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Development of an Internationally Competitive Australian Saffron Industry

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Foreword

Saffron is the world's most valuable spice and most of the product currently used in Australia is imported. Tas-Saff Pty. Ltd., as the first and only significant commercial producer of saffron in Australia, have begun to achieve import replacement and have produced a business and marketing plan with the aim of capturing 25-30 per cent of the local market by 2013. The company has also identified the longer term potential for exporting saffron, particularly at the high quality end of the world market.

In order to achieve these aims, analytical evaluation of Australian saffron and comparison of it to international standards and imported products is a requirement, but no such testing had been previously undertaken. Such assessment of product quality and consistency of quality is necessary for the purposes of determining any present competitive advantage as well as identifying any improvements that could be made through the post harvest treatment methods.

This report identifies the drying process as being a critical and limiting determinant of Australian saffron quality and includes proposed changes to the current drying methodology that would allow production of spice with quality comparable or even superior to the best international product.

The report also details the findings of investigation into the potential for creating new secondary products that might allow value adding to the saffron crop and thus provide further competitive advantage for local growers.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

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Abbreviations

APCI	Atmospheric Pressure Chemical Ionisation
FID	Flame Ionisation Detector
GC	Gas Chromatography
LC-MS/MS	Liquid Chromatography Mass Spectroscopy (with Ion Trap)
HPLC	High Pressure Liquid Chromatography
HCC	4R-hydroxy-β-cyclocitral
MS	Mass Spectroscopy
RH	Relative Humidity

Contents

Executive Summary

What the report is about
This report describes research conducted for the purposes of:

- Evaluating the quality of saffron spice produced in Australia
- Determining how that quality may be improved through altered post harvest methodologies
- Investigating the potential for new product development from the waste flower parts and corms currently discarded.

Who the report is targeted at
This project was initiated and supported by Tas-Saff Pty. Ltd., the only significant producer of saffron in Australia. The findings in this report are therefore primarily targeted at Tas-Saff and the growers contracted to them.

Background
Saffron is produced from the careful separation and drying of the stigmas from the flowers of *Crocus sativus* and is (w/w) the world's most valuable culinary spice. It is also currently receiving renewed worldwide interest for its medicinal properties.

Saffron has traditionally been used in Chinese medicine, and is now the subject of scientific research for its use in anti-cancer, anti-inflammatory, anti-depression and pro-memory/learning treatments. Some of this work has reached the clinical trial stage. At present the cost of saffron spice is prohibitive to its use as a drug but the potential world market growth for saffron based products in coming years is clear.

The presence of colchicine – a compound used commercially as a drug to treat gout and in plant genetics – has also been reported in the corms (bulbs) of *Crocus.*

Australia imports approximately 800Kg of saffron annually at a value of $14m. Tas-Saff is the first and only significant commercial producer of saffron in Australia. The growth of the local industry has been restricted by the labour intensive nature of the harvesting and post-harvest processing procedures and competition with imports from countries with much lower labour costs. The local industry has therefore identified the need to gain market advantage over imports through optimisation of saffron quality.

This report details a research project aimed at providing the analytical assessment of the quality and consistency of spice product from the Australian saffron growers in relation to world standards and determination of:

- How the quality of the local spice product may be improved.
- How the industry may achieve value adding and increased competitiveness through identification, development and commercial introduction of new products from the saffron crop.

These aims were more specifically detailed through the following objectives.

Objectives

- To develop management strategies and chemical analysis protocols to maximize saffron quality and ensure the Australian product meets ISO standard
- To identify potential perfume products from waste flowers after stigma removal and establish viable extraction protocols for these products
- The 2 objectives above to be achieved through a detailed knowledge of the chemistry of carotenoid degradation in Crocus. The objective therefore, is to apply both current knowledge of this chemistry in the stigmas (leading to saffron biosynthesis) and any new knowledge gained from the study of this chemistry in the petals
- To investigate the content of colchicine and its glucoside in waste bulbs and then if viable, establish viable extraction protocols for commercial use
- To work closely with the Industry Partners to apply new technology to the current product and facilitate introduction of new products to the market.

These combined objectives were aimed at facilitating the growth of the local industry by providing the means to gain market advantage through improved quality of the current product – and to allow value adding through new products from the saffron crop.

Methods used

Application of International Standards Organisation (ISO) testing procedures and comparisons with other analytical extraction and chromatographic techniques were used to compare the local product to the ISO standard and competitor's imported products. Comparisons were also made between individual Australian grower's samples to gauge the range and consistency of quality within the local industry.

Application of different conditions using commercial food dryers, ovens and then a purpose built apparatus were applied to stigmas for drying experiments during the 2004 harvest period. A purpose built drying apparatus was designed and constructed to better study these conditions during the 2005 harvest. Application of the same analytical techniques described above, were used to measure quality parameters in both years and then to apply these findings to commercial scale drying during the 2006 harvest. Further to this an investigation of the effect of applied humidity and thus manipulation of the water activity of the stigmas during drying was completed during this 3rd harvest.

Waste flowers, with or without rejected stigmas, were collected during the harvest periods for distillation and extraction trials with some processed then and the remainder frozen for later work. A variety of different post harvest treatments were applied to both fresh and frozen flowers that were then either hydro-distilled or solvent extracted. Products were assessed organoleptically and by chromatographic analysis.

Extractions of *Crocus* corms were performed at intervals over the dormancy period and analysed chromatographically for identification of any colchicine content.

Results and findings

<u>ISO and analytical testing of quality parameters</u>

Full ISO testing procedures showed that local samples of saffron selected for sale from the 2003 harvest met all criteria for ISO, category 1 product, but that potential for significant aroma improvement existed. Samples of competitor's imported products fell short of the standard for the colour and bitterness measurements.

Individual grower samples from 2003 and 2004 were tested 6 months after harvest and 2 of these were retested again after 12 months extra storage. It was revealed that most (90%) of the samples had moisture samples above the 12% standard with a significant number (30%) more than 5% over this, indicating that a significant problem with insufficient drying was occurring.

For the key colour, bitterness and aroma strength measurements 15% of the samples were deficient (for ISO standard) in the former 2 properties, as well as both those re-tested after a further12 months. Storage with excessive moisture leaves the saffron prone to enzymatic degradation of the colour components and this was apparent from the re-tested samples, though colour loss can also occur prior to or during the drying process.

Comparison of the grower samples to a variety of imported products showed that while the local saffron generally compared well for colour to those from Iran and an Italian sample, the latter products had higher aroma strength measurements, except where the local products had greatly degraded in colour. None of these samples compared well with an analysis of the Spanish product (from the region renowned having the world's best saffron) that had both high aroma and colour. The differences were even greater when results for solvent extraction and GC measurement of the aroma compound, safranal were compared as an alternative to the ISO test. The Spanish sample had at least twice the safranal content of all other samples and 3-8 times the content all those where colour was not low. It also had colour strength higher than all other samples. This analysis suggested that for the local product, considerable improvement in the aroma content should be possible, without excessive colour loss.

It was shown that the ISO test procedure for aroma strength correlated very poorly with (non-polar) extraction and analytical GC analysis. The failure of the ISO method to reflect large differences in safranal content is consistent with the recent observations of several other authors – and so for all later work the analytical method was used.

Drying experiments

The stigma drying experiments conducted during the 2004 and 2005 harvest indicated that high temperature drying procedures could enhance aroma formation. Highly significant increases in safranal content were achieved when compared to lower temperature ranges (used currently by the local industry) and with improved colour retention. There was also evidence that the use of significant air-flow; an integral and unavoidable aspect of the use of the current commercial apparatus/method, causes significant loss of safranal.

In response to these results, and the difficulty encountered with the use of conventional ovens in accurately applying precise high temperatures for the relatively short periods required, a purpose built apparatus was designed and constructed to allow proper isolation of the effects of different conditions by using the same device for all treatments. It also served as a prototype for a potential new commercial dryer. This device incorporated heating via blacked out conventional light bulbs controlled by a PID temperature controller, a cylindrical aluminium structure to reduce thermal inertia and a fan with speed control.

The prototype dryer was successfully used for a series of stigma drying experiments during the 2005 harvest. The results indicated that at the elevated temperatures, significantly more safranal was generated and colour components retained, than at lower temperatures. Drying at high temperature for approximately 25 minutes gave the highest result with an 8-10 fold increase in safranal content over the lower temperature treatment currently used by the industry. Furthermore, it was confirmed that the use of air-flow causes reduced safranal levels, probably due to evaporative loss, and that this effect was significant even at relatively low airspeeds. This air-flow effect occurred at both high and low temperatures, although it was more pronounced at the former.

