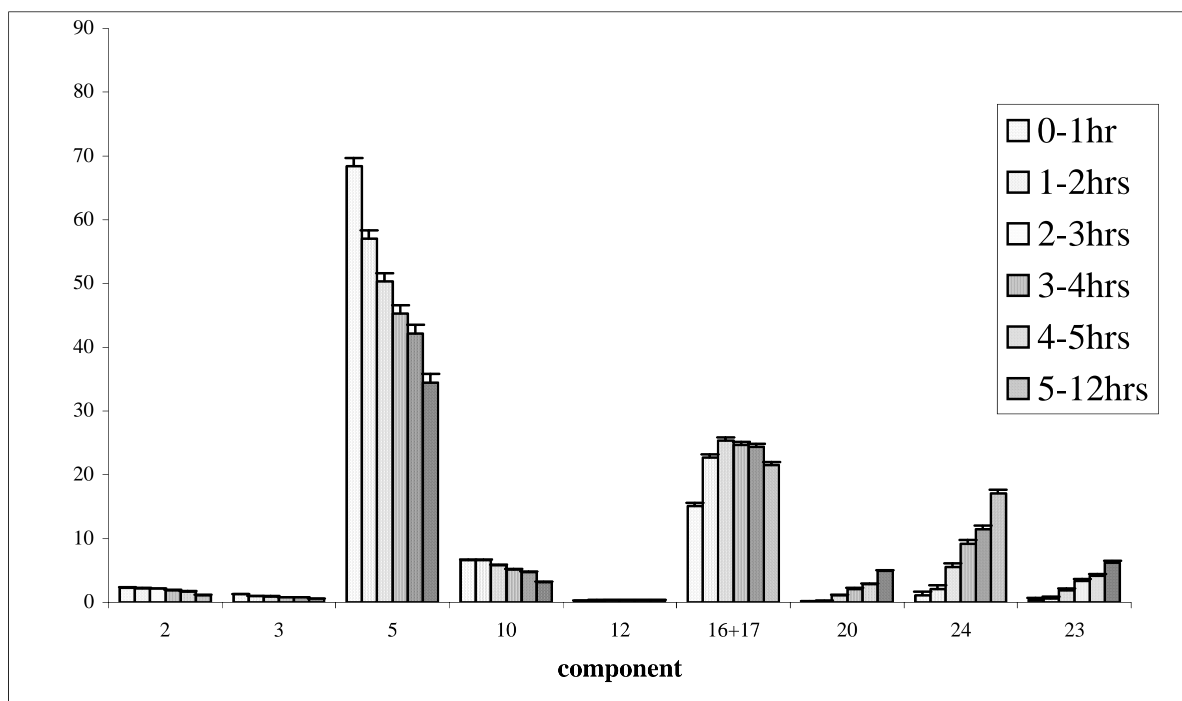


Figure 4.14 Changes in composition of essential oil from ground celery seed with increasing duration of distillation. Experiment 2



Key to components

2 – β-pinene	3 – myrcene	5 – limonene	10 – n-pentylcyclohexadiene
12 – *trans*-pinocarveyl acetate		16 – β-selinene	17 – α-selinene
20 – n-butyl phthalide		24 – sedanolide	23 – sedanenolide

4.2.4 Comparison of distillation of fresh and frozen herb

Table 4.5 shows the yield and composition of oil from celery herb distilled either fresh or frozen. The results for the frozen herb are more variable as shown by the larger standard errors but there is otherwise no difference between the methods. Both the yield and composition of the oil are similar from the fresh and frozen herb. Freezing will provide a suitable means of storing herb before distillation but will not provide a means of improving the release of oil from the herb.

Table 4.5 The yield and composition of essential oil from celery distilled either fresh or frozen (standard deviations in parentheses)

		Herb distilled fresh	**Herb distilled frozen**
yield		0.70% (0.08%)	0.61% (0.14%)
Component		**% components in oils**	
2	β-pinene	3.68% (0.004%)	3.29% (0.58%)
3	myrcene	1.45% (0.02%)	2.08% (1.17%)
5	limonene	67.95% (1.36%)	66.71% (1.83%)
10	a n-pentylcyclohexadiene	3.41% (0.67%)	3.24% (0.44%)
12	*trans*-pinocarveyl actetate	trace	less than 0.319%
16+17	α– and β-selinene	4.16% (0.99%)	4.58% (1.20%)
20	n-butyl phthalide	0.62% (0.01%)	0.79% (0.17%)
24	sedanolide	7.79% (1.43%)	7.76% (3.58%)
23	sedanenolide	2.82% (0.78%)	2.68% (0.58%)

Table 4.6 shows the results of the chloroform extracts of the marc from the distillations. Again, there is little difference between the two treatments. Freezing the herb perhaps decreased the weight of oil retained in the marc but the composition of the two extracts is similar.

Table 4.6 The yield and composition of chloroform extract of marc from celery distilled either fresh or frozen (standard deviations in parentheses)

		Herb distilled fresh	**Herb distilled frozen**
yield		7.55% (1.30%)	5.60% (0.93%0
Component		**% components in oils**	
2	β-pinene	0.049% (0.010%)	0.045 (0.029%)
3	myrcene	0.020% (0.004%)	0.022% (0.001%)
5	limonene	1.122% (0.167%)	1.014% (0.345%)
10	a n-pentylcyclohexadiene	0.084% (0.016%)	0.065% (0.024%)
12	*trans*-pinocarveyl actetate	0.014% (0.002%)	0.018% (0.002%)
16+17	α– and β-selinene	1.007% (0.241%)	0.997% (0.337%)
20	n-butyl phthalide	0.181% (0.040%)	0.175% (0.038%)
24	sedanolide	1.711% (0.664%)	1.196% (0.411%)
23	sedanenolide	1.700% (0.078%)	1.442% (0.159%)
total volatiles		10.946% (1.391%)	10.520% (1.336%)

The yield of oil obtained by chloroform partitioning of the cohobation water, expressed as a percentage of the dry matter distilled was 0.09% (st. dev. 0.02%) for the fresh samples and 0.18% (st. dev. 0.10%) for the frozen material. The composition of the extracts was extremely complex, containing many fatty acids and other components, which made interpretation of the chromatograms difficult. It was not deemed worthwhile to proceed with the evaluation of these data since preliminary investigation highlighted no differences between the two treatments.

4.2.5 Whole seed with and without a mid-distillation break

Figures 4.15 and 4.16 show the incremental and cumulative yields of oil obtained from the distillation of celery seed, with and without a mid-distillation break. Any differences detected are expected after the first two hours as there is no difference in distillation treatment before this time: the break in distillation was at two hours. The incremental curve shows a higher yield at the four hour mark for the distillation with the break but the differences between the two treatments are lost by six hours. The cumulative yield curve shows no difference between the treatments. Oil composition for the two treatments is presented in figures 4.17 and 4.18. It is similar for both treatments although there does appear to be a slight increase in the concentration of limonene in the oil immediately following the break compared with the standard distillation. This is concurrent with a slight decrease in concentration of sedanolide in the oil.

There is a decrease in limonene and an increase in selinene concentration in the final oil fractions of the distillation with a break compared with the distillation without a break, but this is not a valid comparison. The distillation with a break continued for 9.5 hours and the other treatment for only 7.5 hours. Since the selinene and phthalide concentrations increase with increasing duration of distillation while the limonene concentrations fall, this effect is due to the longer duration and not to the applied treatment.

Figure 4.15 Incremental essential oil yield for celery seed distilled with and without a mid-distillation break

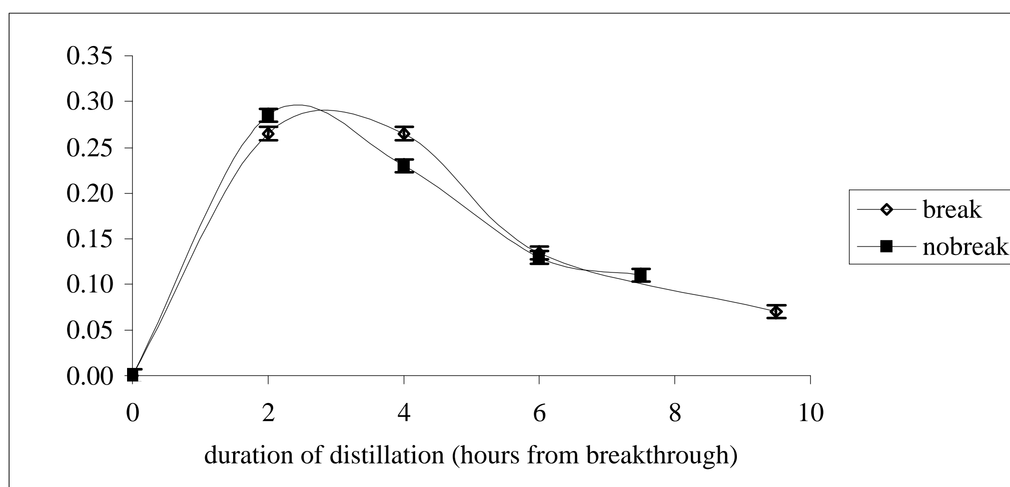


Figure 4.16 Cumulative essential oil yield for celery seed distilled with and without a mid-distillation break

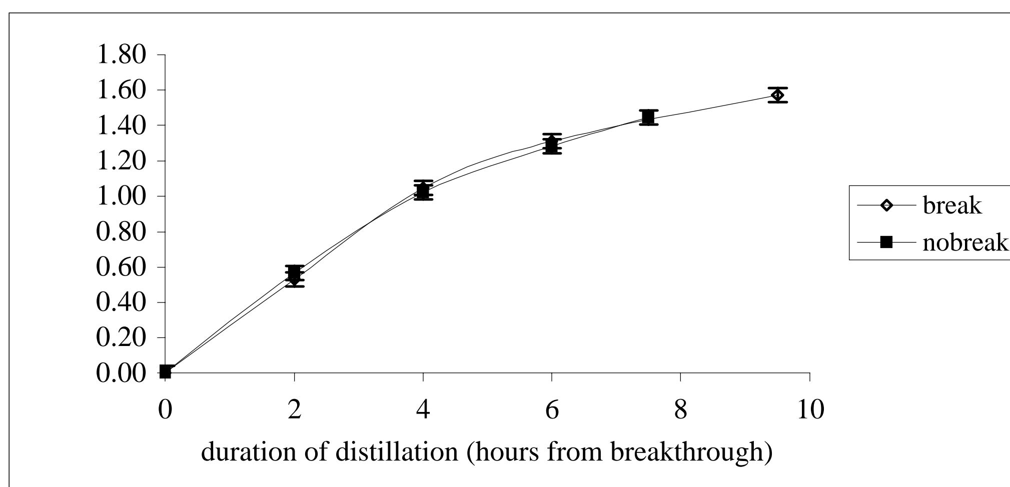


Figure 4.17 Changes, with increasing duration of distillation, in the composition of essential oil from celery seed

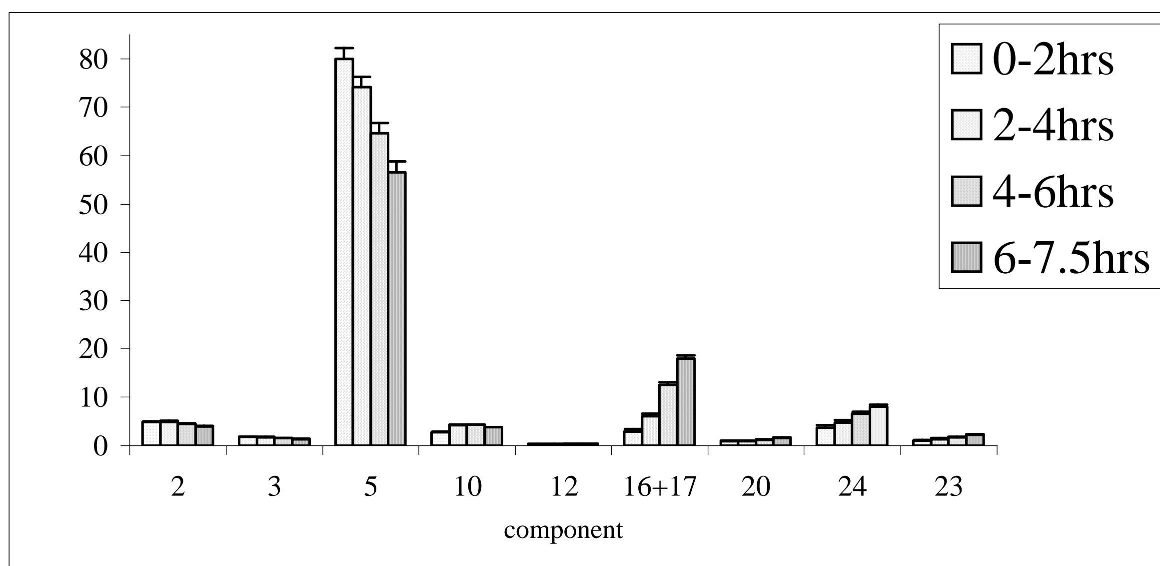
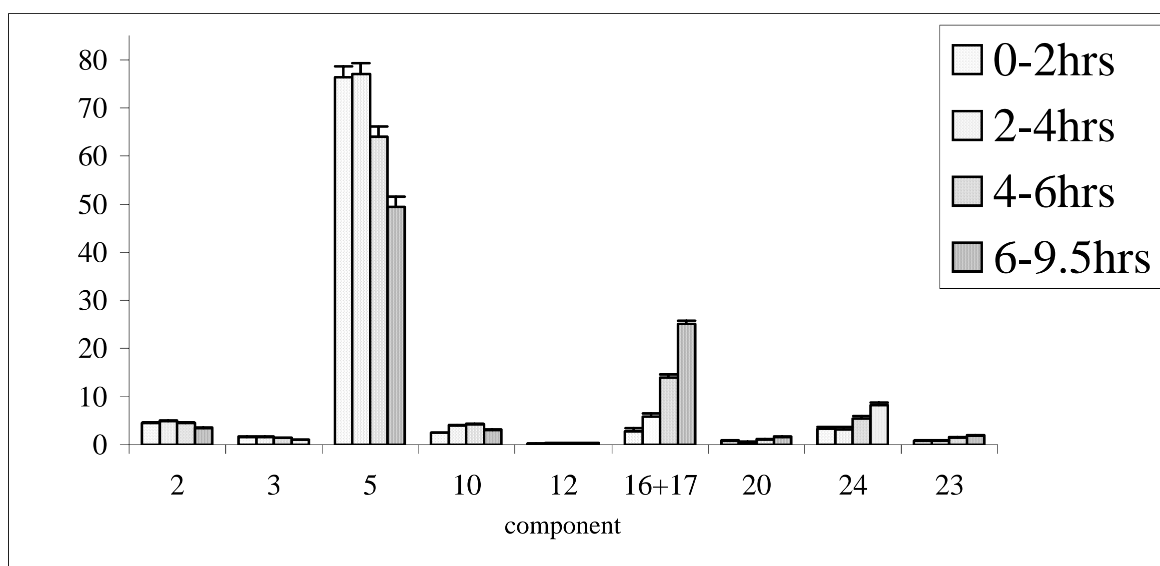


Figure 4.18 Changes, with increasing duration of distillation, in the composition of essential oil from celery seed, distilled with a mid-distillation break after two hours



Key to components

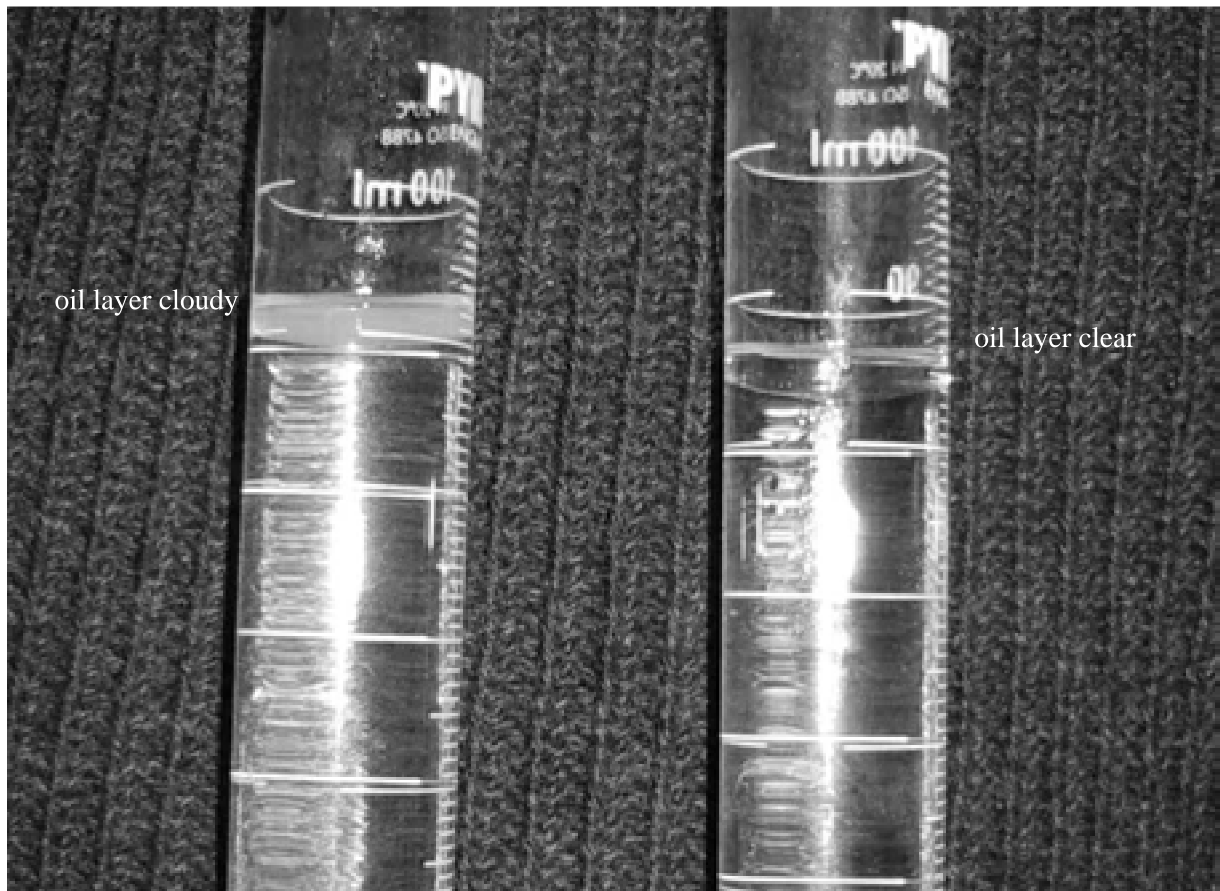
2 – β-pinene	3 – myrcene	5 – limonene	10 – n-pentylcyclohexadiene
12 - *trans*-pinocarveyl acetate		16 – β-selinene	17 – α-selinene
20 - n-butyl phthalide		24 – sedanolide	23 - sedanenolide

4.2.6 Optimum separator temperature

The assessment of separator temperature was primarily a subjective assessment. Plate 4.1 portrays the result of one comparison after 10 mins separation time.

It was observed that under cool conditions (30°C) the oil droplets rose slowly within the water column. There was a tendency for the oil to cling to the sides of the cylinder and the oil layer remained cloudy. At a temperature of 60°C the oil separated much more quickly and cleanly and the final oil layer was clear.

Plate 4.1 Effect of temperature on separation of celery oil from water. Cylinder on left is at 30°C and cylinder on right is at 60°C



4.2.7 Solvent extraction of seeds

The results of the comparison of solvents and preparation for extraction by grinding seeds are presented in figures 4.19 and 4.20. Figure 20 illustrates the yield of the total extract and both the total yield of components and proportional composition of the extracts. The first in each pair of bar graphs in this figure shows the relative proportion of the component in the extract and the second shows the yield of component relative to the amount of plant material extracted.

The total yield of extract (figure 4.19) is much greater from ground seed than whole seed and also greater with chloroform than hexane. The treatment combinations are all significantly different at the 0.1% level as is the interaction between the solvent and the preparation treatment. Chloroform coupled with grinding produces the greatest yield of extract.Grinding the seed produces an extract with a greater proportion of total volatiles, as does extracting with hexane (figure 4.20a). When examining the efficiency of obtaining volatiles from the seeds (figure 4.20b) chloroform is slightly better than hexane but grinding the seed allows a 10-fold increase in the weight of volatiles obtained.

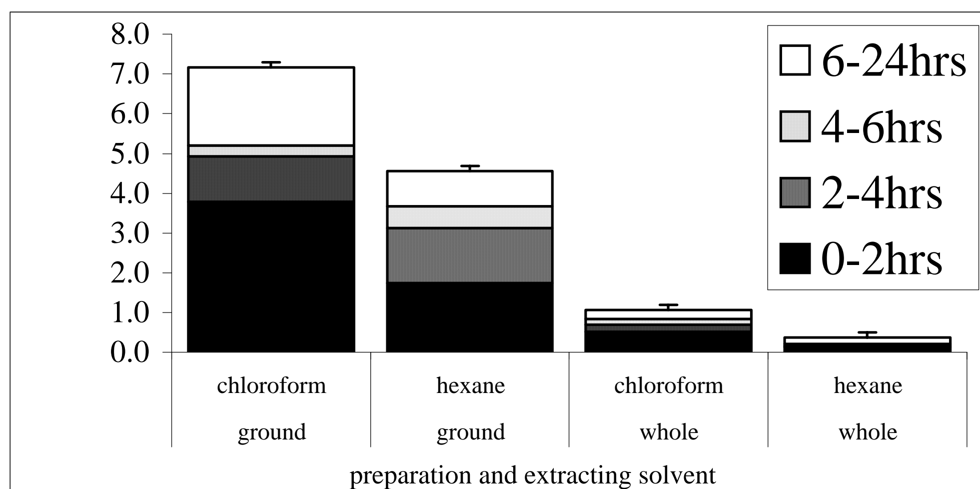
Figures 4.20c to 4.20j show the results obtained for the individual components of interest. The pattern of extraction is very similar for all. With the exception of n-butyl phthalide grinding increases the concentration of the components of interest in the extracts. Grinding had no significant effect upon the concentration of n-butyl phthalide in the extract.

For all components except sedanenolide, there was a statistically significant interaction at the 5% level between the solvent and the preparation technique. Hexane produced the highest concentration of β-selinene and sedanolide in the extracts of ground seed but chloroform produced higher concentrations in the extracts of whole seed. The solvent had no effect on the concentration of n-butyl phthalide in the extracts of ground seed but hexane produced a higher concentration from whole seed. Hexane produced higher concentrations of sedanenolide for both preparations; there was no significant interaction between solvent and preparation technique.

A comparison of treatments was also made in the extraction of absolute amounts of each component from seed. For all components, grinding provided a five to ten-fold increase in the weight of component obtained per weight of plant material extracted. Generally, chloroform extracted a significantly greater weight of component, with the exception being sedanenolide where, although the means followed the same pattern, the results were not statistically significant.

