Leaf Wax Extracted from Cauliflower Waste Shows Antitranspirant Efficacy

Gee-Sian Leung
Suprex
Ray Marriott
Bangor University
Michele Faralli
Fondazione Edmund Mach Centro Ricerca e Innovazione
Minuka Weerasinghe
Harper Adams University
Fiona Corke
Aberystwyth University
Melville Miles
Geencore
Peter Kettlewell (pskettlewell@harper-adams.ac.uk)
Harper Adams University https://orcid.org/0000-0001-9499-3629

Research Article

Keywords: epicuticular wax, solvent extraction, oilseed rape, canola, glycerol monostearate.

Posted Date: February 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-210747/v1

Leaf wax extracted from cauliflower waste shows antitranspirant efficacy

Gee-Sian Leung[a], Ray Marriott[a 1], Michele Faralli[b 2], Minuka Weerasinghe[b], Fiona Corke[c], Melville Miles[d 3], Peter Kettlewell[b *]

[a]*Suprex Limited, Unit 10, Zone 6, Cibyn Industrial Estate, Caernarfon, Gwynedd, LL55 2BD, UK*

[b]*Drought Mitigation Group, Agriculture and Environment Department, Harper Adams University, Newport, Shropshire TF10 8NB, UK*

[c]*Institute of Biological and Environmental Sciences, National Plant Phenomics Centre, Aberystwyth University, Aberystwyth, SY23 3EB, UK*

[d]*Freshtime Limited, The Found Riverside Industrial Estate, Marsh Lane, Boston PE21 7PJ, UK*

*Corresponding author: pskettlewell@harper-adams.ac.uk

Present addresses:

[1]BioComposites Centre, Bangor University, Bangor, Gwynedd, LL57 2UW, UK

[2]Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, 38010 San Michele all'Adige (TN), Italy

[3]Greencore, UK Centre, Midland Way, Barlborough, Chesterfield, S43 4XA, UK

Abstract

Purpose: Excessive transpiration of water from plant leaves can damage crop productivity during droughts, but commercial antitranspirants are expensive. The aim of this research was to characterise extracted wax from brassica leaf waste, and determine its antitranspirant efficacy and economics.

Methods: Yield of wax extracted with dichloromethane from six types of brassica waste was measured and the highest yielding waste was selected for bulk extraction with supercritical

CO_2. Wax was compared with a commercially-available terpene antitranspirant (di-1-*p*-menthene) for efficacy in reducing leaf water vapour loss, measured as stomatal conductance, in three experiments on rapeseed and in one experiment on wheat. Cost of wax under different production scenarios was calculated.

Results: Cauliflower leaf waste gave the highest wax yield, with the concentration varying from 1.31% (m/m) to 5.85% (m/m) in different batches of dried leaves. Nonacosane was the main component of the wax. In two of the three rapeseed experiments and in the wheat experiment, stomatal conductance was significantly reduced to similar extents by wax and by di-1-*p*-menthene, despite the wax being formulated and applied at a much lower concentration. Economic analysis showed that a high wax concentration in the cauliflower leaves would be needed to produce a commercially-viable leaf wax antitranspirant.

Conclusion: The results demonstrate biological efficacy as an antitranspirant of extracted cauliflower leaf wax. Further research is needed on variation in wax yield to reliably source high wax concentration leaves and reduce cost of production, and also to understand the greater efficacy of wax than di-1-*p*-menthene.

Keywords: epicuticular wax, solvent extraction, oilseed rape, canola, glycerol monostearate.

Declarations

Funding

This work was supported by the Food Processing Waste and By-Products Utilisation Network (FoodWasteNet) of the UK Biological Sciences and Biotechnology Research Council (BBSRC) (grant number POC15_02).

Conflicts of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and material

Raw data is available from the Corresponding Author. Extracted wax is no longer available.

Code availability

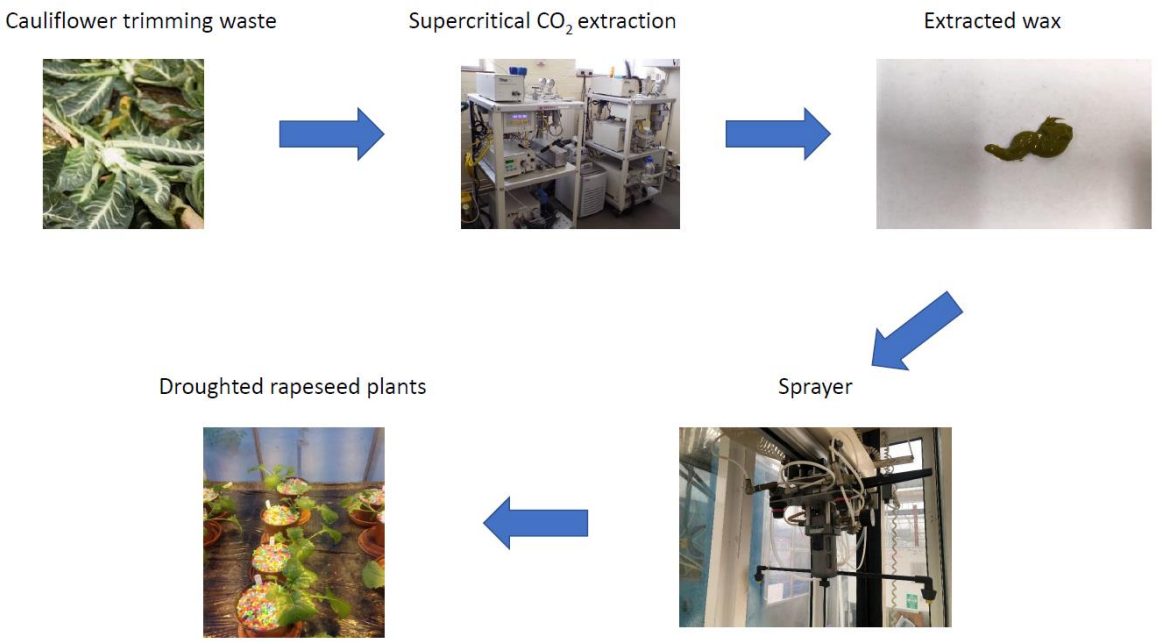
Not applicable

Authors' contributions

CRediT author statement

Gee-Sian Leung: Investigation, Methodology, Writing – Original Draft, Visualization. **Ray Marriott**: Conceptualization, Funding Acquisition, Project Administration, Supervision. **Michele Faralli**: Formal Analysis, Investigation, Methodology, Writing – Original Draft, Writing – Review and Editing. **Minuka Weerasinghe**: Formal Analysis, Investigation, Writing – Review and Editing. **Fiona Corke**: Supervision, Writing – Review and Editing, **Melville Miles:** Resources. **Peter Kettlewell:** Conceptualization, Formal Analysis, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft, Writing – Review and Editing.

Graphical abstract



Statement of Novelty

Cauliflower trimming waste currently has no financial value to fresh produce processors, and this study is the first to demonstrate the potential for valorisation of this waste by extracting wax and formulating it as an antitranspirant i.e. a spray for droughted crops to reduce transpiration and damage to yield.

1. Introduction

Water shortages throughout the world, exacerbated by climate change, are accelerating the adoption of technologies to help crop production use less water [1,2]. One little-used technology is the retardation of water vapour loss from stomata on the leaves of crop plants by application of polymers, referred to as film antitranspirants (ATs) in this context. These polymers are used on ornamental plants, but not yet widely-used on major food crops [3]. Recent research has revealed the potential for these polymers to reduce food crop yield loss from drought if application is timed to drought-sensitive stages of development (e.g. wheat [4,5] rapeseed [6]. Appropriately-timed AT applications under drought have been previously associated with enhanced water saving strategies, lowered abscisic acid accumulation and increase in leaf intrinsic water use efficiency [3,4,5,6] leading to sustained key yield components under distinct reduced water availability patterns [3,6]. However, the current commercially-available film AT products are expensive and a cheaper film AT is needed to facilitate use in crop production.

One possibility for producing a cheaper product may be to extract leaf surface wax, which plants have evolved as a natural barrier to reduce water vapour loss from the leaf cuticle covering the majority of the leaf surface [7]. Brassica species have a substantial layer of leaf wax [e.g. 8], and currently brassica leaf trimming waste is disposed of in the UK as anaerobic digestion or livestock feed with no value to fresh produce processors. The hypothesis tested in

91 our study was that brassica trimming waste may have the potential to acquire value as a
92 source of wax that can be formulated into a novel film AT.
93
94 The objectives of the studies described in this paper were:
95 1. To determine the wax concentration and composition after solvent extraction from
96 small quantities of different types of brassica waste;
97 2. To extract wax using supercritical CO_2 ($scCO_2$) from a larger quantity of the type of
98 brassica waste with the highest wax concentration, and to formulate the wax for spraying;
99 3. To evaluate efficacy of this wax formulation in reducing water vapour loss from
100 glasshouse-grown plants of rapeseed (*Brassica napus*) (Expts 1,2,3 conducted at Harper
101 Adams University) and wheat (*Triticum aestivum*) (Expt 4 conducted at Aberystwyth
102 University).
103
104 **2. Methods**
105 *2.1 Solvent extraction of wax and its characterization*
106 Six types of brassica (all *B. Oleracea* L.) waste were sourced by Freshtime: cauliflower
107 leaves, spring green cabbage leaves, broccoli flower heads, savoy cabbage leaves, broccoli
108 stems, green cabbage leaves. Approximately 30 g of each brassica waste were immersed in 50
109 cm^3 dichloromethane for 24 hours and the dichloromethane was subsequently removed *in*
110 *vacuo* and wax yield determined.
111
112 The wax compounds were analysed with an Agilent 6890 gas chromatograph coupled to an
113 Agilent 5973 mass spectrometer (EI detector) equipped with a Zebron ZB5-MS column (30 m
114 x 0.25 mm x 0.25 μm). The GC-MS system was controlled by MSD Chemstation software
115 equipped with NIST/EPA/NIH Mass Spectral Library. The carrier gas was maintained at 1