In critically assessing the new drying apparatus it was found that, while it worked well for the experiments undertaken, for commercial use major adaptations were required to incorporate a capacity for greater volumes of stigmas while limiting the number of drying levels to minimize temperature variations throughout. To this end it was planned to design a new device with a flatter, rectangular shape and side access to allow rapid and easy sample introduction and removal and to test this apparatus during the 2006 harvest. Before this was attempted however, the following findings shed new light on the requirements for this device.

Although not originally planned as one of the described tasks, an investigation of the importance of water activity to safranal production in the drying process was undertaken during the 2006 harvest. This work was begun as part of an ensuing PhD project, but the initial results are included here because of the important implications regarding the optimal drying procedure.

It was shown that by using a container of hot water in an enclosed (but non-pressurised) drying apparatus (oven) set at a specific high temperature to achieve an equilibrated relative humidity (RH) of approximately 50%, the drying rate of the stigmas was significantly slowed. Although seemingly counter-intuitive for a drying process, this actually allowed the stigmas to remain for longer within a critical range of water activity. As the stigma temperature was sufficiently high this allowed significantly greater (x3) hydrolysis of picrocrocin to form safranal when compared to the optimum high temperature treatments (without applied humidity) identified earlier. The colour strength of the saffron produced in this way was also enhanced.

Colchicine assay

LC-MS/MS analysis of *broad-spectrum* methanol extracts of Crocus corms at intervals over the 2003/04 summer dormancy period failed to detect any colchicine. The relatively crude isolation method employed meant that very low concentrations of colchicines or related compounds could have been missed, but in such a case commercial extraction of these would not be viable and so this part of the project was discontinued.

Waste flower products

None of the distillation products from waste flower samples was obtained in sufficient yield (all < 0.1%) to make this type of product potentially viable. It was noted that the best distillation products contained safranal and this was only obtained from flowers containing some reject stigmas.

Application of extraction trials confirmed that safranal was only generated in samples containing some proportion of reject stigmas indicating that its precursor does not occur in the petals or stamens. Products obtained from flowers without stigmas, although of pleasant aroma and attractive bright orange red colour, did not have the aroma impact for a potential fragrance product. The stigma content (w/w) of all later waste flower samples obtained from growers was therefore determined. The average content of stigmas for sample obtained from the 2004/05 harvests was found to be 0.35% by weight.

Trials of different pre-extraction treatments on fresh and frozen flowers combined with organoleptic assessment revealed that the best product was obtained from fresh flowers dried slowly at ambient temperature for 3 days in the dark. This extract was also shown to have the highest yield of safranal per unit wt. of stigmas. Drying of flowers at higher temperatures also resulted in a loss of other non-safranal aroma notes.

A trial of farm scale collection and onsite drying of waste flowers during the 2006 harvest period revealed that air drying of these flowers on horizontal mesh nets in sheds away from direct sunlight was feasible, though slow in poor weather conditions. An alternative to this method was the use of the food dryers at low temperature (30ºC) though this did result in some loss of volatiles including safranal in the extract and involves considerably more of the grower's time to perform. This loss of volatiles may not be critical if reject saffron is used to augment the extract.

Extraction with a solvent mixture of 10% ethanol in hexane gave a higher yield of product (5%) than extraction with 100% hexane (2%), but the latter had a more attractive colour and persistent aroma. This sample was selected for independent expert assessment by a leading international perfumer. The feedback from this was very positive with the following quote summing up the potential:

"Market potential- small and niche. 'People would bite your hand off if it was the right price and available in quantity'. Price suggestion: several hundred £/kg would guarantee a market".

At present there is insufficient weight of saffron flowers produced by the local industry to create a viable market for the extract described above. However, the use of a currently existing stock (of many 100g) of reject saffron (with degraded colour) could be used to greatly strengthen the safranal aroma impact of the product. This and the clearer identification of extract components with established health care properties (of which safranal is one) could attract a much higher price.

The establishment of a small market for a new product such as this could then encourage a more rapid increase in the *Crocus* crop production than has been occurring in recent years. This would allow the extract to be produced in quantities more suitable for potential buyers and part of the crop could be used exclusively for this production ensuring that the high safranal content of the product was maintained.

The other potential means of boosting the safranal content would be to further investigate the chemistry of the safranal conversion from its precursor picrocrocin. The analysis of samples from the drying experiments revealed that even in those samples with the highest safranal contents, there remained a 25-50 fold excess of the precursor. Thus, there is a great potential for very considerable safranal yield gains given the right post harvest treatments and this could lead to an extract product with boosted aroma impact from only a small stigma content such as used in these trials.

Reports in the literature indicate that saffron flowers contain significant contents of glycosidically bound (and therefore water soluble and non- volatile) carotenoids and other potential volatile/aroma compounds. Fermentation treatments such as those used to boost the aroma in products such as tea, tobacco, rose oil and *Boronia*, could potentially be used to cleave off the sugar unit from these molecules in preparations of *Crocus* flowers to release new aroma volatiles and improve the yield and fragrance strength of extracts.

Implications

Saffron quality

Drying methods

The findings of this study show that, given the application of appropriate drying methodology, the quality of Australian saffron can and should be significantly improved. Most of this improvement will come from increased aroma production, but high temperature/humidity drying will also have a preserving effect on the pigment content as enzymatic degradation is prevented. This should result in consistently higher colour strengths that would be maintained in storage.

The saffron filaments produced from this process tend to be too dry and brittle for easy packaging but in the laboratory some moisture, and with it, much of the resilience is returned by equilibrating the filaments at ambient temperature in a darkened room – possibly with a warm container of water nearby to provide a slightly higher localised atmospheric humidity. This equilibration was shown to work well on a commercial scale although some variation with weather conditions (and thus ambient humidity) did have an effect and would need to be taken into account in timing the length of equilibration. The use of a high humidity drying method may decrease the need for this equilibration.

The implementation of the new drying method would best be made using an apparatus such as an oven or incubator that is enclosed so that a high relative humidity can be uniformly achieved within by simply introducing a dish of hot water. The inclusion of precise temperature control (such as PID) would be an advantage and the device should be calibrated with for the temperature settings when used with added moisture in this way. The method has worked well with an oven of 24L capacity and while a larger chamber would be good for increasing the number of stigmas that may be dried per run, if it is too large, uniformity of temperature and humidity will be more difficult to achieve.

Competition and growth of the industry

The local saffron industry is competing with products from countries with considerably lowers labour costs. In this situation there are 2 alternative ways of providing competitive advantage; reducing the local labour costs or producing product that is demonstrably superior in quality and therefore can attract a higher price. The only way that the former could be achieved in Australia would be through the successful implementation of mechanical flower harvesting and stigma separation.

Some documented attempts have been made to design equipment to mechanise the cutting and collection of flowers, particularly in Italy(1) but the results tended to result in excessive loss of flowers, increase impurities and have negative effects on eventual saffron quality. All the methods tried also tended to cut a considerable proportion of the leaves surrounding the emergent flowers which compromises the later growth of the corms resulting in poor flower yields in subsequent seasons.

Saffron flowering also occurs randomly through a crop over several weeks so that flowers need to be harvested on virtually every day over that period with considerable judgement applied to pick flowers at the right stage of maturity. Any successful mechanical system would require much more uniform flowering to accomplish significant labour saving. This would probably require hormonal and/or environmental manipulation of the plants. One reference was found describing work relating to the hormonal application(*2*) but at this stage not enough is known about *Crocus* physiology to achieve uniformity of flowering this way. Recent trials of indoor (glasshouse) environmental control of saffron crops has shown that flowering timing can be controlled to a degree and product yields potentially improved through more reliable flowering, but no improvement in flowering uniformity was described(*3*). Glasshouse based environmental control may be a more efficient method of producing high yielding quality saffron but considerably more research would be required to establish the precise methods for this as well the cost benefits as this form of horticulture is much more capital intensive than the current practises.

