# Losing colour: the discolouration of plants in spirit preserved collections

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**Abstract**

The Research unit of the Haute-École Arc Conservation-Restauration (UR-arc CR), Neuchâtel, Switzerland, is carrying out a research project that aims to understand the discolouration of botanical spirit preserved collections and strives to propose practical solutions to these problems. This article reports on the results of the initial phase of this project, which focused on creating experiment jars of representative plants specimens immersed in fluids and monitored using the following protocol: photography of the jars, colorimetric measurements, and analysis by UV spectroscopy of the fluid as well as closer observations of the specimens. Colorimetric measurements tracked the colour changes over time. UV spectroscopy was used to identify the pigments present in the fluid. Thereby, different behaviours could be observed, depending both on the type of pigment and on the fluid. Specimens containing pigments with strong dying properties tend to opacify the fluid while keeping their colour, whereas other specimens such as colourful flowers or leaves show clear discolouration problems. Depending on the preservative fluid, the leached pigments degrade at different rates. Moreover, the botanical specimens show other alterations: they may not only assume a lighter or darker colour, but can also shrink, stiffen or soften.

**Keywords:** Spirit preserved collections, plants, fluid preserved, botany, discolouration, spectrocolorimetry, UV-Vis spectroscopy

## Introduction

In botany, besides living plants in gardens or greenhouses, specimens are usually dried and pressed in herbaria. However, they can be preserved in other media, to keep or enhance some of their features. For significance (e.g., Guntau, 1996), expressions of nationalistic pride (e.g., Vogel, 2015), and instruments of both formal and public education. Those values have guided how minerals have been collected, organised, and displayed (Kohlstedt and Brinkman, 2004).

For instance, the "fresh" aspect of a plant as well as its structure and spatial placement can be better preserved if stored in fluid. Similarly, if displaying the specimen is not required, keeping it in frozen storage assures the preservation of its genetic material, which often degrades when pressed or preserved in fluid (Williams, *et al.*, 1999).

Fluid preservation, also called wet preservation, is a technique that dates back to the 17th century. It was then mostly used on animal or human specimens for biological, medical, pathological or curiosity collections. For botanical collections however, the practice developed later (early 19th century) and was less common (Moore, 2010). The process can vary but follows generally the same steps described in Figure 1. The specimen is first collected and cleaned. The preparation for storage can include a fixation step, where the specimen is immerged for some time (from a few hours to a few days generally) in an aqueous solution containing a fixative agent. Depending on the specimen and the display concept, it might also be given a special mounting. Finally, it is put in a sealed container filled with a transparent preservative fluid to be stored or displayed. It will not be removed from this fluid except for some studies, analyses, or restoration interventions.

According to a review carried out across Europe, most of the botanical museums and herbaria store their collections in 70% Industrial Methylated Spirit (IMS) or ethanol, sometimes with previous fixation, either with a solution of ~4% formaldehyde or a commercial preparation of Formaldehyde - Acetic acid - Alcohol (FAA), and sometimes with ~1-5% added glycerol. Some other preservatives often used are Kew mixture, Copenhagen solution, Rum 60° or formaldehyde solution (Prakash, 2019). There are several versions of Kew mixture and Copenhagen solution that have been developed,

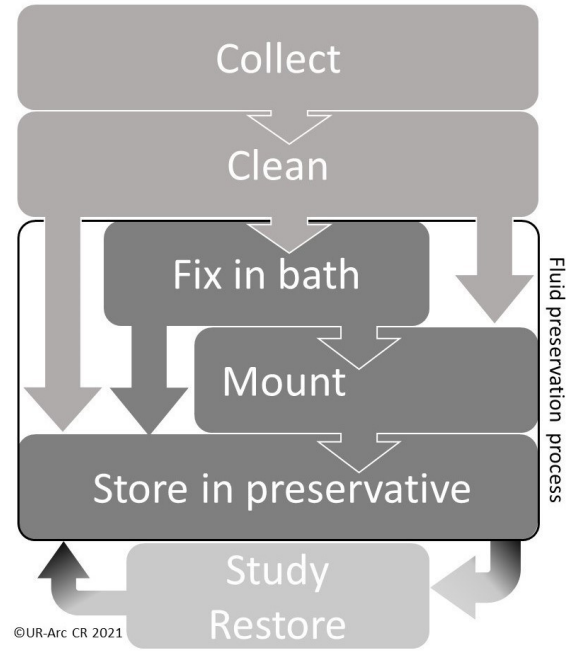



*Figure 1. Preparation of wet specimen from the collect, through the fluid preservation process, to the cycle of interventions once part of the collection. © UR-Arc CR 2021.*

refer to Simmons (2014) and Moore (2010) for details of fluid mixture compositions.

The botanical wet collections are known to be subject to issues related to the presence of vegetal pigments in their specimens. On one hand, the loss of colour in the specimen can go as far as total whitening of the tissues, especially for leaves and flowers. On the other hand, the colouring of the fluid can sometimes lead to an excessive opacification of the jar, especially for darker and pigment-rich specimens. This can occur in both water-based and alcohol-based fluid preservatives, as the pigments or dyes present in the specimens are solubilised by the preservative fluid.

The study presented in this paper is carried out in collaboration with the Botanical Museum of Zurich University and the botanical garden of Neuchâtel and aims at understanding the sensitivity to discolouration of different types of specimens that have undergone various preparation processes. Based on these initial observations, recipes for the targeted preservation of certain pigments (i.e. chlorophylls, flavonoids, betalains, carotenoids and phenolic tannins) will be sought in further steps of the project.

## Methods

*Preparation of the experiment-jars*

A series of tests were carried out within the framework of this study, consisting of several sets of experimental jars that were designed to observe specific aspects of the studied problem. The varying parameters were: the type of specimen and the pigment expected to be leaching, the impact of the fixative used and of the preservative fluid chosen.

Fresh specimens representing different plant types or organs, listed in Table 1, were chosen according to their availability and seasonality. Most of them could be acquired, thanks to the Botanical Garden of Neuchâtel. Set 1 contains leafy specimens, flowers, and fruits with brighter colours knows to show discolouration issues in collections. Set 2 contains more pigment-rich specimens known for their dying properties and often subject to fluid opacification in wet collections.

The preparations chosen, listed in Table 2, were designed to simulate different possible situations observed in collections. Fixation is not always a necessary step in the preparation process. If included, botanical specimens are usually immersed in formaldehyde solution or FAA baths for approximately 24 hours before being

*Table 1. Specimens selected, detailing the colour and main pigment expected to leach into the fluid.*

| Specimen | Type / Organ | Colour | Main pigments of interest | set |
|---|---|---|---|---|
| Lavender | Stalk with flowers | Green / purple | Chlorophylls +anthocyanin flavonoids | 1 |
| Mint | Stalk with leaves | Green | Chlorophylls | |
| Chili pepper | Fruit | Red | Carotenoids | |
| Beetroot | Root - cut | Purple | Betalains | 2 |
| Fresh walnuts | Fruit - cut | Green / brown | Chlorophylls + Phenolic tannins | |
| Dried walnuts | Fruit - cut | Brown | Phenolic tannins | |

transferred into the preservative fluid (Moore, 2010). A 4% w/v formaldehyde solution buffered pH 6,9 (Merck KGaA) and a commercial FAA histological fixative containing (v/v) 40-50% ethanol, 10-15% formaldehyde, 5-10% acetic acid and 3-5% methanol (VWR™ chemicals) were selected for this study. A condition assessment carried out on collections of the Botanical Museum of Zurich University showed that some specimens were preserved in formaldehyde solutions for many years (Dangeon, 2016). Therefore, 4% w/v formaldehyde solution was also used as preservative fluid on some test specimens.

A solution of 70% v/v ethanol made from absolute ethanol (VWR™ chemicals) and demineralized water was selected. Ethanol or IMS are the main preservatives used in museums, but commercial rum is sometimes used as a substitute, either as a temporary "on field" solution or permanent preservative (Grant, 2019). Bacardi® White Rum (37.5% vol alc) was selected.

Finally, glycerol is sometimes added in a small percentage to preserve flexibility of the fixed tissues, especially in collections where the specimen might be manipulated for study. It is less

*Table 2. List of preparations combining fixative and preservative. Code of preparation for experimental phase.*

| Fixative [250ml] | | Time | Preservative [350ml] | Code | Comment |
|---|---|---|---|---|---|
| No fixation bath | | | 70% Ethanol | OE | Main preservative fluid used in museums |
| 4% w/v formaldehyde solution | | 24h | | FE | Fixative the most used in museums |
| FAA | | 24h | | AE | Commercial fixative broadly used for botanical specimen |
| No fix. | White Rum | 7 d | | OR_E | Temporary storage with readily accessible alcohol during "on field" campaign |
| No fixation bath | | | White Rum (37.5% alcohol) | OR | Used as alternative preservative or as temporary "on field" solution. |
| No fixation bath | | | 4% formaldehyde solution | OF | Formaldehyde solution used as preservative fluid |
| FAA | | 24h | | AF | |
| No fixation bath | | | 70% Glycerol | OG | Less hazardous preservative fluid |
| 4% formaldehyde solution | | 24h | | FG | |
| FAA | | 24h | | AG | |

hazardous than formaldehyde and ethanol both for people and storage, and it has also been used as a preservative fluid (Van Dam, 2018). In this study, glycerol (VWR™ chemicals) was selected, with a 70% concentration in demineralized water.

Specimens were weighed before being immersed in fluid. To ensure a similar volume ratio between the specimen and the fluid in the jars, the beetroots and walnuts had to be cut into smaller parts. The specimens that had to be fixed were first put in a 250ml bath of the chosen solution. Eventually, all specimens were put in glass jars with a closed lid (IKEA® Korken 500ml) filled with 350ml of preservative fluid. The monitoring of these experiments started at $T_0$, on the day on the preparation, showed in Table 3.

*Monitoring protocol*

The prepared specimens were stored in the dark in a solvent cabinet with forced air filtration but without climate control, and were monitored frequently for 3 months: daily during the first five days, weekly for the remainder of the first month and every two weeks for the following two months.

The protocol included both visual observation and photography (Canon® EOS 600D – 1/60 F8.0 ISO 100) of the jars in a white lightbox with a reference colour & grey control chart (B.I.G. GmbH) to document the visual changes of the fluid's and specimen's colour through time. To complement this qualitative documentation, the colour of the fluid at each monitoring day was also quantitatively assessed with a portable spectrophotometer (X-rite® Ci62). This instrument acquires the reflectance electromagnetic spectrum, in the visible light range, of an investigated sample. For this project, it was mounted on a vertical stand equipped with a cell for liquid measurements. 6ml of fluid were sampled from the jar with a graduated pipette, three measurements were performed and the average value retained.

From the reflectance spectrum, CIELAB colorimetric values can be extracted. The CIELAB colour space expresses colour as 3 values: *L** represents the lightness scale ranging from 0 (black) to 100 (white); *a** the green-red value from -X (green) to +X (red); *b** the blue-yellow value from -Y (blue) to +Y (yellow). These values are relative to a specific illuminant, defining the white of reference. For this protocol, illuminant D65 (standard day light) was used.