116 $cm^3.min^{-1}$ helium, injector temperature was 250ºC and had a split ratio of 50:1. Mass spectra
117 were recorded in electron impact (EI) ionization mode, scanning m/z 40 to 600 in 1 second.
118 The temperature program was as follows: 60ºC (1.0 min hold), 8ºC/min to 340ºC (20.00 min
119 hold).
120
121 *2.2 ScCO2 extraction of wax and its characterization*
122 Cauliflower was chosen for subsequent extraction trials which were carried out using a Thar
123 SFC-1000 laboratory plant fitted with a 100 ml extractor and a 250 ml separator. At the end of
124 each extraction trial, the leaf wax deposited in the separator was recovered using
125 dichloromethane and the dichloromethane was subsequently removed *in vacuo*. Initially, a
126 two-stage extraction trial was used with the conditions shown in Fig. 1. At the end of the
127 second stage of the extraction trial, the extractor was depressurised at a rate of 1 bar/2 seconds
128 and the yield of the leaf wax was measured and GC-MS was used to analyse the wax
129 composition as described above. In all subsequent extraction trials, the operating conditions as
130 described at Stage 1 in Fig. 1 were used to extract wax from cauliflower leaves.
131
132 A larger-scale extraction trial was carried out using a Thar SFC-1000 laboratory plant fitted
133 with a 2000 ml extractor and a 500 ml separator. Four independent extraction trials were
134 carried out and in each extraction trial, approximately 550 g of air-dried, milled cauliflower
135 leaf was packed into the extractor. Extraction trials were carried out with the conditions
136 shown in Fig. 2, and the total leaf weight is shown for all four extraction trials. At the end of
137 each extraction trial, the extractor was depressurised at a rate of 1 bar/2 seconds. After four
138 extraction trials were carried out, the leaf wax deposited in the separator was recovered using
139 dichloromethane and the dichloromethane was subsequently removed *in vacuo*. The yield of

140 the leaf wax was expressed as % m/m yield and GC-MS as described above was used to
141 analyse the wax.
142
143 A 6% (m/m) cauliflower leaf wax formulation was prepared and supplied to Harper Adam
144 University for *in vivo* plant assessments by adding into hot water wax and glycerol
145 monostearate at a ratio of 1:2 along with 1.0% of Tween 20. This mixture was homogenised
146 at 4000 rpm using a IKA T18 basic Ultra-Turrax until an emulsion was formed.
147 The drying of the leaves was the most time-consuming process during the production of the
148 cauliflower leaf wax and so a final experimental extraction was carried out to evaluate
149 whether the wet leaves could be directly extracted to recover the waxes using the Stage 1
150 conditions in Fig. 1, as previously.
151
152 *2.3 Wax evaluation experiments*
153 *2.3.1 Plant material and experimental design for rapeseed experiments*
154 All the rapeseed experiments were carried out inside an environmentally controlled
155 greenhouse at Harper Adams University. Seeds of rapeseed (cv. Excalibur) were sown in 1 L
156 pots filled with ~ 600 g of John Innes #2 compost at 22 ± 1% volumetric water content
157 (VWC) analysed with a soil moisture probe (ML2X theta probe, Delta-T-device, Cambridge,
158 UK). Three seeds per pot were sown and the pots were thinned to contain one plant at the 2nd
159 leaf stage. The pots were manually watered approximately to saturation on the day that the
160 seeds were sown and no water was applied until the seedlings appeared. After the seedlings
161 appeared, until the application of the watering and AT treatments, the pots were manually
162 watered approximately to saturation every other day.
163

All three experiments consisted of a 2 x 3 factorial design with two watering levels (well-watered, WW and water stressed, WS) and three spray treatments (water, di-1-p menthene and wax) in six or eight randomised blocks. The measurements for the three experiments were conducted in November 2016, January 2017 and February 2017 respectively.

2.3.2 Water management and treatment application for rapeseed experiments

The available water content (AWC) in mL of the pots was calculated by plotting a volumetric water content (VWC) - pot weight curve: three pots (filled with ~600 g of compost at 22 ± 1% VWC) were water-saturated and then dried over ten days at 30°C. The VWC by soil moisture probe (ML2X theta probe, Delta-T-device, Cambridge, UK) and the weight by balance (0.1 g resolution, PCB 2500-2, Kern and Sohn GmbH, Balingen, Germany) were recorded daily. For John Innes #2 compost the permanent wilting point and the pot capacity were ~7% VWC and ~45% VWC respectively according to [9]. The total AWC in mL was then calculated as the difference between the weight of the pot at pot capacity (~1000 g) and the weight of the pot at 7% VWC (~650 g) measured by moisture probe.

Before the spray treatments were imposed the surface of each pot was covered by 100 g of plastic beads, so that the water evaporation from the soil surface was minimised. Then the pots were watered until the weight of each pot was 1000 g, so that the pots are at pot capacity. After this date, the plots belonging to the WS regime were not watered. The pots belonging to the WW regime were watered every other day to maintain the pot weight at 1000 g.

The spray treatments were applied at 4th leaf stage, just after the pots of the WS regime were watered (to pot capacity) for the last time. The three treatments were as follows: water (for control); 1% v/v Vapor Gard (di-1-*p*-menthene 96%, Miller Chemical and Fertilizer LLC,

189 Hanover, USA) in water; 1% v/v wax in water + 0.5% v/v Wetcit. For Expts 1 and 2, the
190 adaxial surface of the leaves was uniformly sprayed using a small hand-held sprayer until the
191 surface was fully covered. For Expt 3, the plants were sprayed using a custom-built automatic
192 pot sprayer with nozzles at 50 cm height from the plants, 3 bar pressure at 1 m/s speed using
193 Flat Fan 015 nozzles (Teejet, USA) delivering the equivalent of 200 L/ha.
194
195 *2.3.4 Physiological assessments for rapeseed experiments*
196 Stomatal conductance (water vapour transmission - gs) was measured with a transient state
197 diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). The measurements were taken
198 on several days after spraying. The equipment was calibrated before every use. Three readings
199 for adaxial gs and abaxial gs were taken from the 3rd leaf of each plant, and the mean
200 calculated. Data were collected between 11.00 am to 2.00 pm, in a block wise manner to
201 minimise any diurnal effect on gs.
202
203 Plant water use (WU) was quantified every day from the date of the spray application until the
204 plants were used for growth analyses 10 days after spraying (DAS). The weight of each pot
205 was measured with a balance between 8.30 am to 9.30 am. It was assumed that the beads
206 completely blocked evaporation of water from soil. Therefore, plant water use was considered
207 equal to transpiration. Daily transpiration or water use of each pot belonging to the WS
208 regime was calculated as the difference between the weight of the pot on the day and the
209 weight of the pot after 24 hours. Daily transpiration or water use of each pot belonging to
210 WW regime was calculated as the difference between the weight of the pot on the day after
211 watering (which is ~1000 g) and the weight of the pot after 24 hours, before watering.
212 Cumulative water use of each plant for the period of experimentation was calculated by
213 summing up daily water use.

214

215 *2.3.5 Plant material and experimental design for wheat experiment*

216 Spring wheat seeds (cv. Paragon) were sown on the 10th of October 2016 in trays and
217 germinated in controlled environmental conditions at ~200 µmol $m^{-2}s^{-1}$ of light, 15°C of
218 temperature, 60% relative humidity and watered every two days. After one week from
219 germination the seedlings showing similar growth were transplanted into 3.5 L pots (one plant
220 per pot) containing the same amount (1100 g) of growing substrate (Levington F2, Fisons,
221 Suffolk, UK). The pots were then transferred inside the National Plant Phenomics Centre
222 (NPPC, Institute of Biological, Environmental and Rural Sciences [IBERS], Aberystwyth,
223 UK) and placed onto the NPPC conveyor. Plants were automatically watered through the
224 automatic system every day and soil moisture was maintained above 30% of volumetric water
225 content. A liquid feed (Chempak No. 2 25:15:15 NPK, Thompson and Morgan) was applied
226 just before GS39 to the whole experiment. During the experiment, plants were grown at 17.7
227 ± 1.56°C and ~60% of relative humidity and an average daily photon flux density of 400
228 µmol $m^{-2}s^{-1}$ from natural light supplemented by high-pressure sodium lamps (16-hr/8-hr
229 light–dark photoperiod) system. The experiment was arranged in a randomized complete
230 block 2 x 3 factorial design with two levels of watering regime (well-watered, WW and
231 water-stressed, WS) and three levels of AT application (as in rapeseed experiments) in eight
232 blocks.

233

234 *2.3.6 Water management and treatment application for wheat experiment*

235 Before full flag leaf emergence (GS39, BBCH wheat growth scale) the watering was applied
236 to the pots by the automatic NPPC watering system ensuring pot capacity to all the plants. Pot
237 capacity was ~2,350 g and the available water content (AWC) of ~1100 mL was estimated by
238 subtracting the pot weight at wilting point (~1250 g, from water retention curve) from the pot

239 weight determined with the gravimetric system. In order to estimate plant water use (WU) soil
240 evaporation was minimized by placing 150 g of plastic beads at the top of the pot (and
241 included in the pot target weight). The beads were then kept stationary in the pot by using a
242 lightweight plastic frame fixed with three metal nails.
243
244 Drought and AT treatments were applied at GS41 on the 30th of November 2016. WW pots
245 were maintained at ~2350 g throughout the experiment. Drought was imposed on WS pots in
246 three steps: a first step of complete dehydration (DAS 1 to DAS 4), a second step of low soil
247 moisture maintenance (DAS 5 to DAS 8) where pots were re-watered to 1450 g (if target
248 weight was below that value), and a third step (DAS 9 to DAS 12) of dehydration. Pot weight
249 was recorded in the morning (~8:00) as well as re-watering to WW pots. Pots were fully re-
250 watered to the WW target weight on DAS 13. AT treatments of either water, 0.5% v/v Vapor
251 Gard or 0.5% v/v of leaf wax in water emulsion were applied with a hand sprayer to give
252 complete adaxial coverage.
253
254 *2.3.7 Physiological assessments for wheat experiment*
255 Total adaxial and abaxial gs was measured between 08.30 and 15:00 on the flag leaf of
256 selected tillers on DAS 3 (n=5), 6 (n=5), 9 (n=6) and 12 (n=6) by using a WALZ GFS-3000
257 system (WALZ, Effeltrich, Germany) with a 4 cm^2 cuvette. Daily WU was estimated as the
258 difference in weight after 24 hours. Daily water use was summed to give cumulative water
259 use over the stress period.
260 *2.3.8 Statistical analysis*
261 All the data were analysed using Genstat (18^{th} Edition, VSNi, Hemel Hempstead, UK). Data
262 were checked for normality and homoscedasticity following visual assessment of residuals vs
263 fitted values plots. The sum of adaxial and abaxial gs were analysed with a three factor

(watering regime x AT x time) randomised complete block repeated measures ANOVA. Since there were no significant interactions between AT and time, the means over all assessment dates are presented. Cumulative water use was analysed by a two factor (watering regime x AT) randomised complete block ANOVA.

