Saffron By-products Integrated Valorisation Using Agroresource Refining Concept (ARC)

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Abstract

Our scientific objective consists in a new saffron by-products integrated valorisation approach by applying the agroresource refining concept to the different parts of the plant in order to obtain aromatic and dye products.

On stigmas, the SPME, volatiles extraction method, has been recognized as the more adapted to a better saffron quality classification. Higher boiling point compounds have been extracted, which could constitute new quality markers. The saffron determined, by this way, as low aromatic value could be used as oleoresins in industrial process.

Fresh flowers have been extracted by hexane maceration giving an intense yellow concrete-absolute. The olfactory impact of aroma compounds has been evaluated by GC/O analysis. An important contribution of the honey note, about 50% of the total odours intensity, suggests a potent application in flavour and fragrance industry.

INTRODUCTION

Crocus sativus Linn. is well known for the high value spice obtained from its dried stigmas. The freshly picked stigmas give not the typical saffron flavour, which is developed during the drying process (Basker, 1993; Raina et al., 1996). Saffron is widely used in Mediterranean countries as a condiment for its delicate flavour and intense colour. The production of 1 kg of saffron, requires more than 160,000 flowers, representing 300 kg of floral waste (Kubo and Kinst-Hori, 1999) and also an important quantity of leaves, about 1.5 tones, formerly used as cattle forage.

Our scientific objective consists in a new saffron by-products integrated valorisation approach by applying the agroresource-refining concept (ARC) to the different parts of the plant. ARC has been widely applied on the different parts of the sunflower plant. From seed, sunflower oil has been produced and from the oil cake, cosmetic products (Diot et al., 1991) and fuels (Yorgun et al., 2001), have been made. From the steam and the wastes of flowers, essential oil was extracted and paper pulp and agromaterials with low density like polystyrene (Marechal and Rigal, 1999) have been developed.

Regarding to the few studies carried on *C. sativus* flowers, leaves and bulbs, our objective is to apply this ARC (Table 1) to *C. sativus* in order to investigate and obtain aromatic and dye by-products. Only the aromatic aspect will be presented in this paper and two valorisations will be developed i.e. the stigmas of second choice and the flowers by-products. Our investigations on stigmas, part of the plant the most used, consist in a better understanding of saffron classification. At the moment, saffron quality has been determined by the International Organization for Standardization (ISO) 3632 trade standards (ISO 3632-2, 1993) in quantifying the rate of crocin, picrocrocin and safranal. The evaluation of the saffron aroma impact has only been made by measuring safranal. Our objective is to find good markers using new analytical methods to determine saffron authenticity and quality category.

MATERIALS AND METHODS

Plant Material

The plant used in these experiments was *C. sativus*. Samples of dried stigmas and flowers came from Quercy area (South-West of France).

Treatments

800g of flowers have been extracted by maceration in 3L of hexane. Solvent was evaporated and concentrated to give a concrete. Waxes from concrete were removed using absolute ethanol.

Analytical Methods

Dynamique Headspace (DHS) technique was used to concentrate saffron volatiles. About 0.1g of dried stigmas were placed into a glass cell. Volatiles were trapped on TENAX with a stripping gas (Helium) flow rate of 30ml/min during 15min at room temperature. The same batches of sample were concentrated during 120min by the Solid Phase MicroExtraction (SPME) technique using a carboxen-PDMS stationary phase fibre.

Headspace extract were analysed by a gas chromatograph (HP 5890) coupled to a mass spectrometer (HP 5971) and performed with a BPX5 column (60m, 0.32mm i.d., 1µm f.t.). A CHISA injector (SGE device) has been used for the DHS analysis (Breheret, et al, 1997). Desorption time was 3min. The conditions were as follows: carrier gas, helium at 1.5 bar; temperature program, 40°C, 1min, 5°C/min, 140°C, 3°C/min, 240°C; split 10ml/min; detector, 300°C, injector, 240°C. Compounds were identified by comparison of their spectra with those of the NIST 98 and WILEY library and literature (Winterhalter, 2002; and Cadwallader et al., 1997).

GC-olfactometry (GC-O) system consisted of a Varian 3900 equipped with a flame ionization detector (FID) and a sniffing port. Column effluent was split 1:1 between FID and sniffing port by using a DB5ms column (50m, 0.32mm i.d., 0.52µm f.t.). Helium was used as carried gas at a constant flow rate 1.5ml/min. Oven temperature was fixed at 60°C during 3 min and increased from 60°C to 110°C at a rate of 3°C/min and then at 5°C/min to 280°C during 20min. One µl of extract was injected (split 10ml/min; 200°C injector temperature; 280°C detector temperature). An expert assessor was used for evaluation of absolute in three replicates by GC-O analyses. Odorants were described and the odour intensity was measured using a scale up to five points. Scores across replicates were analysed to give the aroma profiles of absolute.