For most of the results of this experiment the effect of variety was significant but because there was no replication of variety in the experimental design no comparison can be made between the variety means. A further examination of this effect should be considered in the future.

Figure 4.19 Total yield of solvent extract per dry matter extracted



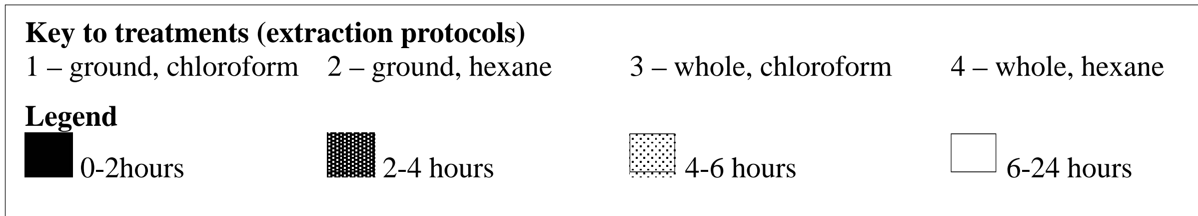


Figure 4.20 a to j Composition of solvent extract of celery seed

Figure 4.20a Total volatiles in extract (mg volatiles/mg extract)

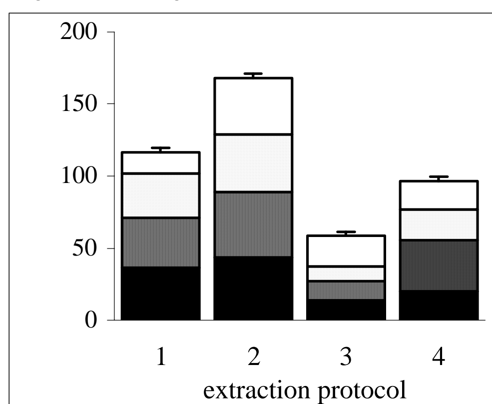


Figure 4.20b Total volatiles in plant material (mg volatiles/g plant extracted)

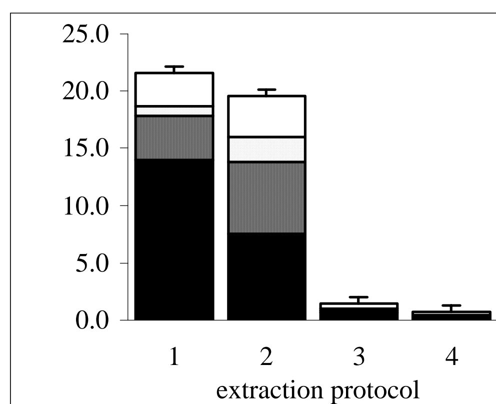


Figure 4.20ç β-selinene in extract (mg β-selinene/mg extract)

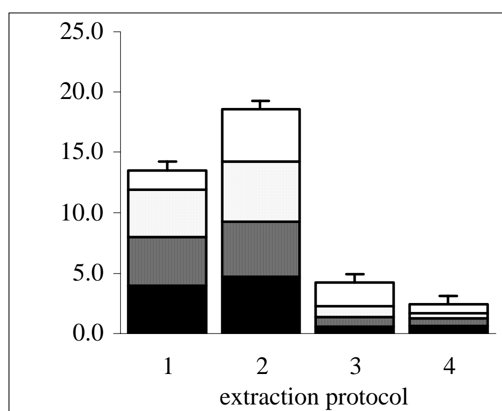


Figure 4.20d β-selinene in plant material (mg volatiles/g plant extracted)

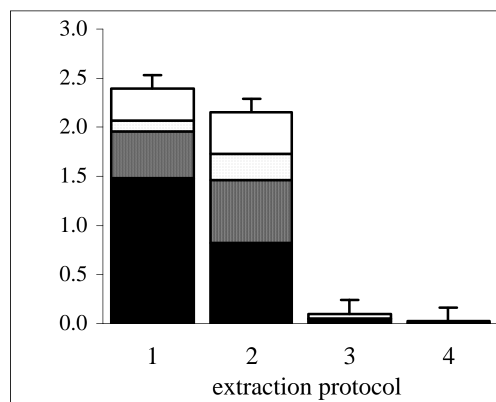


Figure 4.20e n-butyl phthalide in extract (mg n-butyl phthalide/mg extract)

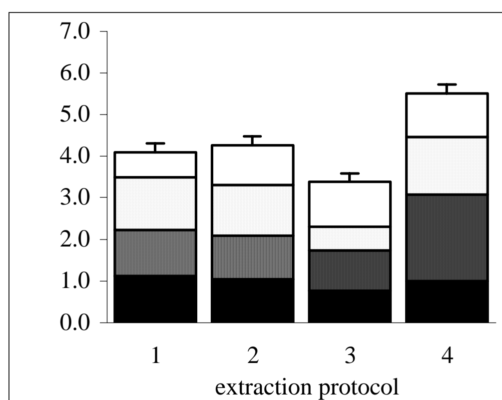


Figure 4.20f n-butyl phthalide in plant material (mg volatiles/g plant extracted)

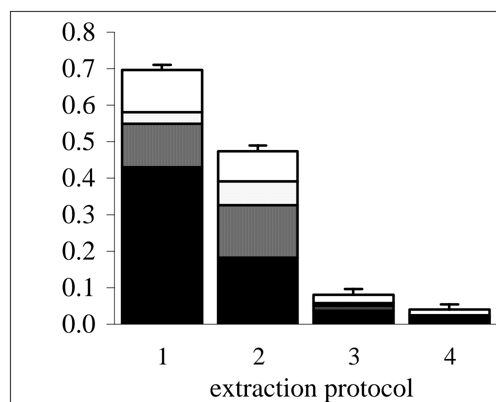


Figure 4.20g sedanolide in extract (mg sedanolide/mg extract)

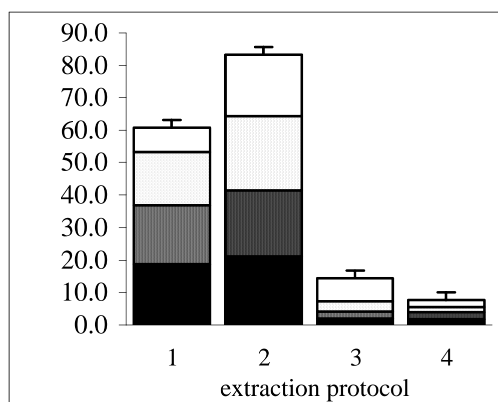


Figure 4.20h sedanolide in plant material (mg sedanolide/g plant extracted)

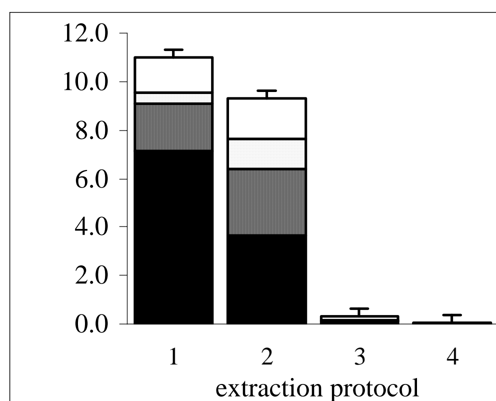


Figure 4.20i Sedanenolide in extract (mg sedanenolide/mg extract)

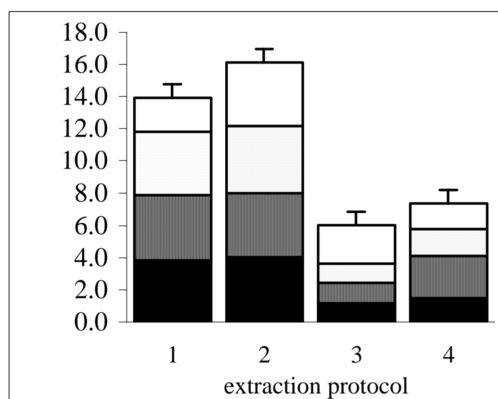
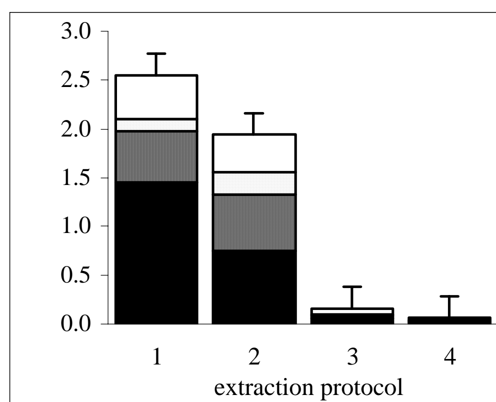


Figure 4.20j Sedanenolide in plant material (mg sedanenolide/g plant extracted)



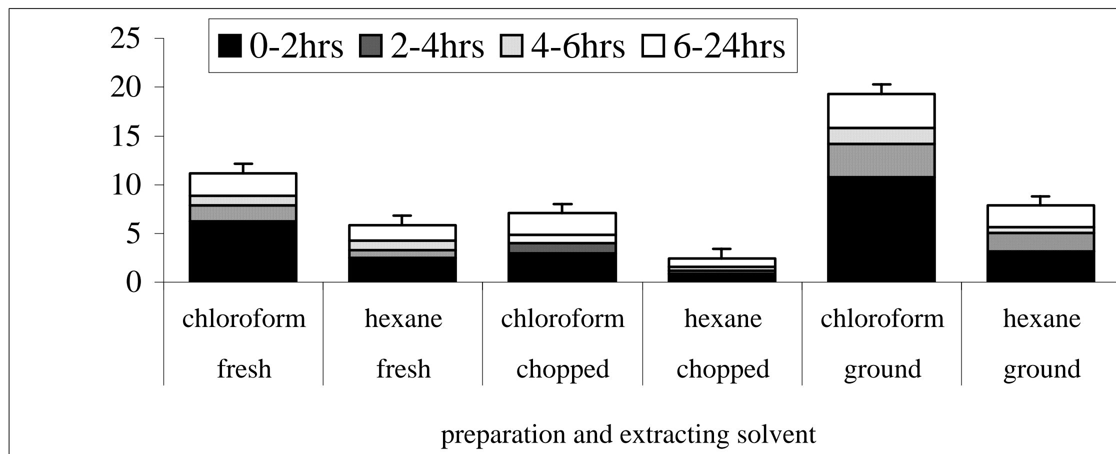
4.2.8 Solvent extraction of roots

This experiment aimed to compare the efficiency of two solvents and three preparation techniques in extracting volatiles from the roots of celery. These treatments were tested across six celery varieties but no comparison can be made between varieties since the experimental design did not include replication at this level. The variety effect was significant for most variables measured, indicating that there are variety differences and potential interactions between variety and extraction method.

Examination of the chromatograms showed that only trace amounts of monoterpenes were present in the extracts prepared by these methods. The components present also differed from the components detected in the tops. In particular, *cis*-neocnidilide was a predominant component in the root extracts but was never detected at greater than trace levels in the above ground portions of the plants. A further phthalide present in the roots was the unknown phthalide with molecular weight 204. The components presented in this section are therefore total volatiles, sedanolide, *cis*-neocnidilide, sedanenolide, n-butyl phthalide and unknown phthalide (mw 204).

The results of the total yield of extract for each treatment are presented in figure 4.21. Both the preparation and the solvent had a statistically significant effect on the yield of extract. Drying and chopping reduced the yield of extract relative to the fresh, chopped material but drying and grinding increased the yield. For all preparation treatments, chloroform produced a greater yield of extract than hexane. In all cases approximately half the total extract obtained was obtained after two hours, *i.e.* the first wash obtained the bulk of the extract on a per wash basis but continuing for 24 hours doubled the yield.

Figure 4.21 Yield of extract from celery roots (mg oil per gram root extracted)



Each treatment combination was compared with respect to both the proportions of components in the extracts and the absolute quantity of each component extracted. The results are presented in figure 4.22 a to l.

The first comparison made was on the total volatiles extracted (figure 4.22 a and b). Both the preparation used and the solvent significantly affected the level of total volatiles in the extract. There was a significant interaction between the solvent and the preparation. The total volatiles in the extract were greater with hexane than with chloroform for the fresh and dry, chopped treatments but solvent had no effect on the total volatiles in the dry ground treatment. The highest total volatiles in the extract were obtained by extracting fresh, chopped roots with hexane.

Figures 4.22 a to l Composition of celery root extracts

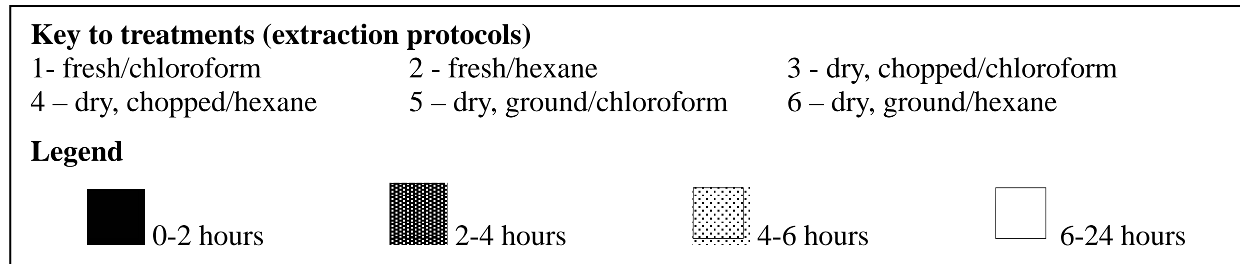


Figure 4.22a Total volatiles in the extract (%)

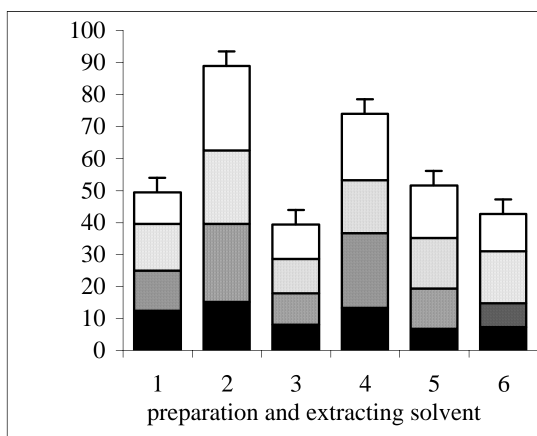


Figure 4.22b Total volatiles extracted from the plant material (ppm)

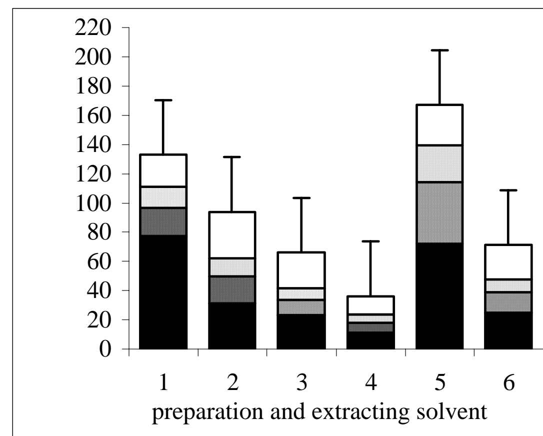


Figure 4.22c Sedanolide in the extract (%)

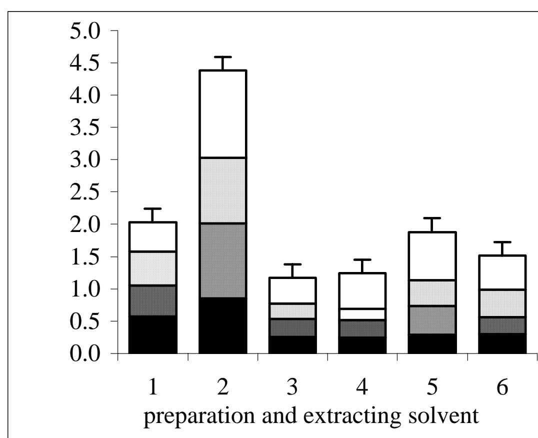


Figure 4.22d Sedanolide extracted from the plant material (ppm)

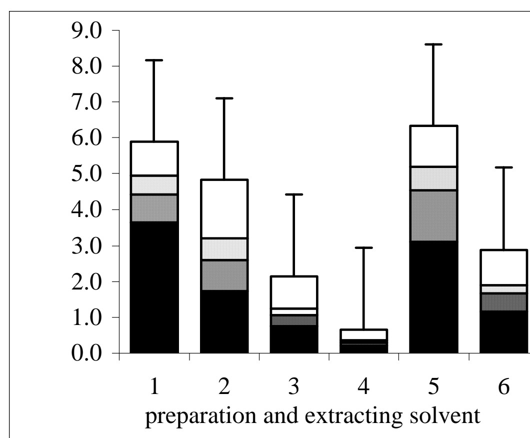


Figure 4.22e Sedanenolide in the extract (%)

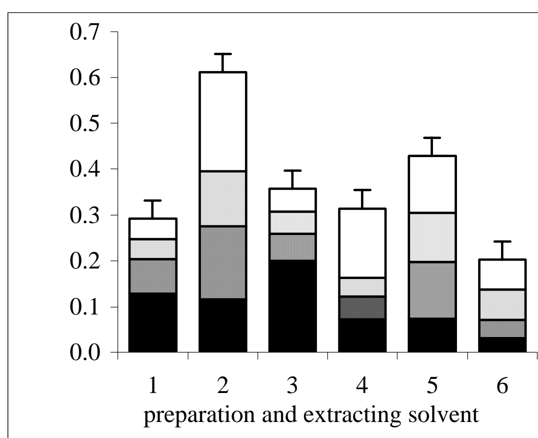


Figure 4.22f Sedanenolide extracted from the plant material (ppm))

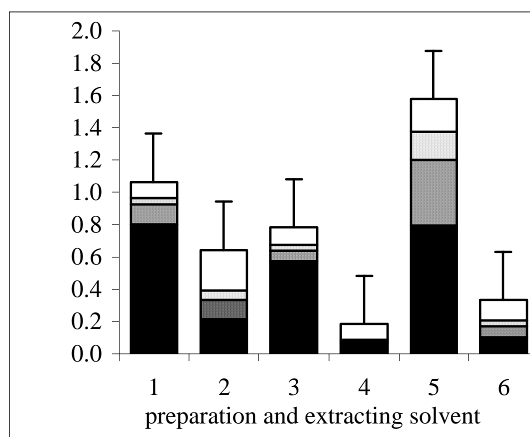


Figure 4.22g n-butyl phthalide in the extract (%))

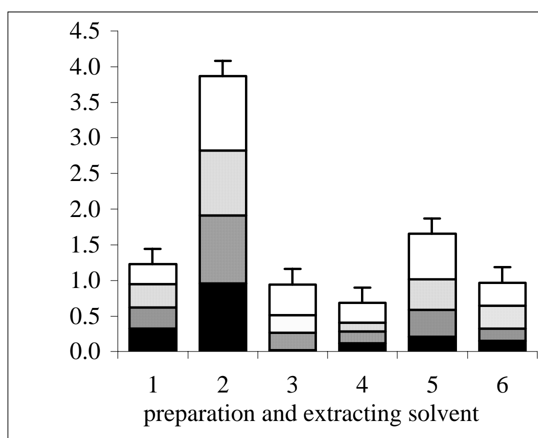


Figure 4.22h n-butyl phthalide extracted from the plant material (ppm)

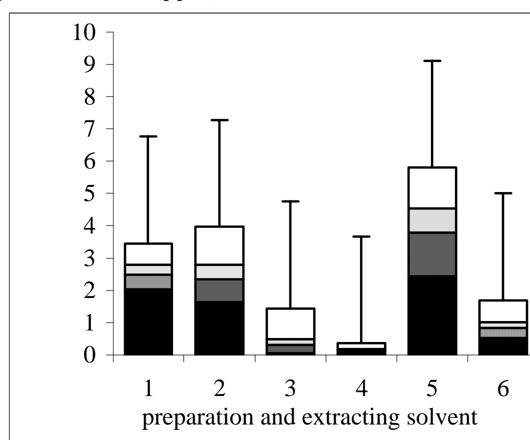


Figure 4.22i cis-neocnidilide in the extract (%)

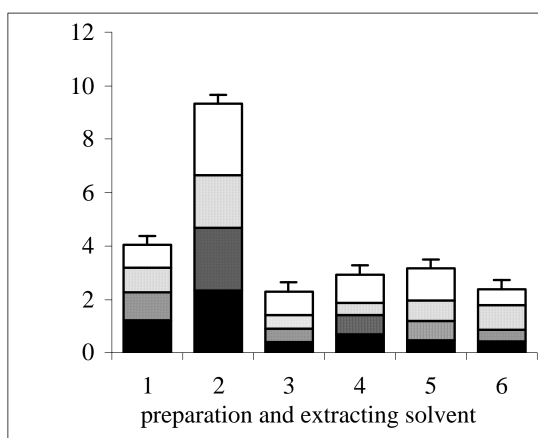


Figure 4.22j cis-neocnidilide extracted from the plant material (ppm)

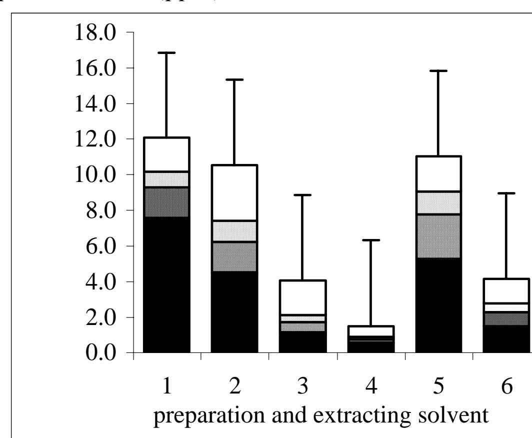


Figure 4.22k unknown phthalide mw 204 in the extract (%)

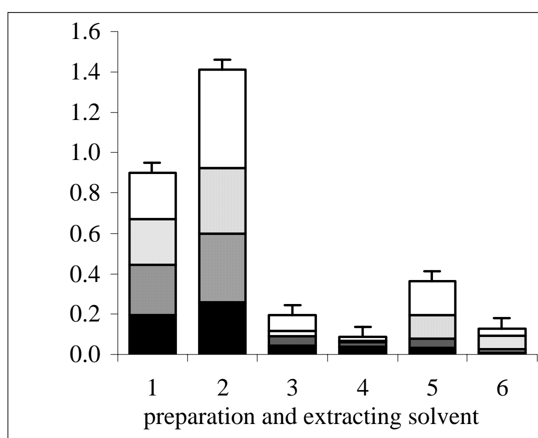
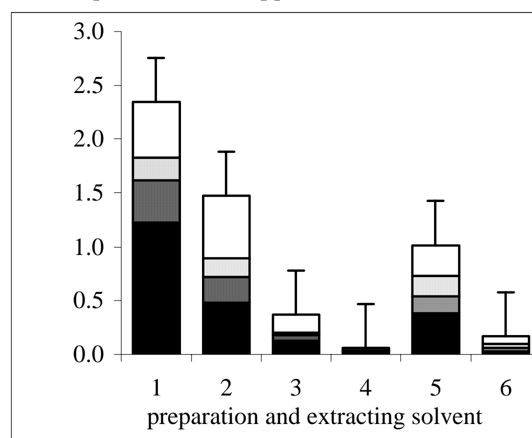


Figure 4.22l unknown phthalide mw 204 extracted from the plant material (ppm)



Chloroform extracted more total volatiles per unit of plant material than hexane for all preparation treatments. Drying the chopped material reduced the yield of total volatiles relative to the fresh material while grinding the material increased the yield of volatiles extracted using chloroform but had no effect on the yield of volatiles extracted using hexane.

