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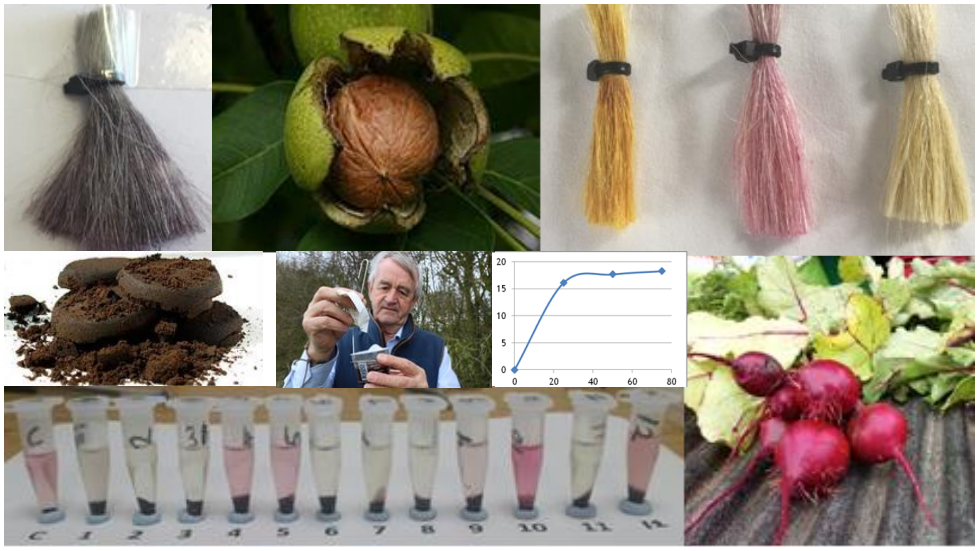
BioBoost

Accelerating biobased horticulture



Pilot Cosmetics

Biotransformation of Coffee, Walnut, Blackcurrant and Blackberry Waste for new Colourants

(WP5)

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NIAB

Eastern Agri-Tech Innovation Hub

BioBoost – Pilot Cosmetics

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1. Summary

BioBoost partner NIAB has led the project activities for valorisation of crop co-products to produce new products for cosmetics. In order to ensure economic outcomes and continuity of the initiative beyond the scope and lifetime of the project, NIAB has worked very closely with SME's in the Eastern Agri-Tech Innovation Hub (EAIH) incubator north of Cambridge. NIAB has worked especially closely with Steve Taylor MD of Celbius Ltd and Zembra Ltd, who is a licensee based at EAIH and an expert in this field. Working with Steve and others NIAB set up a pilot plant at the site and delivered data out of that initiative.

NIAB has set up a pilot plant at the EAIH, as shown in Figure 2, with designated workspace and equipment. NIAB identified crop co-product and waste feedstocks and collaborated with commercial growers; especially, RW Walpole to provide Blackberry and Blackcurrant waste for this project. By enabling contact between Steve Taylor and Bonnie Mitchell, a business consultant with contacts in the health and beauty industry, NIAB has escalated the Route to Market for Celbius Ltd to a much higher level. These contacts are currently sourcing funding to upscale processing based on a pilot to pre commercialisation.

UK Blackberries Growers are facing a new problem to economic production caused by the spotted wing Drosophila, unlike other species of fruit fly, this species lays its eggs within the undamaged fruit. In order to minimise the chance of attack, blackberry farmers must now strip off all remaining fruit and remove it from the environment after the main harvest. This waste is now available to be used in alternative products and has led to the availability of large amounts of blackberry and other fruit waste. Using this crop product the NIAB team has investigated sonic-assisted biotransformation of a purified anthocyanin extract seeking new derivatives, for the future development of new natural safe products, for example as colourants and preservatives in cosmetic products.

NIAB has also been investigating the market for brown colourants in the retail and professional hair product markets. The majority of feedstock's for brown colourants are almost all synthetic chemical products. However, there are a few notable exceptions such as henna, which has a long history of use in this area. This activity within the BioBoost project sought to investigate whether alternative sources of brown colours could be obtained from crop co-products and waste material, with an emphasis on avoidance of toxic solvents and extensive chemical processing. Three potential sources of brown colourant were investigated, namely spent coffee grounds, olive pomace (imports) and UK walnut skins.

A green extraction protocol was demonstrated for the brown pigment in walnut skins using only water as the solvent. The material can readily be purified further by adsorption onto a polyacrylic resin followed by bioethanol desorption. Initial trials for binding to hair met with limited success, but different formulations and methods may solve this in future.

Main findings are:

- Blackberry and current anthocyanin extracts have shown potential for incorporation into oils to give a coloured oil using lipases and ultrasound.
- Hair dyeing trials with anthocyanins and purified brown pigments from walnut met with limited success in getting the pigment to 'stick' to goat hair samples. A suitable chelating agent needs to be investigated, which can be used in conjunction with the pigment and is safe to use on human hair.
- A novel method for increasing anthocyanin stability was demonstrated by a trans glycosylation reaction using starch and amylases.
- There is scope for further research and development into the above.