This allows to quantify the fluid's colour change by computing the difference between the colour values in CIELAB space measured on the fresh preservative solution on the day it was prepared ($T_0$) and the same fluid on the day of the monitoring ($T_x$). This difference is expressed with Delta-E ($\Delta E$), the Euclidian distance between these two points in the colour space. It is calculated using the following equation, where $L_1^*$; $a_1^*$; $b_1^*$ stand for the clean preservative and $L_2^*$; $a_2^*$; $b_2^*$ for the fluid at time of measurement.

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

(Zuppiroli & Bussac 2012)

$\Delta E$ is a strictly positive value, where the two L*a*b* colour values compared are identical if $\Delta E=0$ and diverge as $\Delta E$ grows. A colour difference cannot be perceived by the human eyes when Delta E is less than 1 ($\Delta E<1$), and only becomes clearly noticeable around 10. Above this value, the compared colours are perceptibly different (ViewSonic, 2021). In this paper, any colouration of the fluid of Delta E greater than 10 ($\Delta E>10$) will therefore be considered as a significant colour change.

After 3 months, samples of all jars preservative fluids were collected and analysed with UV-Vis spectroscopy (Nanodrop™ One Thermo Fisher™, range 0-750nm) in order to identify the pigments responsible for their colouration.

*Table 3. All specimens in 70% ethanol with no prior fixation (OE) on the day of preparation ($T_0$). © UR-Arc CR 2021.*

Lavender in OE | Mint in OE | Chili pepper in OE | Beetroot in OE | Fresh walnut in OE | Dried walnut in OE

At the beginning of the second phase of the project, in February 2021, new images and colorimetric measurements were taken, 14 to 18 months after preparation depending on the sets. Colorimetry measurements were also made on some of the test-tubes sampled for UV-Vis analyses to see if any change had occurred.

Finally, investigations were carried out on the specimens to evaluate the structural integrity of the plants. A manipulation test, consisting of light bending and applied pressure, assessed their stiffness and flexibility of the specimens.

## Results and discussion

*Colouration of the preservative fluid*

Table 4 to Table 6 show the $\Delta E$ values for all experiment-jars at different milestones of the monitoring period: after 7 days, 3 months and approximately 1.5 years (14 months and 18 months depending on the set). The greater this value, the more the colour diverges from the clean fluid, correlating to a bigger proportion of pigments leaching into the fluid. A red gradient highlights values the set threshold of $\Delta E=10$. This allows to point at general trends, both related to the type of specimen and the type of preparation fluid. These tables make it clear that specimens for set 2, known for their dying properties, released more pigments in all preparations. Overall, all specimens seem to release more colour in ethanol preservative (Table 4).

After 3 months (Table 5), all samples from set 2, except dry walnuts in ethanol (OE), have passed the $\Delta E=10$ threshold. Regarding set 1, preparations preserved in ethanol all performed poorly, apart from the ones fixed in FAA (AE).

The last measurements taken on both sets after approximately 1.5 years bring some interesting new information (Table 6). Two opposite trends appear:

- $\Delta E$ of some experiment-jars seem to have stabilized or slowly increased. This was observed on the following samples: all
- Lavender jars, Mint in OE, AE, OR_E, OR, OF, AF, OG, FG, AG, Chili in AE, OR, OF, AF, OG, FG, AG, Fresh Walnut in OR, AF, OG, FG, AG and Dry Walnut in OE, FE, OR_E OR, OF, AF, OG, AG.
- $\Delta E$ have significantly dropped for some
- other samples: Mint in FE, Chili in OE, FE, OR_E, All Beetroot jars, Fresh walnut in OE, FE, AE, OR_E, AF, and Fresh Walnut in AE, FG.

By plotting $\Delta E$ /Time for each experiment-jar (Figures 2-7), it is possible to show the rate at which a specimen released pigments in each preparation. Colour changes in the non-alcoholic preservatives increased slowly throughout the monitoring period, whereas the colour changes in the jar containing ethanol started abruptly before stabilizing over time. Moreover, experiment-jars using alcohol (either ethanol or rum) as preservative tend to show a $\Delta E$ slowly decreasing as the fluid returned to a colour closer to the fresh preservative's. This is best observed for the beetroot (Figure 3), where $\Delta E$ for 70% ethanol (OE), white rum (OR) and 70% ethanol with fixation in white rum (OR_E) jars quickly went to high values during the first 1-3 months before decreasing. Similarly, all chili pepper samples (Figure 6) preserved in alcohol not only reached significantly higher $\Delta E$ values but had returned to lower values at the end of the monitoring period. Looking at the photographic documentation of the jars, the behaviour of $\Delta E$ can directly be correlated with the colouration of the fluid.

*Table 4. ΔE after the first week for each specimen in jar. Values above 10 are highlighted with a gradient.*

| ΔE T7 | OE | FE | AE | OR_E | OR | OF | AF | OG | FG | AG | Max | Min |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lavender | 8.23 | 7.44 | 3.76 | 3.06 | 2.82 | 2.46 | 2.07 | 2.66 | 1.18 | 1.65 | 8.23 | 1.18 |
| Mint | 9.84 | 15.67 | 4.66 | 4.23 | 3.91 | 1.67 | 1.33 | 2.00 | 1.22 | 1.85 | 15.67 | 1.22 |
| Chili pepper | 9.33 | 22.54 | 13.69 | 1.31 | 0.59 | 0.45 | 0.68 | 0.44 | 1.12 | 1.02 | 22.54 | 0.44 |
| Beetroot | 36.70 | 39.25 | 22.11 | 37.09 | 38.71 | 29.88 | 25.81 | 41.22 | 12.10 | 9.31 | 41.22 | 9.31 |
| Fresh walnut | 33.61 | 29.90 | 30.46 | 29.32 | 30.75 | 22.68 | 19.94 | 28.45 | 7.65 | 4.96 | 33.61 | 4.96 |
| Dry walnut | 1.78 | 7.20 | 7.16 | 9.88 | 10.04 | 19.01 | 30.33 | 4.40 | 3.21 | 1.26 | 30.33 | 1.26 |
| Max | 36.70 | 39.25 | 30.46 | 37.09 | 38.71 | 29.88 | 30.33 | 41.22 | 12.10 | 9.31 | | |
| Min | 1.78 | 7.20 | 3.76 | 1.31 | 0.59 | 0.45 | 0.68 | 0.44 | 1.12 | 1.02 | | |

*Table 5: ΔE after 3 months for each specimen in jar. Values above 10 are highlighted with a gradient.*

| ΔE T91 | OE | FE | AE | OR_E | OR | OF | AF | OG | FG | AG | | Min |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lavender | 9.76 | 6.32 | 4.40 | 2.89 | 4.26 | 6.78 | 4.40 | 6.04 | 4.51 | 0.99 | 9.76 | 0.99 |
| Mint | 11.12 | 13.78 | 3.82 | 10.00 | 5.00 | 9.81 | 3.82 | 3.44 | 3.64 | 0.70 | 13.78 | 0.7 |
| Chili pepper | 21.30 | 27.40 | 1.64 | 16.36 | 2.66 | 2.60 | 1.64 | 1.96 | 1.60 | 1.90 | 27.4 | 1.6 |
| Beetroot | 44.63 | 35.34 | 25.52 | 25.35 | 35.01 | 32.46 | 25.52 | 37.58 | 34.50 | 21.47 | 44.63 | 21.47 |
| Fresh walnut | 35.01 | 35.16 | 31.44 | 32.52 | 33.08 | 30.84 | 31.44 | 34.40 | 21.15 | 26.23 | 35.16 | 21.15 |
| Dry walnut | 7.27 | 17.34 | 33.47 | 15.95 | 30.12 | 31.90 | 33.47 | 15.10 | 35.27 | 16.34 | 35.27 | 7.27 |
| Max | 44.63 | 35.34 | 33.47 | 32.52 | 35.01 | 32.46 | 33.47 | 37.58 | 35.27 | 26.23 | | |
| Min | 7.27 | 6.32 | 1.64 | 2.89 | 2.66 | 2.60 | 1.64 | 1.96 | 1.60 | 0.70 | | |

*Table 6. ΔE after 14 months (set 2: Beetroot, Fresh and Dry Walnut) or 18 months (set 1: Lavender. Mint. Chili pepper). Values above 10 are highlighted with a gradient.*

| ΔE T455/596 | OE | FE | AE | OR_E | OR | OF | AF | OG | FG | AG | **Max** | *Min* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lavender | 8.47 | 6.76 | 8.42 | 4.19 | 6.83 | 9.82 | 4.73 | 9.31 | 8.09 | 4.46 | 9.82 | 4.19 |
| Mint | 12.49 | 12.58 | 8.98 | 10.65 | 5.91 | 15.60 | 4.82 | 5.27 | 8.31 | 3.69 | 15.60 | 3.69 |
| Chili pepper | 14.64 | 22.87 | 19.66 | 10.35 | 4.62 | 5.12 | 3.44 | 3.12 | 2.97 | 2.23 | 22.87 | 2.23 |
| Beetroot | 17.60 | 29.25 | 16.65 | 6.23 | 22.53 | 29.85 | 22.92 | 34.80 | 31.98 | 21.14 | 34.80 | 6.23 |
| Fresh walnut | 34.84 | 34.40 | 32.80 | 31.69 | 33.52 | 31.75 | 30.66 | 36.76 | 29.26 | 32.67 | 36.76 | 29.26 |
| Dry walnut | 19.02 | 28.01 | 30.15 | 28.73 | 31.86 | 34.23 | 34.18 | 32.39 | 33.16 | 18.27 | 34.23 | 18.27 |
| Max | 34.84 | 34.40 | 32.80 | 31.69 | 33.52 | 34.23 | 34.18 | 36.76 | 33.16 | 32.67 | | |
| Min | 8.47 | 6.76 | 8.42 | 4.19 | 4.62 | 5.12 | 3.44 | 3.12 | 2.97 | 2.23 | | |

After 3 months (T91-92), fluid samples from each experiment-jar was sampled to be analysed by UV-vis spectroscopy, in order to detect characteristic peaks of some of the natural pigments thought to have leached from the specimen. According to reference spectra, polyphenolic tannins have peak in UV absorption between 200-300 nm (Grasel & al. 2016), chlorophylls peak twice: around 450nm and 650nm (Taniguchi & Lindsey 2021), betalains peaks at ~480nm and 550nm (Sengupta & al. 2015) carotenoids peak multiple times in a broad range of 400-500nm (Domenici et al. 2014). It was possible to identify the profile of polyphenolic tannins UV-absorption in both the dried and fresh walnuts fluids (Figure 8, Figure 9), as well as in lavender, mint (Figure 10, Figure 11) and maybe Chili pepper (Figure 13). Betalains were clearly identified in beetroot fluids (Figure 12). Chlorophyll peaks were visible in the mint fluids (Figure 11) and some of the Chili pepper peaks can be attributed to carotenoid compounds (Figure 13).

In addition to giving indications of the compounds extracted from the specimen, a clear correlation can be made between high ΔE value (Figures 2-7) and high absorption in the UV-vis spectra (Figures 8-13). For instance, walnuts and Beetroots jars, with a dark fluid at the time of sampling, showed absorptions reaching values up to 20-30 (Figures 8, 9 and 12), whereas the lighter fluids from mint and lavender jars barely exceed an absorption of 5 (Figure 10-11).