The treatments affected the proportions of all the phthalides in the extracts in a similar way (figures 4.22 c to l). In all cases the dry chopped and dry, ground treatments had a lower proportion of phthalides in the extract compared with the fresh, chopped material. For the fresh material, hexane extracts had the higher levels of phthalides. The solvent used did not alter the concentration of phthalide in the dry, chopped extracts while chloroform tended to increase the yield of phthalides from the dry, ground material.

As pointed out previously, *cis*-neocnidilide was the predominant phthalide present in the roots and was found at approximately twice the concentration of its stereoisomer, sedanolide. The two sedanolide isomers and n-butyl phthalide formed the bulk of the phthalides present in the root extracts.

4.3 Assessment of varieties

The results of the preliminary assessment of the varieties by solvent extraction of small samples of seeds are presented first. It was decided from this initial assessment that no varieties should be excluded from further testing and all those for which sufficient seed was available were tested in the field trial.

The seasonal changes in oil yield and composition of the varieties are then presented followed by a subjective assessment of canopy structure and harvestability. Oil yield and composition of the semi-commercial quantities of herb and seed oils for each variety are compared.

Finally, the results of the semi-commercial trial on the varieties Salad Green and Tendercrisp, including the assessment of the commercial harvest are presented.

4.3.1 Preliminary extraction of seeds

Table 4.7 shows the results of the preliminary assessment of the potential varieties. These results are based upon duplicate samples, except for Green Crunch for which only one sample was available.

The main components of interest are the phthalides. The predominant phthalide is sedanolide in all varieties but the concentration of sedanolide and the ratio of sedanolide to the other phthalides varies between varieties. Tendercrisp has the highest level of n-butyl phthalide while Excelsior has the highest level of sedanolide. USA Green Stringless, Green Crunch and Salad Green have the highest concentrations of sedanenolide.

The variety USA Green Stringless has approximately equal proportions of sedanolide and sedanenolide and these are at approximately double the proportion of n-butyl phthalide.

The varieties Green Crunch and Salad Green have approximately equal proportions of n-butyl phthalide and sedanenolide. These are approximately one quarter the concentration of sedanolide.

The extracts of the remaining three varieties, Darklet, Excelsior and Tendercrisp have relatively little sedanenolide compared with the other phthalides. The ratio sedanolide concentration to n-butyl phthalide concentration for Tendercrisp is approximately 2:1, while for Darklet it is 4:1 and Excelsior it is 9:1.

Table 4.7 Yield and composition of hexane extracts of seed samples of celery varieties

	variety	*USA Green*	*Green Crunch*	*Salad Green*	*Darklet*	*Excelsior*	*Tendercrisp*
	yield (%)	11	8	7	13	11	7
% component in oil	β-pinene	1.33	0.47	0.85	0.52	0.69	1.34
	myrcene	0.32	0.29	0.31	0.40	0.52	0.33
	limonene	34.25	24.42	32.02	34.60	31.55	42.42
	a n-pentylcyclo-hexadiene	trace	trace	0.38	0.17	trace	0.26
	pinocarveyl acetate	trace	trace	0.19	0.20	0.2	0.23
	β-selinene	15.40	9.11	11.24	11.84	5.61	7.34
	n-butyl phthalide	6.83	8.80	7.81	8.03	5.72	13.69
	sedanolide	18.16	36.57	29.01	32.41	47.12	21.46
	sedanenolide	14.13	8.48	7.55	0.57	0.51	2.20

Following the objective assessment of the extracts, an organoleptic assessment was also made. The results of this assessment are presented in table 4.8. Since no seed of the variety Triumph was available for testing, oil from Triumph herb was included in the assessment but was not included in the order of preference ranking.

Table 4.8 Initial organoleptic assessment of seed extracts plus herb oil of Triumph.

Variety	assessment	rank*
Triumph herb	terpenic, resinous	not applicable
USA Green	slightly like salad green, fresh, clean, resinous, similar to celery herb	1
Green Crunch	similar to Tendercrisp, not strong	2
Salad Green	good top-note, nice resinous, similar to celery herb	3
Darklet	good top notes, resinous, seed-like, more pleasant than Tendercrisp	4
Excelsior	more like Tendercrisp, flat	5
Tendercrisp	resinous but flat	6

* Rank refers to the order of preference (1 high, 6 low) by the authors based upon selecting for extracts that are reminiscent of fresh, crushed celery.

No varieties were excluded from the trial work based upon the initial screening. Unfortunately, no further seed of the variety Darklet was available and this variety was excluded from further work on that basis only.

4.3.2 Subjective assessment of varieties in field trial

A subjective assessment of the varieties in the field trial revealed differences in the flowering time of the varieties, amounting to approximately two weeks between flowering of the first and last varieties in the trial. Green Crunch was the earliest flowering variety followed by Salad Green. Excelsior and Tendercrisp flowered at approximately the same time, followed by USA Green and lastly Triumph. There were slight plot differences in time of flowering but generally, all plots of each variety matured at approximately the same rate.

Many of the plots lodged towards the end of the flowering period. This is undesirable in commercial crops but lodging tended to be more associated with the position of the plots in the trial rather than a varietal effect.

An organoleptic assessment was made on one, randomly chosen oil sample from the 4/2/02 harvest of each variety in the trial. This was repeated on all three replicates of each variety. The results are presented in table 4.9. From this assessment, the varieties Salad Green and Tendercrisp were selected for detailed study in the semi-commercial trial.

Table 4.9 Organoleptic assessment of variety trial

variety	first assessment	second assessment
Salad Green	• steely, lacks character, slightly burnt • improved upon dry out to give impression of fresh herb	• pleasant • green celery, peppery
Green Crunch	• celery, strong, peppery	• steely • not reminiscent of celery, more like fennel • paint thinner
Tendercrisp	• seedy • celery, very slightly burnt, peppery	• pleasant • variation between replicates but good
Triumph	• celery, clean, herbaceous	• almost citrus, pleasant but not celery • strong celery but shallow, chlorine
USA Green Stringless	• seedy • meaty, hay, celery	• kerosine, rubbery • green crushed celery but strong off-notes
Excelsior	• pungent, fresh • celery, strong, very slightly burnt	• pleasant, good celery character but off notes, strange • one replicate smells of paint thinner

A further assessment was made on the ease of harvesting each plot when the plants were harvested for herb oil production in the middle of the flowering season. The ease of harvest was somewhat influenced by the level of lodging associated with the plot but it was also influenced by variety. Excelsior was particularly tall and had side shoots with long internodes. This variety was particularly difficult to handle. Triumph, Tendercrisp and USA Green were smaller and easier to harvest than the other varieties.

4.3.3 Serial harvest of field trial

The serial harvest of the field trial began when the earliest varieties were beginning to bolt. The results of the serial biomass measurements are presented in figures 4.23 to 4.26. Figure 4.23 charts the size of the plants, measured by dry weight, from the onset of bolting. The figure shows that for all varieties, the plants rapidly increase in size, until they reach full bloom. Sequential points on the charts in the early part of the flowering season tend to follow a pattern of steady increase. The dry weight then stabilises, although for most varieties there are peaks and troughs in the plant dry weight chart. These perhaps indicate fluctuations in plant dry weight associated with flushes of flowering and changes in plant weight associated with the development of floral organs, petal fall and particularly the fall of fruits which fail to set. It may be associated with set seed shedding although this was not noted as a problem by observation of the crop until late in the season. The possibility that it may be a function of the sample size was examined. Plant size was observed to be more variable following the onset of flowering and therefore although the selection of plants was random within each plot, these variations could be reflected in the mean values. A study of the coefficient of variation of the results across the season did not suggest an increase in the variability of the means with increasing harvest date.

Although the results fluctuate and the relative size of plants varies through the season, Excelsior tends to have the largest plants, while Salad Green and Green Crunch are of intermediate size and Tendercrisp, USA Green Stringless and Triumph are somewhat smaller.

The moisture content of the plant material decreases as the plants mature, as is reflected by the rise in the relative proportion of dry matter throughout the season Figure 4.24. There is little difference between varieties.

The oil content of the plants and the oil yield per unit area are shown in figures 4.25 and 4.26. The oil content per unit of plant matter is expressed as the weight of oil obtained from a 2.5 hour steam distillation of the above ground portion of the plant. The yield curves for this parameter follow closely the curves of plant dry matter production through the season. There is a peak in production for most varieties from late January to early February, followed by a slight decrease.

Oil yield per unit area shows a rapid increase with the onset of flowering for all varieties. Production then peaks and begins to tail off. Peak production occurs first in Salad Green in late January, followed by Excelsior, Green Crunch, Triumph and Tendercrisp in mid-February and lastly USA Green Stringless, which reaches peak production in late February. Three of the varieties, Salad Green, Excelsior and USA Green Stringless showed a possible second increase in production but the final harvest prevented this from being ascertained.

If the varieties are harvested at their peak production, Excelsior will produce the greatest yield of oil per unit area, followed by Salad Green. Green Crunch and USA Green Stringless produce intermediate yields, while Triumph and Tendercrisp have the lowest yields per unit area.

For each variety, oil composition changes over the flowering season in a similar manner. Figures 4.27 a to f show these changes. In each figure, limonene is presented on a second axis to enable the changes in the minor components to be illustrated.

For each variety, limonene increases with the onset of seed set in the secondary umbels. The rate at which this increase takes place differs between varieties. There is a sudden increase in limonene

concentration between mid-December harvest dates for Excelsior, Triumph and USA Green Stringless and thereafter it becomes relatively stable throughout the remainder of the season. For Tendercrisp, there is a very gradual increase in limonene concentration in the oil, throughout the season. The pattern of increase in limonene concentration for Salad Green and Green Crunch is between these two patterns. The increase is relatively fast from mid-December to mid-January and then becomes stable for the remainder of the season.

Figure 4.23 Changes in plant dry weight through the flowering season

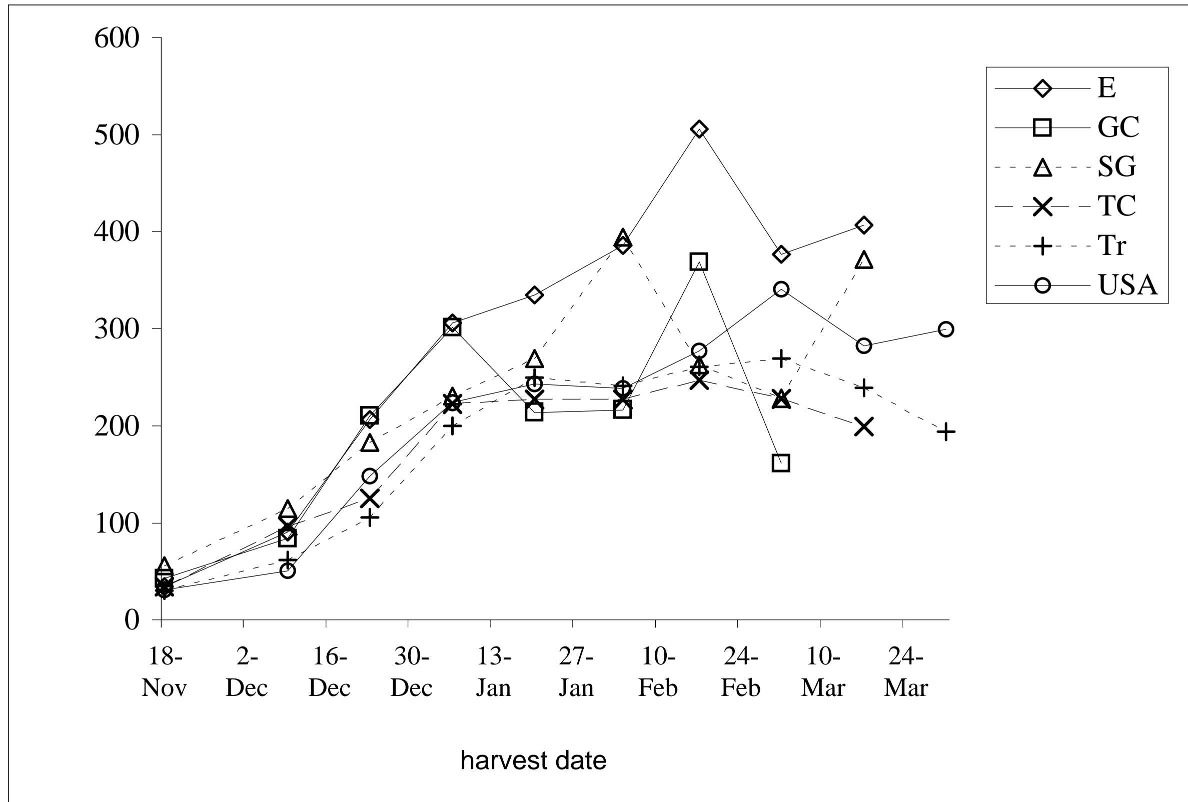


Figure 4.24 Changes in plant moisture content as measured by plant dry matter content through the flowering season

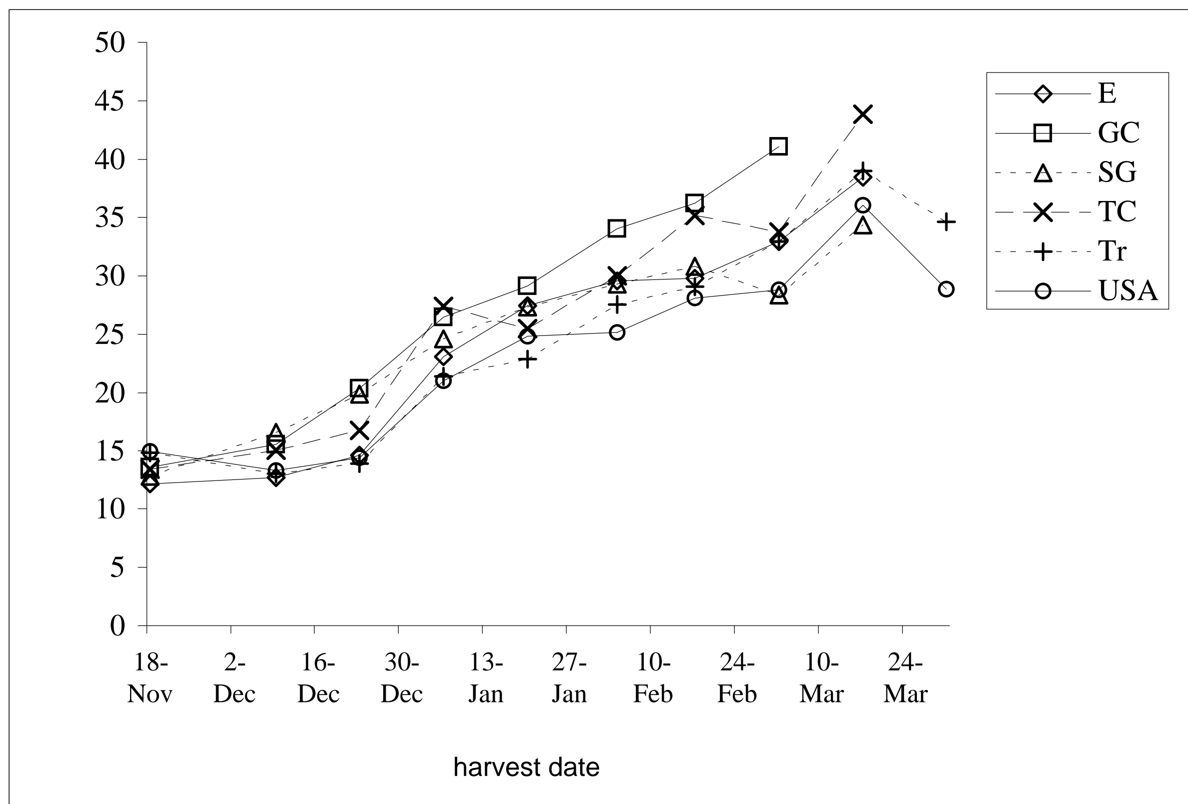


Figure 4.25 Changes in plant oil content (g oil per g dry weight distilled) through the flowering season

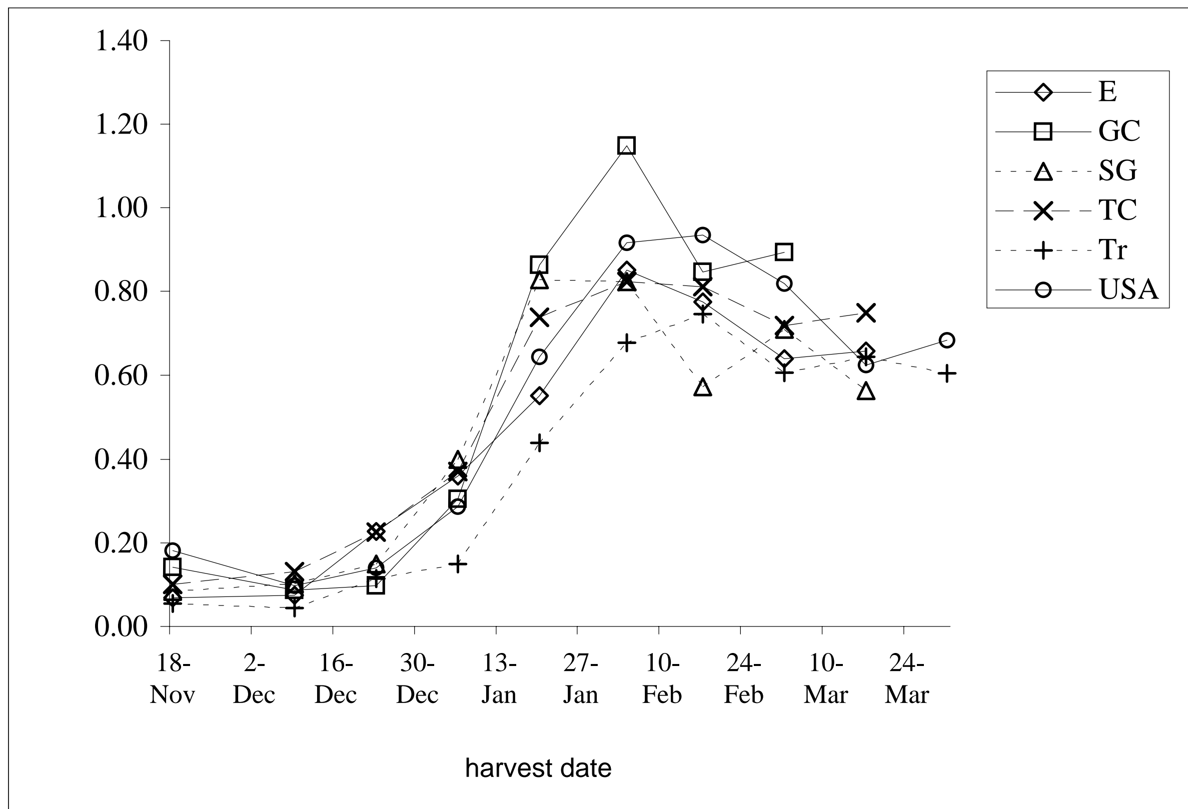


Figure 4.26 Changes in oil yield per unit area (g/m^2) through the flowering season

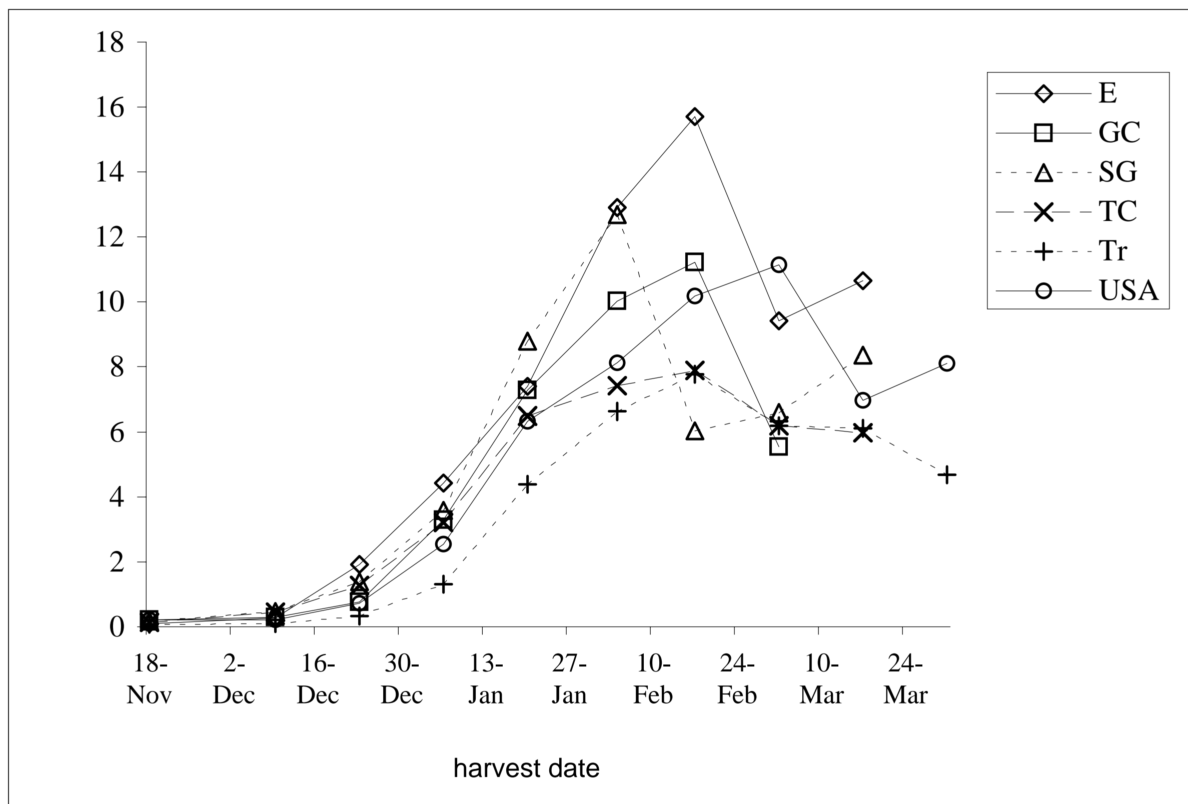


Figure 4.27 a to f Oil composition for each celery variety during the flowering season