Even supposing that mechanical harvesting of a uniform flowering crop could be achieved then that would place considerable pressure on the stigma separation and drying facilities, as the yield of flowers currently processed over several weeks would need to be processed at once. Separation of stigmas from other flower parts was also attempted in the Italian studies(1) by means of fans and wind tunnels but these were not successful because the connection between the base of the style (stigmas) and the other flower parts which tended to remain intact and because the petals tended to wrap around the stigmas unless the flower was totally open and beyond the optimal harvest stage so that quality was compromised. The process also tended to prematurely dry the flowers and result in loss of aroma.

In searching for any information on mechanical harvesting, 2 Indian websites(*4;5*) were found claiming that mechanised systems for saffron harvest, separation and drying had been developed. Attempts to contact the organisation responsible have been unsuccessful to date. There is thus no information available at present on the means of mechanisation or quality of the product from these systems. All the saffron produced in India is for the domestic market in that country and does not have a reputation for high quality(*6*).

Mechanisation of the harvesting separation process may well be feasible, but significant research effort (with a new set of objectives to those investigated here) would first be required to solve all the significant problems associated with the growth and flowering of *Crocus* without compromising

saffron quality. This would require considerable financial input from an industry that has not yet reached the stage of development to justify investment of that scale.

In contrast, the present study has shown that the aroma and colour characteristics of the local product can be (with the appropriate drying technology) significantly and consistently improved, potentially to a quality level that is superior to saffron from competitors anywhere else in the world. If this allows the local Industry to grow through the capture of a larger proportion of the domestic market as well as export developments, then this would be the first step towards establishing the resources to properly investigate mechanical harvesting and processing and alternative ways of growing the crop.

New products

This study has shown that commercially attractive extract product(s) for use as a fragrance can be obtained from the waste flowers as long as those flowers contain at least a small % of stigmas. This has been shown to occur during the normal course of harvesting as stigmas are missed or rejected due to visual defects, but it is possible that the extraction could also be augmented by addition of saffron rejected for sale (due to colour loss) that adds to the aroma (and health care) properties of the product.

In the long term, to properly establish a market for a fragrance product, the overall volume of *Crocus* crop would need to increase significantly to provide sufficient product for buyers. The economic viability of growing some proportion of the crop just for extraction should be assessed, but first more work is required to chemically characterize the extract produced. This should include investigation of the content of components with medicinal/health care properties as well as the best methods of post harvest treatments to optimise these properties and the overall yield.

The encouragement of the growth of the local industry through quality improvement and development of new product(s) with a continuation of the investigative study of the underlying biochemistry, will better position the local saffron industry to take advantage of future trends in international demand for saffron products as the healthcare/medicinal properties of the spice become more commercially utilised.

Recommendations

1. Saffron production from the drying of stigmas should be facilitated by applying high temperature in an elevated humidity without applied air-flow.

2. For this drying it is recommended that growers obtain suitable ovens or incubators that may be adapted to suit drying conditions, or to design and construct a saffron specific apparatus with the following features:

- The drying device should not have a functioning fan but should have a well contained chamber with adequate door seals to allow humidity build up from an open hot water surface in the lower part of the device. Once the hot water is introduced the device should be equilibrated at the specific temperature for at least 30 min prior to introduction of stigmas.

- The drying device should have a good quality temperature controller (such as PID) that prevents thermal inertia from causing temperature overshoot of more than 2-3ºC.

- It is recommended that the volume of the device not exceed approximately 48 litres as too large a capacity would be difficult to heat and humidify uniformly. The chamber should have several rack positions to allow an optimum number of stigmas to be introduced for each drying run, but the distance between racks should be at least 5 cm to allow sufficient circulation and thus temperature/ humidity uniformity throughout. The racks may be covered in nylon mesh to hold the stigmas.

- It is recommended that there should be a narrow lateral port on the side of the device to enable insertion of a thermometer or other temperature measurement probe so that the temperature of the interior can be calibrated when at elevated humidity.

3. To use periods of approximately 1-5 hours after drying for moisture re-equilibration of the saffron filaments. This should be conducted in a slightly humidified, darkened room at ambient temperature. The specific details of this process may be adjusted to suit the drying characteristics of the device (oven) used by each grower - as well as the weather conditions (humidity) on each day of drying. With experience it is relatively easy to judge when the stigmas have reabsorbed sufficient moisture based on feel.

4. That further study of the drying process would be advantageous because of the large excess of picrocrocin and the potential for very significant further safranal yield gains (in saffron and also for new products), and because the effects of drying on the cellular and subcellular structure of stigmas may impact upon the quality of saffron and this is fully not understood yet.

5. That an extracted fragrance product has good commercial potential but that more investigative work is required to refine and chemically characterise it and to determine if this or an altered extract type could also be sold for its health care properties. The flavonoid content in particular should be investigated.

6. To submit larger samples of the extracts from waste flowers, incorporating different contents of added saffron, to recommended perfumers and potential buyers for further assessment.

7. To consider conducting a local consumer and allergen trial of the extract as a fragrant moisturizing cream product to gauge consumer interest for possible local sale as a first step in the marketing process for a new product.

8. To consider the economics of increasing *Crocus* flower production for the purpose of using some of the crop solely for (less labour intensive) extraction purposes. This would allow a greater volume of extract to be available for sale, making it more attractive to potential buyers and provide better economy of scale regarding production costs.

9. To have written reports and proposals distributed and (where feasible) conduct meetings with the growers to fully disseminate the findings and recommendations of this report. This would aid in the uptake of new drying technology.

1. Introduction

1.1 Industry background

The spice saffron is composed of the red-orange filaments of the dried tripartite stigmas from the flowers of Crocus sativus L., a perennial herb of the family Iridaceae. It is highly valued as a culinary spice for its flavouring and colouring properties(1), and for its medicinal use in both traditional treatments(7) and for a range of potential new clinical and pharmaceutical uses.

A growing body of research in recent years has demonstrated that saffron extracts including metabolites such as safranal, crocins and various flavonoids reported from different flower parts, possess chemo-preventative properties against cancer (8-10). Such anti-tumour effects of saffron extracts have been observed in vivo in mice and rats(*11;12*) and in vitro with human carcinoma cells without having any toxic side effects at the doses used(*13;14*). Saffron extracts also been shown to have anti-inflammatory and anti-depressant effects in humans(*15;16*) and to have great potential for promotion of learning and memory in patients with neurodegenerative disorders(17). Abdullaev, one of the chief authorities on this subject indicates that Human clinical trials are proceeding, though the cost of saffron spice is a prohibitive factor to its widespread use in medicine at present(*8;13*).

On average it requires 150-250,000 plants to produce 1 kg of saffron at a value of approximately A$18 000(*1;6;18*) making it the worlds most valuable spice. The annual world production of saffron is an estimated 100-180 tonnes at a value of $700-1300m(*19;20*). Australia imports approximately 800Kg of saffron annually at a value of $14m(20).

Tas-Saff Pty. Ltd. is the first and only significant commercial producer of saffron in Australia with some 34 growers in Victoria and Tasmania at present and has been marketing product locally for 10 years. The company has produced a business and marketing plan (March 2004) that includes the aim of increasing production, via a total of 40 growers, to an estimated 180kg of saffron per year within 10 years. This would equate to an estimated import replacement value of $4m annually(20).

Part of the marketing plan also requires that the saffron consistently meets ISO standards(21), although, prior to the commencement of this project, the Australian product had not been analytically tested for these criteria. Such measurement would provide documented proof of the quality and consistency of the product that could be used as a significant aid to marketing, particularly in a market where Tas-Saff is competing with cheaper but inferior imported product.

The use of the standard ISO and other analytical measurement techniques would also provide a basis for identifying the potential increases in the quality of the product that might be obtained from new post harvest treatment methods.

1.2 Saffron composition

The major colour, flavour and fragrance components of saffron are all thought to be secondary metabolites from the biodegradation of a C40 – carotenoid, zeaxanthin (22)(**scheme 1**). It has been proposed that this oxidative process occurs via a hypothetical 7,8-carotenase enzyme cleaving the zeaxanthin molecule at either end of the polyene chain to produce 1 molecule of crocetin and 2 molecules of picricrocin (*23;24*). The glycosylated forms of crocetin, which are collectively referred to as the crocins, (of which crocin, the digentiobiose ester of crocetin, is the most abundant), are the principal yellow pigments in saffron(*24;25*).