RESULTS AND DISCUSSION

Stigmas volatiles have been identified by the GC-MS analysis (Table 2). SPME and DHS extracting methods were compared by chromatography profiles (Figure 1 and 2). The 3-hydroxy-2-butanone is higher extracted by DHS using TENAX trap. Volatiles are presents after the elution of the safranal, suggesting that higher boiling point compounds have been extracted with SPME: 2-hydroxy-3,5,5-trimethyl-2-cyclohexen-1,4-dione, 4-hydroxy-3,5,5-trimethylcyclohex-2-enone, 2,4,4-trimethyl-3-carboxaldehyde-6-hydroxy-2,5-cyclohexadien-1-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-ol. This technique seemed to be more adaptable to concentrate saffron volatiles and to evaluate saffron quality by giving more information on the volatiles extract. Bad aromatic quality of saffron could be valorised in producing oleoresin (Table 1).

The fresh flowers solvent extraction gave 1.48 g of concrete. The extraction yield was 0.19%. A yellow sweet-smelling absolute has been obtained by removing waxes. The sensory characteristics of flowers absolute were obtained by GC sniffing (Figure 3). The detection of an odour at the sniffing port below an intensity of two was considered as “noise”. Sniffing port analysis revealed that odorants could be classified in seven categories: “burned”, “roasted”, “floral”, “green”, “nutty”, “pungent honey” and “sweet honey” notes. At the beginning of the elution, fresh odours such as “floral” and “green” notes were detected by the assessor followed by a relative intense “nutty” note. In the middle of the analysis strong “honey” notes were perceived and at the end “pyrogeneous” notes were more present. The odour contribution of compounds (Figure 4) showed that “honey” notes and “nutty” notes represent respectively 50% and 20% of the total odours intensity. Sniffing port analysis of absolute gives interesting sensory data. The diversifying note and the important contribution of honey odour suggest that saffron flowers absolute could have a potent application in flavour and fragrance industry.

CONCLUSION

Our investigations consisted in finding new analytical methods to determine quality markers in saffron aroma. SPME method seemed to be more adaptable to extract a wider range of volatiles from saffron. Saffron, evaluated by SPME/GC-MS technique and determined as low aromatic value, could be used in industry to obtain oleoresins.

Flower’s wastes represent a real aromatic interest. The intense “honey” notes from absolute could find an application in fragrance industry.

Further research developments turned towards the application of the agroresource refining concept to the other parts of the plant, style, petals, stamens, leaves, stem and corms are actually underway in order to valorise all the organs of the plant.

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Tables

Table 1. Agroresource refining concept applied to *Crocus sativus*.

Parts of the plant	Extraction	By-products	Application
Stigmas 2nd choice	ethanol extraction	oleoresins	flavour and fragrance industry
style	Ethanol extraction	oleoresins	
stamen	solvent extraction	concrete/absolute	flavour & dye industry
petals	hydrodistillation	essential oil	flavour and fragrance industry
leaves & stem	hydrodistillation	aromatic waters	
corms 2nd choice	hydrodistillation oxidation	essential oil aromatic extract	flavour and fragrance industry

Table 2. Volatiles compounds identified in dried stigmas by DHS/GC-MS and SPME/GC-MS.

No.	Compounds	Mass spectral data [*m/z* (%)]	DHS/ GC-MS Area %	SPME/ GC-MS Area %
1	Acetic acid	43(100),45(88),60(80),28(35), 42(15),44(8)	-	9.4%
2	3-hydroxy-2-butanaone	45(100),43(85),29(23),88(16), 42(12),73(5),55(3)	12.2%	1.8%
3	2-(5H)-furanone/γ-butyrolactone	55(100),42(80),84(73),41(48), 86(40),110(30),109(24)	1.6%	3.3%
4	6,6-dimethylcyclohexane-carboxaldehyde	107(100),125(35),81(33),55(30), 91(28),140(28),122(15)	1.1%	0.4%
5	2,2-dimethylcyclohexane-carboxaldehyde	121(100),105(60),91(55),107(35), 150(33),79(30),151(5)	0.3%	-
6	3,7-dimethyl-1,6-octadien-3-ol (linalool)	71(100),43(85),41(80),93(75), 55(55),69(38),80(30),121(20),136(8)	0.8%	0.3%
7	2-methylene-6,6-dimethyl-3-cyclohexene-1-carboxaldehyde	121(100),91(60),107(45),79(40), 135(30),150(15),166(10)	4.4%	0.6%
8	phenylethylalcohol	91(100),92(50),122(40),65(15), 77(8)	-	0.8%
9	3,5,5-trimethyl-2-cyclohexen-1-one (isophorone)	82(100),138(25),54(10),95(8), 67(6),83(5),139(2)	9.4%	4.3%
10	2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone)	68(100),96(95),39(50),152(48), 109(15),137(13),153(5),81(4)	2.9%	1.5%
11	2,2,6-trimethyl-1,4-cyclohexanedione	56(100),42(95),139(96),154(70), 69(60),70(50),83(15)	3.0%	3.3%
12	2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaledhyde (safranal)	107(100),91(80),121(50),150(45), 105(40),79(25),135(10)	63.6%	50.9%
13	2-hydroxy-3,5,5-trimethyl-2-cyclohexen-1,4-dione	84(100),126(69),168(50),56(48), 153(40),125(38),140(35)	-	2.1%
14	4-hydroxy-3,5,5-trimethylcyclohex-2-enone	98(100),112(50),70(48), 69(46),42(20)	-	0.6%
15	2,4,4-trimethyl-3-carboxaldehyde-6-hydroxy-2,5-cyclohexadien-1-one	109(100),137(95),180(80),152(78), 123(65),91(45),165(35)	-	6.2%
16	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-ol (beta-ionol)	105(100),161(98),91(80),119(80), 121(78),176(45),133(40)	-	0.3%