Figure 4.27 a Excelsior

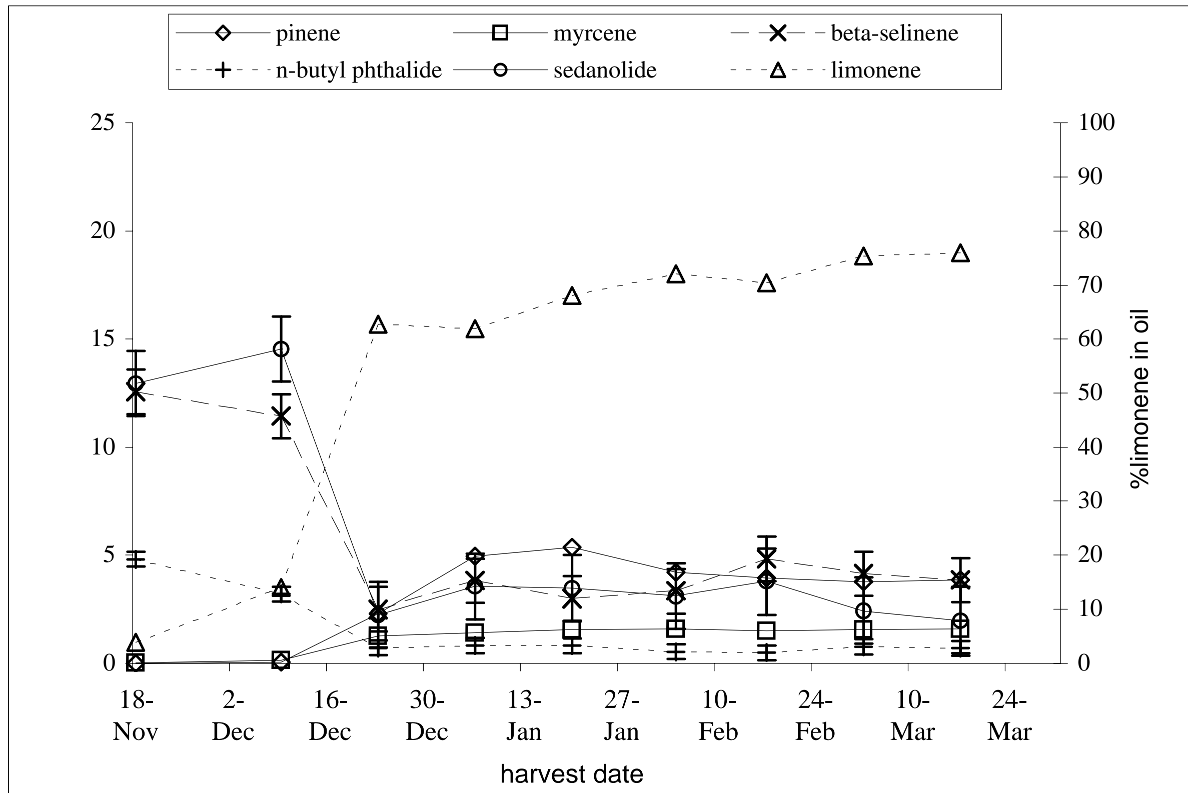


Figure 4.27 b Green Crunch

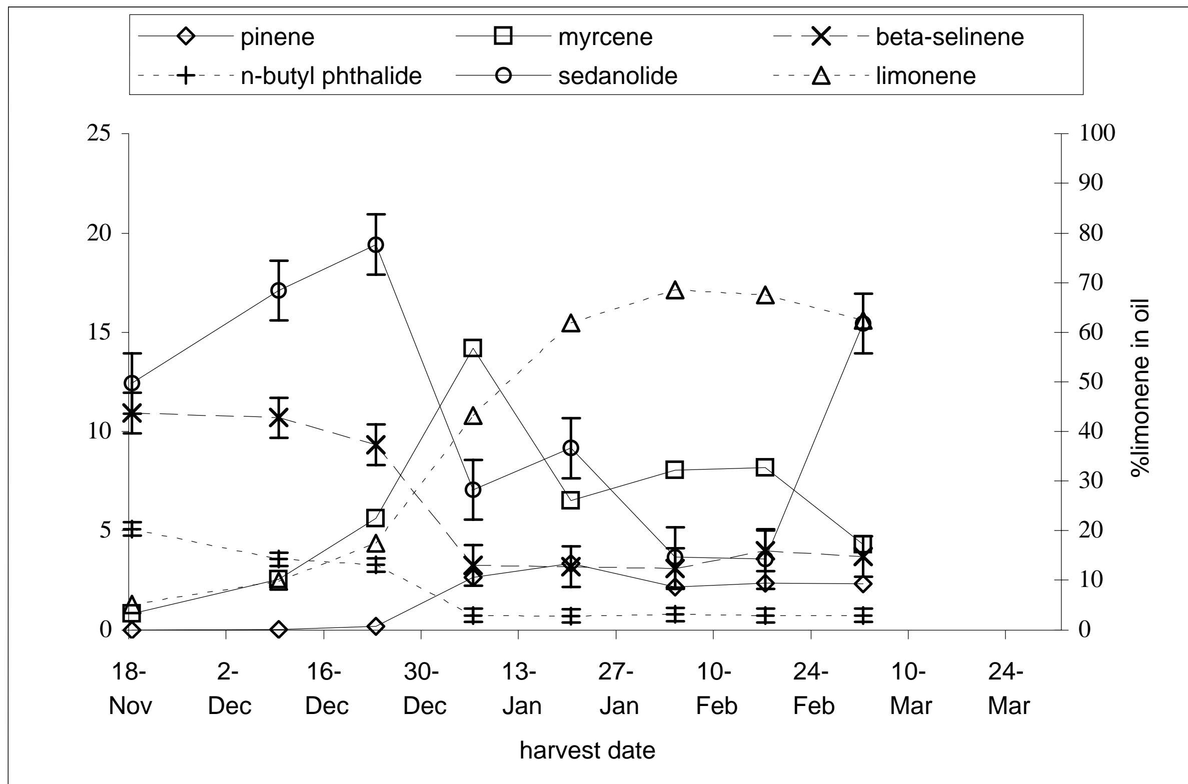


Figure 4.27 c *Salad Green*

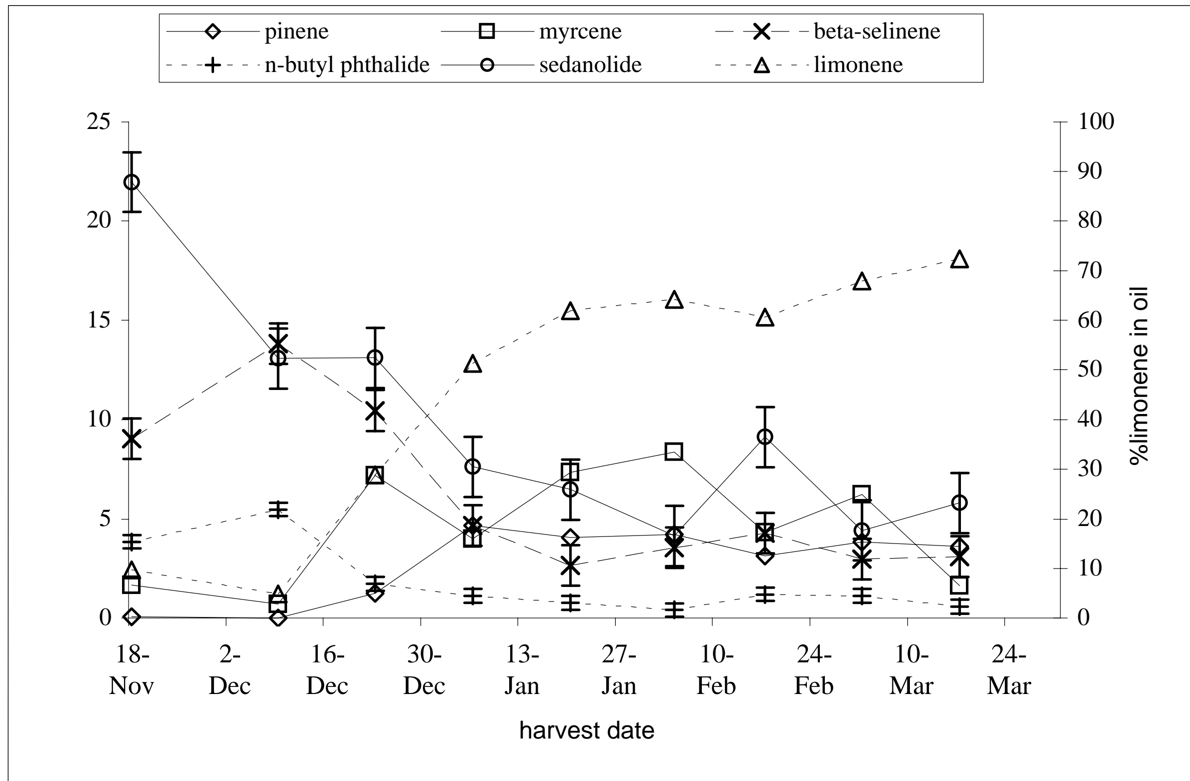


Figure 4.27 d *Tendercrisp*

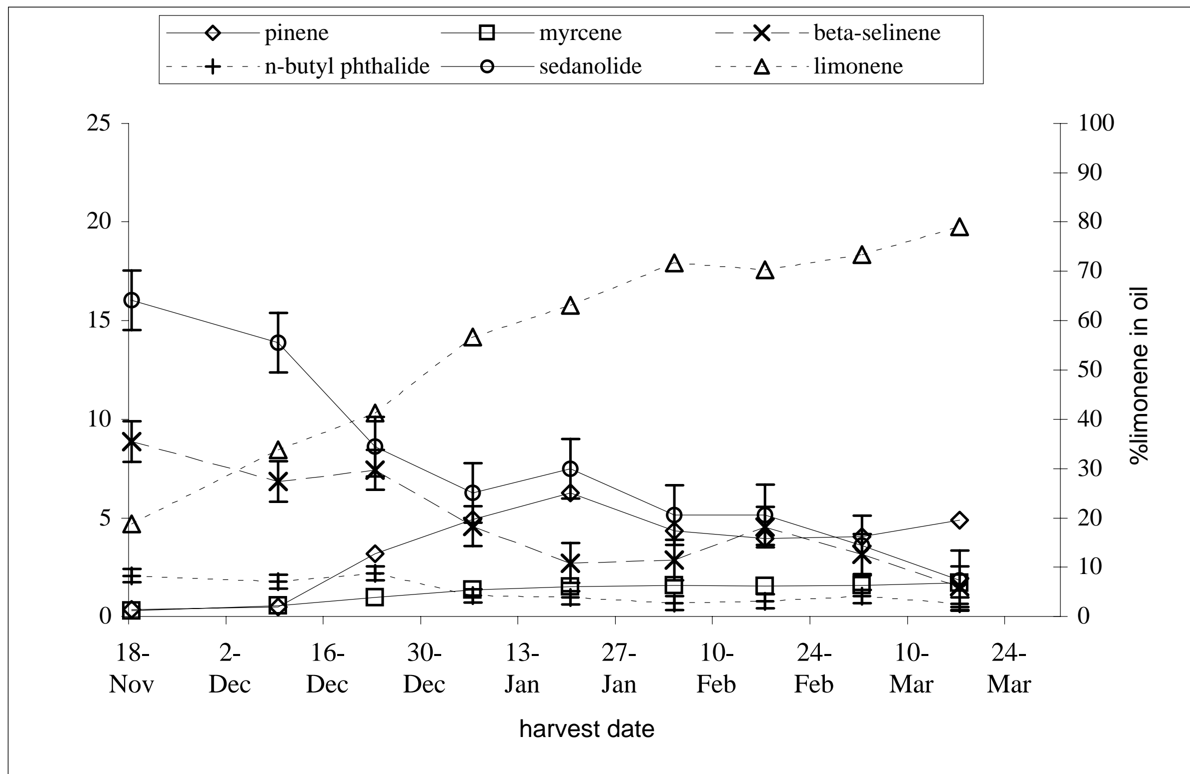


Figure 4.27 e *Triumph*

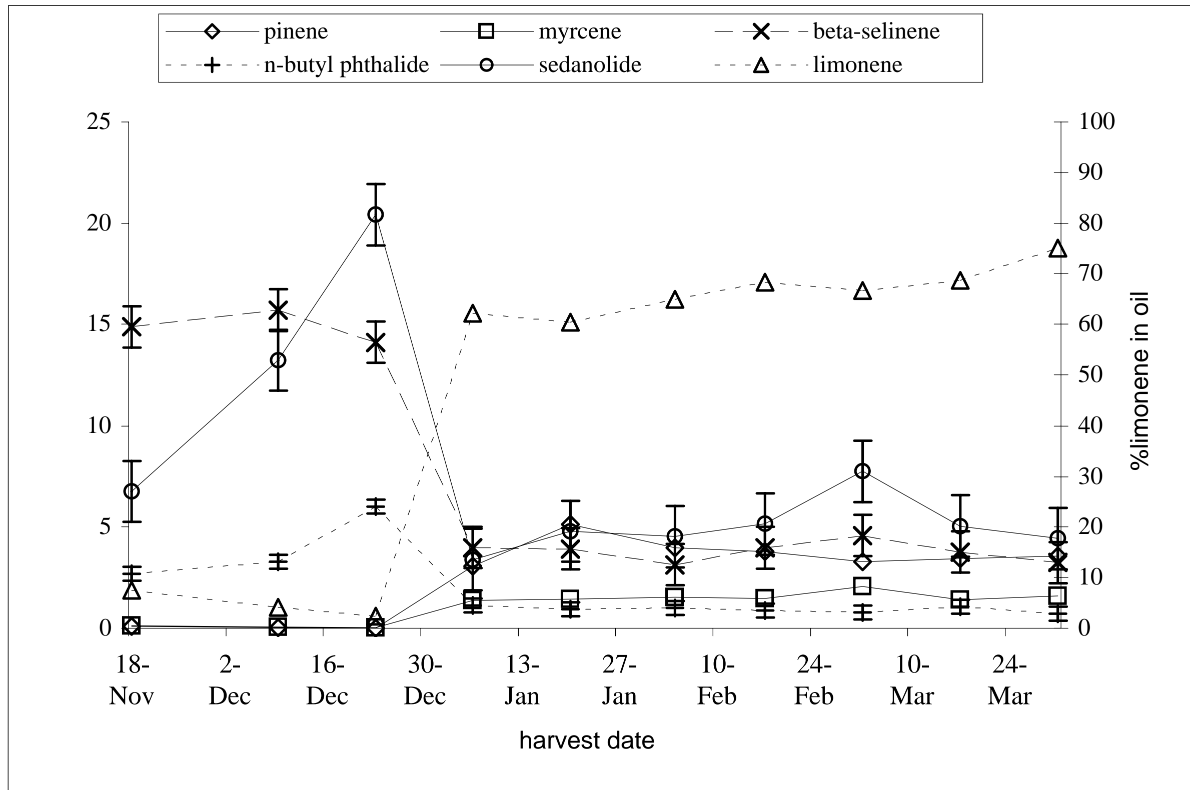
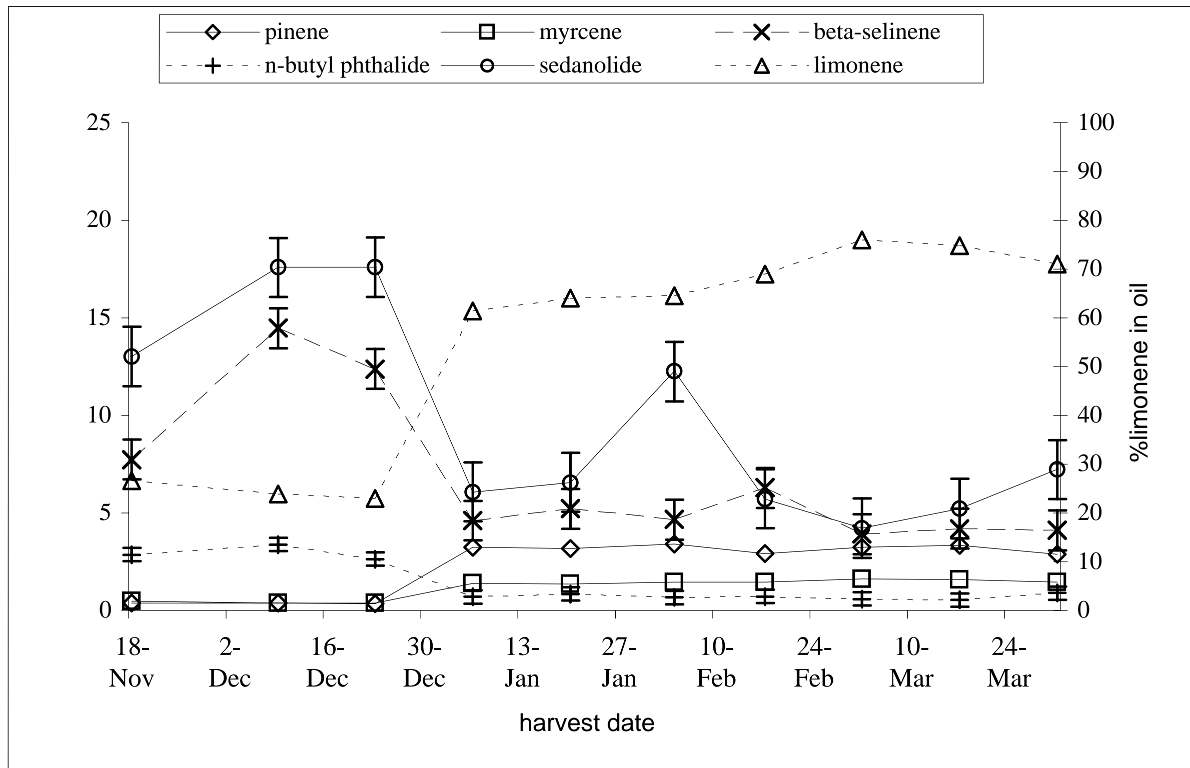


Figure 4.27 f *USA Green Stringless*



Generally, n-butyl phthalide, sedanolide and β-selinene are at higher concentrations in the herb oil early in the flowering season and decrease to stable levels as flowering progresses. For the varieties Triumph, USA Green stringless and Green Crunch (figures 4.27 e, f and b, respectively), the sedanolide levels show a period of increase in the first part of the flowering season before this decrease. The decrease in n-butyl phthalide, β-selinene and sedanolide correspond to the increase in limonene concentration. Both myrcene and β-pinene tend to increase in concentration at approximately the same time as limonene and to then remain at a constant concentration in the oil for the remainder of the season.

4.3.4 Herb oil

The samples of herb for the production of herb oil were harvested on 17/2/02. The results of yield and composition for each variety are presented in table 4.9. The results are not replicated but are based upon pooled samples of the three replicates of each variety distilled in a semi-commercial pilot plant. Because the results are not replicated, comparisons between varieties must be made with caution but the results provide a useful, large-scale comparison with the laboratory distilled serial harvest data.

Table 4.9 Yield and composition of semi-commercial samples of herb oil from each variety

	variety		*Excelsior*	*Green Crunch*	*Salad Green*	*Tendercrisp*	*Triumph*	*USA Green*
	yield per plant (g)		0.451	0.239	0.641	0.310	0.437	0.717
	yield (g/m^2)		1.802	0.956	2.565	1.238	1.747	2.867
	yield (kg/ha)		18	10	26	12	18	29
% component in oil	1	α-pinene	trace	trace	trace	trace	trace	trace
	2	β-pinene	0.52	0.40	1.07	1.07	0.66	0.80
	3	myrcene	0.34	1.15	1.83	0.65	0.34	0.56
	4	p-cymene	trace	trace	trace	trace	trace	trace
	5	limonene	25.88	24.56	30.48	38.56	20.91	33.68
	6	*cis*–β-ocimene	0.73	0.35	0.59	0.55	0.59	0.78
	7	γ-terpinene	trace	trace	trace	trace	trace	trace
	8	fenchone	trace	trace	trace	trace	trace	trace
	9	n-pentylbenzene	0.35	0.19	0.30%	0.58	0.33	trace
	10	a n-pentylcyclo-hexadiene	2.39	2.39	0.17	0.55	2.77	2.20
	11	*trans*-anethole	trace	trace	0.11	trace	trace	trace
	12	trans-pinocarveyl acetate	0.36	0.29	0.38	0.41	0.43	0.26
	13	carveyl acetate	0.09	trace	trace	trace	0.11	trace
	14	caryophyllene	5.05	4.99	3.29	2.28	4.58	2.84
	15	humulene	0.55	0.60	0.37	0.33	0.51	0.38
	16	β-selinene	23.84	19.01	14.60	15.63	18.51	20.63
	17	α-selinene	3.53	2.81	2.19	2.27	2.85	2.94
	18	elemol	0.10	0.21	0.18	0.16	0.11	trace
	19	caryophyllene oxide	1.03	0.81	0.88	1.56	0.92	0.24
	20	n-butyl phthalide	2.78	1.76	2.94	4.15	3.39	1.57
	21	β-eudesmol	0.91	2.12	2.46	0.87	0.81	0.21
	22	α-eudesmol	0.29	1.07	0.67	0.23	0.64	0.84
	23	sedanenolide	17.27	11.57	16.47	9.80	22.88	13.58
	24	sedanolide	6.77	10.95	14.83	8.55	11.93	13.71
	25	neophytadiene	1.15	6.40	1.31	4.80	2.52	2.13

From this assessment of the varieties, USA Green Stringless and Salad Green are predicted to produce the greatest yields per unit area. Excelsior and Triumph are intermediate producers while Tendercrisp and Green Crunch produce the lowest yields.

Based on these results, celery herb will produce oil yields in the range 10-30 kg /ha. However, this oil exhibits a composition indicating that most of the monoterpenes were lost during distillation, thereby reducing the volume of oil recovered. The serial harvest data suggest yields of 30-50kg/ha should be easily attainable. Yields of 80 kg/ha may also be possible. Certificates of analysis for these oils are provided in Appendix B.

4.3.5 Seed oil

Seed yields of 4-6 kg/10m^2 or 4-6 t/ha were obtained from the variety trial. There were no significant differences between varieties.

The seed was distilled in the laboratory to obtain semi-commercial oil samples of the potential varieties. To obtain a potentially higher quality oil, with a lower limonene concentration and higher concentrations of phthalides, the fractions of distilling over in the first hour were kept separate from the fractions which distilled over between one and six hours from breakthrough. The results of yield and composition are presented in table 4.10. The results are the means of three replications of each variety, based upon the three plots of each harvested from the field. The fraction collected between one and six hours will be treated as the potential commercial oil. Certificates of analysis for these oils are included in Appendix B.

The first fraction of oil comprised between one quarter and one sixth of the total oil yield. By discarding this oil, yields were substantially reduced. If the entire oil sample is considered, yields from 45kg/ha to 68kg/ha were achieved whereas if the first fraction was excluded, the yields ranged from 38kg/ha to 56kg/ha.

The ranking order from highest yielding variety to lowest based both on including and excluding the first fraction is the same. The order is Salad Green, Excelsior, Green Crunch, Tendercrisp, Triumph and USA Green Stringless. There were no significant differences between varieties with respect to yield, or the concentrations of the mono- and sesquiterpenes, but there were significant differences between varieties in the concentration of the phthalides. The phthalide concentrations were significantly different at the 5% level for n-butyl phthalide while sedanolide and sedanenolide were different at the 0.1% level.