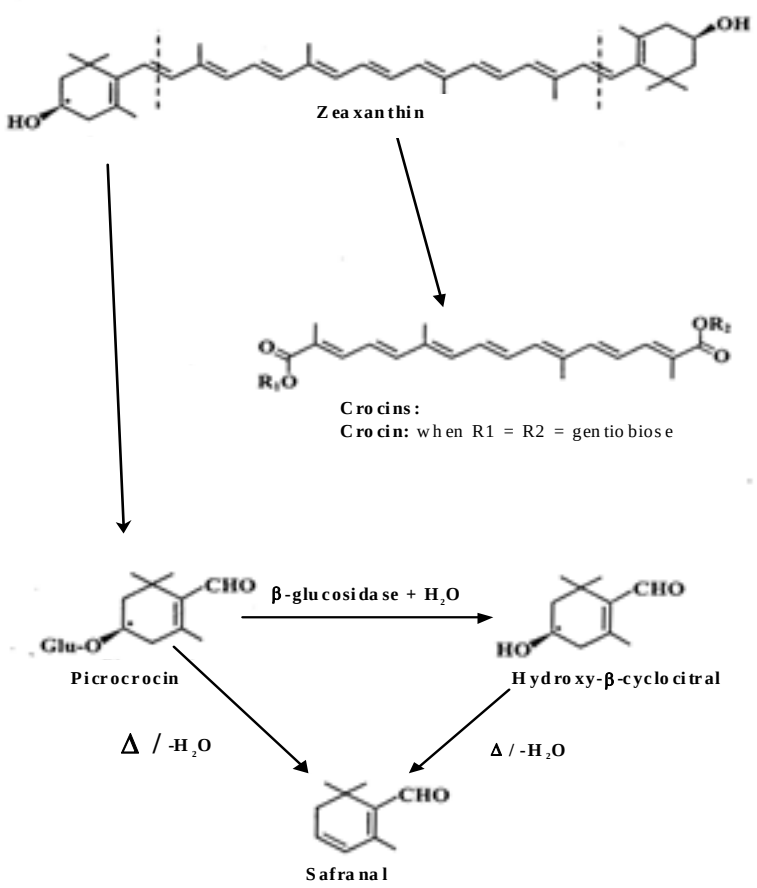
Figure 1: Close-up of fully open *Crocus* flower before picking showing red tripartite stigma, yellow stamens, *lilac* petals and narrow leaves.

The odourless, colourless and water soluble picricrocin, itself a glycoside, has been generally regarded as the main taste component, responsible for the bitter flavour of saffron(*24;26*). While the compound is certainly bitter and would contribute significantly to the taste, Carmona and Alonso(27) rightly highlight the lack of scientific evidence directly correlating picricrocin concentrations with degree of saffron bitterness and that significant levels of other compounds such as flavonoids would also contribute to this taste.

It also generally accepted that picrocrocin is the precursor of safranal, the main odour component of saffron(*24;26;28*) , which is only produced in the stigmas upon drying. This conversion may occur by 2 alternative pathways: either via an intermediate, 4R-hydroxy-β-cyclocitral (HCC), (which is produced by the action of β-glucosidase at moderate temperatures) and which is then readily dehydrated to form safranal, or by direct dehydration of picricrocin to form safranal under more extreme conditions such as high temperatures or very high or low pH(*24;29;30*)(**scheme 1**). The generation of safranal from picrocrocin by this latter process is not completely understood however, as the loss picrocrocin in previously dried stigmas subjected to heat treatments has been shown not to correlate with safranal generation (27), possibly because this conversion is linked to water activity(31).

The quality of saffron is primarily dependent on the actual and relative concentrations of these main 3 main secondary metabolites and these levels are determined by a combination of cultivation/harvesting practises and postharvest treatment(32). The crocin and picricrocin levels peak in the flowers at full bloom and so assuming that the flowers are harvested then, the main determinant of saffron quality is the drying process where picricrocin is hydrolysed to form safranal. The conditions of this drying process including; the temperature, rate of drying, final moisture content and physical air flow characteristics, are all critical to the level of safranal produced and retained in the stigmas as well as any concurrent loss of the colour compounds (crocins) that may occur due to enzymatic or thermal hydrolysis(*29;32-34*).

Scheme 1: Proposed pathway for zeaxanthin biodegradation to produce principal secondary metabolites responsible for colour (crocins), flavour (picrocrocin) and aroma (safranal) in saffron.



Besides these major components there are many other aroma factors in saffron with over 60 volatile compounds having been identified(24). These include 25 carotenoid derived compounds that have been shown to contribute specific notes to the overall odour of the product. These may be produced as either degradation products of safranal or from alternative carotenoid degradation pathways(*28;35;36*). There are also many additional compounds formed through lipid oxidation or hydrolysis of non-carotenoid precursors that have been identified as minor constituents of saffron, but which may contribute to the overall fragrance(24).

Glycoside precursors of both carotenoid and non carotenoid derived volatiles appear to be present in the Crocus stigmas before, and in some cases, after drying(*35;37*), suggesting that there is a complex array of factors which can subtly influence saffron quality.

1.3 Analysis methods

The standard measurement of saffron quality and composition is the ISO-3632 (2003)(21) test methods. Amongst these tests the colour, flavour and aroma components of saffron are measured by a spectrophotometric analysis method that is subject to variation due to; the lack of solubility of safranal in water, interfering absorbance at 330nm due to cis-crocins and post extraction degradation of picrocrocin(*30;33*).

Various other means have been used for measuring the important components of saffron including; thermal desorption GC(*30;38*), HPLC methods with a polarity range capable of measuring the polar crocins and picrocrocin as well as the non-polar safranal from the one polar solvent extract(39) and GC and HPLC analysis of supercritical CO2 extracts(40). Not all these methods are readily available to industry and doubt remains as to whether a single extraction can provide an accurate measure of the true relative levels of all these components(*41;42*).

The literature includes a wide variety of reported safranal concentrations measured by different extraction and analysis methods in saffron of different origins including: approx 800ppm in Indian saffron extracted with 80% ethanol(43), approx 1500ppm in Spanish saffron by supercritical CO2 extraction(40), 1070-3970ppm in Spanish saffron analysed by thermal desorption/GC(30), up to approx. 4000ppm in Indian saffron by simultaneous hydro-distillation/extraction (SHDE)(32), and in Greek saffron up to 1200ppm when cold extracted with diethyl ether or up to 6400ppm in the same material by (SHDE)(*33*), a process where significant extra safranal may be generated due to the heating involved.

The total crocins (as the sum of the glycosyl esters of crocetin) contents of commercial samples of saffron have typically been reported from 5% of dry wt. for sun dried stigmas(43) to 17% of dry wt. for vacuum oven dried samples having poor aroma development(32) although for quality product with good aroma development, values from 6% to 16% of dry wt.(*32; 43-45*) have been given.

1.4 Drying techniques

From the evidence available in the literature there is considerable uncertainty about the ideal conditions for the drying of saffron. Stigmas have traditionally been dried by methods such as sun drying (India and Iran), toasting over hot ashes (Spain) and drying slowly in a darkened room at 30-35°C (Greece)(*1;24;29;32;33*). Of these, the Spanish methods have been regarded as producing the best quality of saffron(*8;24;30;34*) and although recent work has provided detail of the actual conditions involved(29), no precise determination of optimal drying conditions has been provided.

The range of recent scientific studies and reviews available provides contradictory information as to the best drying conditions. In reviewing the topic, Cadwallader (24) in summarizing the work of Riana et al.(32) and others, stated that a temperature range of 35-45°C without air-flow or freeze drying was optimum for good conversion of picrocrocin to safranal without excessive loss of colour. He emphasized that temperatures below this required too long a drying period resulting in excessive enzymatic degradation of crocins, while excessive temperature resulted in thermal degradation of these pigments. Other studies however, have given evidence for quality saffron with aroma from drying conditions such as: 80°C for 30 min in an oven(46) presumably without strong air-flow, 110°C for 2 min and 70°C for 30 min with a strong air-flow(34). The traditional Spanish methods involve maximum temperatures ranging from 75°C to 121°C for periods of 28-55 min.(29). In Australia, Tas-Saff currently uses commercial food dryers with constant non-adjustable air flow at temperatures ranging between 40°C and 55°C. Under these conditions stigmas require drying over a period ranging from 45 to 90 min. to produce saffron with a moisture content of < 10%(6).