3. Results

3.1 Wax yield and composition from solvent extraction of different types of brassica waste

The yield of wax from the six types of brassica trimming waste was: cauliflower leaves 1.51 % (m/m), spring green cabbage leaves 0.21 % (m/m), broccoli flower heads 0.20 % (m/m), savoy cabbage leaves 0.11% (m/m), broccoli stalks 0.06 % (m/m), green cabbage leaves 0.03 % (m/m). Cauliflower leaves had a notably higher wax content compared to other sources of brassica waste and it was thus identified as the most economical source of wax. GC-MS showed that the most abundant compounds in all these samples of waxes were nonacosane, 15-nonacosanone and triacontane (Fig. 3). Other compounds detected in the waxes were free fatty acids, long chain alcohols, long chain diols, long chain alkanes, wax esters and sterols.

3.2 Wax yield and composition from $scCO_2$ extractions

The input and output quantities for the two stage sequential extraction of air-dried cauliflower leaves using $scCO_2$ are shown in Fig. 1. The wax obtained in Stage 1 had shown that nonacosane, 15-nonacosanone, triacontane and γ-sitosterol were the principal components and these compounds were again detected as the most abundant compounds in the wax obtained at Stage 2 of the extraction.

The economics of the process was evaluated and it was concluded from the poor yield obtained using higher operating pressures and temperatures at the second stage, that a second stage was not economic.

In the batch of cauliflower leaves used in the scale-up trial, there was less leaf stalk and leaf blades were larger, leading to a higher dry matter (Fig. 2). A lower yield of wax was obtained

289 in this trial and the principal components were the same as in the two stage trial (Fig. 4). It
290 was seen that there was a decline in the extraction yield of wax throughout the project and this
291 may be due to the seasonal variation of the leaves.
292 When two fresh leaves (92 g) were extracted, the yield was 0.03 g of wax (0.033% m/m), and
293 analysis of this wax showed that the principal components were nonacosane, 15-
294 nonacosanone and triacontane (Fig. 4).
295 *3.3 Wax evaluation experiments*
296 The three rapeseed experiments differed in gs and WU (Fig. 5), probably linked to
297 environmental differences dependent on the time of year. For all three experiments, a
298 significant ($p<0.001$) reduction in both gs and WU from water stress was observed as
299 expected (data not presented). The interaction between spray treatment and watering regime
300 was not significant in all three experiments, therefore only the main effect of spray treatment
301 is presented in Fig. 5. In Expts 1 and 2, both AT and wax reduced gs to similar extents
302 ($p<0.001$). The effects of AT and wax in Expt 3 were not significant ($p=0.267$), possibly
303 because all the plants in this experiment had low values of gs. For WU in Expt 1, AT gave
304 only a small (non-significant) reduction, whereas wax significantly reduced water use by
305 17%. WU was not affected by either AT or wax in Expts 2 and 3.
306
307 For the wheat experiment (Expt 4), the spray treatment and watering regime interaction was
308 significant ($p=0.039$) for gs and the data to show this interaction is presented in Fig. 6. Both
309 AT and wax reduced gs to similar extents in the well-watered plants, but did not reduce gs in
310 the water-stressed plants which had very low values. WU was not affected by AT in Expt 4.
311
312 **4. Discussion**
313 *4.1 Wax characterization and extraction economics*

The dominance of the composition of our extracted wax by C29 compounds, notably the alkane nonacosane is consistent with previous work on *B. Oleracea* [e.g. 10, 11]. Laila et al. [11] give wax concentration values of 0.15% on a fresh weight basis, and re-calculating our single stage extraction result on a fresh weight basis gives a similar value of 0.18%.

Wax formation in brassica species is strongly influenced by environment [e.g. 10], giving differences between leaf samples taken from different locations and at different times of year. In our study this gave more than a fourfold variation in wax yield (from 1.31% m/m to 5.85% m/m) over a 6-month period, which has implications for the economics of the extraction. There appears to be no difference in composition linked to wax yield, consistent with the findings of Baker et al. [10], so the highest concentration should always be selected. Further research to decide the optimal time of year for leaf collection is necessary.

The cost of extracting functional extracts from biomass is largely determined by product yield, extraction time and the volume to be processed. It appears from our work that the highest wax content is in the leaf blade waste rather than in the stalk or leaf mid-rib, so that there is a clear economic advantage in pre-sorting to remove stalk if possible. The wax yield from fresh trimmings is very low and in addition the high moisture content appears to modify the polarity of the scCO2 further reducing the yield. Fortunately, the leafy material can be easily dried at low temperature (35ºC with high air flow) to give a more easily processed material with a higher wax content. We consider that this step is essential for the process to be economical as only 10% of the biomass is processed with a much higher wax yield compared with fresh material.

The $scCO_2$ process cost is very influenced by volume and given that the end application could require high volumes of extract, commercial costs for large-scale drying and extraction should be considered. If we consider a scenario where 1000 kg of wax extract is required and the dried biomass has a wax content of 5% we need to extract 20 t of dried cauliflower leaf (133 t wet mass). At this scale the cost/ton input material would be approximately £3,000 so the 1000 kg wax would cost £60,000 or £60/kg. If the dried leaf biomass contains only 1.5% then 66.7 t (445 t wet mass) needs to be extracted but this would be slightly lower cost due to the higher mass processed (£2,500/t) but the overall cost/kg wax would rise to £167. Conversely if the required mass needed of wax was to rise to 10,000 kg then extraction cost would fall to approximately £1,500/t and so at 5% wax in dried biomass the wax cost/kg would fall to £30/kg.

There is clearly some optimisation that could be achieved in the biomass selection and preparation and it may be possible to shorten the extraction time a little by optimising the extraction parameters. Using the above estimates for cost per kg, however, an approximate cost of production for a formulated wax AT (based on the wax cost only) at 6% (m/m) as used in this research, would vary from £1.80/l (10,000 kg batch @ 5% wax) to £10.02/l (1,000 kg batch @ 1.5% wax). The active substance (di-1-*p*-menthene) in the commercial AT used in our study is no longer sold in the UK because of high cost relative to other products, but previously the retail price was £20/l (B. Lewis, Intracrop, personal communication). Only at the lowest wax cost in the above range would this allow a commercially viable AT to be produced (S. Adams, Plant Impact, personal communication), and if other costs are added e.g. transport of raw material to processing site, formulation components, the economics may be marginal. Conversely, the economics could be more favourable if the wax extraction was an integrated part of biomass biorefining and electricity generation.

363

364 *4.2 Wax evaluation*

365 The spraying experiments show that wax formulated with glycerol monostearate is generally
366 as effective as di-1-*p*-menthene in reducing water vapour transmission when sprayed at 1%
367 (v/v). This is a much lower concentration of active substance (a.s.) for wax, since the wax
368 before dilution was 6% a.s. whereas di-1-*p*-menthene before dilution is 96% a.s., implying a
369 16-fold greater activity of the wax and probably much lower optimum concentration of wax
370 than for di-1-*p*-menthene. Three possible hypotheses to explain this greater activity could be:

371 1. wax is much more effective than di-1-*p*-menthene at blocking stomata;
372 2. the glycerol monostearate used to formulate the wax for spraying also acted as an AT;
373 3. another plant component co-extracted with the wax had AT activity.

374

375 For the first of these hypotheses, there is some evidence in the literature that a wax could be a
376 more- active AT than a terpene. Davies and Kozlowski [12] compared a petroleum-derived
377 wax product (Folicote) with Vapor Gard on *Fraxinus americana* seedlings and although they
378 found very little difference in transpiration reduction in the first 8 days, thereafter efficacy of
379 both products declined but less for the wax so that it was about five times more effective than
380 Vapor Gard. Anderson and Kreith [13] also included a petroleum-derived wax and a terpene
381 (beta-pinene - similar to di-1-*p*-menthene) and found that the wax was superior by about 20%
382 to the terpene in reducing transpiration in wild herbaceous species relative to a water control.

383

384 For the second hypothesis concerning AT activity of the other formulation component, both
385 the alcohol and acid parts of the ester glycerol monostearate are known to have AT properties,
386 possibly suggesting that the ester may also be an active AT. For example, glycerol reduced

leaf water loss of *Monstera deliciosa* [14] and stearic acid reduced corn and soybean transpiration [15].

Thirdly, it was visually apparent that the wax contained chlorophyll and thus probably also other impurities, some of which may possess AT activity. For example, if the plant growth substance abscisic acid was concentrated in the extracted wax, this could function as a metabolic AT [3] to enhance the film AT activity of the wax.

Although the literature cited gives some support for the hypothesis that wax is innately more-effective than a terpene, this advantage of the wax was much less than the 16-fold difference found in our study, and one or both of the other hypothetical mechanisms may also be responsible for additional enhancement of AT activity of the leaf wax. Further research will be necessary to investigate these hypotheses to understand the greater activity of the wax than of di-1-*p*-menthene.

5. Conclusions

This project has evaluated various sources of brassica leaf trimming waste and identified that cauliflower leaves were the best source of plant waxes. It was concluded that the extraction carried out at 350 bar and 50°C generated the highest yield. Leaf wax concentration was very variable and a concentration of at least 5% (m/m) will be needed to be economically viable. The wax was as effective as a commercial AT at reducing water vapour loss, but at a much lower concentration, implying much greater efficacy.

Acknowledgements

This work was supported by the Food Processing Waste and By-Products Utilisation Network (FoodWasteNet) of the UK Biological Sciences and Biotechnology Research Council

(BBSRC) (grant number POC15_02). We are grateful to Dominic Scicchitano (Miller Chemical and Fertilizer, USA) for providing Vapor Gard.