For all the varieties, the limonene concentration was reduced in the commercial oil fraction compared with the first collected fraction although the extent of this reduction was dependent upon the variety. For USA Green Stringless, the reduction was only 0.1% oil per dry matter from 77.17% to 77.01%, while for Triumph the reduction was 6.85% oil per dry matter, from 78.94% to 72.09%. The upper limit for limonene as set by the ISO standard is 79%. By excluding the first fraction of oil, all the varieties tested fell within this limit.

For all varieties, both β-pinene and myrcene exceed the upper limit of the ISO Standard, while β-selinene concentrations are too low. Only USA Green Stringless and Triumph fall inside the ISO standard specifications for β-selinene and oils of both are close to the bottom limit. Sedanenolide, a critically important component of celery oil, also poses problems for ISO standard compliance by these oils. Green Crunch, Salad Green and USA Green Stringless have concentrations just above the bottom limits while the sedanenolide concentrations in the other oils are too low.

At the completion of the final harvest, samples of the trash remaining were distilled to estimate the yield and composition of the oil that was not recovered by the harvesting method. The results are presented in Table 4.11. No significant differences were detected between varieties with respect to oil yield from the trash, which was between 0.1% and 0.2% of dry matter distilled. The oil was relatively low in limonene (28-47%) and high in sedanenolide (8-21%) compared with the seed oil. Differences were detected between varieties, probably due not so much to differences in harvestability and seed recovery but more due to differences in initial oil composition in the intact plants.

Following distillation of the seed, the marc was recovered and extracted with hexane. The results are presented in able 4.12. The marc yielded approximately 1% extract by weight. The extracts were comprised of fatty acids and other non-volatile components but also substantial quantities of sedanenolide (20-30%). Interestingly, these marcs also included 0.4 – 0.9% by weight of the phthalide identified in section 4.1 as unknown phthalide mw 192.

Table 4.10 Yield and composition of essential oil in the 0-1 hour and 1-6 hour fractions of seed oil from each variety

variety		*Excelsior*		*Green Crunch*		*Salad Green*		*Triumph*		*Tendercrisp*		*USA Green*	
time interval from breakthrough for collection of oil fraction		0-1 hour	1-6 hours	0-1 hour	1-6 hours	0-1 hour	1-6 hours	0-1 hour	1-6 hours	0-1 hour	1-6 hours	0-1 hour	1-6 hours
	seed yield (kg/ha)	5.493		5.103		6.053		4.437		4.668		3.890	
	oil yield (% of dry matter distilled)	0.1987	0.8683	0.2033	0.9147	0.2027	0.9127	0.2520	0.8717	0.2350	0.9810	0.1780	0.8260
	oil yield (kg/ha)	11.0	47.7	10.7	46.3	12.3	55.7	11.3	38.3	11.0	45.3	7.0	38.0
	total oil yield (kg/ha)	58.3		57.0		68.0		50.0		56.3		45.0	
peak	component	% component in oil											
2	β-pinene	4.62	4.78	3.20	3.09	4.75	4.73	4.77	4.58	5.60	5.51	3.50	3.85
3	myrcene	1.58	1.61	1.60	1.53	1.67	1.60	1.48	1.44	1.66	1.65	1.45	1.57
5	limonene	83.53	79.00	82.34	77.02	78.72	74.28	78.94	72.09	84.50	77.73	77.17	77.01
10	a n-pentylcyclo-hexadiene	1.88	3.35	1.89	3.25	1.89	3.51	3.20	5.39	1.66	3.29	1.23	2.15
12	*trans*-pinocarveyl acetate	0.20	0.20	0.19	0.19	0.24	0.27	0.27	0.28	0.17	0.18	0.16	0.19
16	β-selinene	1.32	3.64	2.03	3.95	2.63	4.94	1.88	5.59	1.08	4.11	5.30	5.46
20	n-butyl phthalide	0.52	0.49	0.55	0.60	0.79	0.60	0.89	0.71	0.45	0.52	0.73	0.56
24	sedanolide	3.64	3.82	2.91	4.06	2.98	3.47	3.99	4.75	2.46	3.66	3.38	3.80
23	sedanenolide	0.51	0.47	2.53	3.25	2.61	2.87	1.29	1.31	0.65	0.75	2.90	2.79

Table 4.11 Analysis of trash samples from harvest of seed in variety trial

variety	*Excelsior*	*Green Crunch*	*Salad Green*	*Tendercrisp*	*Triumph*	*USA Green Stringless*
yield	0.20%	0.14%	0.19%	0.16%	0.16%	0.11%
Component	% component in oil					
β-pinene	1.92	0.74	2.06	1.04	2.23	0.27
myrcene	0.82	4.17	7.16	0.58	1.07	0.44
limonene	40.23	28.28	38.51	32.06	46.42	29.88
a n-pentylcyclo-hexadiene	4.77	2.17	2.03	2.37	3.90	0.79
caryophyllene	6.34	7.26	5.69	7.09	6.67	7.90
trans-pinocarveyl actetate	0.46	0.44	0.34	0.40	0.34	0.19
β-selinene	15.03	13.82	8.65	14.76	11.45	21.35
neophytadiene	3.94	9.70	7.51	4.15	4.10	5.76
unknown phthalide (mw 192)	0.22	0.22	0.16	0.32	0.28	0.43
n-butyl phthalide	2.92	2.04	1.95	2.86	2.22	1.51
sedanenolide	11.81	14.17	13.67	20.69	9.07	8.34
sedanolide	0.44	0.53	0.57	0.45	0.50	1.28

Table 4.12 Assessment of yield and composition of the hexane extracts of marc from seed distillation

variety	*Excelsior*	*Green Crunch*	*Salad Green*	*Tendercrisp*	*Triumph*	*USA Green*	standard error
yield of extract (%w/w)	1.17	1.26	1.36	1.31	1.29	1.05	0.09
component	**% component in extract**						
limonene	0.41	0.51	0.45	0.58	0.35	0.73	0.14
β-selinene	0.59	0.72	0.60	0.73	0.38	0.79	0.15
n-butyl phthalide	2.13	1.54	1.76	2.11	1.92	1.84	0.15
unknown phthalide (mw 192)	0.85	0.68	0.50	0.69	0.74	0.47	0.07
sedanenolide	29.19	23.01	21.46	29.40	27.88	23.58	1.31
sedanolide	0.82	6.14	6.37	3.29	2.37	5.24	0.86

4.4 Assessment of semi-commercial crop

4.4.1 Assessment of commercial harvest of semi-commercial crop

The commercial harvest was scheduled by serial pre-harvest sampling (figures 4.28 to 4.33). Figures 4.28 to 4.31 show the changes plant biomass while figures 4.32 and 4.33 show the changes in oil yield and composition of the two varieties. It is figures 4.31 to 4.33, in particular, which were used to determine the harvest date. For logistical reasons, associated with the hiring of harvesting contractors and the availability of the commercial distillation unit, both the varieties, Tendercrisp and Salad Green were harvested concurrently, despite Salad Green maturing perhaps two weeks earlier than Tendercrisp.

Figure 4.28 Dry matter production through the season, in the semi-commercial trial of Tendercrisp and Salad Green

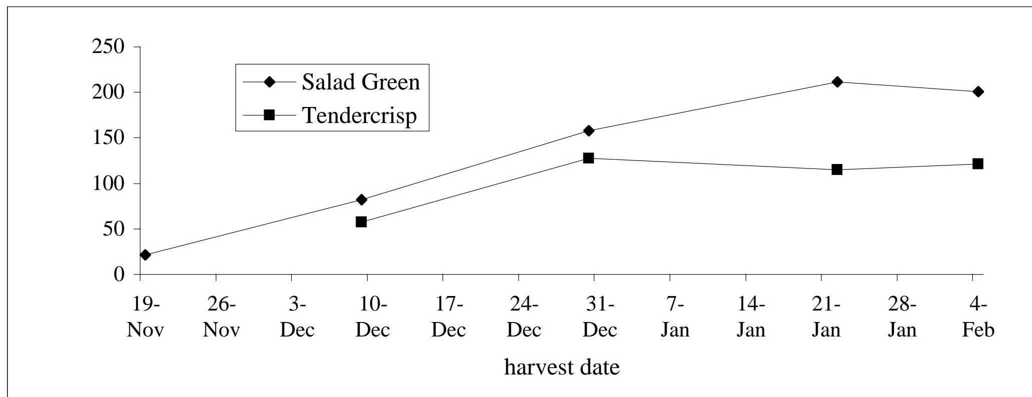


Figure 4.29 Changes in moisture content, as expressed by percent dry matter, through the season in the semi-commercial trial of Tendercrisp and Salad Green

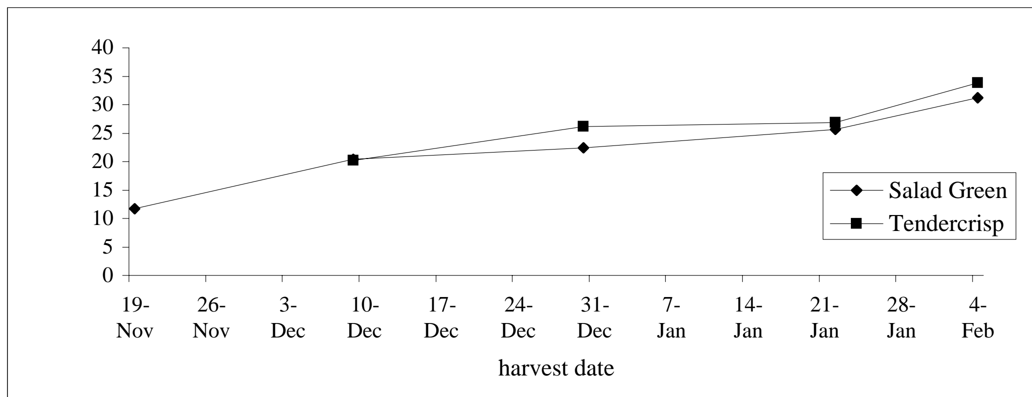


Figure 4.30 Changes in oil content through the season, in the semi-commercial trial of Tendercrisp and Salad Green

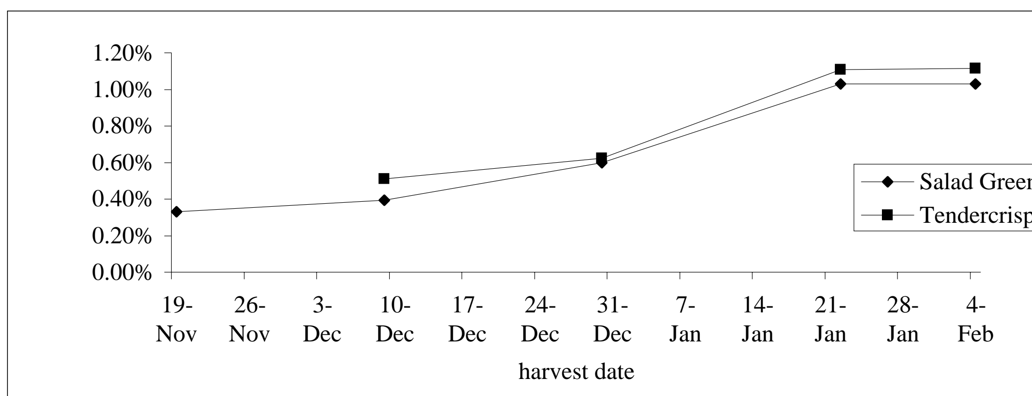


Figure 4.31 Changes in oil production per unit area through the season, in the semi-commercial trial of Tendercrisp and Salad Green

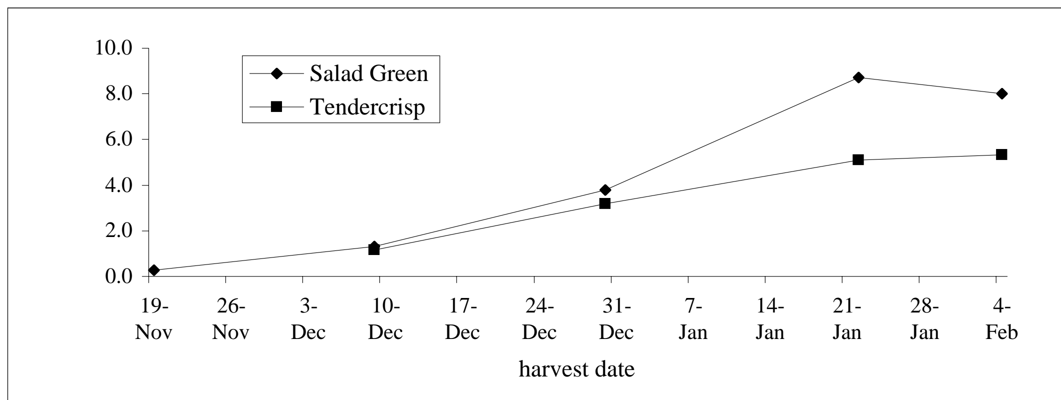


Figure 4.32 Changes in oil composition through the season for the semi-commercial trial of the variety Tendercrisp

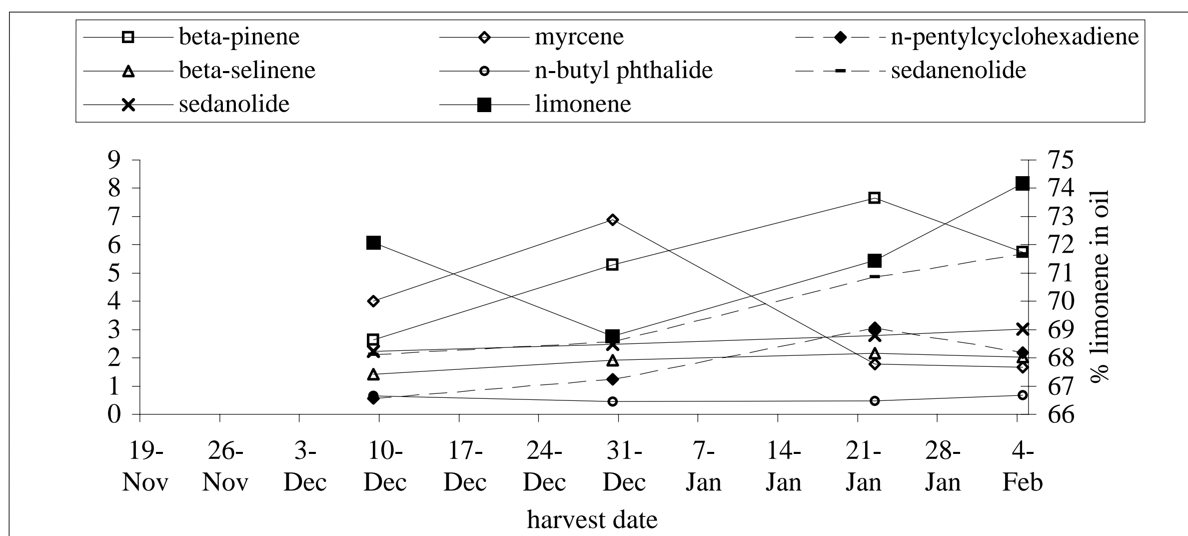
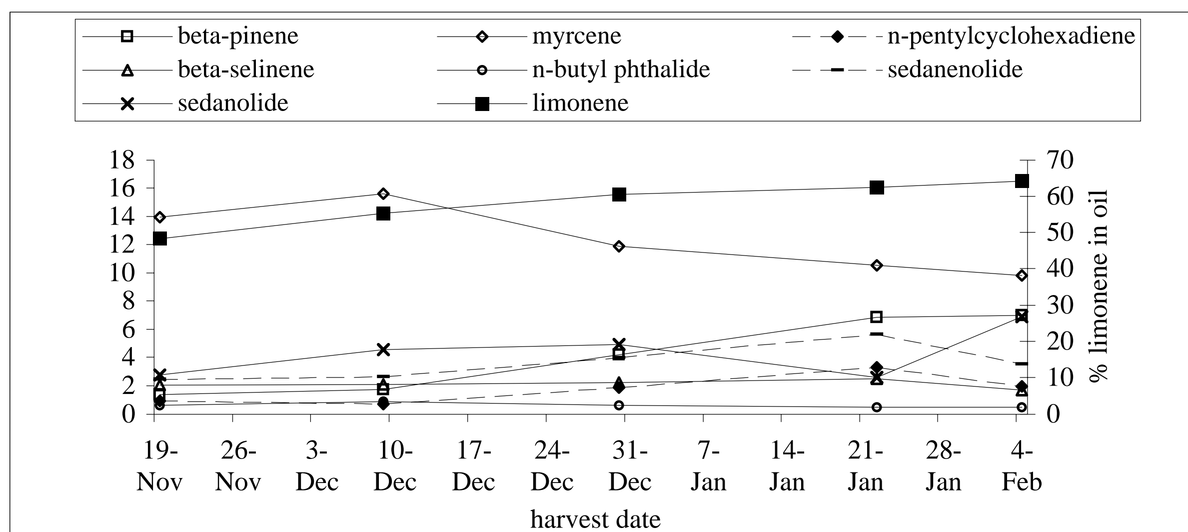


Figure 4.33 Changes in oil composition through the season for the semi-commercial trial of the variety Salad Green



The pre-harvest sampling from the semi-commercial trial commenced for each variety when bolting commenced. The results show a pattern of oil accumulation similar to that of the variety trial (section 4.3.3). Harvest was scheduled once the rapid oil accumulation phase was complete.The changes in oil composition for these varieties at this site were somewhat different from the pattern of changes detected in the variety trial. The oil composition was much more stable in the commercial planting and the rapid increase in limonene concentration was not detected, despite sampling commencing at approximately the same point of physiological development.The commercial harvest resulted in approximately ~3/4 of a vat of material from each variety. The total harvested area was approximately 0.9ha divided equally between the two varieties.

Data for the pre-harvest sampling of the root material are presented in table 4.13. These data indicate that oil yield from the root material decreases through the flowering season. Observation indicated that the root mass increased with plant development. The percentage of volatile components in the extracts decreased through the season.

Table 4.13 Changes through the season in yield and composition of hexane extractable components from celery roots

variety	harvest date	% yield of extract	% component in extract			
			unknown phthalide mw 204	sedanenolide	sedanolide	*cis*-neocnidilide
Salad Green	19-Nov	1.58	0.030	0.000	0.095	0.083
	9-Dec	0.71	0.036	0.120	0.105	0.150
	30-Dec	0.45	0.053	0.095	0.101	0.140
	4-Feb	0.33	0.000	0.000	0.123	0.224
Tendercrisp	9-Dec	0.90	0.013	0.143	0.075	0.120
	30-Dec	0.55	0.000	0.000	0.010	0.065
	4-Feb	0.38	0.000	0.052	0.065	0.010
standard error		0.096	0.019	0.031	0.018	0.018

4.4.2 Assessment of bulk root extracts

Extractions of bulk root material were made for each of Tendercrisp and Salad Green from root material collected at the completion of the commercial trial. Unfortunately the root material deteriorated very quickly in the field, compared with the material from the variety trial and therefore the quality of the root material was lower than expected.

The yield of extract from Salad Green was 0.21% while the yield of extract from Tendercrisp was 0.16%. The primary components in these extracts were fatty acids and some phthalides. Trace amounts of the unknown phthalide mw 204 and sedanenolide were detected in both varieties while the principal phthalides present were n-butyl phthalide, sedanolide and *cis*-neocnidilide. These were present in the extracts at 3%, 3% and 6% respectively in Salad Green and at 7%, 4% and 5% respectively in Tendercrisp. Table 4.14 shows the yield of each component, relative to the weight of root material extracted.

Table 4.14 Composition of extracts of bulk root samples

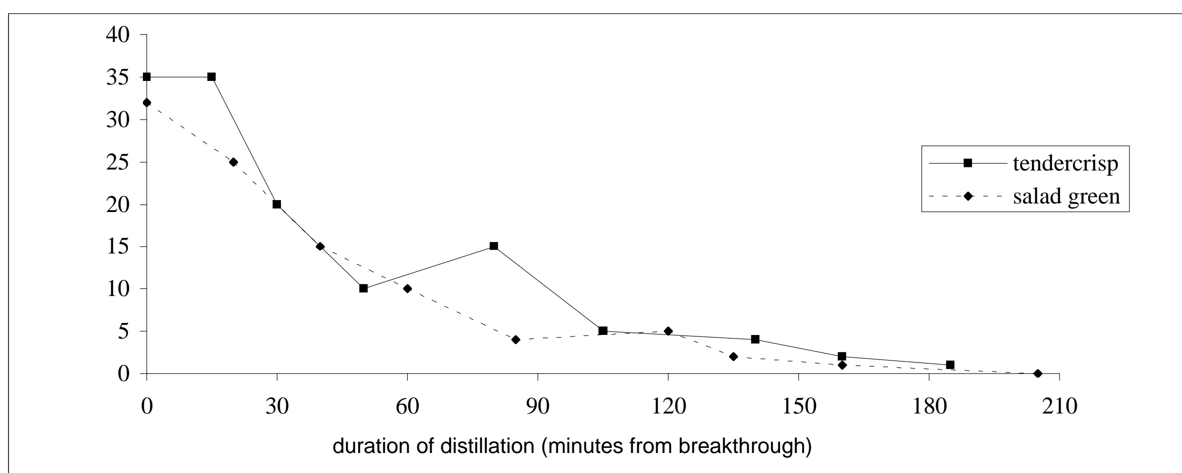
	weight of component per weight of root extracted (mg/g)					
variety	n-butyl phthalide	unknown phthalide mw 204	sedanenolide	sedanolide	*cis*-neocnidilide	total volatiles
Salad Green	2.50	0.85	0.38	2.80	5.01	93.18
Tendercrisp	5.76	trace	0.86	3.59	4.07	88.96

4.4.3 Assessment of the commercial distillation of the semi-commercial trial

Prior to the commencement of distillation, sub-samples of the material from each vat were collected for distillation in the laboratory. The results are presented in table 4.15

The duration of the commercial distillation for Salad Green was 3 hrs 25 mins and for Tendercrisp was 3 hrs 5mins. Distillation was monitored by regularly measuring the volume of oil per litre of distillate at the outlet from the condenser. Distillation was terminated when this volume of oil was less than one millilitre. The results of these assessments are presented in figure 4.34. This figure shows that the oil flow from the condenser was most rapid at breakthrough and declined steadily from that point on. A slight increase in the oil flow rate of the Tendercrisp distillation at 70 minutes was related to a slight increase in the distillate flow rate due to fluctuations in the condenser temperature and steam production through fluctuations in the boiler temperature. The results suggest that the bulk of the oil was obtained in the first hour of distillation.

Figure 4.34 Rate of oil flow from condenser during commercial distillation



Sub-samples of oil were collected for analysis of composition at each sampling time. These results are presented in figure 4.35 for Salad Green and 4.36 for Tendercrisp. These figures show that for both varieties the myrcene and limonene concentrations in the oil fall with increasing duration of distillation, while the levels of the other monoterpenes are relatively constant between increments. The concentrations of the more desirable components *i.e.* β-selinene and the phthalides increase as the distillation progresses.