Figure 1 Steve Taylor from Celbius shows cosmetic project for BioBoost to local MP Lucy Frazer

Figure 2 Steve Taylor, Celbius demonstrates the cosmetic pilot facility at NIAB

Figure 3 Display of cosmetic applications at NIAB Sophi Taylor Agri-Tech Week event – 'Saving waste in Horticulture'

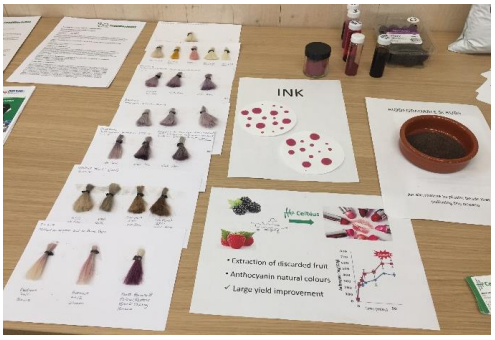

Figure 4 NIAB outreach on behalf of BioBoost at Festival of Plants – Cambridge University Botanic Garden event

Figure 5 NIAB and Celbius collect frozen blackberry waste from Paul Walpole's farm in Norfolk

Figure 6 Fruit trees at RW Walpole Ltd **https://www.rwwalpole.co.uk/**

Figure 7 NIAB and Celbius collect frozen blackberry waste from Paul Walpole's farm in Norfolk

2. Project objectives and results

2.1 Objective: Raw material production

Working together, NIAB and Celbius have sought to apply sonic extraction and purification processing to provide multigram quantities of purified blackberry anthocyanins for further modification.

Initial trials for extracting anthocyanin from blackberries, by blending the fruit and adding water ran into problems from excessive viscosity, mainly due to the release of pectin's, which is a documented problem. The use of pectinase enzymes partially solved this problem, but a better solution was to introduce very small quantities of solvent. Methanol was rejected as an option due to its toxicity; acetone and ethanol were evaluated as potential extraction aids. Acetone proved to be more efficient and consistently yielded around 1.5g of anthocyanin (expressed as the major component cyanidin-3-glucoside) per kg of fresh blackberries. This was determined by developing an absorbance assay based on the differential pH method, monitoring absorbance at pH 1 and pH 4.5 as widely reported in the literature. Two other solvents were tested: isopropanol and ethanol. Isopropanol was marginally less effective than acetone, whilst ethanol was significantly worse. Removal of the bulk biomass by sieving through a fine mesh was found to be easy and effective.

Following optimisation of the solvent volume used the extraction was tested in the presence of 20 kHz ultrasound, delivered directly into the stirred fruit/solvent mixture by a probe. The energy delivered (equating to 37 kWhr/tonne biomass) was around the maximum that could practicably delivered at large scale using a flowcell in recirculation mode without resorting to unrealistic amounts of capital investment. However, at these power levels there was no significant improvement over the silent experiment. The literature reports many cases of ultrasonic improvement, but often using excessive levels of energy. Also, the need for using ultrasound may be very dependent on the physical state of the fruit- a fresh soft fruit may be easier to extract compared to a solid dried biomass where the pigment is in more bound form.

A purification protocol was investigated, involving solid phase adsorption followed by solvent release. Activated charcoal adsorbed the pigment well and was able to remove 98% of the colour from the pigment extract. Recovery of the pigment from the charcoal by solvent elution using ethanol gave a modest recovery of 34% on the first attempt, and this might be worth revisiting in future given the low cost and range of charcoals available; for example, good adsorption was achieved with coconut charcoal but poor results were obtained with bamboo charcoal. Four different hydrophobic Zeolites were tested but these were not effective at binding the pigments and were abandoned. Finally, two different hydrophobic polymeric resins were tested and found to be suitable giving good pigment uptake and a high yield (generally 75-90%) recovery of the pigment into solvent.

Batches of blackberry extract were then produced as needed using a sequence of maceration, solvent stir, filtration, adsorption, elution and finally solvent concentration. This gave a pigment extract largely free of sugars, salts and proteins, and with some further refining is ready to scale up.
The extraction protocols were extended successfully also to blackcurrants.
Milestone 1 "Production of multigram quantities of anthocyanin raw material. Needed to support the experimental biotransformation work." was achieved.

2.2 Objective: Enzyme screening for anthocyanin derivatives

The aim was to expose anthocyanin extracts to a range of carboxylic acids and esters in the presence of several robust lipases, with ultrasonic stimulation to see if new derivatives could be formed.

New products were initially aiming for cosmetic applications and therefore it was necessary to avoid using toxic polar solvents such as pyridine and tert-butanol. These are frequently used for polar

water-soluble substrates in organic media. To allow rapid screening and easy visualisation a TLC system was chosen for development. A TLC assay was therefore developed using literature methods that allowed simple assessment by observing the mobility of coloured spots. A model system was successfully developed for a sugar esterification whereby glucose was esterified with lauric acid and palmitic acid utilising CALB lipase and acetone as the solvent. Using this methodology, a range of 11 lipases, including several used typically in industrial biocatalysis (examples from Candida, Pseudomonas, Rhizomucor, Thermomyces) were tested for the fatty acid acylation of anthocyanin mixtures in acetone with lauric and palmitic acids.

Unfortunately, despite several attempts at different temperatures and use of sonication, it was not possible to demonstrate any reaction. As an alternative approach, instead of using isolated fatty acids, transesterification using a triglyceride as both solvent and substrate was tried. In these experiments screening was conducted with three oils (coconut, olive and castor oil as typical cosmetic carrier oils) with the anthocyanin present in minimal water or glycerol for mobility and solubility. This allowed for potential partial hydrolysis or glycerolysis of the oil which may assist solubilisation of substrates and products as emulsions. Screening reactions were conducted at 45°C and incubating the screening reactions in the presence of 40 kHz ultrasound to maximise mass transfer. An example is shown in the figure to the right (blackberry in olive oil). After centrifugation significant colour was evident in the oil with one example (Candida antarctica lipase B) giving significantly more colour than the control. Further work is needed to see if the colour can be intensified and characterised.