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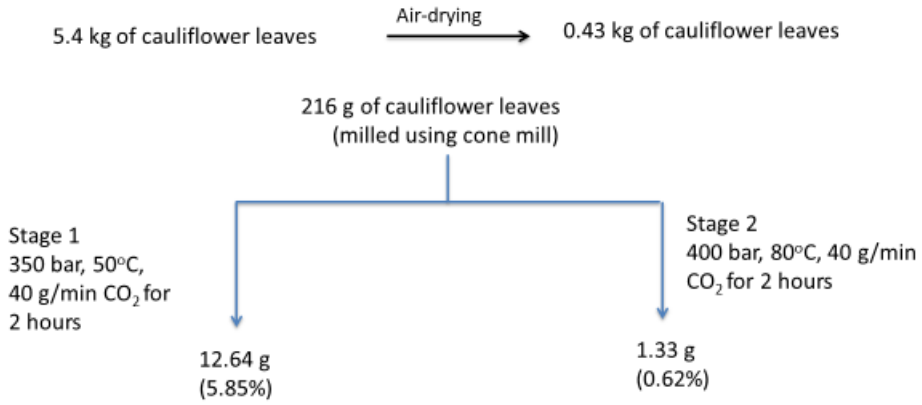
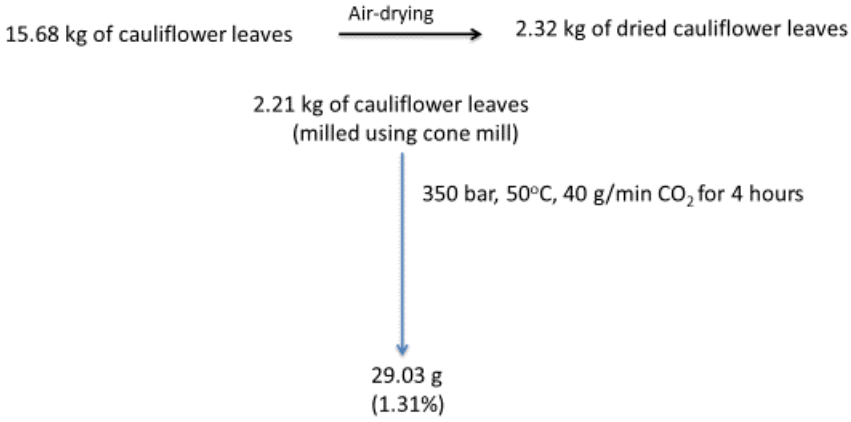


480

481 **Fig. 1** Input and output quantities for the two stage sequential extraction of cauliflower leaves

482 using $scCO_2$

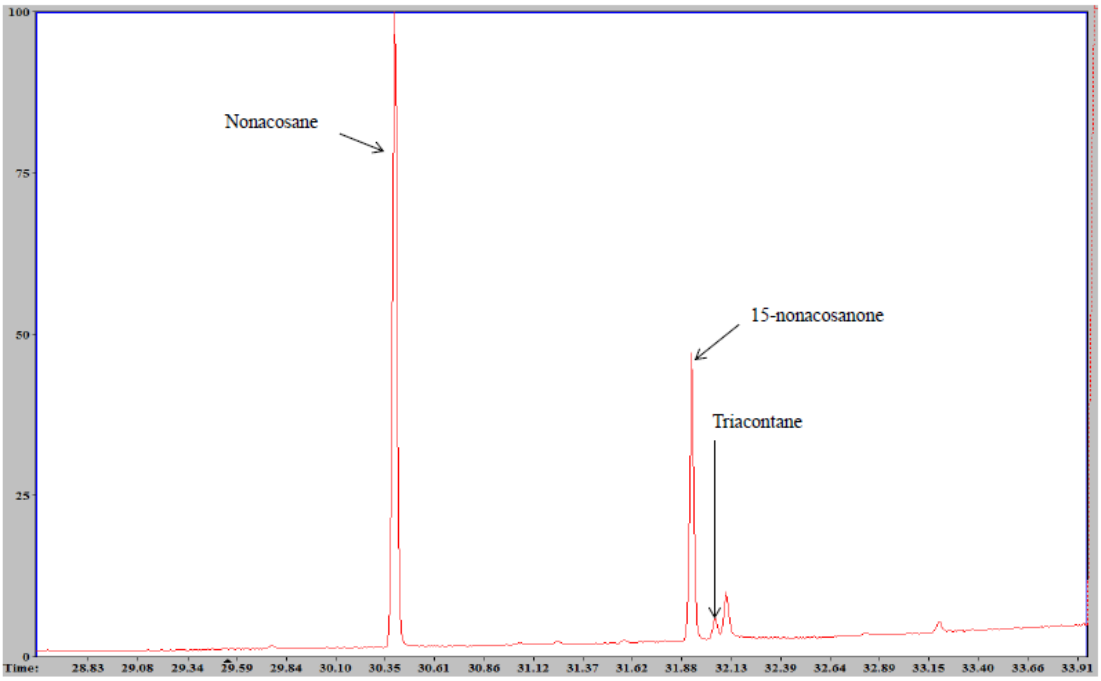


483

484 **Fig. 2** Input and output quantities for the single-stage extraction process to extract waxes

485 from cauliflower leaves using $scCO_2$

(a)



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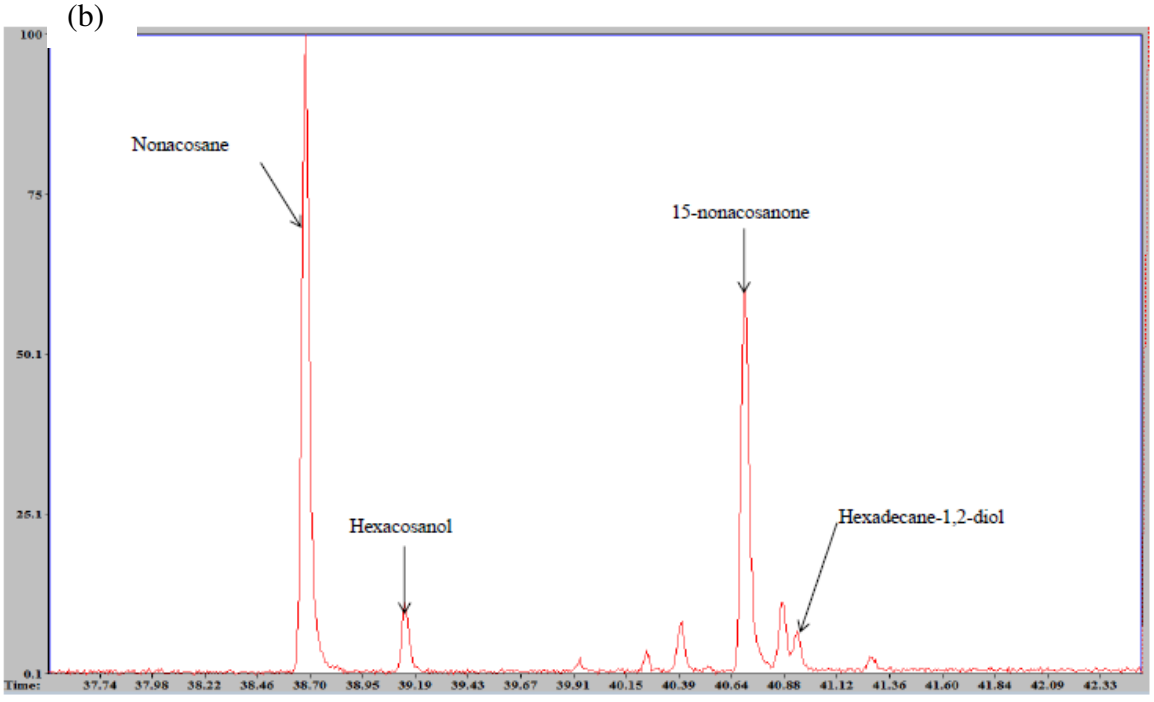
489

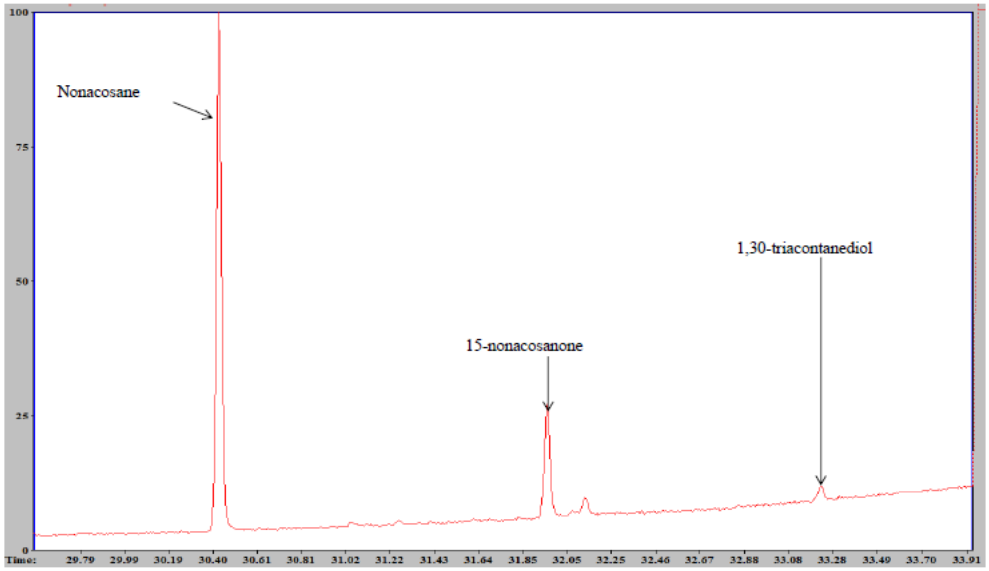
(b)