Figures

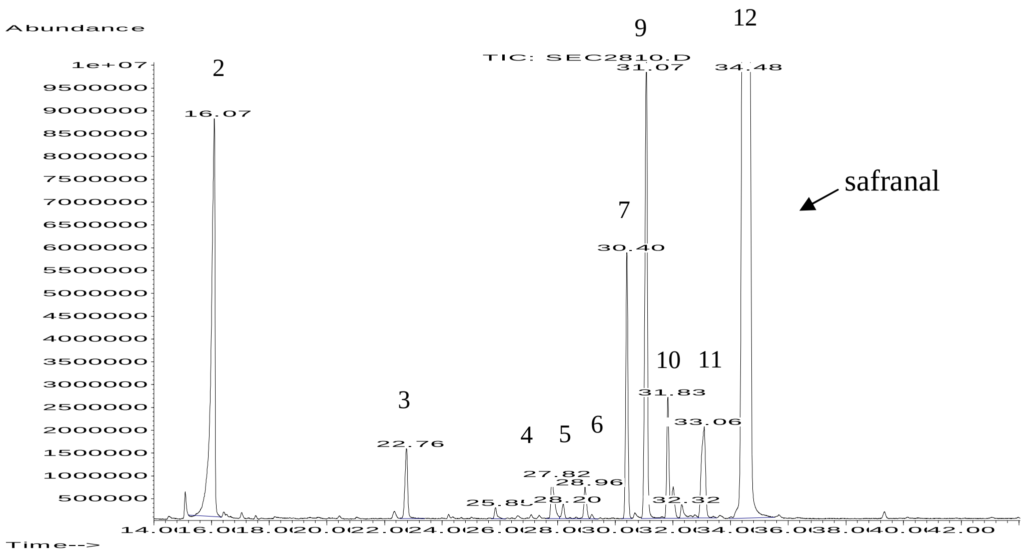

Fig. 1. Chromatogram of saffron obtained by DHS/GC-MS on dried stigmas. See table 2 for picks code.