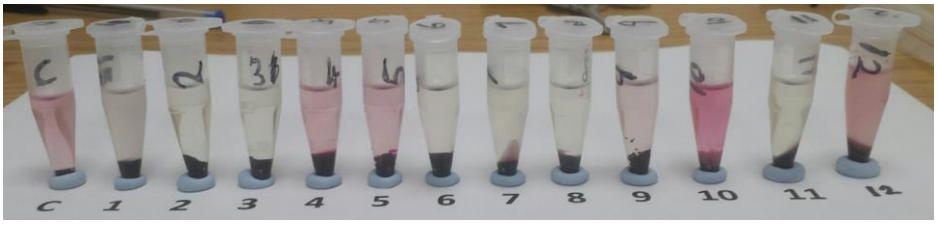

Figure 8 Blackberry in olive oil

A broader selection of enzymes will also be screened, together with the use of co-solvents to see if better, faster reactions can be achieved. Aim "*Demonstration of the ability of the new extract to colour materials such as fats hitherto difficult to mix with standard anthocyanins*." This was achieved albeit requiring further improvement and characterisation.

2.3 Objective: Stability testing

The first aim (Aim 1) was to monitor the stability of new product mixtures.

As a stability trial, screening panels with coloured triglycerides were left static at 4°C and 22°C. It was noted that after 5 days the colour had reduced and there was evidence of material settling. This may suggest that the colour formed was a result of emulsification rather than formation a truly oil-soluble new pigment. Another possibility is that acylation occurred and that the pigmented products were themselves not very soluble in the triglycerides and acted as emulsifying agents. It does however warrant some further investigation to see if the colour can be intensified and stabilised.
The literature suggests that increased glycosylation of anthocyanins may improve stability[1]. A potentially simple approach to glycosylation (with glucose as the sugar) is to utilise the industrial enzyme amylase in the reverse direction with starch as the glycosyl donor. To see if the purified

[1] Structure–activity relationships of anthocyanidin glycosylation

anthocyanin extract could be stabilised by this approach it was reacted with a range of amylases in the presence of starch at elevated temperature (50°C) and the colour monitored over time by following absorbance at three wavelengths and calculating the chromatic index. Two fruit extracts- blackberry and blackcurrant were tested in this way with the results shown in the graphs below.

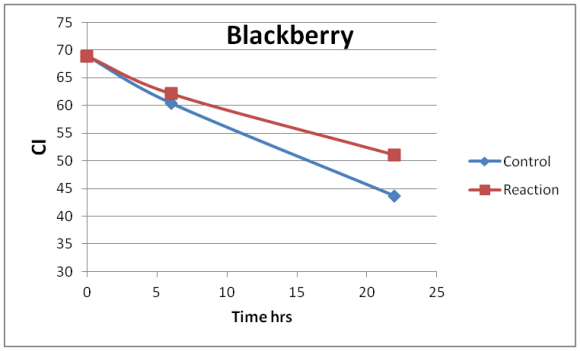

Figure 9 Blackberry anthocyanin stability improved (~20%) by transglycosylation

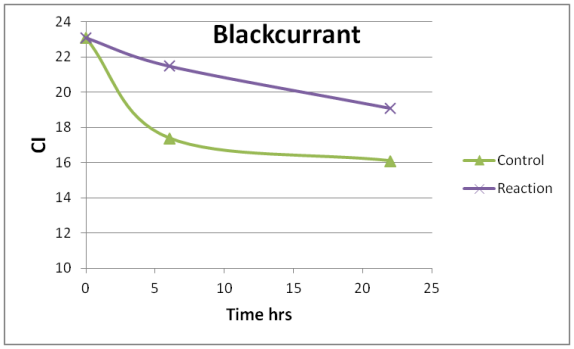

Figure 10 Blackcurrant anthocyanin stability improved (~10%) by transglycosylation

A fungal amylase produced the best results. This looks potentially very promising and needs further investigation. Literature searching revealed that glucose alone in solution does not stabilise anthocyanins (indeed it marginally decreases stability) suggesting that glycosylation may have occurred and this needs verifying, though time was not available within this project. The stabilising effect seems to be significant- in the case of blackcurrant, the degradation rate was apparently nearly halved.

Aim 2. To *demonstrate a tractable biotransformation to derivatise a purified anthocyanin extract into a new entity*. This has been achieved though requires further characterisation.

Aim 3. To *demonstrate enhanced stability properties of the novel derivative extract*. This aim was achieved although by an unexpected approach of transglycosylation, which needs further characterisation.

3. Pigment Extraction Trials – Brown Shades

3.1 Waste By Product - Spent coffee grounds

3.1.1 Background

There are very large volumes of spent coffee grounds available globally. Estimates derived from the internet indicate that in the UK we consume around 95 million cups of coffee each day producing around 500,000 tonnes of used coffee grounds each year. Unfortunately, much of this waste is landfilled from coffee processing and the drinks industry. To some extent there have been efforts to derive commercial products from such waste, for example the company Biobean who have developed a solid fuel briquette. Others have tried to exploit the relatively high residual oil content, extracting it and transforming it into biodiesel. A large volume of this material is, however, still wasted.

We performed a series of experiments to assess the viability of a simple extraction of the residual colour in the materials, sourcing material from a local coffee shop for trials.

Figure 11 Waste coffee grounds

3.1.2 Investigation of colour extraction from spent coffee grounds

Coffee grounds from a domestic coffee machine were air dried overnight at ambient temperature such that a free flowing material was obtained. This was used as the basis for three trials.