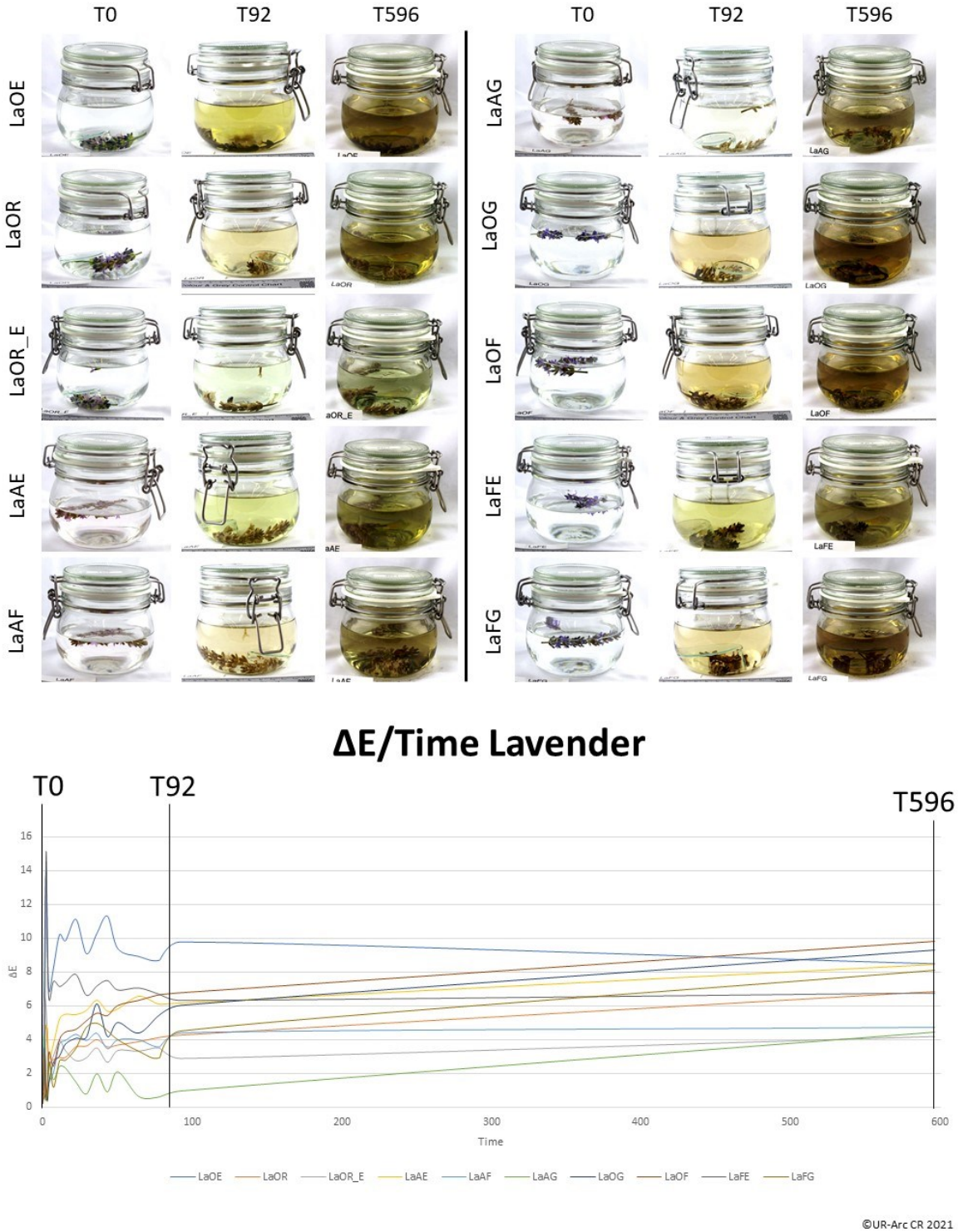
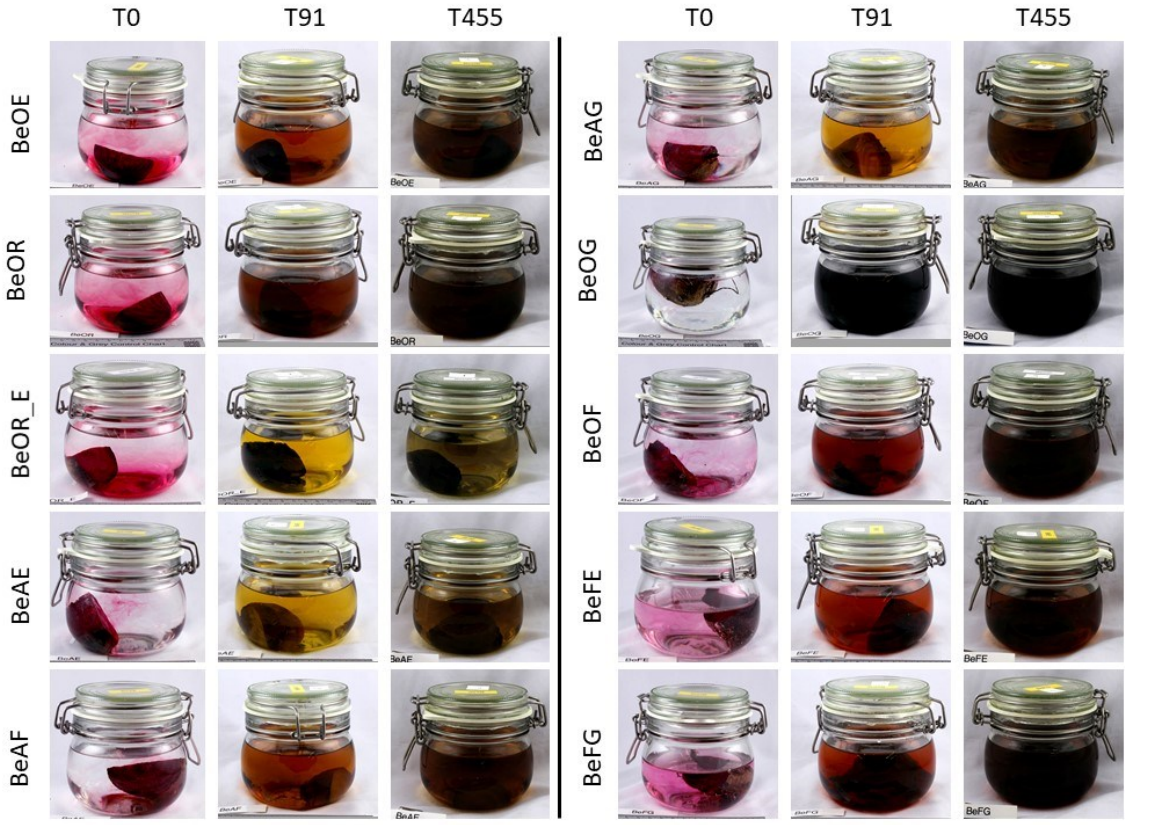



*Figure 2: ΔE/time and photos at time of preparation, 3 months and 18 months for Lavender. © UR-Arc CR 2021.*



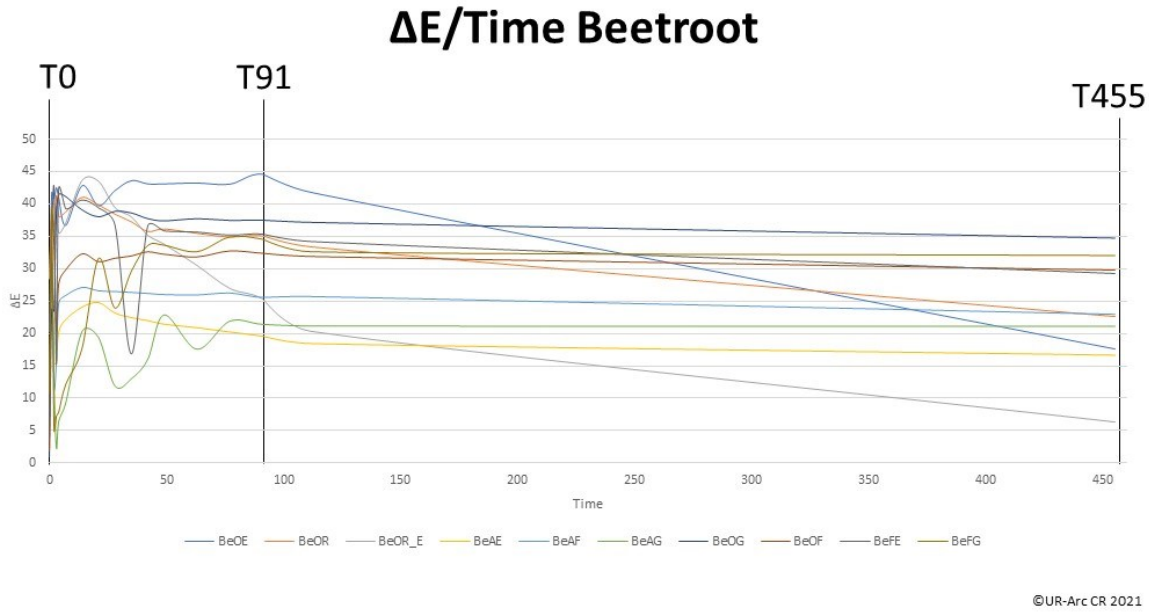




*Figure 3: ΔE/time and photos at time of preparation, 3 months and 14 months for Beetroot. © UR-Arc CR 2021.*