At the completion of distillation, the oil of each variety was collected and the volume measured. The yield of Salad Green was 19.5L, which produced a final weight of 15.2kg after drying and filtering. The yield of Tendercrisp was 13L, which produced a final weight of 10.2kg after drying and filtering.

At the completion of distillation, a sub-sample of the marc from each vat was also taken for further distillation in the laboratory. These results are included in table 4.15. They show that 90% of the oil that was obtained by laboratory steam distillation of the herb was removed by the commercial distillation. Of the individual oil components, the monoterpenes, particularly limonene, were more readily extracted in the commercial distillation. The oil obtained by laboratory distillation of the marc from the commercial distillation was relatively rich in β-selinene and phthalides compared with the oil obtained from the herb.

Each commercial distillation began with the inner skin of the separator empty. Water began to flow to waste after 45mins for Tendercrisp and after 50 mins for Salad Green. The yield and composition of the oil in the waste water is presented in table 4.16. These data show that the oil that is lost, has a relatively high concentration of the desirable components. However, the actual volume of oil lost is

insignificant in the context of the complete distillation. Flow rates vary owing to unavoidable fluctuations in both the rate of steam production and the temperature of the condenser. However, estimates collected at each sampling time show flow rates of around 12L per minute are a reasonable estimate. Using the maximum oil concentration in waste water of 0.21 ml/L, this amounts to a loss of 2.5mL per minute. Water flowed to waste for 155 minutes in the distillation of Tendercrisp resulting in the loss of 390ml of oil. Since the total volume of oil collected was 13L for Tendercrisp, this is a loss of 3%. The inner skin of the separator contains approximately 1000L of water. At up to 0.24mL/L of oil this amounts to a loss of a further 240ml of oil or 1.8% of the final oil collected.

Table 4.15 Yield and composition of oil from laboratory distillation of the herb and marc from the commercial distillation of Salad Green and Tendercrisp

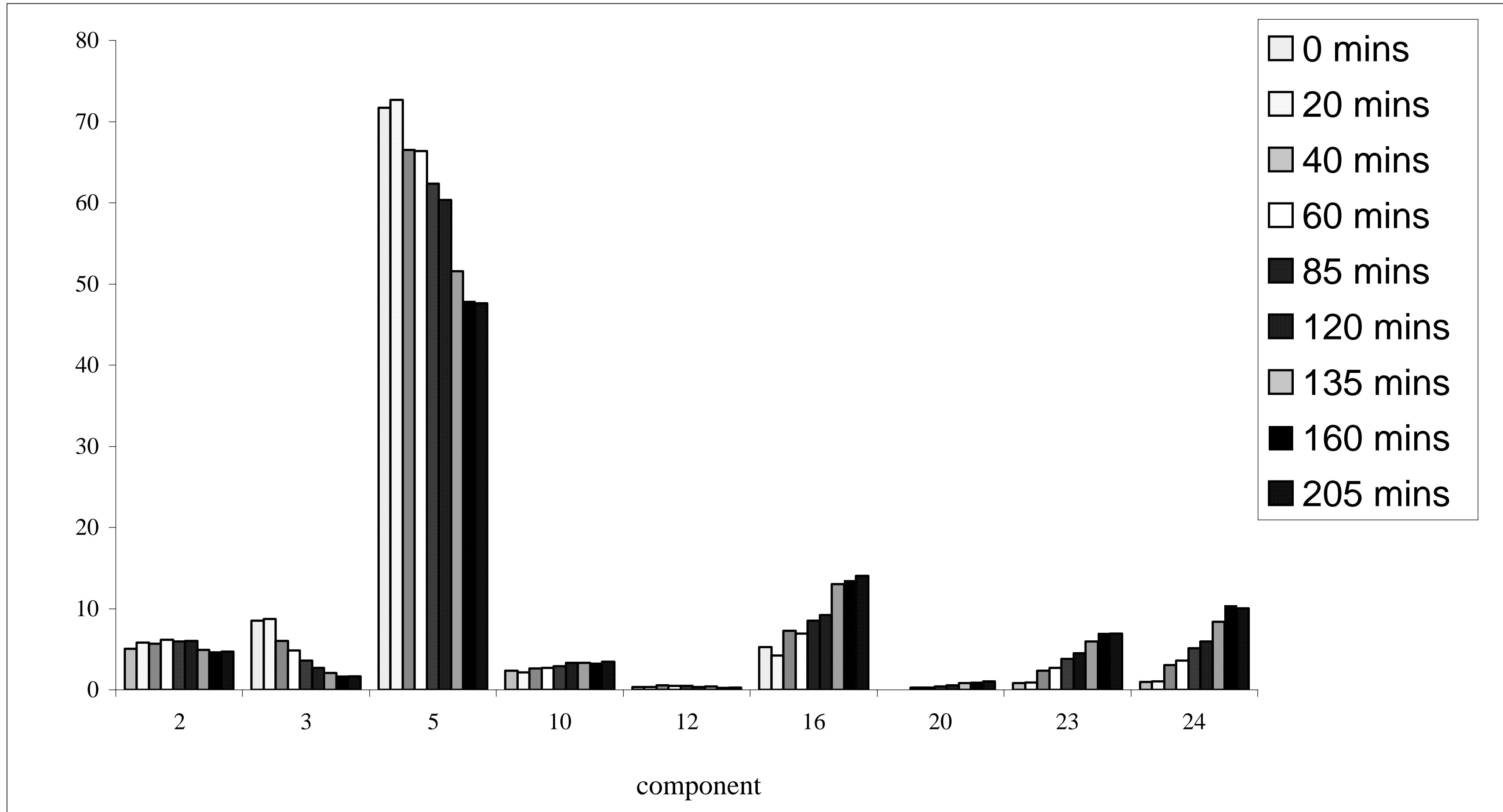
variety	*Salad Green*		*Tendercrisp*	
plant material	herb	marc	herb	marc
oil yield (% of dry matter distilled)	0.68%	0.08%	0.66%	0.09%
	%component in oil			
β-pinene	5.34	1.83	6.15	1.77
myrcene	6.19	0.61	2.23	0.56
limonene	65.69	20.79	72.50	23.65
a n-pentylcyclohexadiene	2.53	1.52	2.27	1.64
trans-pino carveyl acetate	0.37	0.38	0.29	0.22
β-selinene	5.89	21.46	4.17	16.72
n-butyl phthalide	0.54	2.62	0.69	4.20
sedanenolide	2.83	11.34	3.69	20.50
sedanolide	4.60	21.41	2.88	14.76

The composition of final commercial products of Salad Green and Tendercrisp were analysed by GC using both the Innoowax and HP1 columns. The results are presented in table 4.17 and a certificate of analysis for these oils is provided in Appendix B.

Table 4.16 Yield and composition of oil collected from waste water in commercial distillation.

variety	*Salad Green*				*Tendercrisp*			
time from beginning of flow to waste	0	60	140	-	0	60	155	-
	beginning of flow to waste	mid-distillation	end of distillation	water collected from inner-skin of separator	beginning of flow to waste	mid-distillation	end of distillation	water collected from inner-skin of separator
g oil/L waste water	0.13	0.18	0.21	0.24	0.11	0.20	0.20	0.21
component	**% component in oil**							
β-pinene	1.0	1.0	0.4	0.4	1.4	0.7	0.5	0.4
myrcene	1.3	0.6	0.2	0.2	1.7	0.2	0.1	0.2
limonene	23.0	17.2	6.3	6.1	32.3	10.2	6.0	6.9
a n-pentylcyclo-hexadiene	0.7	0.8	0.3	0.3	1.3	0.3	0.1	0.3
trans-pino carveyl acetate	1.4	0.8	0.9	1.1	0.9	0.6	0.6	0.7
β-selinene	13.7	6.5	3.2	2.4	9.4	2.7	0.8	2.1
n-butyl phthalide	1.9	3.6	4.3	4.3	1.8	4.8	5.0	5.5
sedanenolide	21.4	31.4	42.4	44.1	27.0	53.7	62.6	59.7
sedanolide	17.8	27.8	35.4	36.2	10.3	18.6	19.0	19.8

Figure 4.35 Changes in essential oil composition with duration of distillation for the commercial distillation of Salad Green

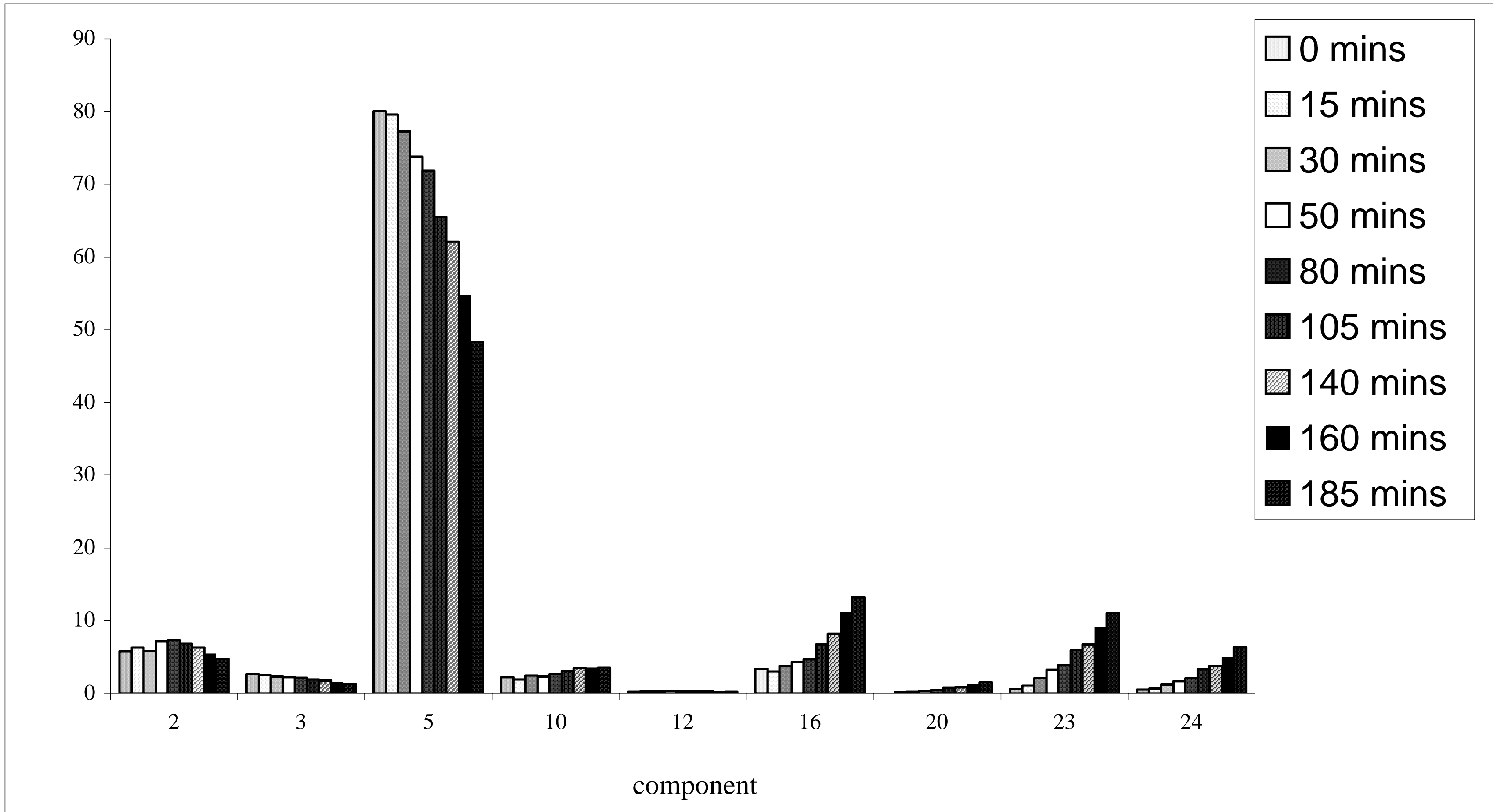


key to components

2 - β-pinene; 3 - myrcene; 5 - limonene; 10 - n-pentylcycloexadiene; 12 - *trans*-pinocarveyl acetate

16 - β-selinene; 20 - n-butyl phthalide; 23 - sedanenolide; 24 - sedanolide

Figure 4.36 Changes in essential oil composition with duration of distillation for the commercial distillation of Tendercrisp



key to components

2 - β-pinene 3 - myrcene 5 - limonene 10 - n-pentylcycloexadiene 12 - *trans*-pinocarveyl acetate

16 - β-selinene 20 - n-butyl phthalide 23 - sedanenolide 24 - sedanolide

Table 4.17 Composition of the final commercial oils of Tendercrisp and Salad Green assessed on both the HP1 and INNOWax columns

variety		**Salad Green**		**Tendercrisp**	
GC column		**HP1**	**INNOWax**	**HP1**	**INNOWax**
		% of FID area			
1	α-pinene	0.26	NI	0.31	NI
2	β-pinene	5.70	5.36	6.76	6.28
3	myrcene	6.43	6.05	2.23	2.10
4	p-cymene	0.16	0.28	0.24	0.26
5	limonene	68.88	69.58	77.75	77.43
6	*cis*-β-ocimene	1.09	0.97	1.03	1.06
7	γ-terpinene	0.16	NI	0.05	NI
8	fenchone	trace	NI	trace	NI
9	n-pentylbenzene	0.27	0.63	0.44	0.60
10	a n-pentylcyclohexadiene	1.46	0.36	0.42	0.20
11	*trans*-anethole	trace	NI	trace	NI
12	*trans*-pinocarveyl acetate	0.46	0.57	0.38	0.39
13	carveyl acetate	trace	NI	0.04	NI
14	caryophyllene	1.58	1.41	0.63	0.53
15	humulene	0.14	0.13	0.06	0.05
16	β-selinene	6.54	#	4.01	#
17	α-selinene	0.98	#	0.57	#
16+17	β-selinene (mixed peak)	-	7.48	-	3.95
18	elemol	0.05	NI	trace	NI
19	caryophyllene oxide	0.14	0.28	0.17	0.19
20	n-butyl phthalide	0.36	0.67	0.64	0.95
21	β-eudesmol	0.37	0.43	0.09	0.11
22+28	α-eudesmol (mixed peak)	0.12	-	0.04	-
22	α-eudesmol	†	0.07	†	trace
23	sedanenolide	1.20	0.19	0.71	0.14
24	sedanolide	2.58	2.50	1.20	1.10
25	neophytadiene	0.18	0.17	0.12	0.11
26	unknown phthalide (mw 204)	ND	ND	ND	ND
27	*cis*-neocnidilide	ND	ND	ND	ND
28	unknown phthalide (mw192)	†	ND	†	ND
29	β-elemene	NI	trace	NI	trace
30	β-farnesene	NI	trace	NI	trace
31	β-himachalene	NI	0.04	NI	0.04
32	*ar*-curcumene (mixed peak)	NI	0.07	NI	0.03
33	carvone	NI	0.25	NI	0.28
34	*cis*-limonene oxide	NI	0.12	NI	0.16
35	-limonene oxide	NI	0.14	NI	0.18
36	*cis*-carveol	NI	0.06	NI	0.09
37	*trans*-carveol	NI	0.15	NI	0.26
38	*trans*-p-mentha-2,8-dien-1-ol	NI	0.10	NI	0.14
39	*cis*-p-mentha-2,8-dien-1-ol	NI	0.07	NI	0.11
40	pinocarvone	NI	0.05	NI	0.05
41	limonene diepoxide (mixed peak)	NI	0.09	NI	0.09
	other unidentified peaks				

NI = not identified on this column
ND = not detected
† coeluting peaks on HP1 column
# coleluting peaks on INNOWax column

4.5 Gross margin analysis

A tentative gross margin analysis is provided in Table 4.18. This is based on estimated from the semi-commercial trial. Two estimates of expected yield are provided as an indication of the expected range. Two conservative estimates of farm-gate price are also included, since this cannot be easily determined prior to the production of marketable quantities of oil.

The expected gross margin of this crop is highly dependent upon both yield and price. The cost of production will be relatively stable.

Table 4.18 Tentative gross margin analysis for the production of celery herb oil

INCOME			
oil yield (kg/ha)	50	30	50
Price ($/kg)	55	55	40
GROSS INCOME	**2750**	**1650**	**2000**
EXPENDITURE			
Establishment costs			
seed	250	250	250
sowing	30	30	30
Fertilizer			
basal	160	160	160
urea	85	85	85
application	30	30	30
Herbicides	70	70	70
application	30	30	30
Fungicides	115	115	115
application	90	90	90
Insecticides	75	75	75
application	15	15	15
Irrigation	125	125	125
Harvesting	75	75	75
Distillation	300	300	300
TOTAL COSTS	**1450**	**1450**	**1450**
GROSS MARGIN	**1300**	**200**	**550**

Chapter 5 Discussion

The aim of this project was to identify a celery variety, for use in local production of steam-distilled herb oil, for the flavour and fragrance market. Initially the product is aimed at the domestic market but later also for export. In addition, the same or a further variety could provide seed oil for use in the therapeutic goods industry. As a natural therapeutic product, the oil must have a high concentration of phthalides. One reference to a commercial product suggests that the total phthalide concentration, of oil for therapeutic use, should be adjusted to a concentration of 85% (http://www.doctormurray.com/articles/celery.htm.).

A key point of interest to potential growers of celery as an essential oil crop, is that such production is six months out of season with celery for the fresh vegetable market. Unlike a vegetable crop, the essential oil crop is required to flower and must be sown in autumn for vernalisation during winter. Bolting commences in the following spring. This can pose some difficulties in the procurement of seed and potential growers for essential oil need to be aware that seed may not be readily available in autumn. Contact should be made with seed companies at least six months before the intended planting date.

A further point of agronomic interest is that for economic viability, the high planting costs of a typical celery vegetable crop must be reduced. Thus, an essential oil crop needs to be sown from seed as a broad acre crop and not established from transplants. This requires careful management but has an advantage in that seed of reduced germination percentage may be used, with the low germination rate being compensated for by a higher sowing rate. Since high germination rates are required by growers of fresh vegetables, essential oil growers may be able to source seed of low germination rates at lower prices.

5.1 Identification of oil components

Initial identification of the components of the oils and extracts was based on comparison of GC/MS TIC traces with those available in the literature. The mono- and sesquiterpenes present were generally readily identified, as they are common to the essential oils of many species. Some difficulty was encountered in defining the minimum levels of components that would be reported. Limonene comprises around 70% of most of the oils examined, while other components are present in concentrations often less than 1% of the oil. Of these, the phthalides, in particular, have very low odour thresholds. The organoleptic properties of the oil are therefore strongly dependent upon components that are present very small quantities. A decision was made to adjust integration of the chromatograms so that peaks for the 40-50 most abundant components were integrated.

Two phthalides not frequently reported in celery oil were detected. These were previously recorded as the unknown phthalide mw 192 and unknown phthalide mw 204. The first of these appears to be 3-butyl-5,6-dihydro-4*H*-isobenzofuran-1-one, which was reported by Nitz, Spraul and Drawert (Nitz et al. 1992) as a sensorially active phthalide from parsley roots. Retention and TIC trace data were a good match for this compound, which was also reported by these authors to be in trace amounts in celery.

The second unknown phthalide (mw204) has previously been reported in fresh celery by Tang *et al* (Tang et al. 1990) but the tentative structure postulated by these authors is incorrect. Accurate molecular weight measured for this component was 204.0800, indicating a formula of $C_{12}H_{12}O_3$ and not $C_{13}H_{16}O_2$.

Neither of these phthalides was detected in the seed and herb oils at greater than trace levels. The mw 192 phthalide was a significant component of the solvent extracts of the steam-distilled seed marc. The mw 204 phthalide was a significant component of the solvent extracts of roots.

Quantification of the components of essential oils is frequently based upon the FID response and here this allowed easy quantification of the mono- and sesquiterpenes. The phthalides presented more difficulty. After comparing the two columns, HP1 and HP-INNOWax, over a range of oils and extracts, it was found that the INNOWax column gave greater separation and allowed clearer identification of the monoterpenes and alcohols, allowing the identification and quantification of an additional 13 peaks. However, six components previously identified on the HP1 column were not identified on the INNOWax column. The HP1 column gave the best peak resolution and separation of the phthalides.

The phthalides are relatively polar components and elute from the polar INNOWax column at relatively high temperatures. Thus, when they elute there is a significant amount of column bleed, which interferes with the resolution of the phthalide peaks. In addition, the phthalides tend to be somewhat unstable. As stipulated in the results section, the unknown phthalide mw 192 was particularly unstable on the INNOWax column and co-eluted with α-eudesmol on the HP1 column, making quantification difficult. This was not a problem in analysis of the seed and herb steam distilled oils since mass spectroscopy showed these oils to contain only trace quantities of this phthalide.

FID peak area was used in this research to quantify all components relative to an equivalent hydrocarbon. No attempt was made to determine response factors but for the phthalides the FID response will be approximately 0.8 that of the equivalent hydrocarbon, due to the ester functional group (Scanlon and Willis 1985; Jorgensen et al. 1990). Therefore, the concentrations of the phthalides presented in this report are underestimated. ISO standards are based upon FID, assuming a response factor of unity. Relative concentrations of components are measured, as in the results presented in this report.

FID peak area for the phthalides, when measured on the INNOWax column is also inaccurate. The commercial oils were assessed on both columns and for most components the calculated concentrations in the oils agreed between columns. Minor changes are expected when concentrations are expressed as percentages, owing to differences in the total number of components included in the overall percentage, but for a few components, the results are markedly different. For example, n-pentylbenzene appears higher when the oil is assessed on the INNOWax column and the n-pentylcyclohexadiene isomer appears to be lower. Sedanolide levels agree between columns but n-butyl phthalide levels are higher on the INNOWax and sedanenolide levels are higher on the HP1. It was shown by Nitz *et al* (1992) that the mw 192 phthalide is converted to sedanenolide and n-butyl phthalide on a Carbowax column. SIM traces on GC/MS confirmed this. It is also probable that on the INNOWax column, sedanenolide is converted to n-butyl phthalide; thus, the INNOWax column over estimates the concentration of n-butyl phthalide and underestimates the concentration of sedanenolide.

The ISO standard for celery seed oil does not specify concentrations of either sedanolide or n-butyl phthalide, which are both components critical to the organoleptic nature of celery. These components also appear to be of significance in the pharmaceutical activity of celery. These components are therefore included with those from the ISO standard when oils are assessed.

At the time of initial procurement of seed for the variety trial there was an indication from the suppliers that the varieties Green Crunch and USA Green Stringless may be synonyms. The oil composition data obtained for these two varieties do not support this and it is believed that they are in fact different varieties.