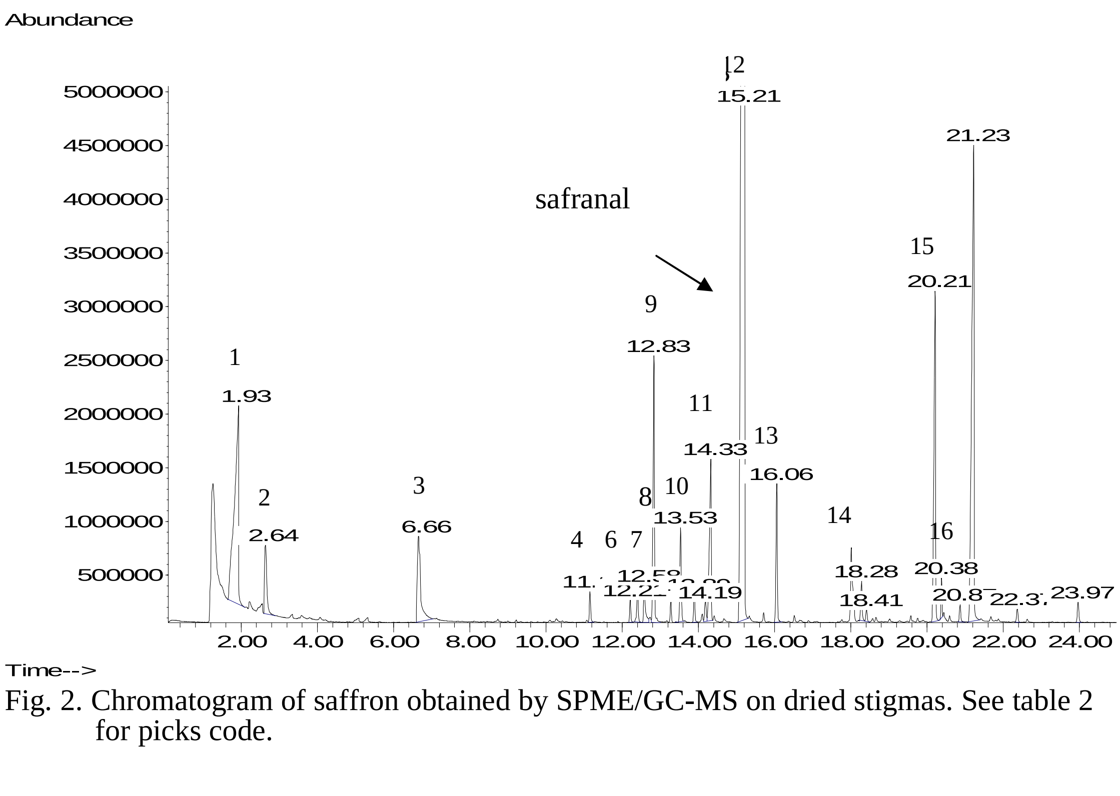

Fig. 2. Chromatogram of saffron obtained by SPME/GC-MS on dried stigmas. See table 2 for picks code.

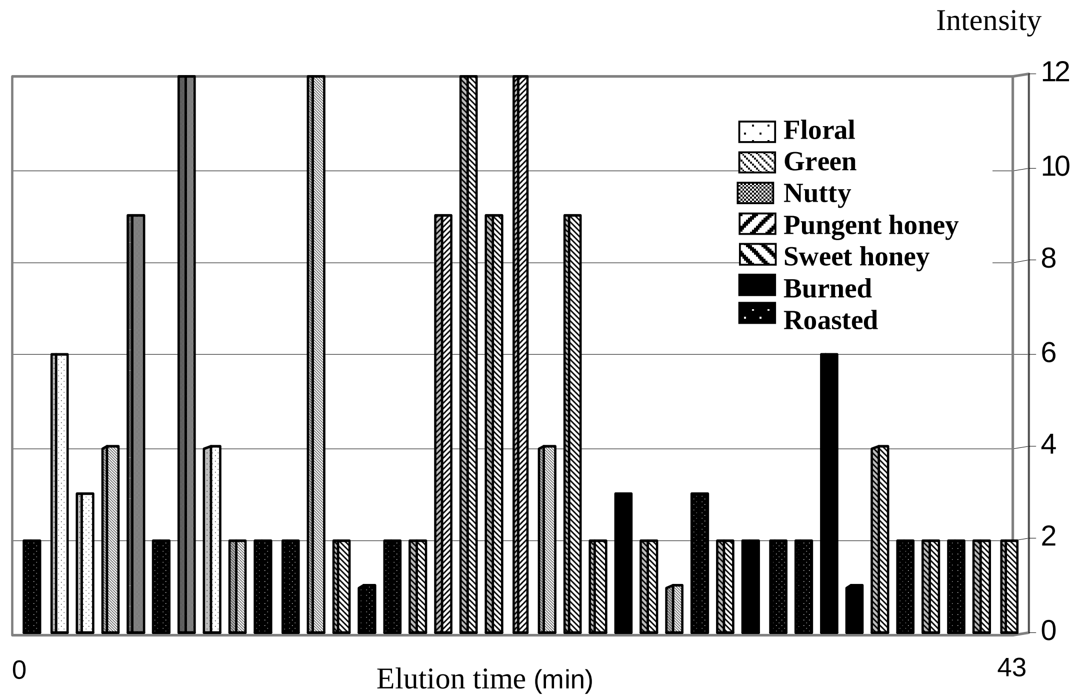

Fig. 3. Aroma profile of the absolute.

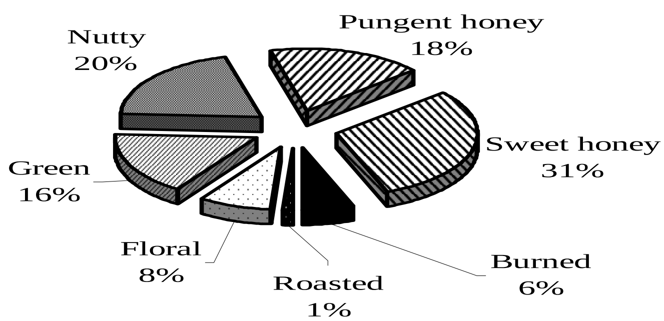

Fig. 4. Odour contribution of compounds.