The major focuses of this part of the project were thus:

- To apply the ISO-3632 (2003)(21) test procedures to Tas-Saff saffron from a range of their growers to establish whether the product meets the ISO standard parameters, to get an indication of the range of quality being produced and to compare it to the imported product from their competitors.

- To develop and trial analytical methods designed to measure the actual levels of the important secondary metabolites in *Crocus* stigmas and dried product and to compare these methods to the ISO test measurements.

- To conduct experiments designed to investigate alternative drying procedures to the current method employed by Tas-Saff and in so doing establish optimum post harvest treatment conditions for the production of saffron. Factors for investigation include; drying temperature, duration and rate of drying, air-flow and final moisture content.

- Once optimum conditions have been determined it was then the intention to adapt the drying apparatus/ technology to best incorporate these conditions and to test the new methods on a commercial scale during a harvest period.

1.5 Value adding potential

The production of saffron from *Crocus* flowers utilises < 5% of the dry wt of the flowers picked. The propagation of *Crocus* corms is also far in excess of what is required for crop expansion and renewal even with expansion of the local industry(6). Any new secondary products from the saffron crop, if saleable, would allow value adding and thus provide further competitive advantage for local growers. The following possibilities requiring further investigation were identified.

1.5.1 New flavour or fragrance products from waste flowers

No reference could be found in the literature to the content of crocins, picricrocin, safranal or other aroma active compounds in *Crocus* flower parts other than stigmas, particularly the petals and stamens, which though discarded as waste in the normal production of saffron, constitute a much greater weight than the stigmas (>95%). The fresh flowers have a delicate but pleasant aroma.

In response to a request from Tas-Saff, preliminary steam distillation and (hexane) extraction trials were undertaken with waste petals from the 2002 harvest. The steam distillation product, although of low yield, had an attractive, complex, delicate and highly persistent aroma. Furthermore, GCMS analysis identified safranal and a low level of the intermediate precursor, hydroxy-β-cyclocitral in this oil.

The solvent extract of the fresh (undried) waste flowers gave a product of vivid red-orange colour with only a trace level of safranal, but with significant content of hydroxy-β-cyclocitral. Heating (80°C) then extraction of the resulting marc then gave a product with further hydroxy-β-cyclocitral and a low concentration of safranal indicating that some thermal conversion from the polar precursor (picricrocin) had occurred. The colour of these extracts suggested that significant levels of carotenoid pigments were present in the waste flowers.

This postharvest treatment of the waste flowers revealed that potentially valuable products could be produced from this material. A further major focus of this project was thus to investigate these

distillation and extraction products and determine the best methods for optimal yield, aroma and colour characteristics including conversion of precursors to safranal. Identification of any other aroma volatiles from both alternative carotenoid degradation pathways and non carotenoid sources would also be undertaken.

1.5.2 Colchicine related compounds from corms

The alkaloid colchicine and its glucoside, colchicoside are chemicals with commercial value for their use in plant genetics for inducing polyploidy(47) and in medicine for a variety of uses including the treatment of gout(48-50) and potentially, cancer(51-53). In response to a request from the Industry partners who wished to find out if the *Crocus* corms were safe to eat but also related information that suggested that Crocus corms may contain toxic alkaloids, a literature search was performed. This revealed one report of colchicine and its glycoside, colchicoside detected in *Crocus sativus* corms(54). An additional focus of this project was therefore to make appropriate extractions of a range of corms to determine if they contained these compounds and if so, whether the concentration was sufficient for any potential secondary production in the “off season” for the local saffron industry.

2. Methodology

2.1 Specific methodology

Where specific technology or procedures have been applied to experiments or particular processes, they are described in the relevant section of the results chapter.

2.2 General methodology

2.2.1 ISO analysis of grower industry samples

All measurements described in this report as ISO testing procedures were performed according to the International Standards Organisation (ISO) Technical Specification (TS) 3632-2 Test methods 2003, reference number ISO/TS 3632-2:2003(E).

2.2.2 Collection of stigma material

2.2.2.1 2004 harvest

The experiments were conducted on Crocus stigmas from the same Southern Tasmanian crop at 3 harvest dates of April 8, 18 and 30, 2004. The flowers on each of these days were picked by hand at approximately the same time of day as part of a normal commercial harvest. It should be noted however, that no attempt was made to test for the effect of harvest time in this study and that a variety of factors such as weather conditions and the exact time between flower picking and stigma removal would have varied between harvest dates. All conditions were kept identical between treatments on the same harvest day.

Stigmas for the experiments were separated by hand from a random selection of the picked flowers at 18°C indoors. All stigmas were separated from the flower with the tripartite stigmas intact (connected to style) initially, although separation of some of these did occur through the treatment and extraction processes. For each treatment replicate, 20 stigmas were placed in sealed vials and transported to the laboratory in a cooled container (approx. 4°C for 2 hours). The exceptions were the samples of treatments J and K where 100 stigmas per replicate were collected to allow enough material for ISO tests to be performed as well as the extractions.

2.2.2.2 2005 harvest

Experiments were conducted on *Crocus* stigmas from 2 properties, one in Northern Tasmania and the other in the south. Each discrete experiment was conducted on material taken from the same farm on the same day with all other conditions kept identical as for the 2004 harvest except that transport to the laboratory from the Northern farm took approximately 4 hours.

2.2.2.3 2006 harvest

Material was collected as for the 2005 harvest.

2.2.3 Application of treatments

2.2.3.1 Experiments during 2004 harvest

The stigma samples were equilibrated in the vials at room temperature (20°C) for 15 min., weighed and then placed on Teflon trays for drying except the 2 frozen treatments (E and I) that were placed in vials in a -20°C freezer and the fresh sample (A) which was extracted immediately. The drying conditions were then applied to the other treatments using either electric ovens with fans disconnected or a food dryer (Ezidri Snackmaker from Hydraflow Ltd. NZ.) set at either the medium or high temperature setting (46°C and 58°C) with the tray including the stigma samples at the second from top level of a 5 level stack. The air-flow over the stigmas in the dryer at both temperature settings was measured at 2.9 m/sec. The temperatures of the interior of both dryer and ovens were recorded with digital probe thermometers and found to vary within a range of ±3°C of the set temperature. Where a treatment involved a high temperature followed by a lower temperature, the samples where transferred immediately from one oven to another at the appropriate time to avoid a temperature lag period. The frozen treatment samples were thawed for 15 min next day then dried as described above or extracted immediately. Each treatment was replicated 4 times and they are detailed in **table 4**.

2.2.3.2 Experiments during 2005 harvest

For the experiments conducted during this season a custom designed and built drying apparatus was used for all drying. Details of this equipment are included in the relevant results section.

2.2.3.3 Experiments during 2006 harvest

For experiments conducted during this season, treatments were applied as above but with the inclusion of elevated humidity oven drying and stigma temperature measurement, the details for which are included in the relevant results section.

2.2.4 Extraction of saffron samples

The following method was used for experiments conducted during all harvests.

For the determination of the pigments, picrocrocin and comparison of safranal extraction, a polar solvent was required and as for other commonly used methods, methanol was chosen(*29;39;45;55;56*). For comparison of safranal (and HCC) extraction a relatively non-polar solvent was required(*33;46*) and hexane was used in this case.

After treatment, samples were reweighed then divided up with a 4 stigma subsample of each replicate taken, combined with those from the same treatment, weighed, dried at 104°C for 24 hours, and reweighed for mean moisture determination (as per ISO test procedure) used to calculate concentrations on a dry weight basis for all treatments. The remaining stigmas of each replicate were split into 2 subsamples of approximately 8 stigmas, which were each, weighed (to 4 decimal places at 20°C) into separate sealed vials. To each of these treatment replicates, 5 ml of HPLC grade hexane and 0.492 mg of β-cyclocitral (Fluka, 95%) (as an internal standard), were added and the vials sealed. To the remaining subsample from each replicate, 5 ml of HPLC grade methanol was added and the vial sealed.

The extraction method used was adapted from the ultrasound assisted method used by Kanakis et al(*33*) as this did not employ heat which could cause compositional changes. Extraction samples were blended using an ultraturrax fitted with a 10mm shaft before being subjected to ultrasonification for 1 hour in the dark and left on a shaker at 100 rpm for 20 hours in the dark at 15°C. The samples were then allowed to settle out in the dark for 1 hr. before an aliquot of 1 ml of each of the extracts was transferred to 2 ml GC vials and sealed. These samples were then stored in a freezer at -10°C before analysis.