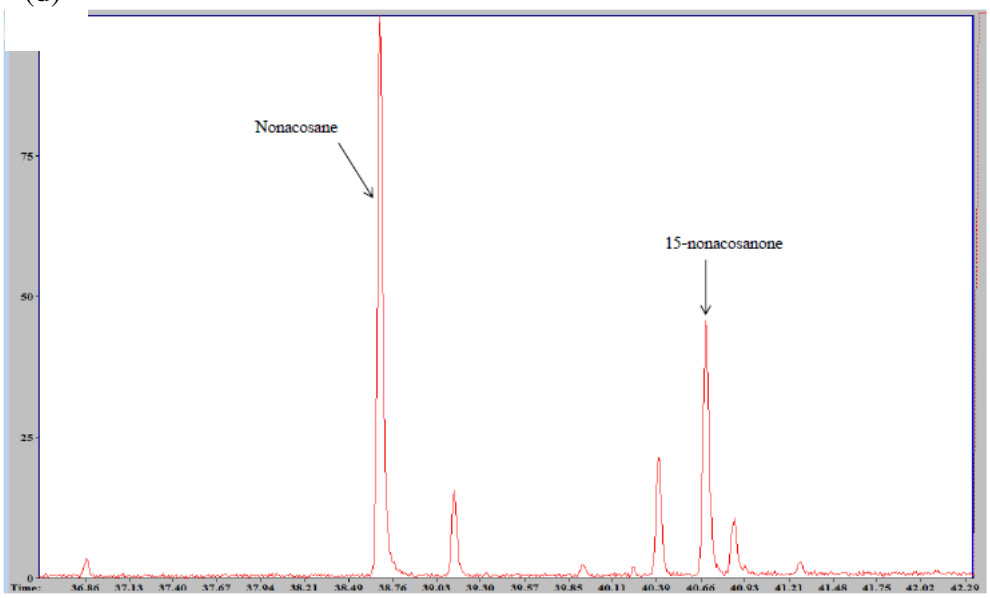
490

491

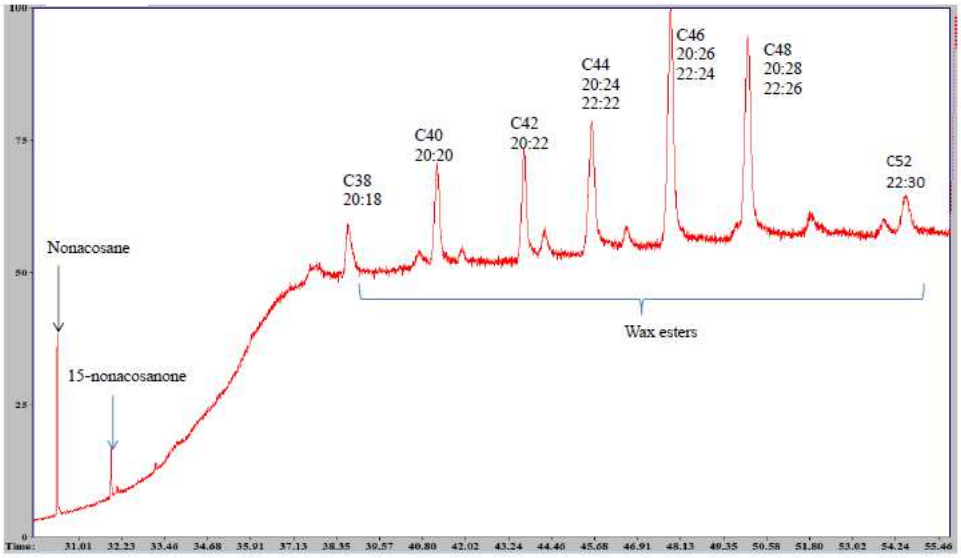
(c)


Nonacosane
15-nonacosanone
1,30-triacontanediol

(d)


Nonacosane
15-nonacosanone

(e)



(f)

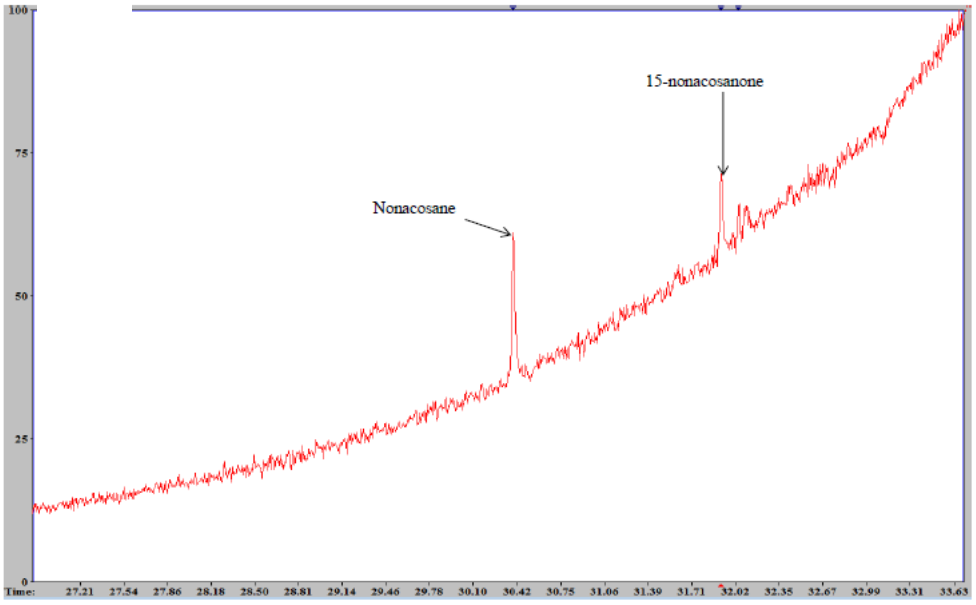
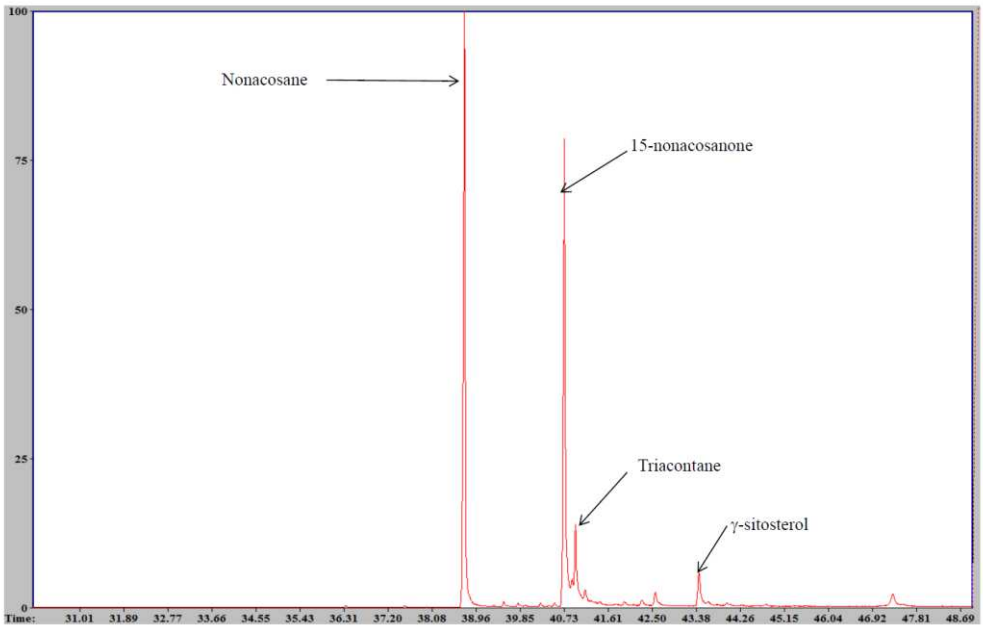


Fig. 3 GC-MS chromatograms of soluble wax compounds found in six types of brassica wax extracted with dichloromethane from (a) cauliflower leaves, (b) spring green cabbage leaves, (c) broccoli flower heads, (d) savoy cabbage leaves, (e) broccoli stalks, (f) green cabbage leaves

(a)


Nonacosane
15-nonacosanone
Triacontane
γ-sitosterol
100
75
50
25
0
Time:
31.01 31.89 32.77 33.66 34.55 35.43 36.31 37.20 38.08 38.96 39.85 40.73 41.61 42.50 43.38 44.26 45.15 46.04 46.92 47.81 48.69

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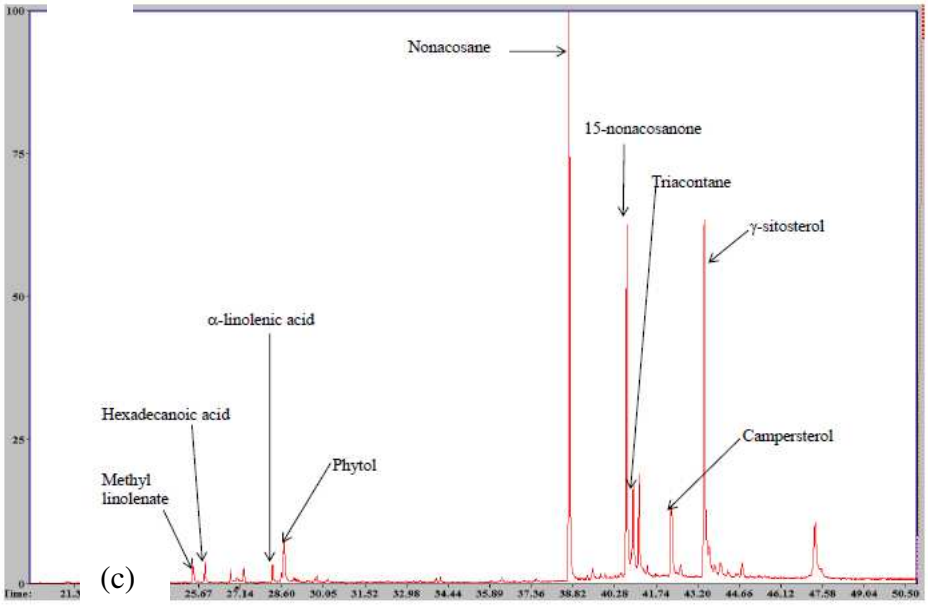
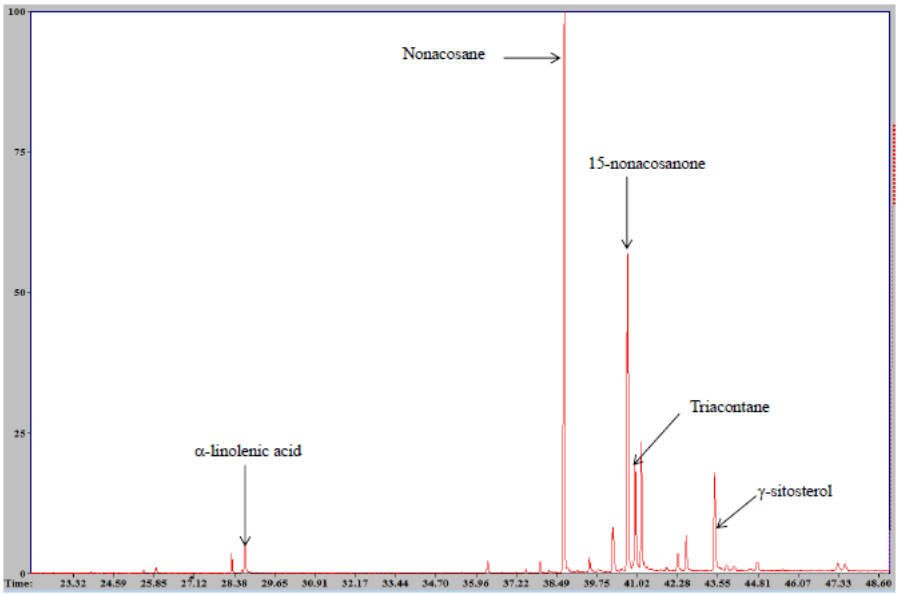
526

527

(b)



528 (c)



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(d)