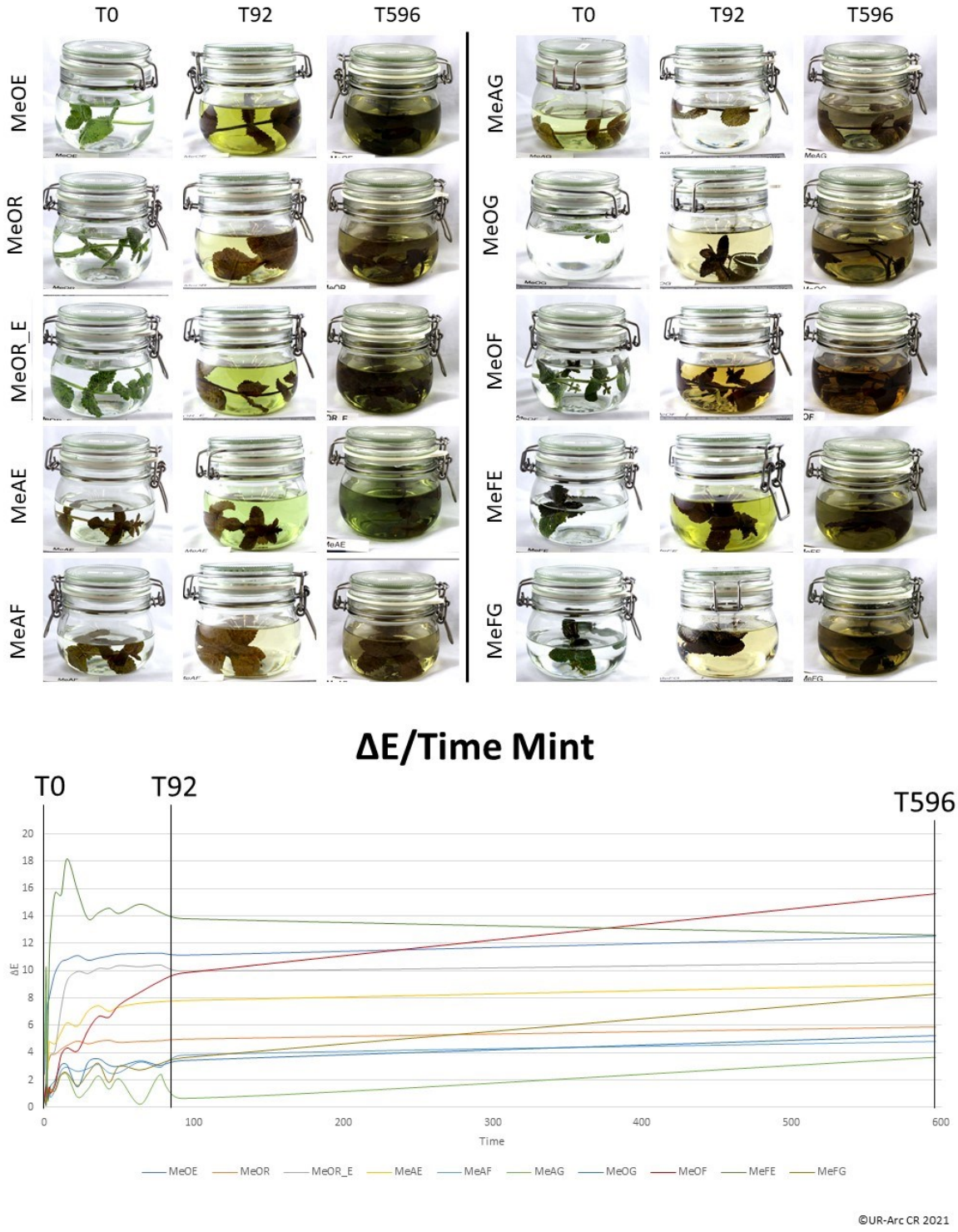



*Figure 4. ΔE/time and photos at time of preparation, 3 months and 18 months for Mint. ©UR-arc CR 2021.*

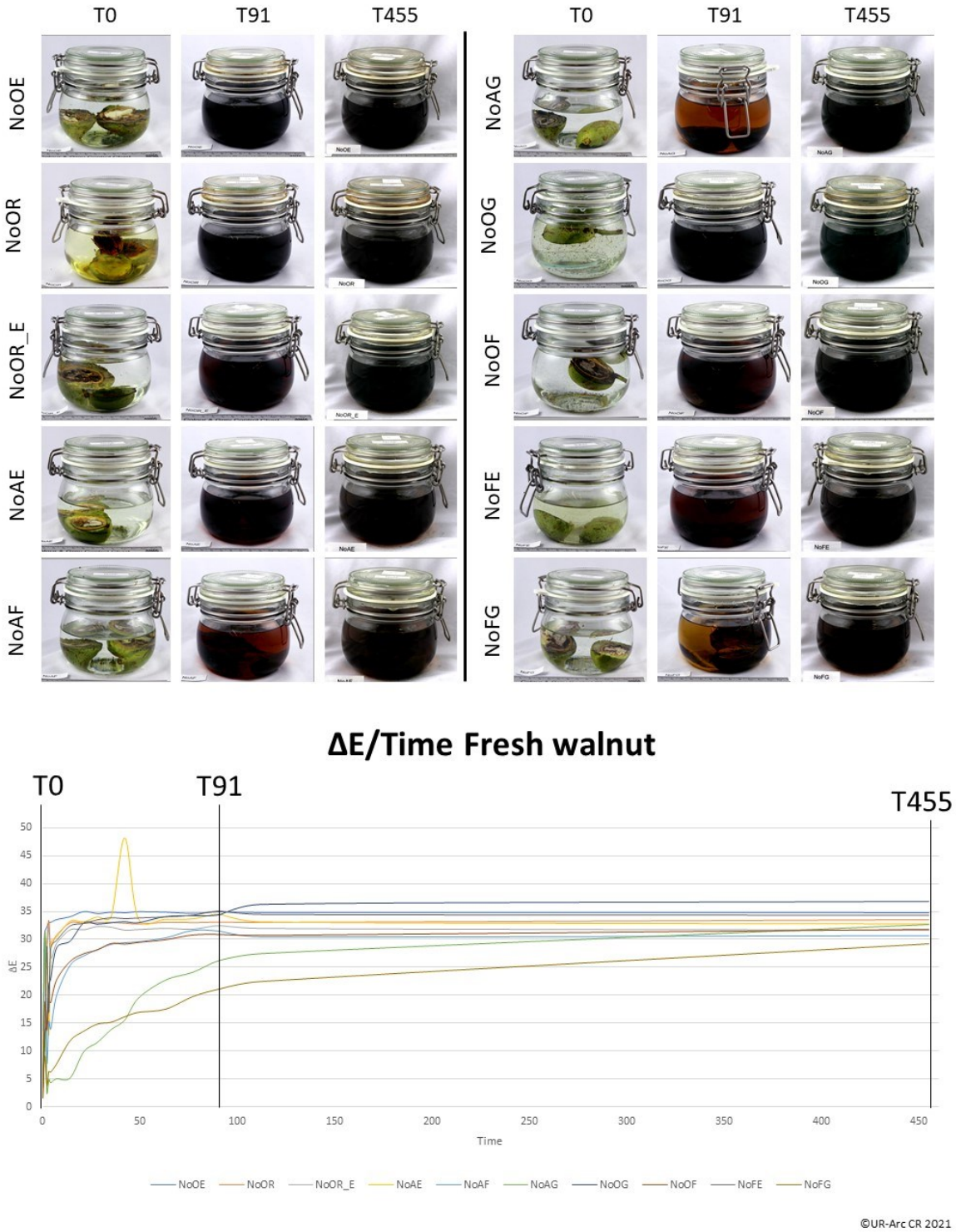



*Figure 5. ΔE/time and photos at time of preparation, 3 months and 18 months for Fresh Walnut. © UR-Arc CR 2021.*

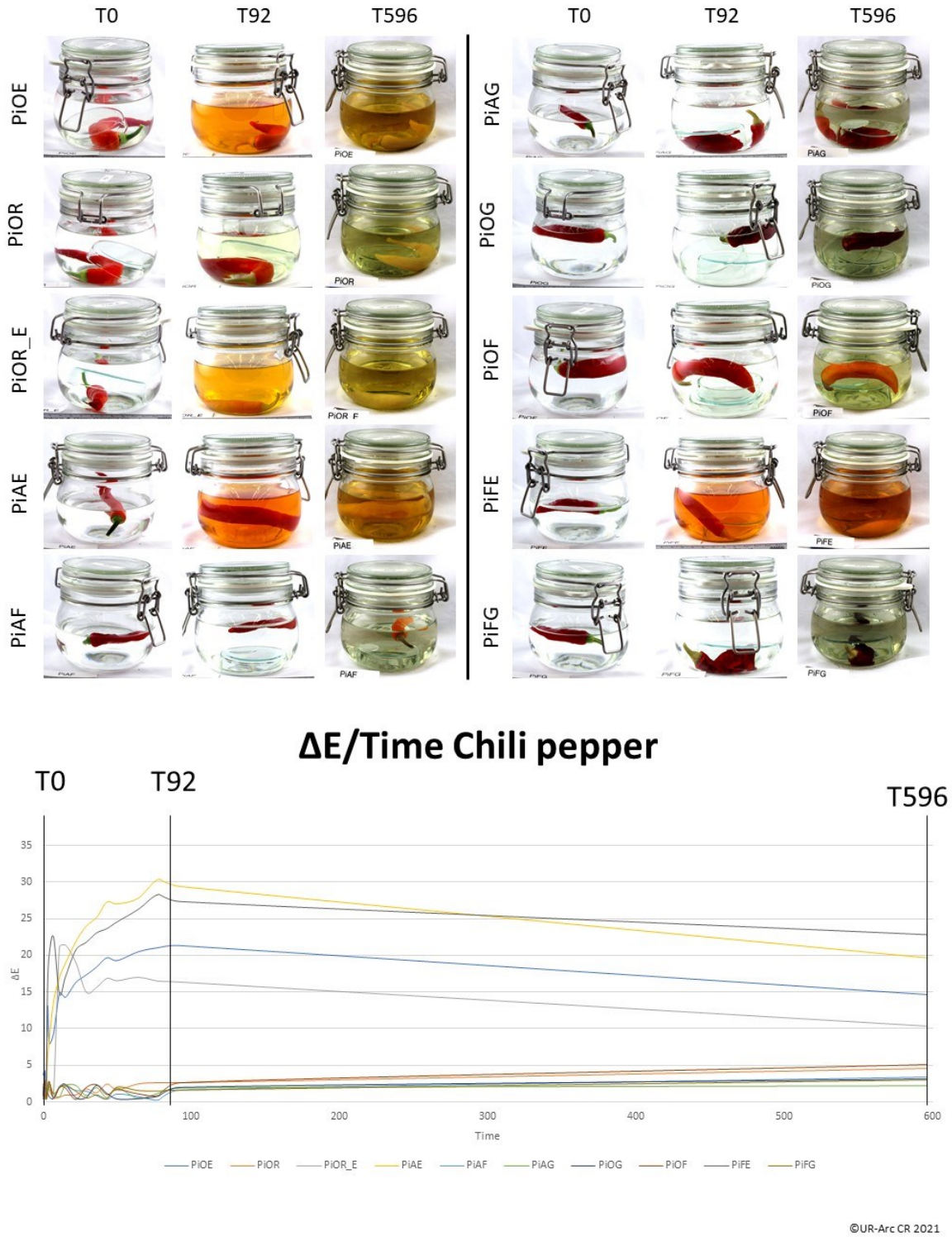
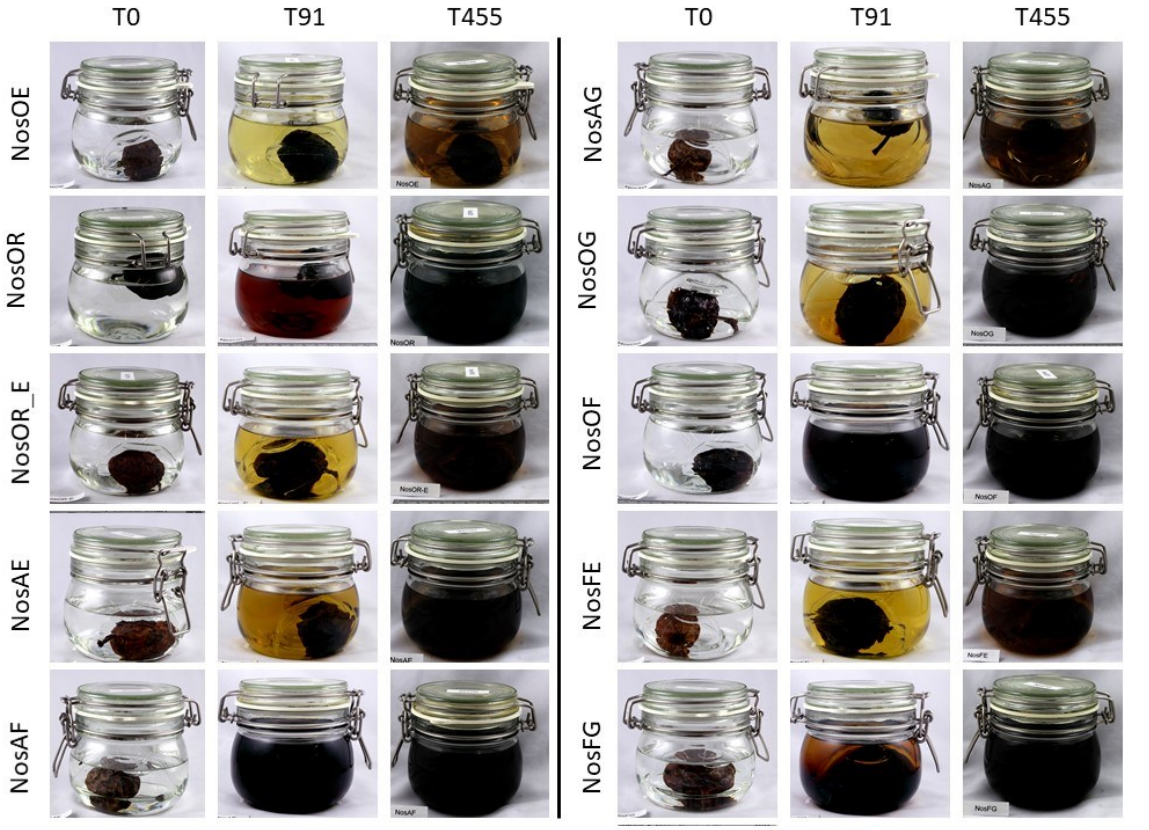