Trial 1: Water extraction with heating
10g coffee grounds was stirred in 40ml deionised water magnetically, heating to 80°C. After stirring the slurry for 2 hours a sample was centrifuged. The supernatant was a pale milky brown- the colour intensity was poor and there was evidence of a heavy suspension or emulsion caused by residual oil that had leached off the granules. This was not studied further.

Trial 2: Use of ultrasound pre-treatment
To attempt to draw more colour from the material ultrasonic energy was applied to the sample prior to heating to fragment the material. 10g coffee grounds was stirred in 40ml deionised water magnetically. Sonication was applied at 20 kHz and 20W power for 5 mins prior to heating. After heating to 80°C with stirring for 2 hours a sample was centrifuged. The supernatant had a visually similar appearance to the first trial with milkiness from oil that had leached off the granules. This was abandoned- this didn't look any different to the first trial.

Trial 3: Use of ultrasound during extraction
The third trial used a higher energy of ultrasound for a longer period. 10g coffee grounds was stirred in 40ml deionised water magnetically, heating to 80°C. Sonication was applied at 20 kHz and 40W for 30 mins at 80°C. After stirring for 2 hours a sample was centrifuged. The supernatant was once again a pale milky brown with similar appearance to previous trials.

At this stage it was decided to terminate this study since colour extraction was relatively poor with an aqueous solvent. A second problem in this system was the relative ease of release of residual oil that would necessitate a defatting stage either prior to processing or after extraction. It seems that the colour is tightly bound to the coffee ground particles and that stronger solvents might be needed. However, even if an organic solvent did effectively remove colour it would almost certainly co-extract the oil, necessitating downstream purification. No further work was done due to this added complexity.

3.2 Waste By Product - Walnut skins

3.2.1 Background

Walnut trees are cultivated globally for both their wood and fruit, and oil used in the cosmetic, health and building industries. There are approximately 3.8M tonnes of Walnuts produced globally (2017 figures) with China being the highest producer. The Walnut Company is the main producer in the UK. Walnut skins are left behind during the processing of the whole fruit for their nuts and have largely been discarded with little major application identified. There are reports of their use in textile dyeing though not in an organised commercial manner. This section of work sought to identify a simple extraction protocol for the key colour component, which is juglone, a compound that is very similar in structure to the main colourant in henna hair dye.

Figure 12 Walnut husk

3.2.2 Water extraction of walnut skins

Walnuts from a local source were collected (June), left over from the previous year's crop- dry with some limited microbial degradation- and the skins peeled off. The dark brown fragments were briefly blitzed in a coffee grinder- 16.1g. The walnut solids were stirred magnetically in 200 ml deionised water, heating to 80°C. The increase in absorbance was followed at three wavelengths, diluting x 20 in water then monitoring at 420 nm 520 nm and 620 nm. The supernatant became increasingly brown and was eventually an intense brown colour. Data was as follows:

Time mins	420 nm	520 nm	620 nm	CI
0	0	0	0	0
35	0.258	0.133	0.069	9.2
75	0.604	0.256	0.133	19.86
120	0.762	0.338	0.164	25.28
180	0.849	0.363	0.167	27.58
225	0.967	0.425	0.199	31.82
290	1.023	0.449	0.202	33.48
320	1.053	0.469	0.218	34.8

NB CI = Chromatic intensity- sum of absorbance with respect to dilution.

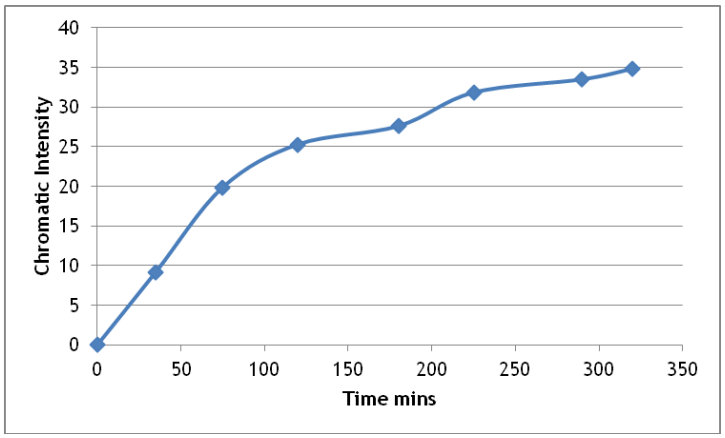

Figure 13 Monitoring colour extraction by increased absorbance

After about 5 hrs the colour intensity had reached a maximum, with little further increase. The composition of the colour was assessed, as in the table below.

Time mins	420 nm	520 nm	620 nm
35	56.1%	28.9%	15.0%
75	60.8%	25.8%	13.4%
120	60.3%	26.7%	13.0%
180	61.6%	26.3%	12.1%
225	60.8%	26.7%	12.5%
290	61.1%	26.8%	12.1%
320	60.5%	27.0%	12.5%

When the % of each wavelength contribution to the colour is calculated, apart from an initial shift it does not change throughout the extraction.

After 5.5 hrs the mixture was cooled and allowed to stir overnight. Next morning, A420=1.160, A520=0.535, A620=0.268. CI= 39.3. A limited amount of further extraction occurred overnight.
The mixture was filtered with aid of Dicalite- this was sluggish. This yield 105ml filtrate to which 5g diluting support (confidential) was added. The solution was evaporated under vacuum (50°C, 100 mbar then reduced to 15 mbar)- became a bit sticky so added another 5.83g support. The dried weight was 11.88g, so with 10.83g support added the residue extract was 1.05g.
A sample was sent to a professional hair dressing for testing and results are awaited. This sample was also tested for uptake onto goat's hair by NIAB.