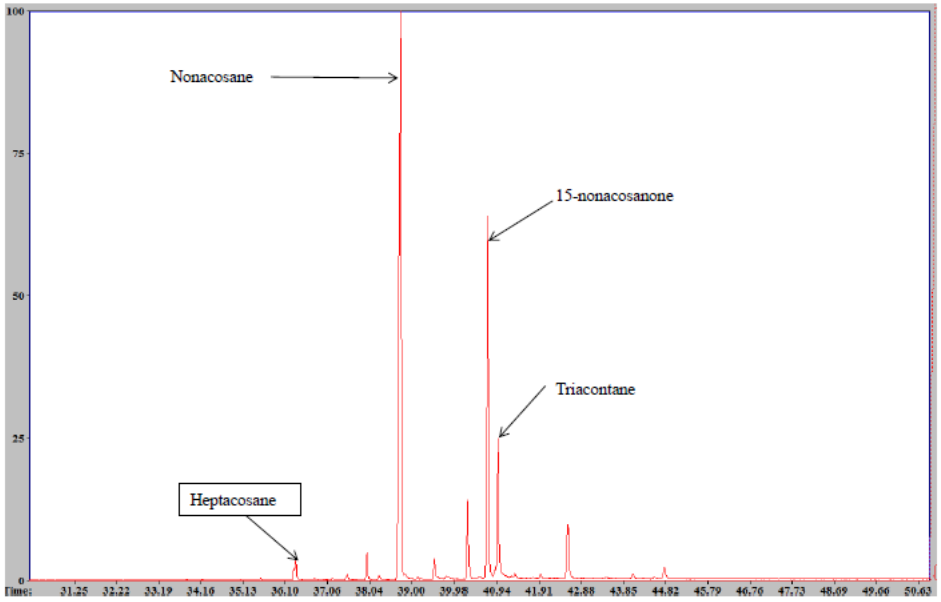

Fig. 4 GC-MS chromatograms of soluble wax compounds in cauliflower leaves extracted using $scCO_2$ from (a) first stage fraction, (b) second stage fraction, (c) single-stage trial, (d) fresh leaves

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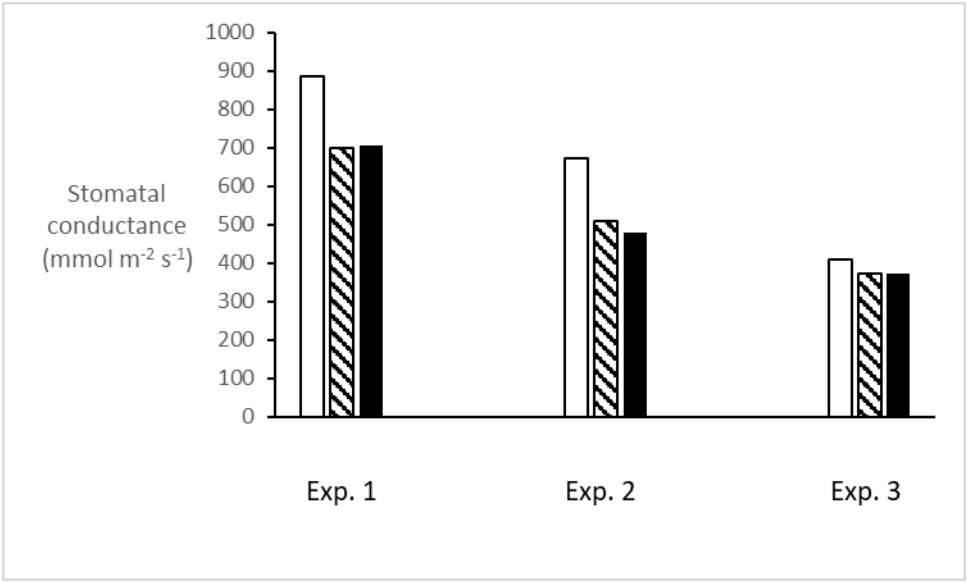
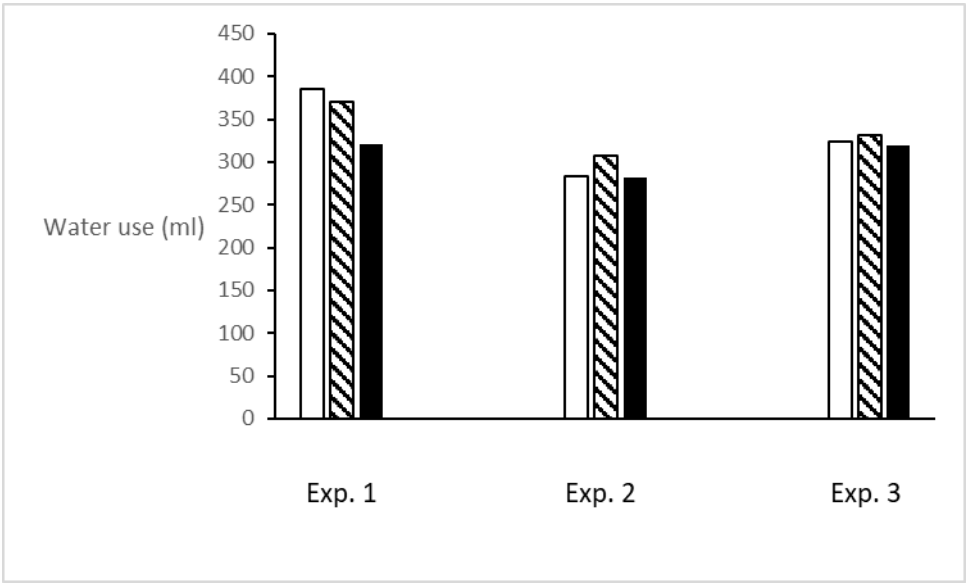
559 (a)



560

561 (b)

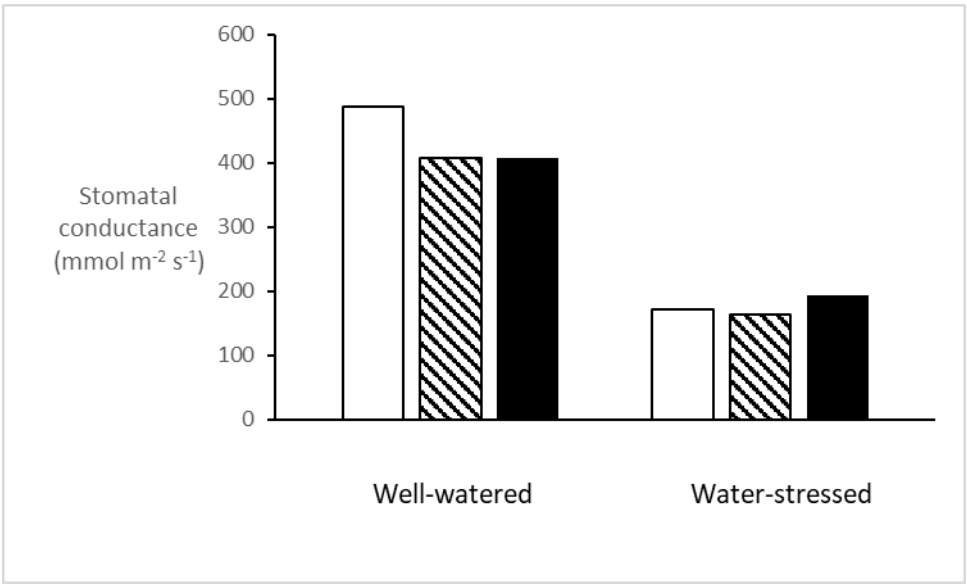


562

563 **Fig. 5** Comparison of sprays of water (unfilled bars), AT (hatched bars) and wax (filled bars)
564 in three rapeseed experiments on (a) summed abaxial and adaxial stomatal conductance (mean
565 over several dates up to 10 days after spraying) and (b) cumulative water use over 10 days
566 after spraying. SEDs (DF) are (respectively for Expts 1, 2 and 3): stomatal conductance 18.2
567 (25), 28.4 (35), 25.8 (25); water use 15.0 (25), 14.7 (35), 13.9 (35)

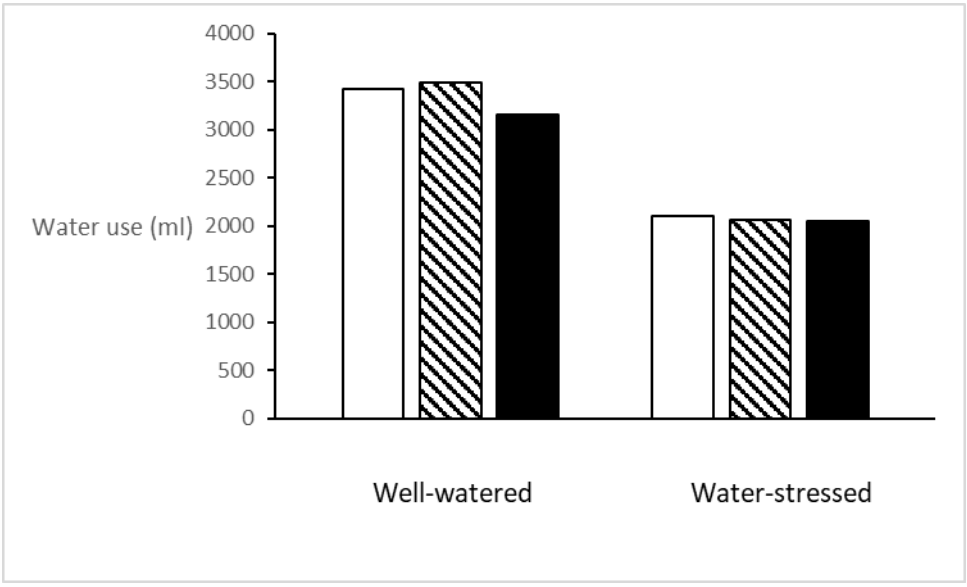
568

569 (a)



570

571 (b)



572

573 **Fig. 6** Comparison of sprays of water (unfilled bars), AT (hatched bars) and wax (filled bars)
574 for two watering regimes in wheat on (a) summed abaxial and adaxial stomatal conductance
575 (mean over several dates up to 10 days after spraying) and (b) cumulative water use over 10
576 days after spraying. SEDs (DF) are: stomatal conductance 1.25 (25); water use well-watered
577 219 (14), water-stressed 65 (14) (variance heterogeneity prevented a combined ANOVA of
578 well-watered and water-stressed data)