2.2.5 Gas chromatographic analysis

A Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionisation detector (FID), a split injection system and a HP-1 cross linked methyl silicon gum column (30m C 0.32mm id., 0.33mm film thickness) was used. Injections of 2ml were made with an injector temperature of 250°C in splitless mode with purging resuming after 2 min. Carrier gas was N2 at a column flow of 1.8ml/min., a head pressure of 8 psi and a split ratio of 1:50. The oven temperature program was 50°C for 2 min. before rising at 9°C/min to 290°C and held for 11.3 min. The detector temperature was 295°C.

In order to accurately quantify the response of safranal by GC-FID, calibrations involving determination of the standard curves for the response of 8 concentrations of safranal (Fluka, 75%, cat. No.17306) between 26 and 500 mg/ml in both hexane and methanol were run. The determination of HCC was made relative to the response of safranal in hexane, as this compound is structurally very similar. The internal standard was used only as a check of overall response of the GC-FID.

GC-MS Analysis. Samples were analysed by GC-MS to confirm the safranal and HCC identities using a Varian 3800 GC coupled directly to a Varian 1200L triple quadrupole mass spectrometer. A Varian Factor-Four VF5-MS (25m x 0.25mm x 0.25 micron film) with helium as carrier gas at a flow rate of 1.2mL/min was used. 2 microlitre aliquots were injected into a Varian 1177 injector using the split mode (15:1) at a temperature of 210°C, and the column oven was held at 6°C for one minute and then ramped to 14°C at 5 degrees per minute to 140 C and then to 28°C at 20 degrees per minute. The m/z range from 35 to350 was scanned every 0.3 seconds. A reference spectrum of HCC was available from an in-house specialized terpene library. Analyses of waste flower extracts were also conducted under these conditions.

2.2.6 HPLC/UV-Vis analysis

HPLC coupled to a Waters 996 Photo-Diode Array detector. Trans-4-crocin, trans-3-crocin, cis-4-crocin, trans-2-crocin, cis-3-crocin and trans-2-crocin were measured at 440nm and picrocrocin was measured at 250 nm. Separation and identification of these compounds was made with reference to the methods and results reported previously by of Castellar *et al*(24) and Lozano *et al*.(15). A Waters Nova-Pak C18 column (3.9mm x 150mm) and an Alltech Econosphere 5 micron C18 guard cartridge at a flow rate of 1 ml/minute were used. Solvent A was methanol and solvent B was 1% acetic acid in Milli-Q water. The gradient was 20%-A to 80%-A at 45 minutes, then to 100%-A at 45.01 minutes and this was held to 52 minutes. Re-equilibration between runs was 10 minutes. The known molar absorptivities of crocins and picrocrocin (in water and alcohol) were used to calculate concentrations of these compounds in the extracts, using the known conversion factor between molar absorptivity and raw PDA peak area at this flow rate based on a β-carotene standard. The molar absorptivities were those stated by Castellar *et al*.(56) or calculated from the extinction coefficients (E1%/1cm) at each wavelength, given by Davies(57). For crocin (used for all the crocins which possess the same chromophore) this was e440 = 133750 M-1cm-1 while for picrocrocin, e 250 = 10100 M-1cm-1 and for β-carotene the E1%/1cm at 440nm = 2620 and thus e440 = 140432 M-1 cm-1.

2.2.7 Extraction of *Crocus* corms

The procedure for extraction of saffron corms was adapted from the method described by Poutaraud and Girardin(48).

Samples of approximately 100g of corms were blended in a stainless steel processor with 500ml of ethanol and extracted at ambient temperature overnight with shaking at 100rpm. The mixture was then

allowed to settle for 1 hour and the liquid decanted. This was then centrifuged 4000rpm and the supernatant decanted, filtered through a 0.45µm Millipore filter and dried down on an RVE at 30°C.This extract was then re-dissolved in 10 ml of methanol , chosen as the best solvent for a wide range of polarities and a subsample of this taken for LC-MS analysis.

2.2.8 LC-MS/MS analysis of corm extracts

Samples were run on a Waters Novapak C18 (3.9x150mm) column using a gradient from 50% acetic acid/methanol to 100% methanol at 25 minutes at a flow rate of 1 ml/min.. A Finnigan LCQ (LC-MS/MS) was operated in APCI mode with data-dependent MS/MS scans from the most intense ion.

First principle searches for compounds with the molecular weight of colchicines and colchicoside were made to make possible identifications before any library searches for ion patterns were conducted.

2.2.9 Extraction and distillation of waste flowers

Flower samples were distilled in cohobation units comprising 18 litre stainless steel vats fitted with glass Liebig condensers. Due to the low volume yield of distillate, the product was collected into 15ml hexane traps. The sample was recovered from this by first drying the hexane of any residual water with sodium sulphate before filtering through #4 Whatman filter paper and drying off the hexane in an RVE at 30°C.
Solvents used for extractions including hexane, petroleum ether (hexane/pentane 20/80), ethanol and chloroform were all AR grade, redistilled to ensure purity. Extraction of flower samples were made using an initial wash of solvent (at 6 times v/w), subjected to ultrasound for 1 hour and then left on a shaker at 100 rpm in the dark overnight. The solvent was then collected and the flowers washed with 2 further washes of 1 hour each with shaking. The combined solvent was then filtered through glass wool to remove any particulates and dried down in a RVE at 30°C.

Conversion of extracts (concretes) to absolutes was achieved by first dissolving the extract in 5 times v/w of warm (50°C) ethanol. This solution was then gradually cooled to 4°C with gentle agitation and bore chilling to –10°C and filtering through a pre cooled Buchner apparatus using #4 Whatman filter paper to remove precipitated waxes. The ethanol was then dried off at 30°C in a RVE.

2.2.10 Statistical analysis

Linear regression analyses of the standard curve plots of safranal GC/FID response were performed using Microsoft Excel 2000. Statistical analysis of the comparison of the treatments was carried out using the procedures of the SAS statistical package, version 6.12, 1989-1996, SAS Institute Inc., N.C. USA. This included a log transformation of the safranal and HCC data sets before analysis of variants (ANOVA) as this provided a closer fit to a normal distribution. It should be noted that ANOVA was performed independently for each harvest time with the 2004 drying experiments. Error bars on all graphs represent standard deviations.

3. Results and findings

3.1 ISO and analytical testing of quality parameters

Full ISO testing procedures showed that local samples of saffron selected for sale from the 2003 harvest met all criteria for ISO, category 1 product, but that potential for significant aroma improvement existed. Samples of competitor's imported products fell short of the standard for the colour and bitterness measurements.

Individual grower samples from 2003 and 2004 were tested 6 months after harvest and 2 of these were retested again after 12 months extra storage. It was revealed that most (90%) of the samples had moisture samples above the 12% standard with a significant number (30%) more than 5% over this, indicating that a significant problem with insufficient drying was occurring.

For the key colour, bitterness and aroma strength measurements 15% of the samples were deficient (for ISO standard) in the former 2 properties, as well as both those re-tested after a further12 months. Storage with excessive moisture leaves the saffron prone to enzymatic degradation of the colour components and this was apparent from the re-tested samples, though colour loss can also occur prior to or during the drying process.

Comparison of the grower samples to a variety of imported products showed that while the local saffron generally compared well for colour to those from Iran and an Italian sample, the latter products had higher aroma strength measurements, except where the local products had greatly degraded in colour. None of these samples compared well with an analysis of the Spanish product (from the region renowned having the world's best saffron) that had both high aroma and colour. The differences were even greater when results for solvent extraction and GC measurement of the aroma compound, safranal were compared as an alternative to the ISO test. The Spanish sample had at least twice the safranal content of all other samples and 3-8 times the content all those where colour was not low. It also had colour strength higher than all other samples. This analysis suggested that for the local product, considerable improvement in the aroma content should be possible, without excessive colour loss.

It was shown that the ISO test procedure for aroma strength correlated very poorly with (non-polar) extraction and analytical GC analysis. The failure of the ISO method to reflect large differences in safranal content is consistent with the recent observations of several other authors – and so for all later work the analytical method was used.