3.2.3 Uptake on Goats Hair Method

At NIAB, the goat hair sample was first washed to remove any impurities. Walnut extraction uptake was tested in two ways:

1) Extract was mixed with fresh Aloe Vera gel (which acts as a binding agent) (see figure 14)
2) Extract only (see figure 15)

The samples were brushed onto the hair ensuring full and even coverage, wrapped individually in silver foil and left for 2 hours for pigment uptake. After 2 hours, the samples were washed and left to dry in the air naturally. The results show that there was very little uptake of colour on either sample. Figure 14 using Aloe Vera also shows that some colour from the gel has given the hair a green/yellow tint compared to the sample using Walnut pigment only.

Figure 14 Uptake of Walnut extract with Aloe Vera

Figure 15 Uptake of Walnut extract only

3.2.4 Trial purification

5 ml of the initial brown filtrate was shaken with 1g resin A and B, monitoring the residual filtrate to see if colour was taken up by the resins. Samples were diluted x 50 into deionised water for measurements.

Time mins		420 nm	520 nm	620 nm	CI
0		0.556	0.295	0.16	50.55
30	A	0.309	0.165	0.084	27.9
30	B	0.332	0.182	0.091	30.25
270	A	0.228	0.121	0.069	20.9
270	B	0.304	0.163	0.08	27.35
O/N	A	0.234	0.14	0.083	22.85
O/N	B	0.237	0.136	0.073	22.3

About half of the colour has been taken up, and both resins showed about the same capacity. This is worth investigating further with a higher resin loading.

The extraction method was repeated using fresh unripe green walnuts that were collected in July. 153g fresh walnut skins was smashed with a hammer then blitzed in 300 ml deionised water. The mixture was heated with constant stirring to 85°C for 2 hours. The mixture went brown- but the colour was not sufficiently intense. The walnut pigment was not fully formed and this method was abandoned.

3.2.5 Further process development

Further work was conducted to improve the previously developed methodology. Walnut husks were procured from Dyeing Crafts- as a dried and granular substrate. The walnut solids (100g) were stirred magnetically in 500ml DI water, heating to 80°C. The increase in absorbance was followed, diluting x 50 in water then monitoring at 420nm 520nm and 620nm. The supernatant became increasingly brown.

Time mins	420 nm	520 nm	620 nm	CI
0	0	0	0	0
5	0.389	0.153	0.055	29.85
25	0.861	0.331	0.148	67
55	1.195	0.471	0.204	93.5
80	1.22	0.494	0.212	96.3

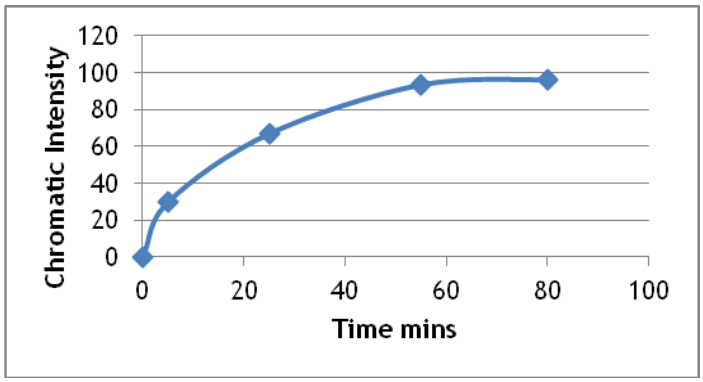

Figure 16 Monitoring colour extraction by increased absorbance

At higher substrate loading, the colour release was faster. However, as the mixture was heated it became viscous. After 2 hours the mixture was cooled to 40°C and 1 ml of pectinase added (Biocatalysts). This reduced the viscosity slightly, but the viscosity was still too high for effective processing.

The colourant mixture was diluted at a four to one ration to reduce the viscosity and the mixture was filtered through a sinter with Dicalite, which was slow and incomplete after two hours.
The solution was evaporated URP to yield two samples:

Conc 1 0.719 0.305 0.140 x250 CI = 290
Conc 2 0.444 0.188 0.091 x250 CI = 181

These were tested for uptake onto goat's hair, but again uptake was not sufficiently intense.

Resin purification was attempted- 30ml of the original extract was shaken with 3g acrylic resin and colour absorbance monitored over time.

Resin uptake:

Time mins	420 nm	520 nm	620 nm	CI
0	0.412	0.172	0.093	33.85
45	0.389	0.153	0.055	29.85
90	0.28	0.143	0.071	24.7
1150	0.171	0.091	0.048	15.5

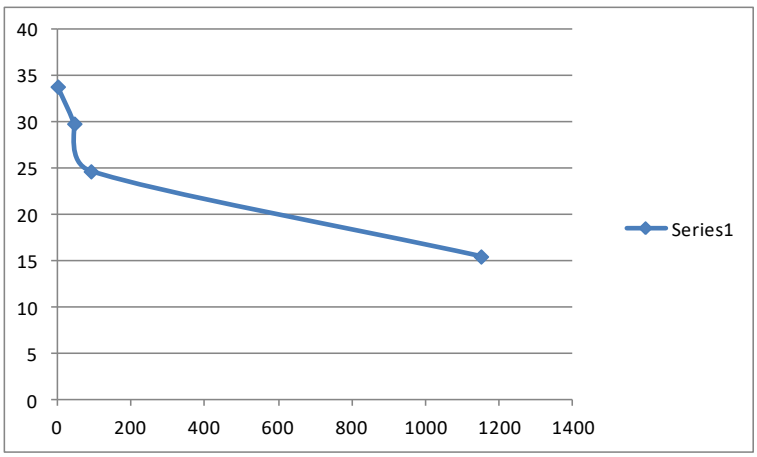

Figure 17 Monitoring colour uptake onto resin by decreased absorbance

Another 3g resin was added and the mixture shaken for 48 hrs.