*Figure 6. ΔE/time and photos at time of preparation, 3 months and 18 months for Chili Pepper. © UR-Arc CR 2021.*



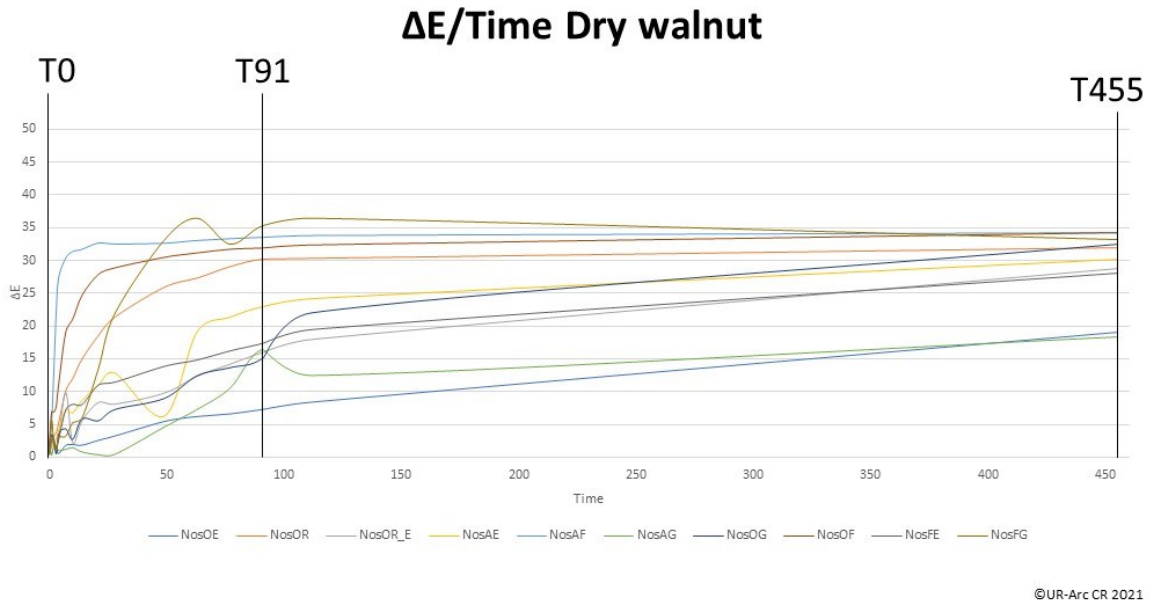






*Figure 7. ΔE/time and photos at time of preparation, 3 months and 18 months for Dry Walnut. © UR-Arc CR 2021.*