3.2 Drying experiments

The stigma drying experiments conducted during the 2004 and 2005 harvest indicated that high temperature drying procedures could enhance aroma formation. Highly significant increases in safranal content were achieved when compared to lower temperature ranges (used currently by the local industry) and with improved colour retention. There was also evidence that the use of significant air-flow; an integral and unavoidable aspect of the use of the current commercial apparatus/method, causes significant loss of safranal.

In response to these results, and the difficulty encountered with the use of conventional ovens in accurately applying precise high temperatures for the relatively short periods required, a purpose built apparatus was designed and constructed to allow proper isolation of the effects of different conditions by using the same device for all treatments. It also served as a prototype for a potential new commercial dryer. This device incorporated heating via blacked out conventional light bulbs controlled by a PID temperature controller, a cylindrical aluminium structure to reduce thermal inertia and a fan with speed control.

The prototype dryer was successfully used for a series of stigma drying experiments during the 2005 harvest. The results indicated that at the elevated temperatures, significantly more safranal was generated and colour components retained, than at lower temperatures. Drying at high temperature for approximately 25 minutes gave the highest result with an 8-10 fold increase in safranal content over the lower temperature treatment currently used by the industry. Furthermore, it was confirmed that the use of air-flow causes reduced safranal levels, probably due to evaporative loss, and that this effect was significant even at relatively low airspeeds. This air-flow effect occurred at both high and low temperatures, although it was more pronounced at the former.

In critically assessing the new drying apparatus it was found that, while it worked well for the experiments undertaken, for commercial use major adaptations were required to incorporate a capacity for greater volumes of stigmas while limiting the number of drying levels to minimize temperature variations throughout. To this end it was planned to design a new device with a flatter, rectangular shape and side access to allow rapid and easy sample introduction and removal and to test this apparatus during the 2006 harvest. Before this was attempted however, the following findings shed new light on the requirements for this device.

Although not originally planned as one of the described tasks, an investigation of the importance of water activity to safranal production in the drying process was undertaken during the 2006 harvest. This work was begun as part of an ensuing PhD project, but the initial results are included here because of the important implications regarding the optimal drying procedure.

It was shown that by using a container of hot water in an enclosed (but non-pressurised) drying apparatus (oven) set at a specific high temperature to achieve an equilibrated relative humidity (RH) of approximately 50%, the drying rate of the stigmas was significantly slowed. Although seemingly counter-intuitive for a drying process, this actually allowed the stigmas to remain for longer within a critical range of water activity. As the stigma temperature was sufficiently high this allowed significantly greater (x3) hydrolysis of picrocrocin to form safranal when compared to the optimum high temperature treatments (without applied humidity) identified earlier. The colour strength of the saffron produced in this way was also enhanced.

3.3 Colchicine assay

LC-MS/MS analysis of *broad-spectrum* methanol extracts of Crocus corms at intervals over the 2003/04 summer dormancy period failed to detect any colchicine. The relatively crude isolation method employed meant that very low concentrations of colchicines or related compounds could have been missed, but in such a case commercial extraction of these would not be viable and so this part of the project was discontinued.

3.4 Waste flower products

None of the distillation products from waste flower samples was obtained in sufficient yield (all < 0.1%) to make this type of product potentially viable. It was noted that the best distillation products contained safranal and this was only obtained from flowers containing some reject stigmas.

Application of extraction trials confirmed that safranal was only generated in samples containing some proportion of reject stigmas indicating that its precursor does not occur in the petals or stamens. Products obtained from flowers without stigmas, although of pleasant aroma and attractive bright orange red colour (**Figure 3**), did not have the aroma impact for a potential fragrance product. The stigma content (w/w) of all later waste flower samples obtained from growers was therefore determined. The average content of stigmas for sample obtained from the 2004/05 harvests was found to be 0.35% by weight.

Trials of different pre-extraction treatments on fresh and frozen flowers combined with organoleptic assessment revealed that the best product was obtained from fresh flowers dried slowly at ambient temperature for 3 days in the dark. This extract was also shown to have the highest yield of safranal per unit wt. of stigmas. Drying of flowers at higher temperatures also resulted in a loss of other non-safranal aroma notes.

A trial of farm scale collection and onsite drying of waste flowers during the 2006 harvest period revealed that air drying of these flowers on horizontal mesh nets in sheds away from direct sunlight was feasible; though slow in poor weather conditions. An alternative to this method was the use of the food dryers at low temperature (30ºC) though this did result in some loss of volatiles including safranal in the extract and involves considerably more of the grower's time to perform. This loss of volatiles may not be critical if reject saffron is used to augment the extract.

Extraction with a solvent mixture of 10% ethanol in hexane gave a higher yield of product (5%) than extraction with 100% hexane (2%), but the latter had a more attractive colour and persistent aroma

3.4.1 Independent organoleptic assessment of extract

A subsample of the extract was sent to an agent in the United Kingdom who forwarded the sample to a leading perfumer, Dr J. Heffernan,(*58*). The following are his comments from an organoleptic assessment of the product:

"Quite green in dilution, vegetal, with a heavy floral back note but the green-ness is the defining factor. It has a sweet coumarin note to it as well'.

In concentration it has a floral top note - heavy floral, exotic, reminiscent of ylang ylang and jasmine. The top note isn't very 'toppy', the odour seems to kick in as a 'heart'. Exotic, narcotic flower and honey notes, and the green-ness subordinate. The green-ness is light and fresh, giving a fresh aspect to the product in concentration. Almost 'cucumbery' in its freshness, very nice.

Quite sweet and exotic in concentration. Very like French Narcissus absolute.

Market potential- small and niche. 'People would bite your hand off if it was the right price and available in quantity'. Price suggestion: several hundred £/kg would guarantee a market. You could approach SRS Aromatics, or another agent for florals to sell it. Is there a health aspect with saffron?

It could be used with synthetics to give a broader, more natural base to the perfume. In all-naturals it would give a green fresher sensation, which you could build other florals on top of, the saffron would be the heart of the floral."

Although this assessment is very much preliminary and from only one extract produced – without any replication, this expert opinion would suggest that there is a potential for a fragrance product from the waste saffron. Considerably more work is required to isolate the aroma notes in this extract type and application of GC-organoleptic (sniffer-port) techniques would allow this with complementary GC-MS analysis providing the chemical identities.

Figure 3: Waste flower extract from 2006 drying trial illustrating the bright red/orange colour of the extract product.

4. Implications

4.1 Saffron quality

4.1.1 Drying methods

The findings of this study show that, given the application of appropriate drying methodology, the quality of Australian saffron can and should be significantly improved. Most of this improvement will come from increased aroma production, but high temperature/humidity drying will also have a preserving effect on the pigment content as enzymatic degradation is prevented. This should result in consistently higher colour strengths that would be maintained in storage.

The saffron filaments produced from this process tend to be too dry and brittle for easy packaging but in the laboratory some moisture, and with it, much of the resilience is returned by equilibrating the filaments at ambient temperature in a darkened room – possibly with a warm container of water nearby to provide a slightly higher localised atmospheric humidity. This equilibration was shown to work well on a commercial scale although some variation with weather conditions (and thus ambient humidity) did have an effect and would need to be taken into account in timing the length of equilibration. The use of a high humidity drying method may decrease the need for this equilibration.

The implementation of the new drying method would best be made using an apparatus such as an oven or incubator that is enclosed so that a high relative humidity can be uniformly achieved within by simply introducing a dish of hot water. The inclusion of precise temperature control (such as PID) would be an advantage and the device should be calibrated with for the temperature settings when used with added moisture in this way. The method has worked well with an oven of 24L capacity and while a larger chamber would be good for increasing the number of stigmas that may be dried per run, if it is too large, uniformity of temperature and humidity will be more difficult to achieve.

4.1.2 Competition and growth of the industry

The local saffron industry is competing with products from countries with considerably lowers labour costs. In this situation there are 2 alternative ways of providing competitive advantage; reducing the local labour costs or producing product that is demonstrably superior in quality and therefore can attract a higher price. The only way that the former could be achieved in Australia would be through the successful implementation of mechanical flower harvesting and stigma separation.

Some documented attempts have been made to design equipment to mechanise the cutting and collection of flowers, particularly in Italy(1) but the results tended to result in excessive loss of flowers, increase impurities and have negative effects on eventual saffron quality. All the methods tried also tended to cut a considerable proportion of the leaves surrounding the emergent flowers which compromises the later growth of the corms resulting in poor flower yields in subsequent seasons.