Figures

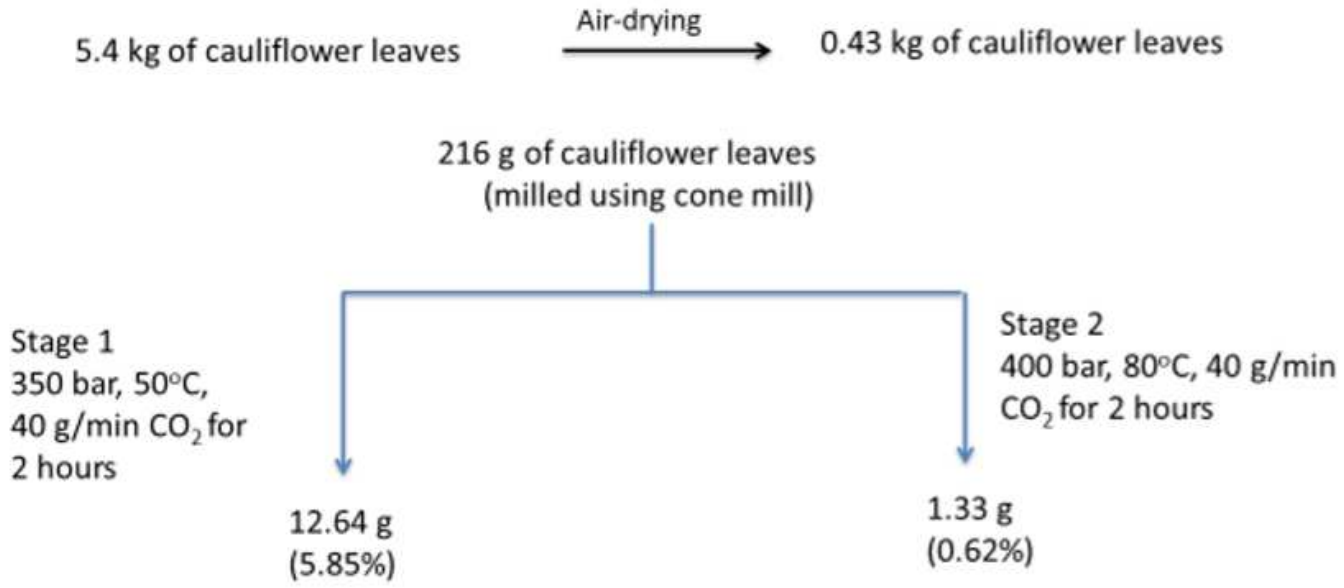


Figure 1

Input and output quantities for the two stage sequential extraction of cauliflower leaves using scCO2

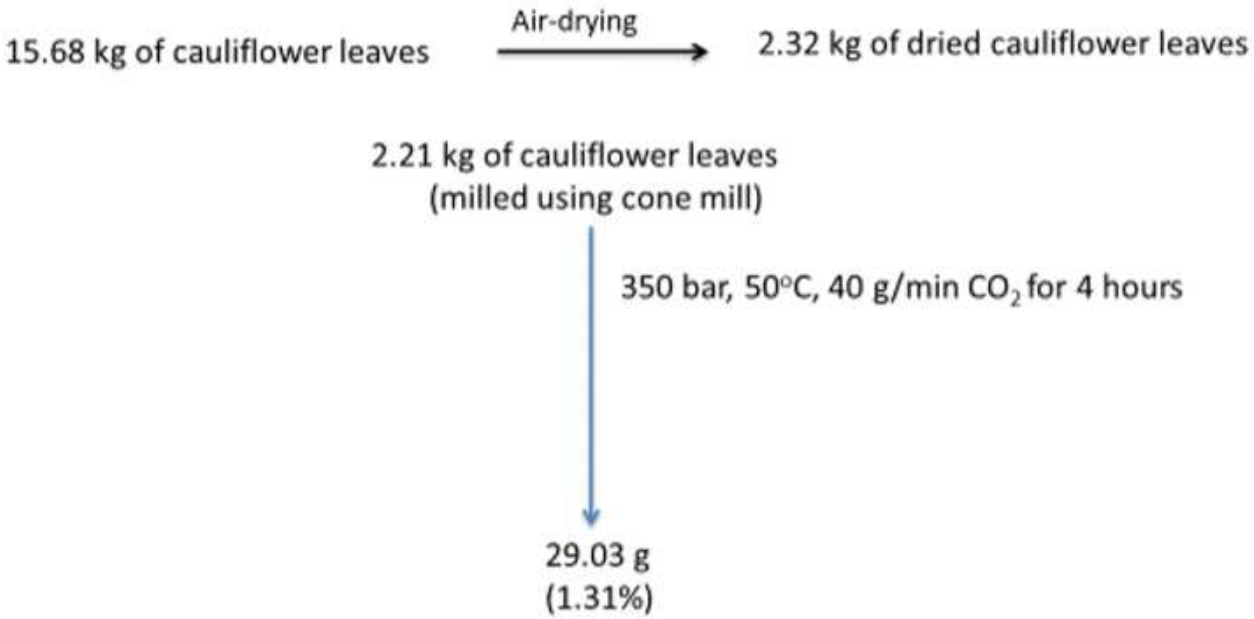


Figure 2

Input and output quantities for the single-stage extraction process to extract waxes from cauliflower leaves using scCO2

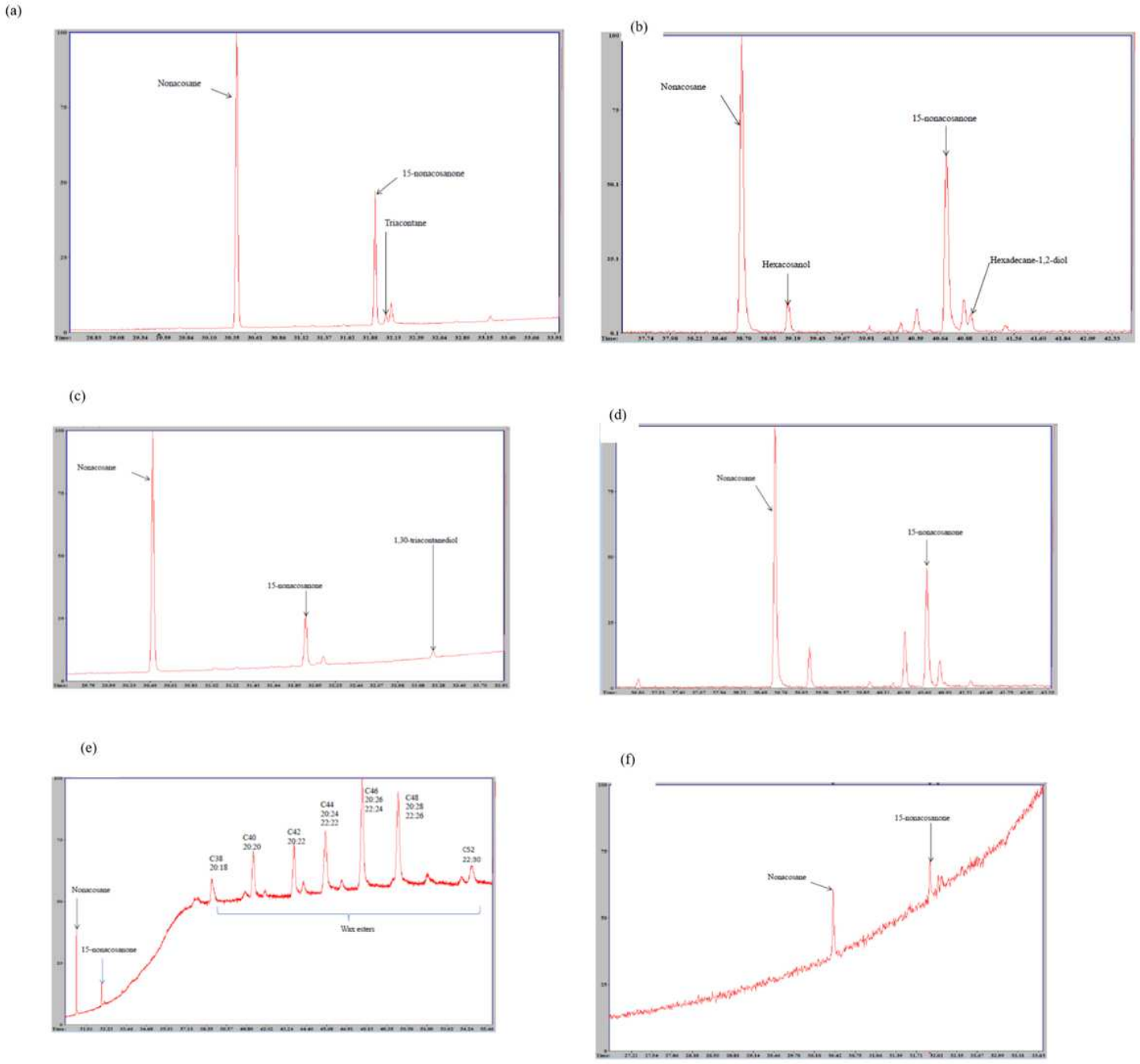


Figure 3

GC-MS chromatograms of soluble wax compounds found in six types of brassica wax extracted with dichloromethane from (a) cauliflower leaves, (b) spring green cabbage leaves, (c) broccoli flower heads, (d) savoy cabbage leaves, (e) broccoli stalks, (f) green cabbage leaves