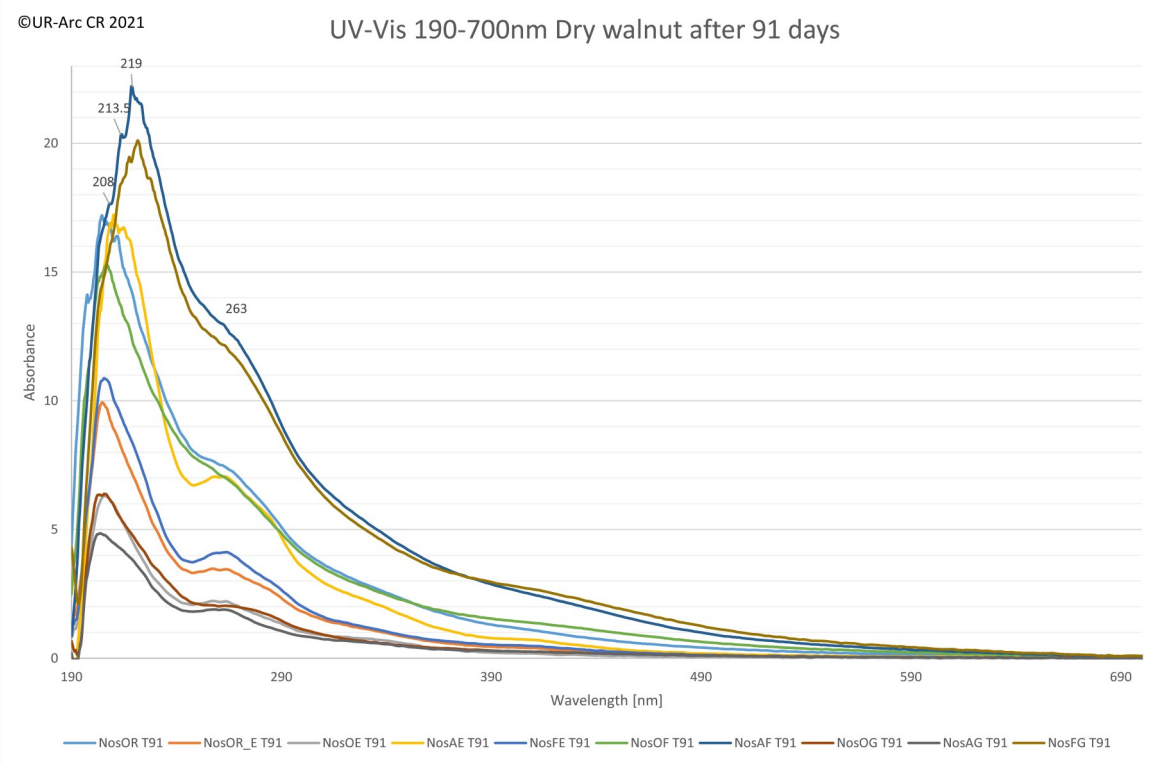



*Figure 8. UV-Vis absorption spectra on Dry walnut fluids. © UR-Arc CR 2021.*

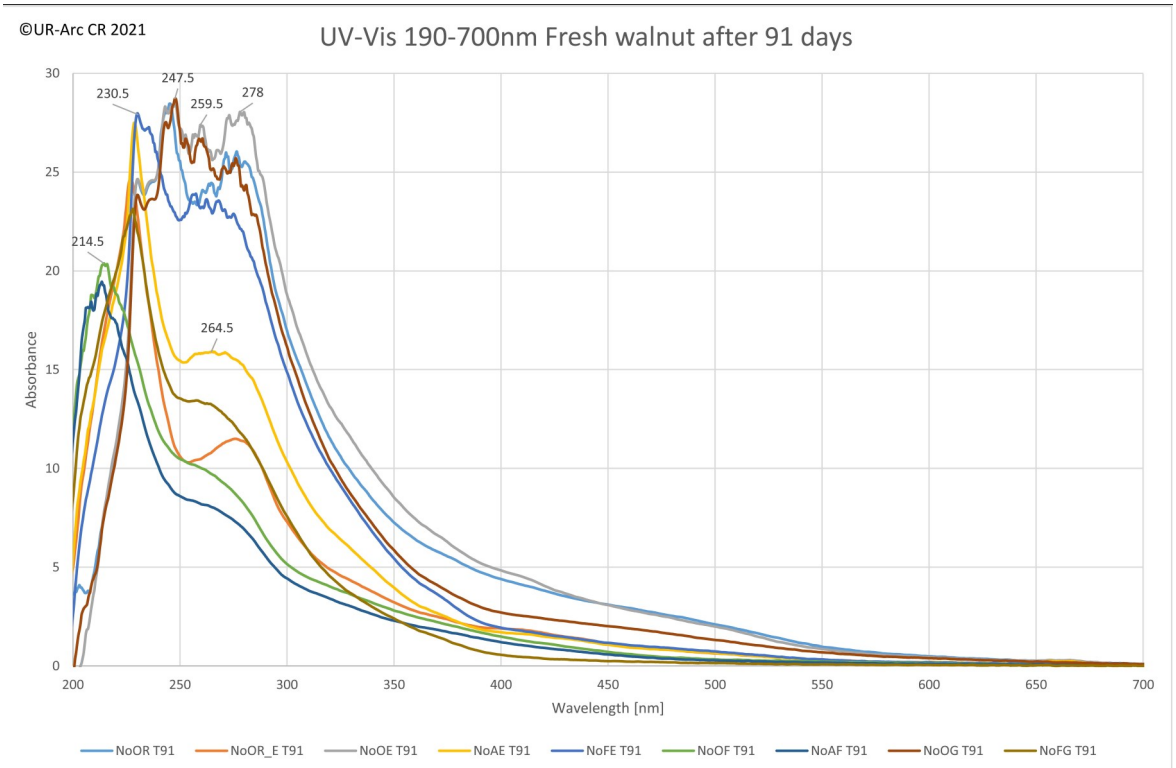



*Figure 9. UV-Vis absorption spectra on Fresh walnut fluids. © UR-Arc CR 2021.*

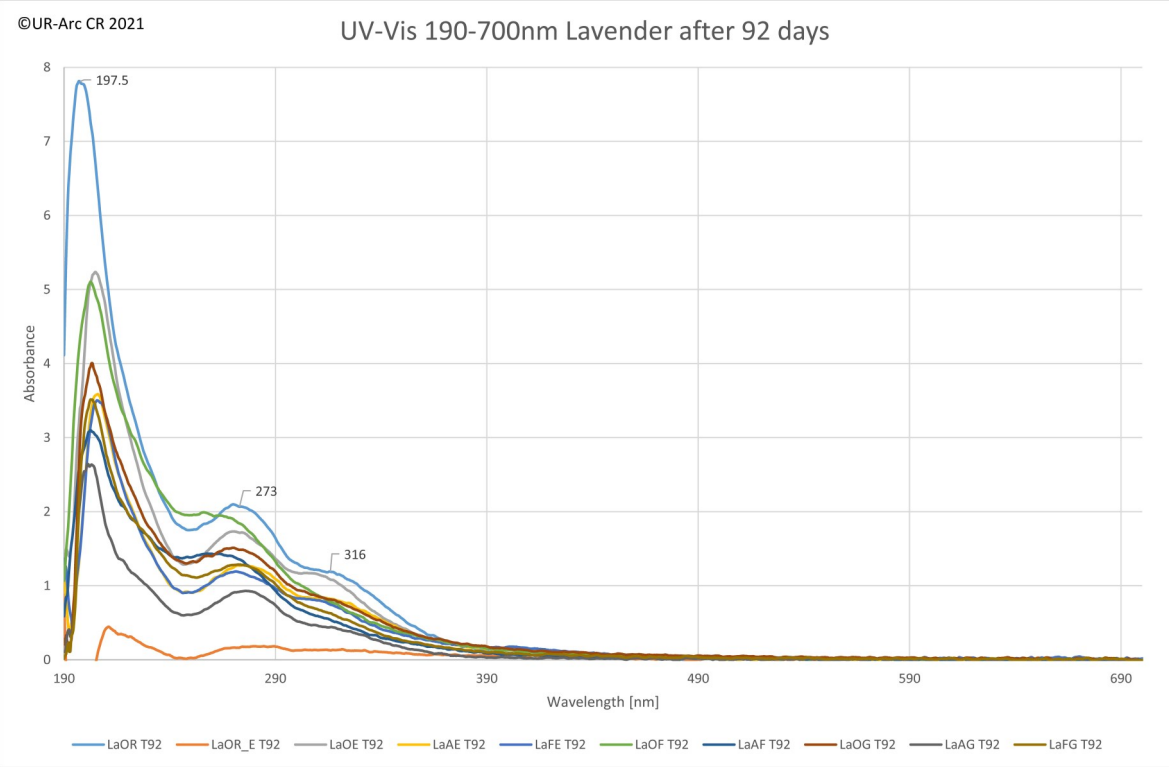



*Figure 10. UV-Vis absorption spectra on Lavender fluids. © UR-Arc CR 2021.*

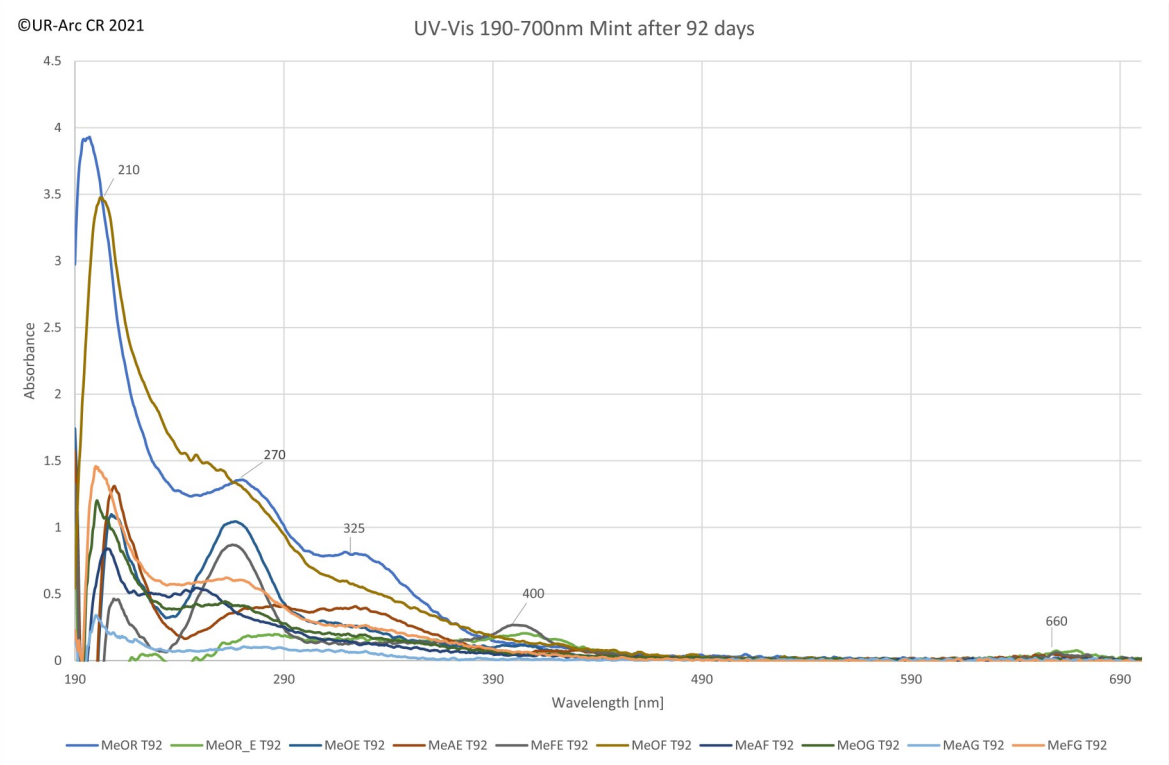



*Figure 11. UV-Vis absorption spectra on Mint fluids. © UR-Arc CR 2021.*

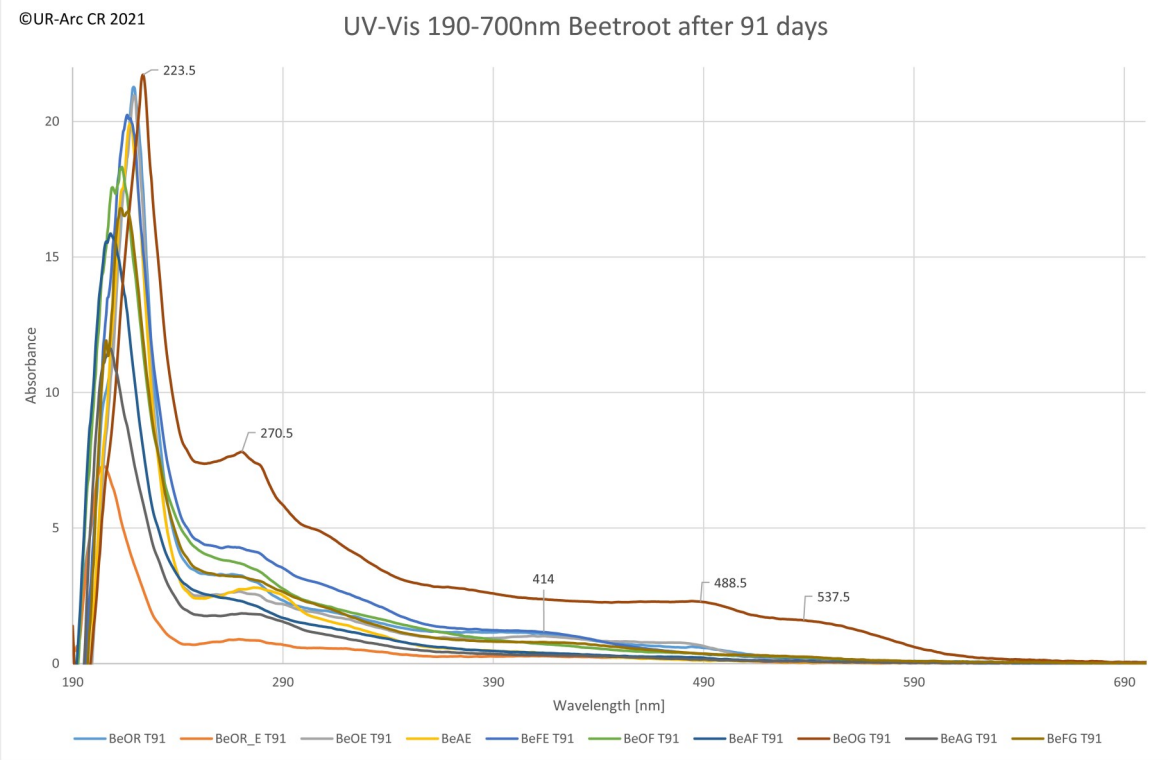



*Figure 12. UV-Vis absorption spectra on Beetroot fluids. © UR-Arc CR 2021.*

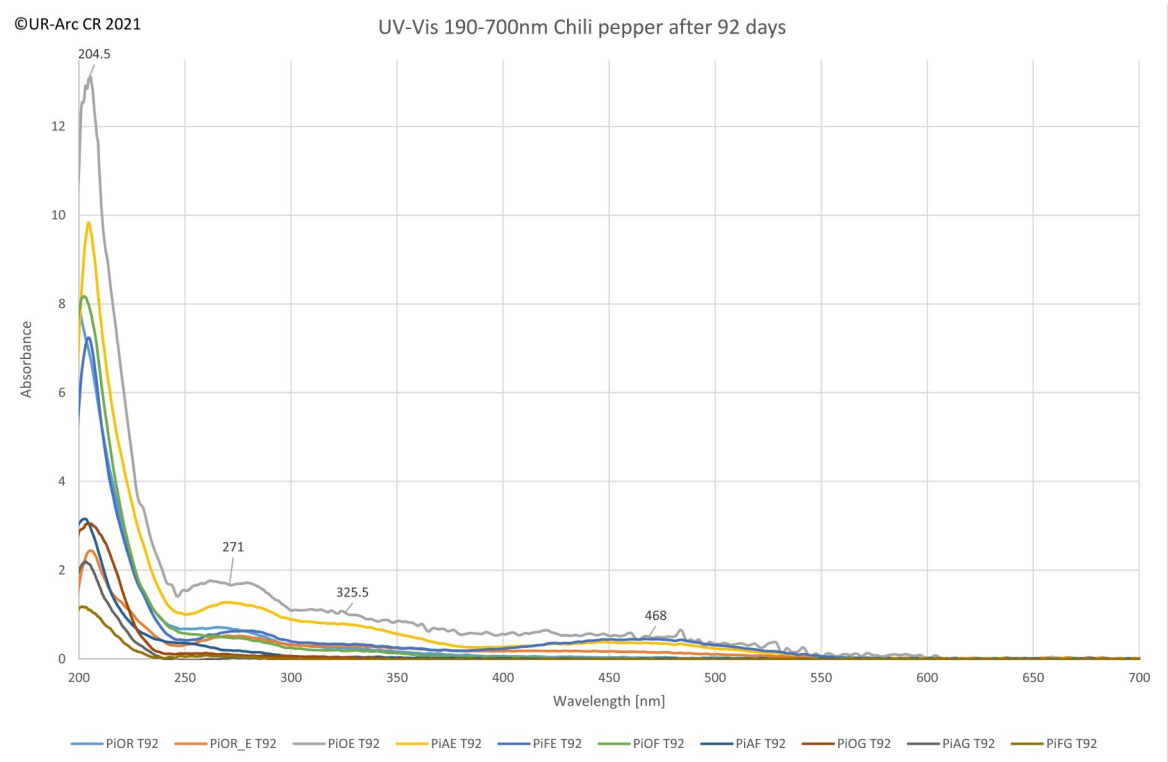



*Figure 13. UV-Vis absorption spectra on Chili pepper fluids. © UR-Arc CR 2021.*

*Degradation of the leached pigments in the fluids*

Photographs taken on the same day of colorimetry measurements allow us to link an increasing ΔE to the leaching of colour from the specimen into the fluid, and a decreasing ΔE to the overall discolouration of the tainted fluid (Figures 2-7). The hypothesis was that the pigments were degrading once inside the fluid, and that this process was mostly happening with alcohol-based solutions. To test this, the samples that were collected from the jars for the UV-Vis analysis were kept in test tubes conserved in the dark and re-measured with the spectrophotometer after 14-18 months. The specimen could not contribute to the concentration of pigments in the fluid anymore, allowing observations to be made in regard to the interactions between pigments and fluid.