5.2 Laboratory protocols for assessment of the oils

Laboratory protocols for the assessment of oil yield and quality are not required to mimic the commercial processes exactly, but the important selection criteria do need to be correlated with the results of commercial production. Steam distillation using cohobation stills in the laboratory will produce slightly different oils to the flow-through stills used commercially and the duration of

distillation will be markedly different. However, laboratory distillation provides a good indication of the steam-distillable essential oil present in the plant material.

The nature of the duration of distillation curve is such that there is an initial phase of rapid release of oil components followed by an asymptotic release that will continue for many hours. In order to obtain data on oil yield and composition that are repeatable it is necessary to distil for sufficient time after breakthrough in order to pass the phase of rapid oil release. During the rapid phase, a small change in distillation time will make a large difference to the volume of oil obtained. Once past this point, slight errors in the length of duration will translate to minimal error in estimation of oil yield. For celery herb, the initial phase is concluded within approximately one hour from breakthrough, though there are statistical differences between the increments to three hours. For celery seed, the nature of the distillation curve is influenced by the preparation treatment and further discussion will be presented below.

Change in oil composition with duration of distillation is a further criterion for consideration in defining a standard duration of distillation for the assessment of samples. For all herbs, the more volatile components distil over more rapidly and appear at higher concentrations in the early increments. The less volatile components appear at higher concentrations in the later increments. For celery herb, the limonene concentration falls rapidly during the first three one hourly increments. As distillation progresses the limonene concentration continues to decrease with each increment but the changes are smaller. Corresponding to the fall in limonene concentration is an increase in concentration in the phthalides and β-selinene.

Based upon both the oil yield and composition data a cut-off point of 2.5 hours was arbitrarily set for regular examination of celery herb in the laboratory. This point was chosen as being of great enough duration to avoid the rapid phase of oil release and yet allow sufficient samples to be processed in a day.

Celery herb samples can be stored frozen at –18°C prior to distillation, without adverse affects on either oil yield or quality.

Distillation of celery herb using a hexane trap improves the recovery of oil and potentially the recovery of sedanenolide, however since this is not a desirable method for commercialisation, and the benefits were slight, the use of a hexane trap was not deemed appropriate for use in this project.

Distillation of dry seed often poses a problem because the seed coat can have low permeability to the steam and be resistant to the release of oil. For this reason when the trial to establish a duration of distillation curve for seed was undertaken, a comparison was also made of ground and whole seed. Guenther (1950) states that the seed should be crushed before distillation and it was hypothesised that the yield from ground seed would be higher and that the oil would be extracted more quickly. Grinding did in fact reduce the lag phase at the onset of distillation with a greater proportion of the total oil from each treatment being obtained more quickly from the ground seed. However, grinding substantially reduced the total yield of oil from ground seed. When the duration of distillation was extended to 40 hours, the oil yield from ground seed was less than half the yield obtained from the whole seed. Much of this loss can be attributed to the volatilisation of the monoterpenes, particularly limonene, from the ground seed prior to sealing in the distillation vat.

A cursory re-examination of the grinding process revealed the loss of some oil in the grinding apparatus, but not sufficient to account for the entire difference between the ground and whole seed. Grinding of the seed is not recommended and if it is undertaken a more rapid method of packing the stills must be found. If grinding is undertaken, the ground seed can be frozen at -18°C in a sealed container, without exacerbating the loss of volatiles. Other methods of preparing the seed, such as crushing or rolling, may be tested in the future, but any method which exposes the oil to the atmosphere before the charge can be sealed in the distillation vat, risks the loss of volatiles.

As an estimation of the total oil present, the total yield of volatiles obtained from seed by solvent extraction was compared with the total oil yield from whole distilled seed. Both were in the order of 2%, indicating that steam distillation was able to extract most of the oil, but that this requires a distillation of at least 24 hours.

The arbitrary cutoff point for laboratory distillation of dry seed is 6 hours. The cumulative oil graph still has a relatively steep slope beyond this point but the slope is declining. The logistical difficulties in handling a large number of samples, with distillation durations exceeding 6 hours for each, outweigh the benefits of continuing distillation beyond this point. For samples where a greater accuracy is required, the trial of distillation with and without a mid-distillation break, shows that the distillation can be paused overnight and then continued without major effect on the final yield and composition. This research assessed the oil in one hourly increments and based upon these results the distillation should be continued for a minimum of one hour past the break. The first increment after the break showed a slightly higher limonene concentration than the other increments. No other differences were observed.

Tang *et al* (1990) showed that fresh celery has very few glycosidically-bound volatiles. It has been postulated in the past that observed flushes of oil following a break in distillation, *e.g.* as in the commercial distillation of fennel (*Foeniculum vulgare* Mill.) are due to the breakdown and release of glycosidically-bound volatiles.

5.3 Selection of varieties for essential oil for flavour and fragrance

As stated earlier, the development of this product attempts to mimic the marketing success achieved with the development of parsley herb oil.

The criterion of primary importance in the selection of varieties for this use is the organoleptic assessment. Although researchers can make preliminary selections in this area by selecting oils that are reminiscent of fresh, crushed celery, of necessity the final selection must be undertaken by the end-user. From the assessment of the oils from the variety trial, two varieties, Salad Green and Tendercrisp, were selected as being the most promising of the six varieties tested. These were therefore chosen for further analysis in the semi-commercial trial. The oils finally chosen for commercial production may be either seed or herb oils. Quantities of each from all the varieties have been made available for the industry partner to undertake market research. Ultimately the varieties produced for flavour and fragrance will depend upon the results of this market research.

Volumes of the herb oils, large enough for market analysis, were produced in a semi-commercial pilot-scale distillation plant. When the herb oils produced in the pilot are compared with the ISO standards for seed oil these oils fall just outside the requirements. The β-pinene concentration for Green Crunch is low compared to the ISO standard while the β-selinene concentration is slightly too high for the variety USA Green Stringless. For all varieties but Tendercrisp, the sedanenolide concentration is also fractionally too high. However, the component with the greatest discrepancy between the ISO standard and the concentration in the oil is limonene, for which the concentration in the oil is only at about half that required by the ISO standard for all varieties. Since this is not one of the major therapeutic components, this may not pose a marketing difficulty.

The herb oils produced in the pilot plant are surprisingly low in limonene, and high in selinene and phthalides compared with other oils produced during the course of this research. Interestingly, the laboratory-distilled herb oils from the serial harvest of the variety trial are very different in composition to that distilled in the pilot plant. This is true, even for the material collected for the serial harvest at the same time as the bulk sample. A sub-sample of the bulk Tendercrisp material from the pilot-plant distillation was distilled in the laboratory and had similar concentrations of components to the serial harvest material. Additionally, the commercially produced oils from the final field trial on Salad Green and Tendercrisp showed a greater conformity with the laboratory-distilled oils. These comparisons suggest that the method of distillation in the pilot plant leads to a loss of monoterpenes

from the oil. The pilot-plant has a large condenser and separator, relative to the volume of the plant material that can be distilled. The relative surface area of these is also large when compared to both the commercial units and the laboratory cohobation stills. Variety selection will therefore be based upon the results of composition analysis from the serial harvest. Oils of similar composition to the herb oils from the pilot plant, should be easily produced by fractionation of commercial oils of the corresponding variety, if these are the market requirements.

The herb oils from the lab distillations of the mid-February harvest fit reasonably well with the ISO standards for seed oil, although generally the β-selinene concentrations tend to be low. Herb oils produced from the pilot plant distillation are low in limonene and high in phthalides compared with the ISO standard for seed oil. Sedanenolide is the dominant phthalide but this is probably an artefact of the pilot-plant distillation, as explained earlier.

Since the variety for herb oil production is intended for the flavour and fragrance market, this selection is perhaps best done by organoleptic assessment. Oils most reminiscent of fresh, crushed celery were sought and the two varieties Tendercrisp and Salad Green, selected for the final field trial, are the preferred varieties.

The components important in the organoleptic properties of the oil are likely to be β-selinene, n-butyl phthalide, sedanolide and sedanenolide. Some authors have described the aroma of these components using sniffing-port GC. McLeod and Ames (1989) describe myrcene as unpleasant, β-selinene as green and fragrant, n-butyl phthalide as sweet and fruity and both sedanolide and sedanenolide as celery and pungent. *Cis*-neocnidilide is described by these authors as celery, less pleasant, camphor and petrol. *Cis*-neocnidilide is not significant in the aroma of the herb but may be important if components from root extracts are to be used in this context. Uhlig, Chang and Jen (1987) separated the components of celery using HPLC and assessed their importance to the celery aroma after dilution in water. Sedanolide was found to be important while n-butyl phthalide and sedanenolide were not. Nitz, Spraul and Drawert (Nitz et al. 1992) provide not only a description of the aroma of the components but also threshold values. Sedanolide (*cis* isomer) had the aroma of celery and a threshold of >200ng/stimulus, while sedanenolide was described as celery, spicy with a threshold value of 500ng/stimulus. The threshold for n-butyl phthalide was 700ng/stimulus but of particular interest is the threshold value of 0.5ng/stimulus for the 192 mw phthalide. At such a low value, this component is no doubt contributing to the overall aroma of celery, even when present at only trace levels in the oil.

Both the absolute values and the ratios of these components are potentially important. Preliminary comparisons between the organoleptic assessments and the oil composition data suggest that the ratio of β-selinene to n-butyl phthalide may be a key factor in determining the organoleptic properties of the oils. Although sedanolide and sedanenolide are sensorially active compounds, they do not have particularly low odour thresholds. Insufficient data were available in this research project for a full assessment of this observation and it is unlikely that the ratio of two components will determine the acceptability of a fragrance or flavour product. However, this ratio may prove a useful reference point in later studies. The component n-butyl phthalide does not appear as part of the ISO standard but concentration of this component is recommended for inclusion in quality assurance assessments. The absolute value of sedanenolide and the ratio of sedanolide to sedanenolide also appear to have a bearing on organoleptic quality.

The physical properties of the oils are supplied in the Certificates of Analysis in Appendix B. Literature values are drawn from Parry (1918) and Guenther (1950) as follows:

Celery seed oil (Parry, 1918)

- Specific gravity 0.860 to 0.895
- Optical rotation +60° to +82° (rarely +40°)
- Refractive index 1.4780 - 1.4860

Celery herb oil

- Specific gravity 0.848 to 0.880

Optical rotation +41° to +60° (rarely +40°)
Refractive index 1.4780 - 1.4810

Guenther (1950) quotes the physical properties reported by many authors. These values fall in the following ranges

Celery seed oil

Specific gravity(15°C) 0.866 to 0.914
Optical rotation (20°C) +46° to +76°
Refractive index (20°C) 1.478 – 1.488

Celery herb oil

Specific gravity 0.872 to 0.891
Optical rotation +65° to +77° (rarely +40°)
Refractive index 1.4803 – 1.4841

The physical properties provided on the ISO standard for seed oil are:

Specific gravity (20°C) 0.867 to 0.908
Optical rotation (20°C) +48° to +78°
Refractive index (20°C) 1.478 – 1.488

The specific gravity values of the seed oils of all varieties fit the range defined by the ISO standard. For all varieties except USA Green Stringless, the specific gravities of the herb oils produced by distillation in the pilot-plant are too high. This is probably because the oil composition is skewed towards a higher content of the heavier molecular weight components. The specific gravities for the two commercial oils of Salad Green and Tendercrisp both fit the ISO standard specifications.

The refractive indices for all the oils are within the range of the ISO standard.

The optical rotation for each of the laboratory distilled seed oils and the pilot plant distilled herb oils are outside the ranges specified by both the ISO standard and the literature, however the commercially distilled oils both fit within the specified range.

5.4 Selection of varieties for therapeutic oil production

Organoleptic properties are not relevant to selection of a variety for this market, since the oil will be encapsulated prior to consumption. In this instance, the criteria of importance are oil yield per unit area and the concentration of physiologically active components *i.e.* β-selinene and the phthalides. The component n-butyl phthalide appears in the medical literature as a particularly important therapeutic component (Craig, 1999). The other phthalides are also of importance but it is unlikely that all have the same level of physiological activity. As research into these components progresses, others may be deemed more or less important. Salad Green and Triumph produce the highest levels of n-butyl phthalide while Salad Green and Green Crunch produce the highest levels of sedanenolide. Salad Green is low in sedanolide. Although consideration is given to these rankings, since no specific requirements have been made available by the industry partner, at this stage the recommendation is to select the variety producing oil with the highest concentration of total phthalides. Total phthalide concentration will be higher in the vegetative plants but oil yield at this time will be very low. The varieties in order of maximum total phthalide concentration at the point of greatest oil yield per unit area are USA Green Stringless, Salad Green, Triumph, Tendercrisp, Green Crunch and Excelsior. Salad Green also produces the highest yield of seed oil per unit area. When the characteristics of oil yield per unit area and total phthalide production are considered, Salad Green would appear to be the best of the varieties tested for the commercial production of essential oil for the therapeutic market.

Even if the crop is taken through to mature seed before distillation, the seed should not be cleaned from the stem material before distillation. Analysis of the waste material from the variety trial indicated that the stem material contained high levels of phthalides, particularly sedanenolide.

Removing this material may reduce distillation costs by decreasing the volume of material to be distilled but this must be weighed against reduced oil quality through lost phthalides.

5.5 Determination of optimum harvest date

For flavour and fragrance oils, the optimum harvest time will be determined by the development of the appropriate organoleptic properties of the oil, through the season. Harvest should take place when the best flavour and fragrance is noted by organoleptic assessment. The graphs of the serial harvest indicate that there is a marked change in oil composition as the plants begin to flower, after which, the oil composition becomes relatively stable. Subtle changes in oil aroma relating to the relative levels of the phthalides were discussed previously. With the level of knowledge at this time, these changes are best analysed for each crop by organoleptic assessment of pre-harvest samples.

Sedanenolide appears to be produced in the stems and vegetative canopy, therefore it is present at a higher concentration in the younger plants because in these it is not diluted by the generative canopy.

Yield is also important. Economies of scale under Australian production conditions are such that a very small quantity of high quality oil is not likely to be profitable for the grower to produce in the commercial distillation plant. Therefore, harvest must take place when higher yields can be achieved, *i.e.* after the commencement of flowering.

There is a trend in all varieties for oil yield to peak around mid-February and then oil yield per unit area begins to decline. This may, however, be partly due to the extraction technique. A standard distillation of 2.5 hours was used to distil all samples in the experiment. This was based upon the duration of distillation curves developed for herb. Towards the end of the flowering season the seeds begin to mature and dry and at some point will begin to follow the duration of distillation curves for dry seed. The duration of distillation of seed curves, show only 0.6% oil yield at 2.5 hours but 2% yield at 40 hours. Since these curves, which are based upon the varieties Excelsior and Salad Green, show a percent yield in the order of 2% of dry matter after 40 hours of distillation while the maximum yield from the serial harvest graphs is 1.2% it is suggested that these curves should in fact not show this decline. It is difficult to adjust the duration of distillation to compensate for this effect as the serial harvest experiment progresses, because this would require repeated duration of distillation experiments through the season. A standardised duration of distillation was used but this needs to be kept in mind when assessing the yield curves.

The commercial distillation of the varieties in the semi-commercial trial was taken to exhaustion. The oil composition resulting from this distillation was very similar to that predicted by the 2.5 hour laboratory distillation of the herb collected from the vat prior to the commercial distillation. Therefore although there may be changes in yield not detected by a 2.5 hour distillation of serial harvest samples, the oil composition is a valid assessment tool.

Oil yields of between 30 and 60kg/ha are predicted when the herb is harvested from mid-to late February. The varieties producing the higher yields of herb oil are Excelsior, Green Crunch and USA Green Stringless. Tendercrisp and Triumph were the lowest producers. Salad Green was an intermediate variety. Early in the season, it appeared to be a high oil yielding variety but then production fell. This suggests seed shedding but this was not measured. Since Salad Green is the preferred variety based upon the analysis of oil composition, consideration should be given to determining whether shedding was indeed a problem or whether because it is an early variety, yield is perceived as decreasing due to the early transition from herb to seed material.

5.6 Distillation to maximise the extraction of the valuable components

The herb material from the semi-commercial trial was wilted in the paddock before distillation in the commercial facility. This is a standard commercial practice for other Apiaceae and results in distillation that is more effective.

Seed material can be distilled either as cleaned seed harvested with a header, or as whole, forage harvested plants distilled in the same manner as herb material. Distillation of whole plants is recommended since the stem and trash material has oil that is high in sedanenolide.

Consideration must be given to breaking the seed coat prior to distillation to allow better penetration of the steam. Guenther (1950) recommends that the seed be crushed before distillation and that it be immediately transferred to the distillation vessel to present loss of oil by evaporation. Laboratory work in this project showed that the risk of loss to evaporation was significant and it is not recommended that the seed be ground. Protocols could be put in place to minimise this loss, but under commercial conditions, the risk of loss is great. Crushing or rolling the seed are two possible solutions but any method which exposes the oil to the atmosphere will be subject to the risk of the loss of volatiles.

The curves showing cumulative oil yield as a percentage of total yield were used to give a comparison of the rate of oil release between the distillation of whole and ground seed. For both the experiments conducted during this research the ground seed had a significantly faster rate of oil release in the first hour compared to the whole seed, but after the initial hour, the rate of release of oil from the ground seed began to fall. Between two and five hours, the slope of the curves began to come together and after 6 hours, there was no difference between the two.

The decision to grind the seed or to distil whole seed must be made based upon economic considerations of the cost of grinding or other preparation, the cost of a longer distillation, particularly fuel and labour, and the value of the oil lost due to volatilisation of exposed oil before the charge can be sealed into the vat.

The duration of the commercial distillation is a further significant consideration in the production of oil. Guenther (1950) recommends distillation of celery seed for 10-12 hours and that the distillation waters be cohobated. The commercial distillation in this research reached exhaustion after approximately 3 hours. For optimum oil quality, the distillation should be carried out to exhaustion since the more valuable components are distilled over late. The duration of the distillation must take into account the costs of fuel, the volume of oil being produced and also the composition and therefore the quality of the oil.

It is recommended that under commercial conditions, that the operator separate the first 30 minutes of oil from the remainder. This will improve the quality of the remaining fraction by decreasing the concentration of the monoterpenes and increasing the concentration of the valuable components. Based on the results of the commercial distillation in this project, this fraction will be roughly one fifth of the total volume of oil. This point was chosen because there is a noticeable shift in composition due to a higher concentration of phthalides in the oil collected at this point in the commercial distillation of the semi-commercial trial. It may be possible to rectify this oil by fractionation and blend the valuable components back into the bulk oil. However, Guenther points out that rectification using slightly superheated steam at atmospheric pressure can lead to the oil developing a note reminiscent of burning rubber. This note was observed in some samples in this project, particularly those stored for a period of time. It is possible that the taint is somehow due to the high temperature degradation through isomerization or dehydrogenation of the phthalides, perhaps to *cis*-neocnidilide for example. This theory is only supposition and needs to be tested.

Long commercial distillations may be undertaken over several distillation periods, incorporating mid-distillation breaks. Mid-distillation breaks were not found to affect the final oil yield or composition.

It is recommended that distillation commence with the inner skin of the separator empty and that the separator temperature be maintained as high as possible to allow fast, clean separation of the oil from the water. It was shown in this project that a low separator temperature allowed the oil to cling to the sides of the separator, reducing oil recovery rates.

5.6 Seed oleoresin

Oleoresin, produced by solvent extraction of the seed, is another potential product from celery. Guenther (1950) describes the oleoresin as having more of the body of celery than the oil, due to the presence of more of the less volatile components. This product would have potential as either a flavour or fragrance product but perhaps more particularly in the therapeutic goods market.

The important criteria for selection of the optimum protocol for extraction of the oleoresin are the yield and quality of the extract. There are two yield factors to consider, the total yield of extract and the yield of the individual components. The quality of the extract depends primarily on the concentration of β-selinene and the phthalides in the extract, with a high quality extract having a higher concentration of these relative to the other components. Two aspects of the potential protocols were compared, the preparation of the seed and the extracting solvent.

The two extracting solvents compared were the polar solvent, chloroform and the non-polar solvent, hexane. Chloroform extracted a greater total yield of product as well as more of the individual components, than did hexane. Although the yield of extract was greater with chloroform, the quality of the extract was higher with hexane, because the proportion of phthalides was greater in the hexane extracts.

Grinding had a dramatic effect on the efficiency of release of solvent extractable components from the seed. The total weight of extract obtained from ground seed is in the order of six times the yield from whole seed. The effect was less pronounced on the yield of total volatiles and varied with the individual components. Grinding more than doubled the yield of β-selinene, sedanolide and sedanenolide. There was a significant interaction between the solvent used and grinding on the yield of n-butyl phthalide, such that the increase in efficiency of extraction by chloroform over hexane was much greater for the ground seed than the whole seed.

An interesting comparison can be drawn between the composition of the seed oleoresins and the steam distilled seed oils. The oleoresins are low in monoterpenes, despite being extracted from the same seed sources as the steam distilled oils. As described above, the whole seed was resistant to extraction and no components were well recovered by either solvent. However, the ratio of monoterpenes to phthalides concentration was also skewed, compared to the steam distilled oils. The monoterpene concentrations were so low that they were frequently detected only at trace amounts. For this reason these data were not analysed statistically. Two possible causes for the low monoterpenes concentrations are suggested. Either the monoterpenes were lost during the dry down process or the phthalide components were more accessible to the solvent, perhaps being in oil that was stored closer to the seed surface than the oil rich in monoterpenes.

The extracts of the ground seed had higher concentrations of the monoterpenes but again the ratio of monoterpenes to phthalides was skewed compared to the steam distilled oils. In this instance, it is expected that the monoterpenes were lost due to volatilisation between grinding and extraction, as was found in the distillation of ground seed. The fact that the monoterpenes were present at measurable levels in these extracts and that a standard method of dry down was used, makes it most likely that monoterpenes loss during dry down was minimal. It is therefore hypothesised that there is discontinuity between oil storage structures within the celery seed.

The polar solvent, chloroform, produced statistically greater yields but it is not an acceptable commercial solvent due to its hazardous nature. Chloroform was chosen to test the effectiveness of a polar solvent in the laboratory experiments, but not intended to be used commercially. As a test solvent in the laboratory, it has the advantage of being easy to handle. It is immiscible with water and has a low boiling point, allowing straightforward clean-up of the extracts using an RVE. An extracting solvent such as ethanol or methanol will extract more unwanted components, such as sugars, requiring more complex extraction protocols.

Although statistically different, the increased yield using chloroform as opposed to hexane may not be enough to be economically worth the extract complications of using a polar solvent. The costs associated with extract clean-up and solvent recovery may outweigh the economic value of the additional yield.

Further aspects of solvent extraction are the number and duration of the washes. Although further oil was extracted with each wash, each additional oil increment is of similar size, indicating that the duration of each wash is not as important as the partition coefficients of the oil components. Many short duration washes will be more effective than a few of long duration.

5.7 Extraction of the marc from seed oil production

The difficulties in distilling oil from dry seed have already been discussed. Two main problems are the resistance of the seed coat to steam penetration and the difficulties in obtaining even penetration of the steam through the charge. Grinding, crushing or rolling the seed as described previously can overcome the resistance of the seed coat, but there are risks associated with the loss of the more volatile components. Solvent extraction to produce oleoresin gave good recovery of the phthalide components but also resulted in low recovery of the more volatile components.