Saffron flowering also occurs randomly through a crop over several weeks so that flowers need to be harvested on virtually every day over that period with considerable judgement applied to pick flowers at the right stage of maturity. Any successful mechanical system would require much more uniform flowering to accomplish significant labour saving. This would probably require hormonal and/or environmental manipulation of the plants. One reference was found describing work relating to the hormonal application(*2*), but at this stage not enough is known about *Crocus* physiology to achieve uniformity of flowering this way. Recent trials of indoor (glasshouse) environmental control of saffron crops has shown that flowering timing can be controlled to a degree and product yields potentially improved through more reliable flowering, but no improvement in flowering uniformity was described(*3*). Glasshouse based environmental control may be a more efficient method of producing high yielding quality saffron but considerably more research would be required to establish the precise

methods for this as well the cost benefits as this form of horticulture is much more capital intensive than the current practises.

Even supposing that mechanical harvesting of a uniform flowering crop could be achieved then that would place considerable pressure on the stigma separation and drying facilities, as the yield of flowers currently processed over several weeks would need to be processed at once. Separation of stigmas from other flower parts was also attempted in the Italian studies(1) by means of fans and wind tunnels but these were not successful because the connection between the base of the style (stigmas) and the other flower parts which tended to remain intact and because the petals tended to wrap around the stigmas unless the flower was totally open and beyond the optimal harvest stage so that quality was compromised. The process also tended to prematurely dry the flowers and result in loss of aroma.

In searching for any information on mechanical harvesting, 2 Indian websites(*4;5*) were found claiming that mechanised systems for saffron harvest, separation and drying had been developed. Attempts to contact the organisation responsible have been unsuccessful to date. There is thus no information available at present on the means of mechanisation or quality of the product from these systems. All the saffron produced in India is for the domestic market in that country and does not have a reputation for high quality(*6*).

Mechanisation of the harvesting separation process may well be feasible, but significant research effort (with a new set of objectives to those investigated here) would first be required to solve all the significant problems associated with the growth and flowering of *Crocus* without compromising saffron quality. This would require considerable financial input from an industry that has not yet reached the stage of development to justify investment of that scale.

In contrast, the present study has shown that the aroma and colour characteristics of the local product can be (with the appropriate drying technology) significantly and consistently improved, potentially to a quality level that is superior to saffron from competitors anywhere else in the world. If this allows the local Industry to grow through the capture of a larger proportion of the domestic market as well as export developments, then this would be the first step towards establishing the resources to properly investigate mechanical harvesting and processing and alternative ways of growing the crop.

4.2 New products

This study has shown that commercially attractive extract product(s) for use as a fragrance can be obtained from the waste flowers as long as those flowers contain at least a small % of stigmas. This has been shown to occur during the normal course of harvesting as stigmas are missed or rejected due to visual defects, but it is possible that the extraction could also be augmented by addition of saffron rejected for sale (due to colour loss) that adds to the aroma (and health care) properties of the product.

4.2.1 Production estimation and potential

The mean fresh flower weight for all the fresh flowers used in the extraction and distillation trials in this study was 0.99 g per flower. Based on the current production of saffron by Tas-Saff of approximately 3 kg per year(6), where approximately 250 000 flowers are required to produce 1 kg of saffron, then the current annual production of waste flowers would be approximately 750 kg of fresh weight. Given an average moisture content for these flowers of 0.86 % and an extraction yield (from dry weight) of 2.73%, the possible production of extract (concrete) per year would be approximately 3 kg.

This amount of extract may not be sufficient to create a viable market and would need to be priced higher than indicated in the perfumers comments to be worth pursuing as an option for by the Industry. However, there does exist a considerable stock (many 100g) of saffron product that is not of sufficient quality for sale due to degraded colour, but which has relatively high safranal content. The addition of

appropriate quantities of this to waste flowers for extraction could result in product of considerably greater aroma strength that would likely attract a much higher price per kg. Clear identification and quantification of extract constituents with health care properties (alluded to in the perfumer's comments) could also boost the price considerably. It should be remembered that safranal is one such compound.

Successful establishment of an extract as a commercial product could lead to a considerable increase in the volume of the *Crocus* crop with a proportion of it possibly grown exclusively for extract production. There is at present an excess of corm stock and thus a significant increase in production could be achieved relatively easily if the individual growers were given sufficient incentive. If part of the crop were grown just for extract product then the considerable labour involved with the careful separation and drying of stigmas to produce saffron would be removed from the cost of this production as flowers could be picked and dried e*n masse* at ambient temperature before storage and extraction. Use of the rejected saffron stock could be used to boost the aroma strength of the extract (during the initial years of market establishment), before crop production increased enabling the use of a proportion of the crop solely for extraction (and thus maintenance of consistently high safranal content).

Reports in the literature indicate that saffron flowers contain significant contents of potential aroma precursors such as glycosidically bound carotenoid and non carotenoid metabolites(*24;37;59*) that are non-volatile and water soluble). Some form of fermentation treatment similar to those demonstrated in products such tea, tobacco, rose(*60*) and *Boronia*(*61-63*) could be used to cleave off the sugar units from these molecules, thus releasing aglycones as aroma volatiles that may improve the yield and aroma strength of extract products.

The encouragement of the growth of the local industry through quality improvement and development of new product(s) involving investigative study of the underlying biochemistry, will better position the local saffron industry to take advantage of future trends in international demand for saffron products as the healthcare/medicinal properties of the spice become more commercially utilised.

5. Recommendations

In order to facilitate the consistent optimisation of the quality of saffron spice produced in Australia and to realise the potential for new products from waste flower material, the following recommendations are made to the local industry.

1. That saffron production from the drying of stigmas should be facilitated by applying high temperature in an elevated humidity without applied air-flow.

2. For this drying it is recommended that growers obtain suitable ovens or incubators that may be adapted to suit drying conditions, or to design and construct a saffron specific apparatus with the following features:

    - The drying device should not have a functioning fan but should have a well contained chamber with adequate door seals to allow humidity build up from an open hot water surface in the lower part of the device. Once the hot water is introduced the device should be equilibrated at the specific temperature for at least 30 min prior to introduction of stigmas.

    - The drying device should have a good quality temperature controller (such as PID) that prevents thermal inertia from causing temperature overshoot of more than 2-3ºC.

    - It is recommended that the volume of the device not exceed approximately 48 litres as too large a capacity would be difficult to heat and humidify uniformly. The chamber should have several rack positions to allow an optimum number of stigmas to be introduced for each drying run, but the distance between racks should be at least 5 cm to allow sufficient circulation and thus temperature/ humidity uniformity throughout. The racks may be covered in nylon mesh to hold the stigmas.

    - It is recommended that there should be a narrow lateral port on the side of the device to enable insertion of a thermometer or other temperature measurement probe so that the temperature of the interior can be calibrated when at elevated humidity.

3. To use periods of approximately 1-5 hours after drying for moisture re-equilibration of the saffron filaments. This should be conducted in a slightly humidified, darkened room at ambient temperature. The specific details of this process may be adjusted to suit the drying characteristics of the device (oven) used by each grower - as well as the weather conditions (humidity) on each day of drying. With experience it is relatively easy to judge when the stigmas have reabsorbed sufficient moisture based on feel.

4. That further study of the drying process would be advantageous because of the large excess of picrocrocin and the potential for very significant further safranal yield gains (in saffron and also for new products), and because the effects of drying on the cellular and subcellular structure of stigmas may impact upon the quality of saffron and this is fully not understood yet.

5. That an extracted fragrance product has good commercial potential but that more investigative work is required to refine and chemically characterise it and to determine if this or an altered extract type could also be sold for its health care properties. The flavonoid content in particular should be investigated.

6. To submit larger samples of the extracts from waste flowers, incorporating different contents of added saffron, to recommended perfumers and potential buyers for further assessment.

7. To consider conducting a local consumer and allergen trial of the extract as a fragrant moisturizing cream product to gauge consumer interest for possible local sale as a first step in the marketing process for a new product.

8. To consider the economics of increasing *Crocus* flower production for the purpose of using some of the crop solely for (less labour intensive) extraction purposes. This would allow a greater volume of extract to be available for sale, making it more attractive to potential buyers and provide better economy of scale regarding production costs.

9. To have written reports and proposals distributed and (where feasible) conduct meetings with the growers to fully disseminate the findings and recommendations of this report. This would aid in the uptake of new drying technology.

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