The CIELAB values obtained on those test-tubes were very different from the ones obtained on the day the fluid was sampled. This shows that the colour of the fluid changed even in the absence of the specimen. Tables 9, 10 and 11 report the overall change ($\Delta E$), but also in which direction the variation occurred: change in saturation ($\Delta L^*$) or change in hue ($\Delta a^*$ and $\Delta b^*$) between the time of sampling (T91-92) and the second measurement (1.5 year). $\Delta E$ is a strictly positive value. $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ are differential equations ($\Delta X = x - x_0$) (X-Rite Pantone, 2016), where:

$\Delta L^*$
[+] : brighter / less dark
[-] : darker / less bright

$\Delta a^*$
[+] : redder / less green
[-] : greener / less red

$\Delta b^*$
[+] : yellower / less blue
[-] : bluer / less yellow

Overall, alcohol-based preservatives showed the biggest colour change. Samples from the Mint (Me) experiment-jars (Table 9) show very little changes, with a $\Delta E<5$. These changes mainly occurred on the $L^*$ and $b^*$ values. These values translate to a change from brighter green or yellow (Figure 4, T92) to a nearly transparent fluid. To the observer, this scale of change is hardly noticeable. It must be noticed that this trend has not been observed on the experiment-jars themselves, who kept darkening after 18 months due to the presence of the specimen, continuing to release pigments.

Beetroot fluids showed the highest $\Delta E$, ranging from 5 to 22, with the changes mainly occurring in the $a^*$ and $b^*$ values (Table 10). The jars had a wide range of saturation ($L^*$ from 26 to 59) of warm yellow-brown colour at the time of sampling (Figure 3, T91).

By the time of the second measurement, all fluids of unfixed specimens in alcohol-based preservatives, OE, OR, OR_E, had noticeably changed ($\Delta E>10$). For the Chili peppers fluids, only specimens in alcohol-based fluids had a distinct colouration at the time of sampling (Figure 6, T92). They were also the ones undergoing bigger colour changes (Higher $\Delta E$) (Table 11).

*Integrity of the specimen*

The photographic documentation as well as frequent observations of the jars allowed assessments to be made in relation to the conservation state of the specimens during the monitoring period. Multiple alterations have been noticed and classified in 6 groups (Table 12).

## Discussion

The results presented above tend to corroborate the empirical observations made in the Botanical Museum of Zurich University and other collections and clearly show that depending on the specimen, discolouration problems occur in different fluids and at different rates. The classification of specimens in two sets based on their colour preservation problem ended up being relevant. Indeed, when the solubility of the pigment and its location in the cell is considered, the different trends observed during the tests start to make sense. The pigments mainly responsible for the colour of specimens from set 1 are chlorophylls and carotenoids, stored in the chloroplasts (Buchanan, Gruissem, and Jones, 2015). These specimens tend to leach their pigments quickly in alcohol-based preservative such as ethanol independently of the fixation process. Indeed, ethanol is known to be a great extracting agent, since it increases membrane permeability (Goldstein, 1986; Hendry, Houghton, and Brown, 1987). Those same pigments are also known to be very sensitive, explaining why they degraded inside the fluid after a certain time. However, either due to the light colour of the pigments or their lower concentration in the jar, this leaching was not enough to opacify the fluid to the point of masking the specimen from view. In comparison, specimens with strong dying properties from set 2 owe their colour to water soluble pigments stored in the vacuole (Buchanan, Gruissem, and Jones, 2015; Delgado-Vargas, Jiménez, and Paredes-López, 2000). They were all darker and more voluminous than the specimens from set 1, and leached so

*Table 9. Delta E, L* a* and b* for Mint (Me) sampled fluids*

| | Mint | | | |
|---|---|---|---|---|
| | **sample** | **L** | **a** | **b** |
| **JARS T92** | MeOE | 60.93 | -3.81 | 11.52 |
| | MeOR | 59.80 | -1.85 | 5.59 |
| | MeOR_E | 60.61 | -4.29 | 10.08 |
| | MeAE | 60.30 | -4.12 | 7.56 |
| | MeAF | 59.48 | -0.83 | 3.48 |
| | MeAG | 62.44 | -0.60 | 1.86 |
| | MeOG | 61.52 | -0.94 | 4.73 |
| | MeOF | 58.12 | -1.27 | 9.73 |
| | MeFE | 59.14 | -4.62 | 13.73 |
| | MeFG | 62.43 | -1.11 | 5.22 |
| **TUBES T92** | MeOE | 58.39 | -3.14 | 8.52 |
| | MeOR | 58.14 | -1.88 | 6.45 |
| | MeOR_E | 56.93 | -4.07 | 8.73 |
| | MeAE | 58.39 | -4.22 | 7.22 |
| | MeAF | 59.22 | -0.75 | 3.03 |
| | MeAG | 60.74 | -0.57 | 2.14 |
| | MeOG | 59.63 | -0.74 | 4.20 |
| | MeOF | 57.90 | -1.31 | 8.74 |
| | MeFE | 57.98 | -3.98 | 10.11 |
| | MeFG | 59.80 | -0.96 | 4.71 |
| **Tube - jar** | **ΔE** | **ΔL** | **Δa** | **Δb** |
| MeOE | 3.99 | -2.54 | 0.67 | -3.00 |
| MeOR | 1.87 | -1.66 | -0.03 | 0.86 |
| MeOR_E | 3.93 | -3.68 | 0.22 | -1.35 |
| MeAE | 1.94 | -1.91 | -0.10 | -0.34 |
| MeAF | 0.53 | -0.26 | 0.08 | -0.45 |
| MeAG | 1.72 | -1.70 | 0.03 | 0.28 |
| MeOG | 1.97 | -1.89 | 0.20 | -0.53 |
| MeOF | 1.01 | -0.22 | -0.04 | -0.99 |
| MeFE | 3.85 | -1.16 | 0.64 | -3.62 |
| MeFG | 2.68 | -2.63 | 0.15 | -0.51 |

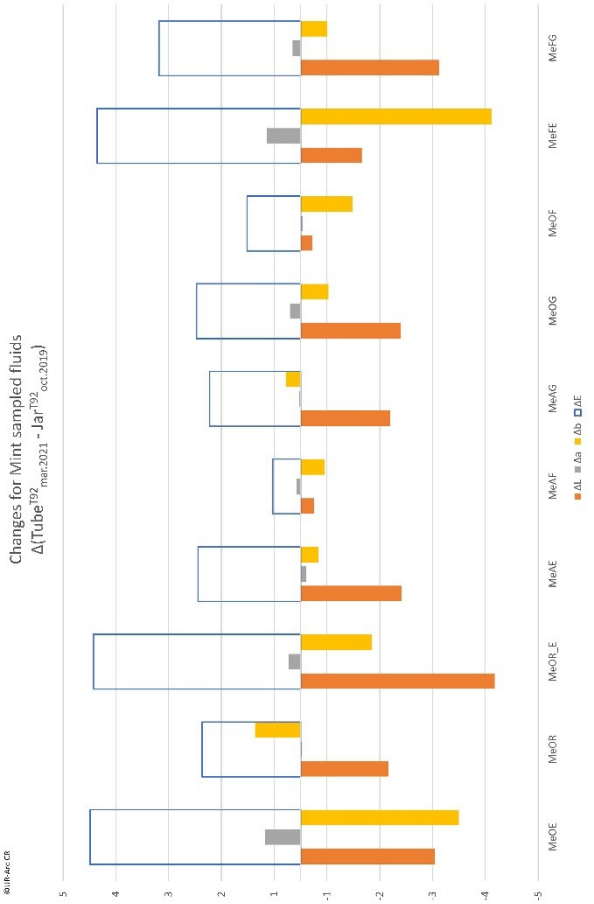
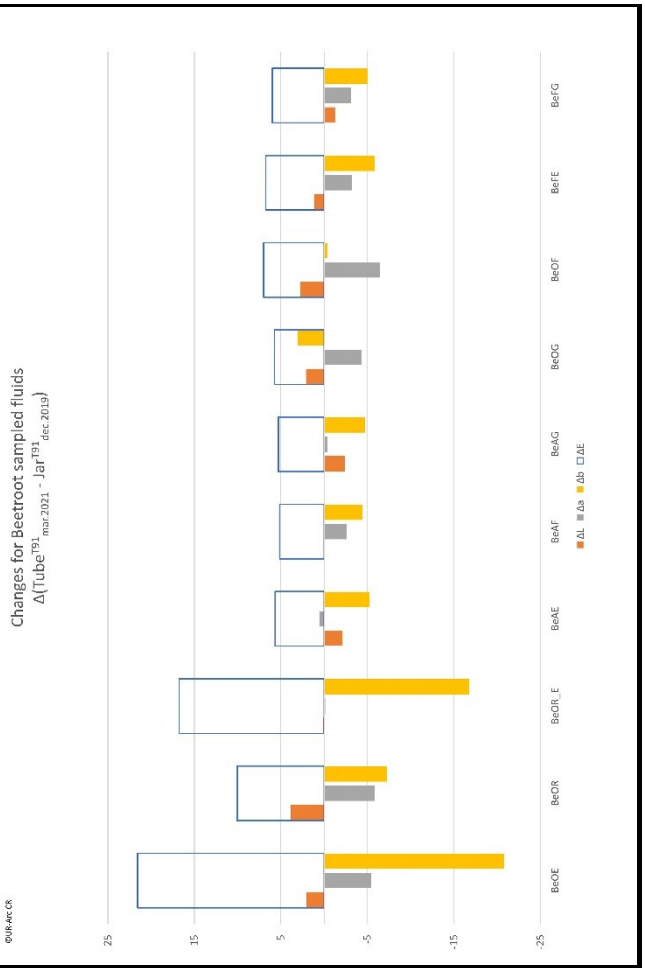


*Table 10. Delta E, L* a* and b* for Beetroot (Be) sampled fluids.*

| | Beetroot | | | |
|---|---|---|---|---|
| | **sample** | **L** | **a** | **b** |
| **JARS T91** | BeOE | 52.44 | 6.40 | 43.95 |
| | BeOR | 46.83 | 8.08 | 31.87 |
| | BeOR_E | 58.84 | -0.75 | 26.10 |
| | BeAE | 59.30 | -1.92 | 20.27 |
| | BeAF | 54.16 | 3.87 | 24.66 |
| | BeAG | 58.04 | 0.33 | 22.57 |
| | BeOG | 26.97 | 9.93 | 3.14 |
| | BeOF | 46.08 | 13.59 | 25.20 |
| | BeFE | 50.43 | 6.87 | 33.39 |
| | BeFG | 47.92 | 11.73 | 30.25 |
| **TUBES T91** | BeOE | 54.47 | 0.96 | 23.18 |
| | BeOR | 50.66 | 2.25 | 24.63 |
| | BeOR_E | 58.93 | -0.87 | 9.34 |
| | BeAE | 57.17 | -1.42 | 15.05 |
| | BeAF | 54.21 | 1.28 | 20.22 |
| | BeAG | 55.68 | -0.06 | 17.85 |
| | BeOG | 29.07 | 5.58 | 6.23 |
| | BeOF | 48.82 | 7.14 | 24.8 |
| | BeFE | 51.55 | 3.64 | 27.57 |
| | BeFG | 46.67 | 8.63 | 25.26 |
| **Tube - jar** | **ΔE** | **ΔL** | **Δa** | **Δb** |
| BeOE | 21.57 | 2.03 | -5.44 | -20.77 |
| BeOR | 10.05 | 3.83 | -5.83 | -7.24 |
| BeOR_E | 16.76 | 0.09 | -0.12 | -16.76 |
| BeAE | 5.66 | -2.13 | 0.50 | -5.22 |
| BeAF | 5.14 | 0.05 | -2.59 | -4.44 |
| BeAG | 5.29 | -2.36 | -0.39 | -4.72 |
| BeOG | 5.73 | 2.10 | -4.35 | 3.09 |
| BeOF | 7.02 | 2.74 | -6.45 | -0.40 |
| BeFE | 6.75 | 1.12 | -3.23 | -5.82 |
| BeFG | 6.01 | -1.25 | -3.10 | -4.99 |




much that the fluid in most jars was completely dark at the end of the monitoring period. These specimens released pigments at a steadier rate when preserved in alcohol. However, they tend to release more pigments when put in contact with formaldehyde, either as fixative or preservative fluid, probably because these pigments are water soluble.