Conc 3 0.156 0.063 0.022 x250 CI = 12.0.

The colour reduced by a further 20% suggesting a multicomponent mixture where colour components were binding with differing affinities to the resin.

Clearly some of the colour is not amenable to uptake. The resin was drained and washed with water twice then steeped in 10 ml 96% ethanol. The solvent intensified with the brown colour, and it looks like the colour adsorbed will readily desorb if needed, though this was not quantified. Thus, a promising purification protocol is evident with uptake and elution of the brown colour demonstrated from the acrylic resin.

3.2.6 Repeat extraction and stability trial (black walnut)

Black walnut husks were procured from Joanna's Garten Detrade as a dried and granular substrate. The walnut solids (108g) were ground in a coffee grinder then stirred mechanically in 600ml deionised water, heating to 75°C. The increase in absorbance was followed, diluting x 50 then x 500 in water then monitoring at 420nm 520nm and 620nm. The supernatant became increasingly and intensely brown.

Time mins	420 nm	520 nm	620 nm	CI
0	0	0	0	0
10	0.506	0.192	0.078	38.8
50	0.12	0.022	0	71
90	0.148	0.05	0	99
1200	0.198	0.096	0.036	165

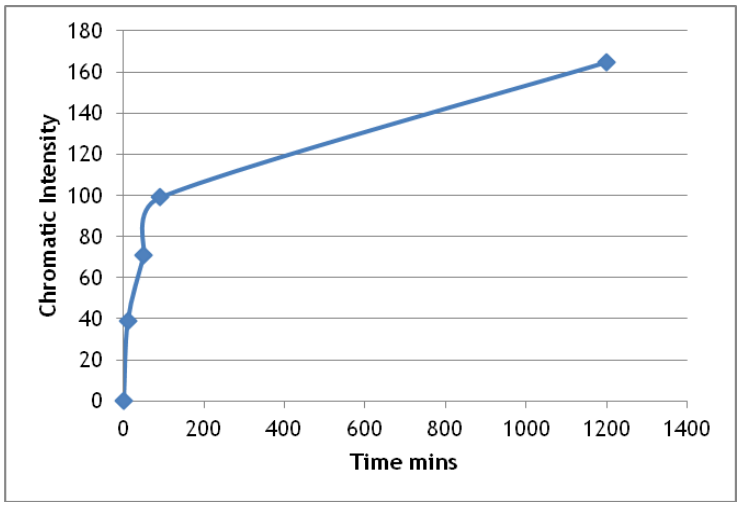

Figure 18 Monitoring colour extraction by increased absorbance

The mixture was partially filtered through Dicalite- to give 88g filtrate (0.198 0.096 0.036 CI = 165) that was concentrated under vacuum to 27g.

The remainder was passed through 82um mesh filter- this was effecive. (0.3 0.158 0.067, CI = 263)- This yielded 149 g that was concentrated to 55 g. The concentrate was stored at ambient and 4°C temperature and monitored visually for microbial contamination. Both samples became visibly contaminate over a period of several days. Thus, the concentrate is not biologically stable and requires freezing for stability or further purified to prevent microbial growth.

3.3 Waste By-Product - Olive pomace

3.3.1 Background

Olive pomace is the granular dried material that is left over after olive oil is extracted from olives. It comprises a mixture of residual skin, flesh and fragmented stones. Much of this material is currently burned for heat and/or power, either in loose free-flowing format or after briquetting. This section of work was performed to assess the potential for extracting the brown coloured pigments in this material. Substrates for study were procured from Zembra Ltd., who source the material from olive mills in Italy and Tunisia, where the pomace is formulated into horticultural products.

3.3.2 Extraction of olive pomace

Dried olive pomace was procured from Zembra as a dried and granular material. The pomace (119 g) was ground up to a coarse powder in a coffee grinder then stirred mechanically in 500 ml deionised water, heating to 85°C. The increase in absorbance was followed, diluting x 50 in water then monitoring at 420nm 520nm and 620nm. The supernatant became increasingly brown.

Time mins	420 nm	520 nm	620 nm	CI
0	0	0	0	0
45	0.129	0.049	0.012	9.5
150	0.222	0.109	0.04	18.55
195	0.218	0.103	0.028	17.45

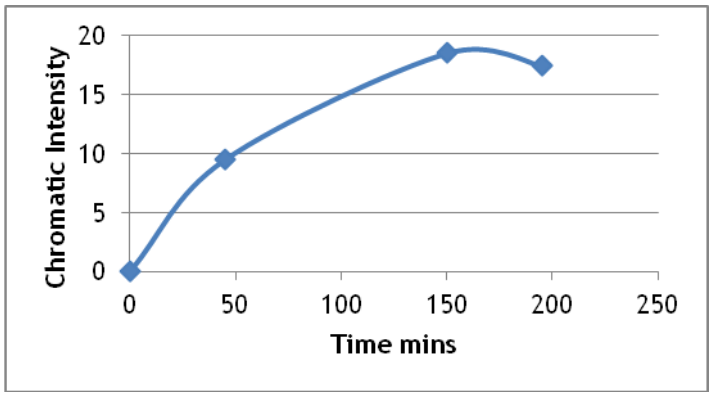

Figure 19 Monitoring colour extraction by increased absorbance

The sample was filtered through a sinter, then concentrated to yield 10 ml of concentrate. Despite a relatively high solid loading in the extraction, the colour yield was modest. Furthermore, the extract had a pungent aroma of phenolic compounds and on the basis of this and the modest colour release was not pursued further.