A further method of extraction to be considered is steam distillation to recover the more volatile components, followed by solvent extraction of the marc to recover the remaining less volatile sesquiterpenes (primarily β-selinene) and phthalides.

The seed oil distilled in the cohobation stills in the laboratory gave yields of oil in the order of 1.0-1.2% of dry matter distilled. When the marc from these distillations was further extracted using hexane, extracts in the order of 1.0-1.2% were also obtained. Approximately 30% w/w of these extracts were comprised of volatile components that are of interest *i.e.* limonene, β-selinene and phthalides. Sedanenolide concentrations were around 20% w/w of extract. The mw 192 phthalide was also a significant component of these extracts, with a concentration of between 0.5 – 0.9% in the extract. Although the FID peak for mw 192 is mixed, in this case, it contains only trace amounts of α-eudesmol, which was well extracted by the steam distillation and it could be satisfactorily quantified. These figures translates to yields of valuable volatile components from the original seed of 0.3 – 0.4% on a dry matter basis.

The marc extracts contain high proportions of valuable components, but the economic value of carrying out this additional extraction is dependent upon the transport costs associated with moving the marc from the distillation plant to a solvent extraction plant, the cost of the extraction and the price that can be obtained for the extract.

5.8 Root extracts

Celery root extracts have potential in the therapeutic goods markets. Root tinctures are mentioned in the literature (Guenther, 1950) and therefore have potential, but at this stage, no information is available as to the required specifications. It is expected that celery root tinctures would be used in much the same manner as other celery therapeutic products and that quality extracts would have high concentrations of phthalides. However, there are substantial differences between the composition of extracts from above and below ground parts of celery and there are phthalides present in the roots of celery that are present only at trace levels in the tops of the plants.

The primary phthalide component in the roots is *cis*-neocnidilide whereas it is the cis-isomer that is the dominant form in the tops. The other major phthalide present is the mw 204 phthalide. The value of the root extracts as therapeutic agents depends upon the activity of the extracted components, particularly *cis*-neocnidilide and mw 204 phthalide.

If market research is favourable, development of this product depends upon the establishment of a suitable extraction protocol from a suitable celery variety. As with the establishment of a seed

extraction protocol, two factors, preparation technique and extracting solvent, were tested in developing an extraction protocol for roots.

The comparison of hexane and chloroform has similar results in the roots as in the seeds. Within the extract, hexane extracted more phthalides relative to other components than did chloroform but for most components the absolute yield relative to plant weight was greater with chloroform.

The preparation treatment “dry chopped” was the least efficient extraction protocol. Components were possibly lost during the drying phase and this combined with the poor penetration of the solvent into the material, lead to low yields.

There was a significant interaction between solvent and preparation treatment and the remaining treatments are assessed as combinations. The combinations, listed in order of extraction efficiency are: “ground chloroform”, “fresh hexane”, “fresh chloroform” and “fresh hexane”. As was discussed in relation to the seed oleoresins, chloroform is not a suitable commercial solvent. The extraction of fresh, air-dried roots with hexane is a suitable protocol. The use of slightly more polar solvents such as isohexane, pentane or light petroleum might be considered.

The yields of extract obtained from the roots of the semi-commercial trial were much higher than the yields from the variety trial. Values of less than 1 mg/g root extracted were achieved from the variety trial compared with yields from the semi-commercial trial of 12mg/g of root for Salad Green and 14mg/g Tendercrisp. It is possible that volatiles, including phthalides were lost in the air-drying process of the variety trial. The drying process was more efficient on the small samples used in the variety trial compared with the bulk samples from the semi-commercial trial.

This research identified potential varietal differences and variety by extraction protocol interactions. Statistically, all the effects tested were significant for all the variables; therefore, there may be variety by treatment interactions that should be tested. Modifications to the protocol may be required to optimise extraction, once a particular variety is chosen. At this stage, extraction of fresh, chopped roots with hexane is the recommended extraction protocol.

The size of the roots relative to the size of the plants was quite small. There are two points to note from this: the yield of plant material per unit area will be small relative to for example the yield of parsley or fennel roots and the second point is that the roots should be relatively easy to recover using a modified potato digger or similar implement.

It is predicted that yields of approximately 100ppm total phthalide will be obtained from root extractions. These yields are relatively low when compared with the yields from the tops of the plants but if a suitable price for the can be achieved, extraction of the roots will lead to a greater return from the crop. Higher yields than those achieved in either trial from this research project should be attainable. Yields from the variety trial were reduced by the over efficient air-drying procedure on the small samples. Yields from the semi-commercial trial were reduced by the delay in their harvest, after harvest of the tops. It is predicted that yields of root extracts will be maximised by harvesting the roots immediately after harvest of the tops, wilting or air drying for a short period at cool temperatures, chopping with the commercial equivalent of a garden mulcher and extracting with either isohexane or light petroleum.

5.9 Economic viability of the industry

The oil yields obtained from the semi-commercial trial in this research were 15.2kg Salad Green and 10.2kg Tendercrisp. This was a total of 25kg from 0.9ha or 28kg/ha. These yields should be easily increased, taking into account the insights gained through this research.

This trial was sown relatively late, due to the difficulty in procuring seed as discussed in the introduction. A higher planting density should also be examined. The semi-commercial trial was

planted at 3 plants/m^2, with plants spaced at 0.50m square spacings within beds and interbed spacings of 0.8m. A small area was planted at 4 plants/m2, by closing the within row spacing to 0.3m. Plants at this higher density were not noticeably different to plants in the remainder of the trial. Plants sampled from this higher density area at the end of the trial produced oil yields equivalent to the remainder of the trial. No samples were taken from this area through the growing season and the area was not set up as a replicated trial, so no statistical comparisons were made. However, because canopy closure did not take place in the main trial area until late in the season, at approximately the time of anthesis of the tertiary umbels, it is felt that the higher plant density should be used or at least undergo further testing. A higher plant density, coupled with earlier sowing should result in higher yields.

At a price of $50/kg for the oil and with yields of 50kg/ha of steam-distilled oil, the gross margin for this crop makes it comparable to poppy and pyrethrum production. The inclusion of additional products, in the form of either marc or root extracts or both, should increase this gross margin further.

Implications

The researchers are confident that this industry is economically viable. Yields of 50kg oil per hectare should be achievable under commercial conditions and at a farm-gate price of $50/kg for the oil; the gross margin for this crop makes it comparable to poppy and pyrethrum production. The inclusion of additional products, in the form of either marc or root extracts or both, should increase this gross margin further.

With Tasmania's good reputation for quality products it should be possible to add this product to the suite of essential oils traded, thereby diversifying the range of products available and the range of crops that growers can produce. The distillation and extraction technology and equipment already exist for the commercial scale production of essential oils and oleoresins. Adding another crop will spread the overhead costs, adding profit to the industry.

Recommendations

The variety best suited to local production for both herb essential oil for flavour and fragrance and the pharmaceutical industry was Salad Green. After full market analysis is complete and a suite of specifications defined, this recommendation may change. Other varieties may have characteristics that are desirable, but Salad Green has been chosen as the most likely variety for initial market research.

The crop should be sown early in the autumn and pre-harvest sampling for oil quality should commence at full bloom. Harvest should be scheduled by organoleptic assessment of the oils.

The duration of commercial distillation should be extended for as long as economically possible, because the valuable components are distilled over late in the process. Consideration should be given to solvent extraction of the marc, especially if the crop is distilled after seed maturity has been reached.

Hexane is recommended for the production of oleoresins from the seeds or the roots, but a more polar solvent such as pentane or isohexane could be tested.

Appendix A

Nomenclature of the Phthalides

The literature on the composition of celery oils and extracts is confusing and contains many inconsistencies. In this report the term sedanolide was chosen for the component believed to be the cis- isomer because the general convention in the literature has been to refer to this component by this name. Comparison of data generated in this study with the limited available literature on retention time and GCMS spectra, support the premise that the component we identified is the same component identified by other authors.

Most literature refers only to the component identified as sedanolide and does not refer to the other isomer. However, because this study identified a second component as the dominant form in the celery root extracts, there is a need to distinguish between the two. This second isomer could be identified as *trans*-sedanolide because it appears from GCMS data to be a stereoisomer of the component identified as "sedanolide" and because MacLeod and Ames (1989), who also identified both isomers, referred to the pair as *cis*- and *trans*-sedanolide.

Neocnidilide would appear to be a synonym for sedanolide, however there are inconsistencies in the literature.

The structures provided on page 75 of the review by Lawrence (Lawrence 1981-87) are referenced to the thesis of Gijbels (1983). However, these structures are not consistent with the structures published by the same author (Gijbels) in 1987. The structures in Lawrence (Lawrence 1981-87) are in fact isomers of the component referred to by Fischer and Gijbels (1987) as cnidilide. The compound cnidilide has the registry number 3674-03-1.

In Chemical Abstracts, the structure of the compounds neocnidilide (registry number 4567-33-3) and *cis*-neocnidilide (registry number 124815-25-4) agree with those published by Fischer and Gijbels (1987). No structure is given in the database for the trans- isomer although one is provided by Fischer and Gijbels in the quoted publication.

The system of naming cis- and trans- isomers used by Fischer and Gijbels (1987) is the same as that used by Cocker (1966). The stereoisomers are assigned as cis- and trans- based on the positions of the hydrogen ions attached to the 3 and 3a carbons. Basing the nomenclature on the IUPAC rules would see the assignation reversed. The IUPAC rules name such stereoisomers by assigning priorities to the attached functional groups. Since the butyl group has a higher priority than the hydrogen atom, the cis/trans assignation would be based upon the position of the butyl functional group.

The primary difficulty is then in establishing which isomer is eluted first by the chromatography methods used in the current study. It is not possible to determine this by either retention time or by mass spectrometry. When only GCMS and retention time are available, the order of elution is normally established by comparing the data obtained by these methods with data obtained from the literature.

Fischer and Gijbels (1987) clearly state that *trans*-neocnidilide elutes first, followed by *cis*-neocnidilide on non-polar columns. If the nomenclature of Fischer and Gijbels (1987) is accepted, then the compound referred to in our study as "sedanolide" is in fact "*trans*-neocnidilide".

MacLeod and Ames (MacLeod and Ames 1989) refer to the publication of Cocker (1966) as the source of their nomenclature. The structures drawn agree with those drawn by Fischer and Gijbels (1987) and equate *trans*-sedanolide with *trans*-neocnidilide and *cis*-sedanolide with isocnidilide. Unfortunately, MacLeod and Ames (MacLeod and Ames 1989) show *cis*-sedanolide eluting before the *trans*-isomer.

Nitz , Spraul and Drawert (1992) show *trans*-sedanolide eluting first on both polar and non-polar columns. These authors provide registry number for *cis*- and *trans*-sedanolide as 2550-44-9 and 3553-29-5 respectively. Their trans- structure has the same stereochemistry as *cis*-neocnidilide.

In conclusion, the situation with regard to the nomenclature of these compounds is ambiguous and inconsistent. Clarification is believed to be beyond the scope of this project and report, since the aim was to provide a field and laboratory assessment of celery varieties in order to provide information on variety selection for the development of an oil and extract industry. To this end, the authors will continue to refer to the first eluting peak as sedanolide, believing from the literature that the isomer identified as being the most abundant in the steam-distilled oils of seed and herb is the same isomer identified as sedanolide by most other authors writing on the volatile components of celery seed and herb. The later eluting peak will be identified as *cis*-neocnidilide, in accordance with Fischer and Gijbels (1987) since these authors provide NMR data and are therefore likely to have documented the correct stereochemistry in their figures, even if the nomenclature used is unusual.

Appendix B

Certificates of analyses

The following pages contain certificates of analyses for each of the oils that were prepared for market analysis.

These oils were herb and seed oils of each of the varieties: USA Green Stringless, Excelsior, Green Crunch, Triumph, Salad Green and Tendercrisp.

Also included are certificates of analyses for the two oils produced by commercial harvest and distillation of the semi-commercial trial of Tendercrisp and Salad Green.

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb and Seed Oil var "USA Green Stringless"

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Herb Oil	Seed Oil
1	α-pinene	trace	0.18%
2	β-pinene	0.80%	3.93%
3	myrcene	0.56%	1.51%
4	p-cymene	trace	0.04%
5	limonene	33.68%	79.14%
6	*cis* -β-ocimene	0.78%	0.07%
7	γ-terpinene	trace	trace
8	fenchone	trace	trace
9	n-pentylbenzene	trace	0.43%
10	n-pentylcyclohexadiene	2.20%	0.58%
11	*trans* -anethole	trace	trace
12	*trans* -pinocarvyl acetate	0.26%	0.26%
13	carvyl acetate	trace	trace
14	caryophylene	2.84%	0.21%
15	humulene	0.38%	trace
16	β-selinene	20.63%	5.88%
17	α-selinene	2.94%	0.80%
18	elemol	trace	trace
19	caryophylene oxide	0.24%	0.09%
20	n-butyl phthalide	1.57%	1.25%
21	β-eudesmol	0.21%	0.05%
22	α-eudesmol	0.84%	trace
23	sedanenolide	13.58%	1.55%
24	sedanolide	13.71%	2.74%
25	neophytadiene	2.13%	0.10%
	other unidentified peaks	3%	1%

Physical Properties

(all results are means from duplicate measurements)

	Herb Oil	Seed Oil
Specific Gravity (10 ml at 20°c) =	0.889	0.870
Refractive Index (20°c) =	1.482	1.482
Optical Rotation (20°c, 1dm photolength) =	-164°	-103°

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb and Seed Oil var "Excelsior"

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Herb Oil	Seed Oil
1	α-pinene	trace	0.23%
2	β-pinene	0.52%	4.55%
3	myrcene	0.34%	1.05%
4	p-cymene	trace	0.09%
5	limonene	25.88%	76.34%
6	*cis* -β-ocimene	0.73%	trace
7	γ-terpinene	trace	trace
8	fenchone	trace	trace
9	n-pentylbenzene	0.35%	0.15%
10	n-pentylcyclohexadiene	2.39%	0.90%
11	*trans* -anethole	trace	0.15%
12	*trans* -pinocarvyl acetate	0.36%	0.24%
13	carvyl acetate	0.09%	0.25%
14	caryophylene	5.05%	0.12%
15	humulene	0.55%	trace
16	β-selinene	23.84%	4.08%
17	α-selinene	3.53%	0.52%
18	elemol	0.10%	trace
19	caryophylene oxide	1.03%	0.41%
20	n-butyl phthalide	2.78%	1.83%
21	β-eudesmol	0.91%	0.10%
22	α-eudesmol	0.29%	trace
23	sedanenolide	17.27%	0.05%
24	sedanolide	6.77%	0.43%
25	neophytadiene	1.15%	trace
	other unidentified peaks	6%	9%

Physical Properties

(all results are means from duplicate measurements)

	Herb Oil	Seed Oil
Specific Gravity (10 ml at 20°c) =	0.941	0.886
Refractive Index (20°c) =	1.495	1.482
Optical Rotation (20°c, 1dm photolength) =	-167°	-101.8°

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb and Seed Oil var "Green Crunch"

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Herb Oil	Seed Oil
1	α-pinene	trace	0.17%
2	β-pinene	0.40%	3.17%
3	myrcene	1.15%	1.37%
4	p-cymene	trace	0.05%
5	limonene	24.56%	78.71%
6	*cis* -β-ocimene	0.35%	0.05%
7	γ-terpinene	trace	trace
8	fenchone	trace	trace
9	n-pentylbenzene	0.19%	0.80%
10	n-pentylcyclohexadiene	2.39%	0.06%
11	*trans* -anethole	trace	trace
12	*trans* -pinocarvyl acetate	0.29%	0.30%
13	carvyl acetate	trace	trace
14	caryophylene	4.99%	0.34%
15	humulene	0.60%	0.05%
16	β-selinene	19.01%	4.33%
17	α-selinene	2.81%	0.62%
18	elemol	0.21%	trace
19	caryophylene oxide	0.81%	0.30%
20	n-butyl phthalide	1.76%	1.90%
21	β-eudesmol	2.12%	0.36%
22	α-eudesmol	1.07%	0.06%
23	sedanenolide	11.57%	0.58%
24	sedanolide	10.95%	3.27%
25	neophytadiene	6.40%	trace
	other unidentified peaks	8%	3%

Physical Properties

(all results are means from duplicate measurements)

	Herb Oil	Seed Oil
Specific Gravity (10 ml at 20°c) =	-	0.869
Refractive Index (20°c) =	1.481	1.487
Optical Rotation (20°c, 1dm photolength) =	-170°	-104.5°

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb and Seed Oil **var "Triumph"**

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Herb Oil	Seed Oil
1	α-pinene	trace	0.24%
2	β-pinene	0.66%	4.79%
3	myrcene	0.34%	1.39%
4	p-cymene	trace	trace
5	limonene	20.91%	73.73%
6	*cis* -β-ocimene	0.59%	0.09%
7	γ-terpinene	trace	trace
8	fenchone	trace	trace
9	n-pentylbenzene	0.33%	1.05%
10	n-pentylcyclohexadiene	2.77%	1.74%
11	*trans* -anethole	trace	trace
12	*trans* -pinocarvyl acetate	0.43%	0.50%
13	carvyl acetate	0.11%	0.06%
14	caryophylene	4.58%	0.76%
15	humulene	0.51%	0.08%
16	β-selinene	18.51%	6.25%
17	α-selinene	2.85%	0.99%
18	elemol	0.11%	trace
19	caryophylene oxide	0.92%	0.29%
20	n-butyl phthalide	3.39%	1.71%
21	β-eudesmol	0.81%	0.16%
22	α-eudesmol	0.64%	trace
23	sedanenolide	22.88%	2.75%
24	sedanolide	11.93%	1.46%
25	neophytadiene	2.52%	0.08%
	other unidentified peaks	4%	2%

Physical Properties

(all results are means from duplicate measurements)

	Herb Oil	Seed Oil
Specific Gravity (10 ml at 20°c) =	0.920	0.868
Refractive Index (20°c) =	1.489	1.483
Optical Rotation (20°c, 1dm photolength) =	+177°	-108.9°

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb and Seed Oil var "Salad Green"

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Herb Oil	Seed Oil
1	α-pinene	trace	0.24%
2	β-pinene	1.07%	4.86%
3	myrcene	1.83%	1.46%
4	p-cymene	trace	0.05%
5	limonene	30.48%	76.09%
6	*cis* -β-ocimene	0.59%	0.06%
7	γ-terpinene	trace	trace
8	fenchone	trace	trace
9	n-pentylbenzene	0.30%	0.84%
10	n-pentylcyclohexadiene	0.17%	0.19%
11	*trans* -anethole	0.11%	trace
12	*trans* -pinocarvyl acetate	0.38%	0.38%
13	carvyl acetate	trace	trace
14	caryophylene	3.29%	0.45%
15	humulene	0.37%	0.06%
16	β-selinene	14.60%	5.23%
17	α-selinene	2.19%	0.78%
18	elemol	0.18%	0.07%
19	caryophylene oxide	0.88%	0.41%
20	n-butyl phthalide	2.94%	1.60%
21	β-eudesmol	2.46%	0.53%
22	α-eudesmol	0.67%	0.09%
23	sedanenolide	16.47%	0.78%
24	sedanolide	14.83%	2.79%
25	neophytadiene	1.31%	0.13%
	other unidentified peaks	5%	3%

Physical Properties

(all results are means from duplicate measurements)

	Herb Oil	Seed Oil
Specific Gravity (10 ml at 20°c) =	0.947	0.873
Refractive Index (20°c) =	1.492	1.482
Optical Rotation* (20°c, 1dm photolength) =	-173°	-104.7°

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb and Seed Oil var "Tendercrisp"

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Herb Oil	Seed Oil
1	α-pinene	trace	0.29%
2	β-pinene	1.07%	5.64%
3	myrcene	0.65%	1.39%
4	p-cymene	trace	0.06%
5	limonene	38.56%	78.50%
6	*cis* -β-ocimene	0.55%	0.04%
7	γ-terpinene	trace	trace
8	fenchone	trace	trace
9	n-pentylbenzene	0.58%	0.84%
10	n-pentylcyclohexadiene	0.55%	trace
11	*trans* -anethole	trace	trace
12	*trans* -pinocarvyl acetate	0.41%	0.26%
13	carvyl acetate	trace	trace
14	caryophylene	2.28%	0.20%
15	humulene	0.33%	trace
16	β-selinene	15.63%	4.40%
17	α-selinene	2.27%	0.61%
18	elemol	0.16%	trace
19	caryophylene oxide	1.56%	0.31%
20	n-butyl phthalide	4.15%	1.62%
21	β-eudesmol	0.87%	0.05%
22	α-eudesmol	0.23%	trace
23	sedanenolide	9.80%	0.31%
24	sedanolide	8.55%	0.70%
25	neophytadiene	4.80%	0.04%
	other unidentified peaks	7%	5%

Physical Properties

(all results are means from duplicate measurements)

	Herb Oil	Seed Oil
Specific Gravity (10 ml at 20°c) =	-	0.870
Refractive Index (20°c) =	1.49	1.48
Specific Optical Rotation (20°c, 1dm photolength) =	-155°	-100.7°

	MEMORANDUM
TO:	**Essential Oils of Tasmania**
FROM:	**Dr Linda Falzari, Research Fellow** **School of Agricultural Science, University of Tasmania**
Subject:	**Celery Oil, Certificates of Analysis**
Date:	**31-Jul-04**

Tasmanian Celery Herb Oil

Certificates of Analysis

Peak No.	Component Identified	% of Total FID Area	
		Salad Green	Tendercrisp
1	α-pinene	0.26	0.31
2	β-pinene	5.70	6.76
3	myrcene	6.43	2.23
4	p-cymene	0.16	0.24
5	limonene	68.88	77.75
6	*cis* -β-ocimene	1.09	1.03
7	γ-terpinene	0.16	0.05
8	fenchone	trace	trace
9	n-pentylbenzene	0.27	0.44
10	n-pentylcyclohexadiene	1.46	0.42
11	*trans* -anethole	trace	trace
12	*trans* -pinocarvyl acetate	0.46	0.38
13	carvyl acetate	trace	0.04
14	caryophylene	1.58	0.63
15	humulene	0.14	0.06
16	β-selinene	6.54	4.01
17	α-selinene	0.98	0.57
18	elemol	0.05	trace
19	caryophylene oxide	0.14	0.17
20	n-butyl phthalide	0.36	0.64
21	β-eudesmol	0.37	0.09
22	α-eudesmol	0.12	0.04
23	sedanenolide	1.20	0.71
24	sedanolide	2.58	1.20
25	neophytadiene	0.18	0.12
	other unidentified peaks	0.89	2.11

Physical Properties

(all results are means from triplicate measurements)

	Salad Green	Tendercrisp
Specific Gravity (25 ml at 20°c) =	0.860	0.866
Refractive Index (20°c) =	1.480	1.479
Optical Rotation* (20°c, 2dm photolength) =	+71°	+78°

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