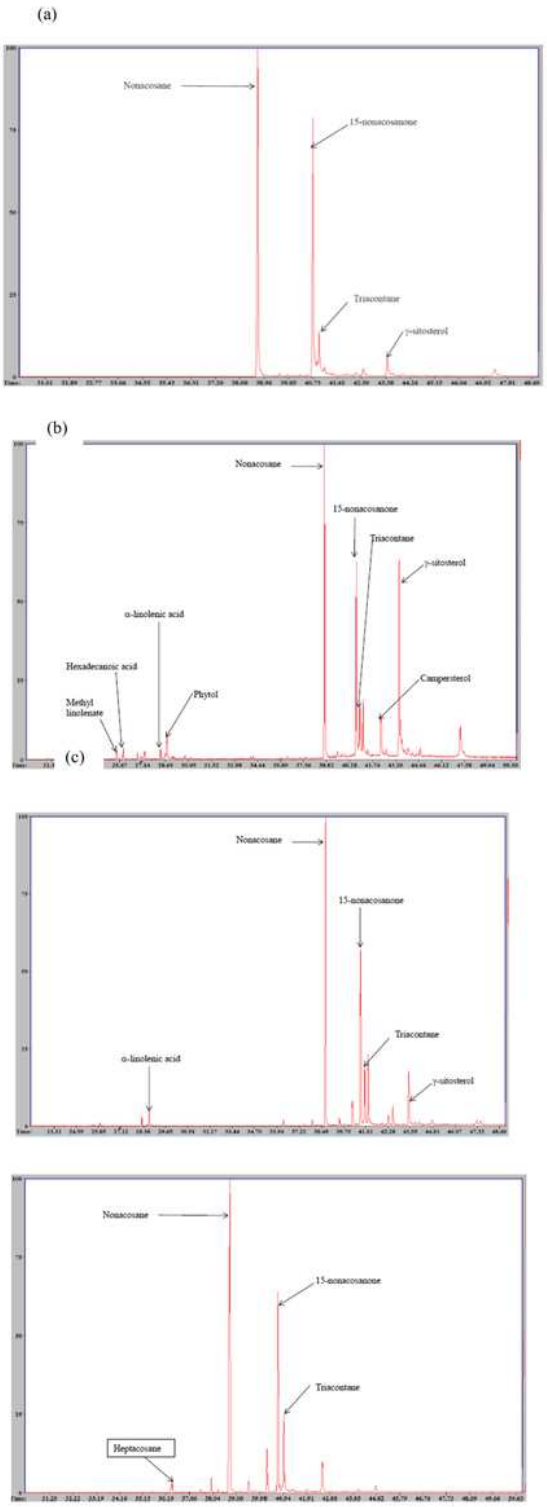
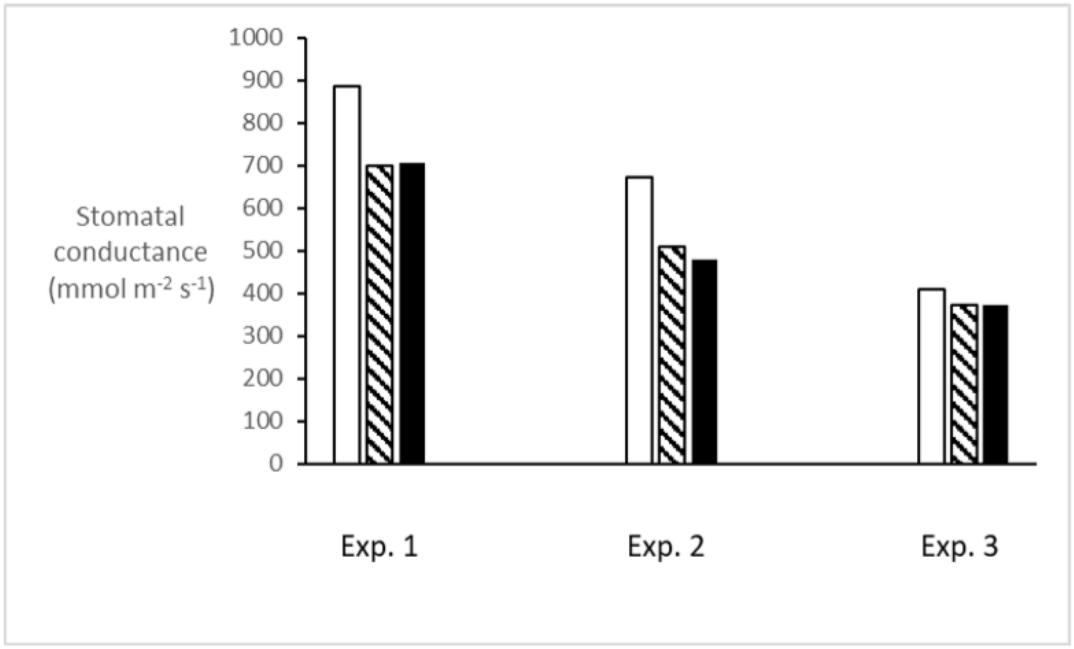


Figure 4

GC-MS chromatograms of soluble wax compounds in cauliflower leaves extracted using scCO2 from (a) first stage fraction, (b) second stage fraction, (c) single-stage trial, (d) fresh leaves

(a)



(b)

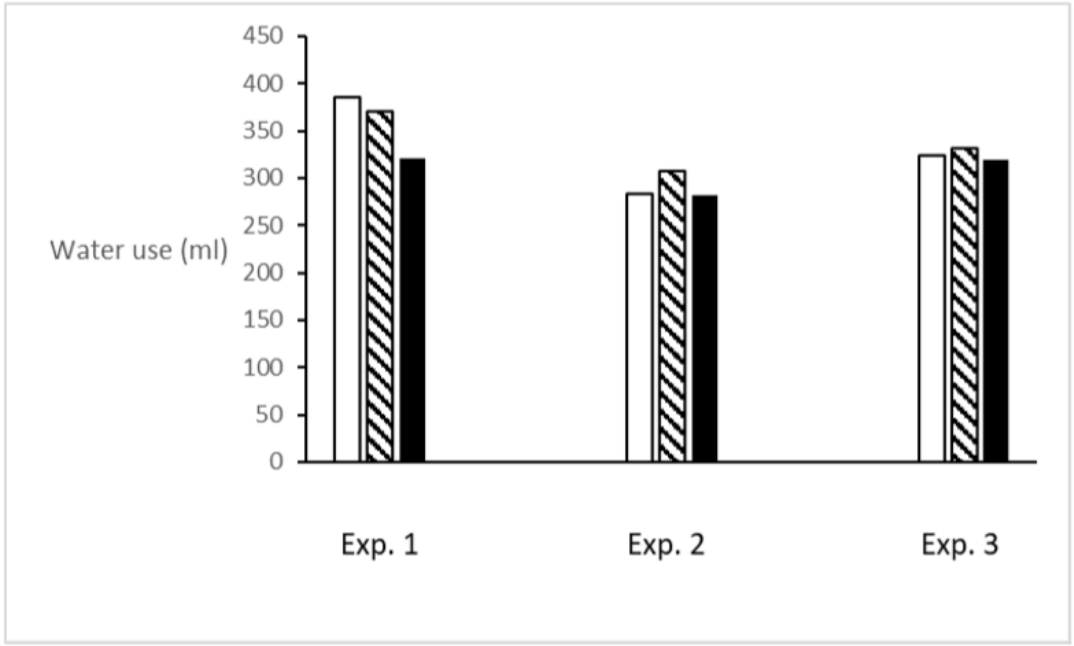


Figure 5

Comparison of sprays of water (unfilled bars), AT (hatched bars) and wax (filled bars) in three rapeseed experiments on (a) summed abaxial and adaxial stomatal conductance (mean over several dates up to 10 days after spraying) and (b) cumulative water use over 10 days after spraying. SEDs (DF) are (respectively for Expts 1, 2 and 3): stomatal conductance 18.2 (25), 28.4 (35), 25.8 (25); water use 15.0 (25), 14.7 (35), 13.9 (35)

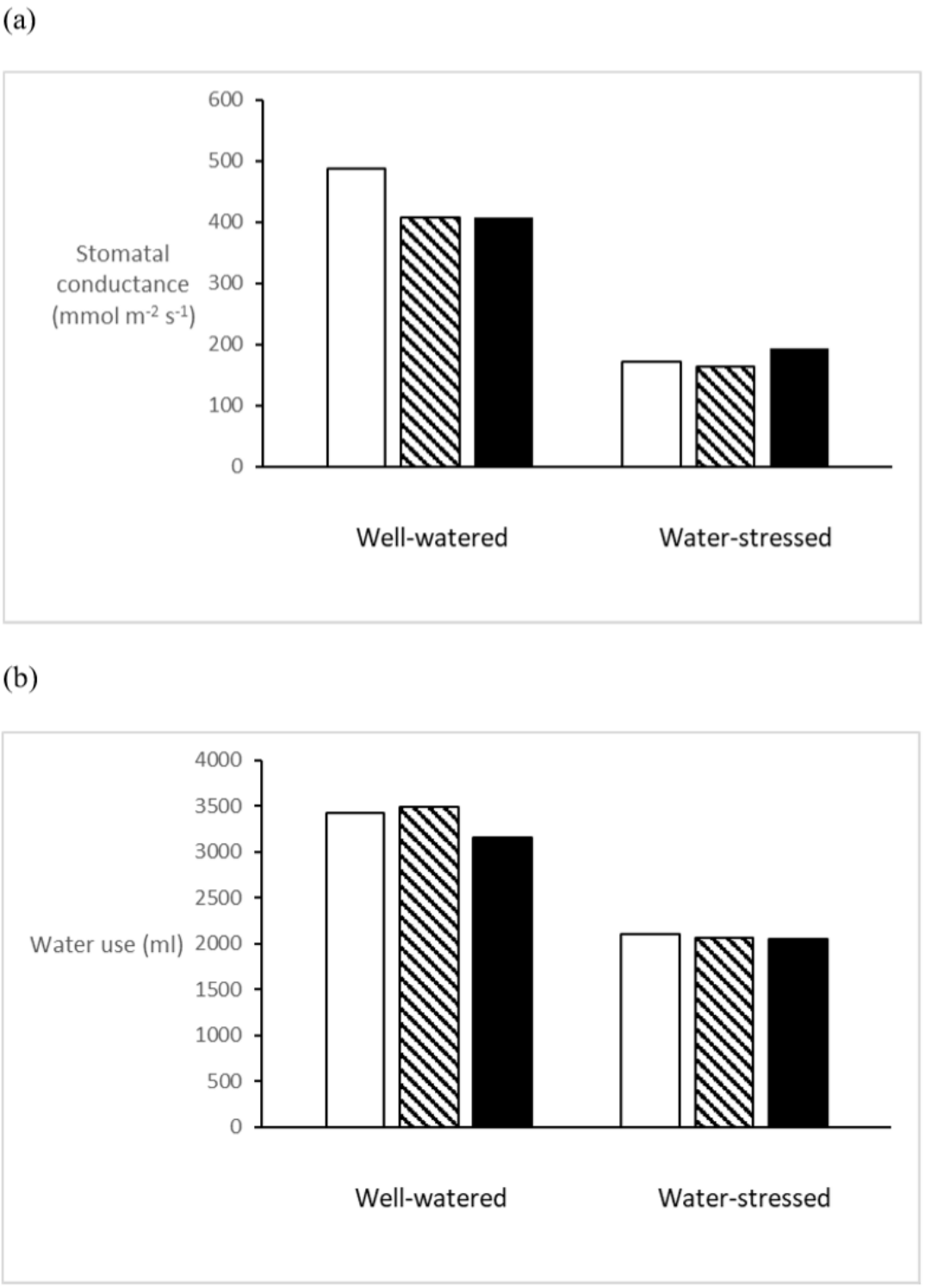


Figure 6

Comparison of sprays of water (unfilled bars), AT (hatched bars) and wax (filled bars) for two watering regimes in wheat on (a) summed abaxial and adaxial stomatal conductance (mean over several dates up to 10 days after spraying) and (b) cumulative water use over 10 days after spraying. SEDs (DF) are: stomatal conductance 1.25 (25); water use well-watered 219 (14), water-stressed 65 (14) (variance heterogeneity prevented a combined ANOVA of well-watered and water-stressed data)