4. Extraction of food and agricultural residues for hair colourants: purple/red colours

4.1 Introduction

As a follow on to earlier work conducted on the extraction of anthocyanins from berry fruits, beetroot was investigated as a substrate for purple to red colourants. This represents a different class of colourant, where the colour arises from the betalain class of compounds. Beetroot represents an abundant source of raw material, for example from the grading process, from peelings and from solutions left over after pickling and cooking. This section of work was conducted to assess the potential of beetroot extracts for hair colourants.

4.2 Extraction of fresh beetroot

Beetroot was procured from a local industry (G's Fresh Produce Ltd) (figure 20) then topped/tailed and scrubbed clean. 695g fresh beetroot was chopped up and homogenised with 1 l deionised water to give a slurry at pH 6.8. This was lowered to pH 4.6 by addition of 3 ml of conc. HCl. The mixture was stirred mechanically and heated to 75°C over a period of 50 mins then allowed to cool to 40°C.

Figure 20 Beetroot raw material

The total slurry yield was 1274g, (absorbance A420 0.172 A520 0.737 A620 0.06, a sample of supernatant after solids removal was diluted x 50 into deionised water.)
The total chromatic yield was CI = 48.45, or 61725 CIg (total amount of colour = CI x weight) for 695g beetroot, equivalent to 88.8 CI/g beetroot.

The slurry was filtered to 100um (squeezing the pulp through a single layer of muslin) to yield 1274 g. This was partially filtered through a Dicalite bed on a sinter- this was rather slow.
655g was filtered (582g was not filtered and was frozen.)

The 655g batch was acidified with concentrated HCl to pH 1.8. This was then slurried with 75 g untreated polyacrylic resin and the total colour uptake monitored by diluting samples into deionised water.

Time mins	420 nm	520 nm	620 nm	CI
0	0.068	0.463	0.035	28.3
15	0.01	0.084	0.001	4.75
25	0.001	0.012	-0.005	0.4

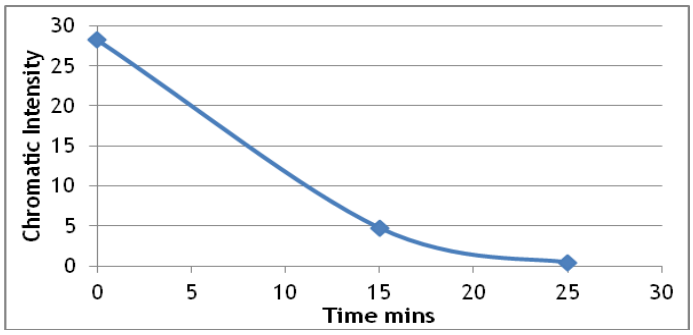

Figure 21 Monitoring colour uptake onto resin by decreased absorbance

Colour uptake was efficient and fast. The resin beads were drained through a 100um sieve, then washed sequentially with 100 ml then 50 ml deionised water. Three elution's with an alcoholic solvent (confidential) were done, totalling 150 ml, 150 ml, then 100 ml. Samples were diluted x 500 then x 50 into deionised water.

Eluate	420 nm	520 nm	620 nm	CI
1	0.022	0.149	0.002	86.5
2	0.13	0.494	0.023	32.35
3	0.072	0.191	0.004	13.35

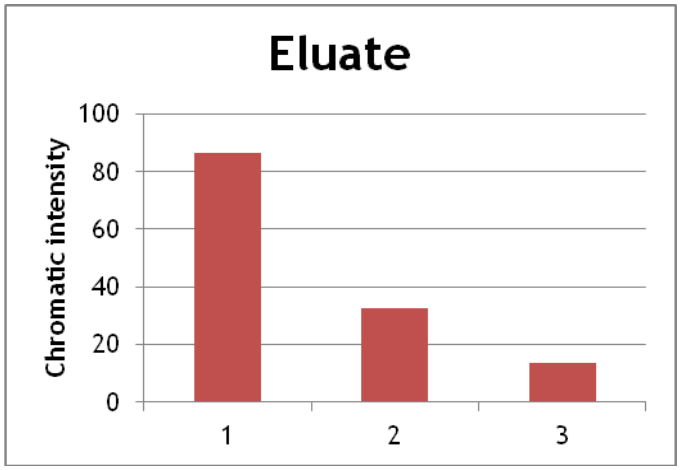

Figure 22 Relative product yield in product fractions

The eluates were evaporated under pressure to yield 12.72 g of a deep purple mobile homogeneous concentrate.

4.3 Comparative extraction- beetroot powder from Phil Metcalfe

As a comparison, dried beetroot powder was extracted to compare yields from a dried beetroot source. Extraction of the dry powder with a range of solvents (ethanol, acetone, methyl ethyl ketone, isopropanol) yielded no substantial colour directly. Extraction with deionised water was as follows: 4 g dried beetroot powder was stirred in 40 ml 0.1% HCl, heating to 75°C for 5 mins, then cooling to ambient temperature. Samples were centrifuged and diluted x 50 into deionised water.