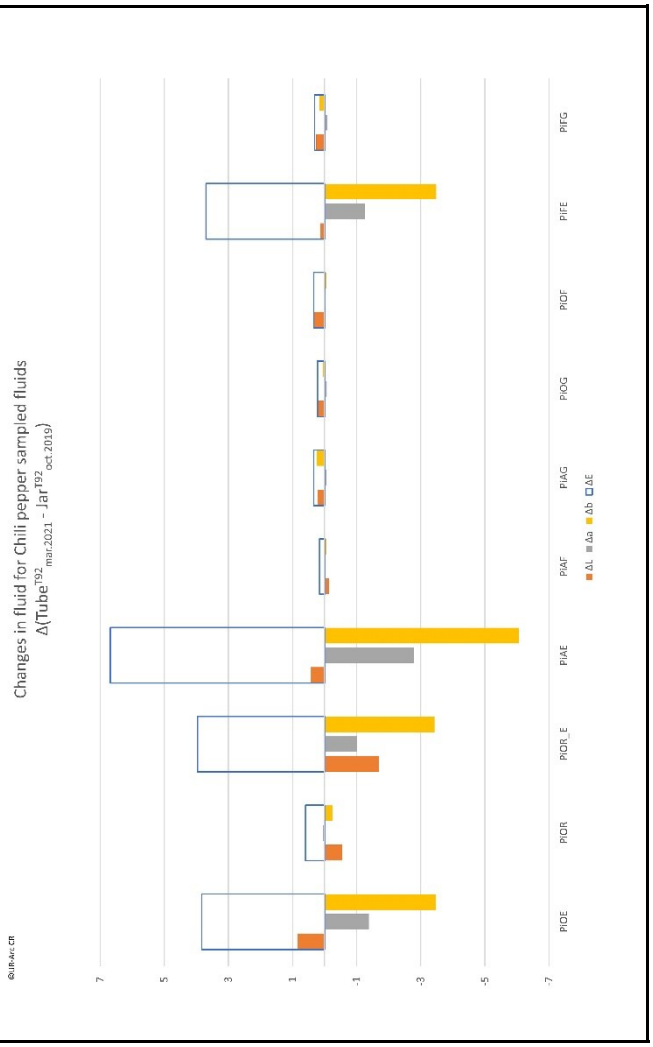
The interpretation of the UV-Vis spectra was carried out by comparison with data found in the literature and a good indication of the type of pigments could be obtained. Moreover, it was possible to correlate the intensity of the absorption spectra with the importance of the colour change ($\Delta E$), as well as confirm the degradation process of the leached pigments in alcohol-based preservatives.

Specimens from both sets showed, overall, less discolouration when fixed and then preserved in 70% glycerol. However, colour is not the only feature to be preserved, and glycerol at such high concentration caused severe shrinkage in fleshy specimens. Moreover, glycerol-preserved specimens require stricter climate control for their storage, in order to prevent moulding issues (Van Dam, 2018). Other changes such as softening and stiffening of the tissues have also been observed. Based on the simple manipulation-test on these two sets, they seem to be fluid-dependent. For instance, alcohol-based preservatives seem to stiffen all specimens, whereas glycerol seems to cause shrinkage in fleshy specimens (i.e. fruits) and FAA caused softening in thinner tissues. Further investigations are needed, and microscopic observations on the specimens, as well as pH measurements have already been added to the monitoring protocol for the new ongoing sets of tests. Other historical or modern preservatives will also be incorporated in the future experiments.

It now seems evident that grouping the colour alterations under the same "discolouration" label only makes sense with regards to how it affects the preservative fluid, but it gives very little

*Table 11. Delta E, L* a* and b* for Chili pepper (Pi) sampled fluids.*

| | Chili pepper | | | |
|---|---|---|---|---|
| | **sample** | **L** | **a** | **b** |
| **JARS T92** | PiOE | 55.88 | 3.97 | 20.75 |
| | PiOR | 60.72 | -1.15 | 3.39 |
| | PiOR_E | 59.86 | 1.27 | 17.07 |
| | PiAE | 55.03 | 7.18 | 28.40 |
| | PiAF | 60.65 | -0.67 | 1.22 |
| | PiAG | 61.21 | -0.57 | 1.42 |
| | PiOG | 61.13 | -0.58 | 1.61 |
| | PiOF | 59.85 | -0.83 | 1.84 |
| | PiFE | 54.79 | 7.56 | 26.04 |
| | PiFG | 61.51 | -0.53 | 1.48 |
| **TUBES T92** | PiOE | 56.72 | 2.59 | 17.28 |
| | PiOR | 60.18 | -1.11 | 3.15 |
| | PiOR_E | 58.16 | 0.25 | 13.64 |
| | PiAE | 55.45 | 4.40 | 22.34 |
| | PiAF | 60.51 | -0.69 | 1.16 |
| | PiAG | 61.43 | -0.62 | 1.67 |
| | PiOG | 61.33 | -0.65 | 1.67 |
| | PiOF | 60.18 | -0.86 | 1.78 |
| | PiFE | 54.91 | 6.31 | 22.56 |
| | PiFG | 61.77 | -0.61 | 1.63 |
| **Tube - jar** | **ΔE** | **ΔL** | **Δa** | **Δb** |
| PiOE | 3.83 | 0.84 | -1.38 | -3.47 |
| PiOR | 0.59 | -0.54 | 0.04 | -0.24 |
| PiOR_E | 3.96 | -1.70 | -1.02 | -3.43 |
| PiAE | 6.68 | 0.42 | -2.78 | -6.06 |
| PiAF | 0.15 | -0.14 | -0.02 | -0.06 |
| PiAG | 0.34 | 0.22 | -0.05 | 0.25 |
| PiOG | 0.22 | 0.20 | -0.07 | 0.06 |
| PiOF | 0.34 | 0.33 | -0.03 | -0.06 |
| PiFE | 3.70 | 0.12 | -1.25 | -3.48 |
| PiFG | 0.31 | 0.26 | -0.08 | 0.15 |



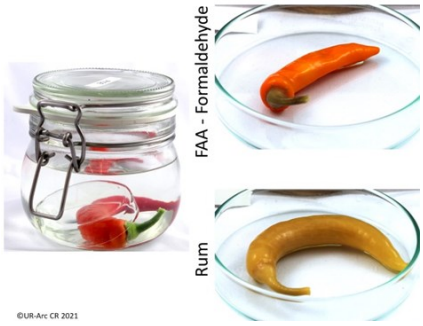

information about what is happening in the specimen. It would be more appropriate to refer to the colour alterations of the specimen in a conservation-assessment report as "lightening" or "darkening". Indeed, in a specimen, some pigments are released in the fluid when others stay in the plant. Therefore, in some cases (set 1), the specimen assumes a lighter colour. The remaining pigments can sometimes undergo molecular modifications, thus changing the specimen's colour once again. This can manifest in the darkening, or yellowing, of leaves or browning of flowers for example. Darkening of the specimen also occurs if the leaching pigment has strong dying properties. This will not only opacify the fluid, but it can also affect other tissues from the specimen, making it look darker. This is what happens to the fresh walnuts, which darkened to a brown tone.

From those results, it appears that monitoring the colour change of a fluid to understand the discolouration problems of a specimen is not sufficient on its own. Only by cross-referencing colorimetric information with photographic documentation and closer observation of the specimen, was it possible to get a clearer picture of what was happening. However, such extended monitoring protocol can be difficult to achieve on the scale of a whole collection, and museums should choose simpler documentation protocols that are easier to maintain.

## Conclusion

This paper presented the first results of an ongoing project carried out at the UR-arc CR of Neuchâtel, Switzerland. The aim was to gather experimental data on the discolouration problems identified in museum collections of botanical specimens. Two sets of experiment-jars containing

*Table 12:.Alterations observed on the specimens.*

| | |
|---|---|
| Discolouration / colour leakage:<br>Release of pigments from the specimen to the fluid. It gives the fluid a noticeable tint. In the most advance stage, this leads to the complete opacification of the fluid, masking the specimen. |  |
| Lightening:<br>Loss of colour. The specimen assumes a lighter colour. This often happened in alcohol-based preservatives but was also noted in other preservatives. The pigments thought to be sensitive to this alteration in the tested fluids are chlorophylls, carotenoids and anthocyanin flavonoids (Set 1). |  |
| Darkening:<br>The specimen assumes a duller colour, usually in the brown tones. The pigments more sensitive to this alteration in the tested fluids are mostly tannins. |  |
| Softening:<br>The specimen becomes soft. When removed from the fluid, it doesn't hold its shape. When manipulated out of the fluid, it is very flexible. In the most advanced stage, the tissues have a jelly texture and can look translucent. (Leaves fixed in FAA fixative)<br><br>Stiffening:<br>The specimen becomes stiff. When removed from the fluid, it holds its shape perfectly. When manipulated out of the fluid, it is very rigid with the potential for break-age. (Leaves in 70% ethanol and formaldehyde) |  |
| Shrinkage:<br>Could be due to dehydration, especially noticeable on fleshy specimens, such as fruits. Moreover, stiffening was often observed on the shrunk specimen. The tissues retracted, causing the specimen to look wrinkled or dry. (fruits and roots in glycerol and sometimes in ethanol) |  |

specimens were prepared following different fluid preservation protocols. The discolouration of the specimens was indirectly monitored by measuring the colouration of the fluid regularly for 3 months and once again after approximately 1.5 years.

Information on the change of colour in the fluid was obtained by spectrocolorimetry measurements in the CIELAB colour space, and expressed by computing ($\Delta E$), the overall perceived difference between the clear fluid and the altered one. Finally, the leached pigments were partially identified by UV-vis spectroscopy. As expected, alcohol-based preservatives promoted the leaching of pigments from the specimens. The two tested fixatives, formaldehyde and FAA, gave different results depending on the specimen and the preservative fluid used. Finally, high concentration glycerol proved to be a good way to preserve colour but, in some cases, caused severe damage to the structural integrity of the tissues.

In conclusion, every specimen has a complex mix of pigments, varying in nature and concentration, even between individuals from the same species. All the pigments were found to have sensitivity to solvents and undergo different chemical degradation processes. Therefore, it is for now not possible to propose a "one fits all" recipe to preserve them. As it is often the case in conservation, one should have a case-by-case approach to the matter. The research continues and allows us to refine our test protocols.

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