Time mins	420 nm	520 nm	620 nm	CI
0	0	0	0	0
25	0.162	0.159	0.002	16.15
50	0.172	0.174	0.008	17.7
75	0.177	0.178	0.011	18.3

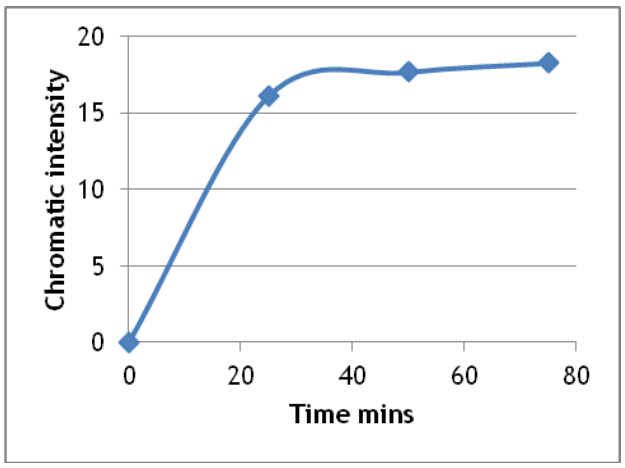

Figure 23 Monitoring colour extraction by increased absorbance

The extract was noticeably redder than the whole beet extraction, which had more of a blueish tint to the purple.

Estimate yield comparison:
From above, total chromatic yield: CI = 48.45, or 61725 CIg (total amount of colour = CI x weight) for 695g beetroot, equivalent to 88.8 CI/g beetroot.

From the powder, total chromatic yield: CI = 18.3, or 732 CIg (18.3x40) for 4g beetroot powder, equivalent to 183 CI/g powder.

Beetroot is about 90% water, so 1 g beetroot powder is equivalent to about 10 g raw beet.
For the powder, 183 CI units/ g powder is therefore about 18.3 CI units/g raw beet- this is about 20% of the value of raw beet. We conclude that this particular method of processing of beetroot, comprising water removal and micronisation to give a beetroot fibre powder has a profoundly adverse effect on the quantity of colour that may extracted from the beet. We conclude that for betelain-colourants, fresh beetroot should be used.

4.4 In-house hair dyeing trials

Beetroot samples extracted at the EAIH were tested for utility as a hair colourant using goat's hair at NIAB.

4.4.1 Uptake Method

The goat hair sample was washed to remove any impurities. Beetroot extraction uptake was tested in two ways:

1) Extract mixed with fresh Aloe Vera gel (which can act as a binding agent) (see 44/2 beetroot sample, figure 24)
2) Extract only (see figure x)

The samples were brushed onto the hair ensuring full and even coverage, wrapped individually in silver foil and left for 2 hours for pigment uptake. After 2 hours, the samples were washed and left to dry in the air naturally.

4.4.2 Results Beetroot

The results show that there was enhanced uptake of colour on the sample using Beetroot extract 44/2 with Aloe Vera (Figure 24) compared to using the extract alone (Figure 25). The Aloe Vera does not seem to have transferred any colour effect on the resulting sample.

Figure 24 Uptake of Extracts with Aloe Vera: Walnut, Turmeric in glycerol, Beetroot, Marigold with castor oil and Marigold with Glycerol - Date: 22.07.17

Figure 25 Uptake of Beetroot Extract with no Aloe Vera compared with a commercial hair dye - Date: 22.07

Blackberry extracts were also tested for pigment uptake on goats hair by NIAB using the same method as above (see figure x). Again, the samples using Aloe Vera showed enhanced uptake.

Figure 24 Uptake of Blackberry Extract with and without Aloe Vera – Samples of the dye were reacted with a range of aromatic compounds such as flavonoids which form new complexes - Date: 07.11.17

5. Main Benefits to SMEs & Follow up

The BioBoost pilot has delivered some positive results for development of natural based cosmetics using crop co-products. These data and methodologies are forming the basis for ongoing work by NIAB and collaborating SMEs and for further R&D and grant applications by NIAB. The extraction technology has been improved and applied to a range of soft berry fruits by Celbius, who were able to produce samples of both blackberry and blackcurrant purified extracts. These were then tested on hair samples using protocols recommended by a hair product manufacturer (Scott Cornwall). Of particular interest was the first demonstration of coloured triglycerides and the use of amylase to increase anthocyanin stability by an as yet unverified mechanism, and this is expected to form the basis of future IP and grant applications.

The Hub facility was a particular benefit, allowing SME's, who have access to limited resources, to operate much more effectively than otherwise might be the case. Contacts such as fruit growers and some potential future commercial contacts in the hair product field provided by NIAB also acted as a stimulus to the SMEs.

NIAB, together with Celbius have shown potential for transforming purified anthocyanin pigments to yield a coloured oil, albeit with limited stability, which needs further optimisation and investigation. In addition, a novel method for increasing anthocyanin stability was demonstrated by a trans glycosylation reaction.

The work is helping to support a business case by Celbius to develop a larger scale pigment extraction plant at a local site. The vision is that this could be a multipurpose facility that can extract pigments from a variety of waste food products, selling them into the food and cosmetic industries. In addition to this, further joint grant applications are envisaged to look at oil-based colourants and to see if the anthocyanin glycosylation effect can be confirmed, understood and commercially exploited. Whilst grant applications are formulated Celbius has indicated it will continue to conduct further research into the results obtained.

Once an extraction plant is established and revenue generating, focusing primarily on colourants in the first instance, the scope for broadening the product range will be considered. This could include other botanical and herbal products, and new innovative products will be developed from these. The potential for exploiting NIAB's agronomy expertise and contacts to establish local raw material supply for botanical, herbal and fruit crops that may be grown in a bespoke manner will be explored.

The vision will be to enable Celbius to develop a business based on a product range that uses raw materials grown and extracted locally using processes that generate minimal waste, and use, where appropriate, discarded crops as raw materials. There is potential for NIAB to explore with Celbius the use of technologies such as thermophilic aerobic digestion for efficient utilisation of solid plant residues post extraction, and for use of low-contaminated water streams from the extraction facility.