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PII: S0963-9969(18)30337-5
DOI: doi:10.1016/j.foodres.2018.04.053
Reference: FRIN 7571

To appear in: *Food Research International*

Received date: 20 February 2018
Revised date: 5 April 2018
Accepted date: 23 April 2018

Please cite this article as: Ana Paula Eskildsen Pagano, Nauman Khalid, Isao Kobayashi, Mitsutoshi Nakajima, Marcos Antonio Neves, Erick Leite Bastos , Microencapsulation of betanin in monodisperse W/O/W emulsions. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Frin(2017), doi:10.1016/j.foodres.2018.04.053

Microencapsulation of betanin in monodisperse W/O/W emulsions

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ABSTRACT

Betanin is the main pigment of the food color beetroot red (E162). Due to the fair heat and light stability of E162, this pigment is mainly used in minimally processed packaged food products. Encapsulation increases the stability of betanin, but detailing on the effect of different sources of betanin on the properties and stability of multiple emulsions are scarce. Here we describe the encapsulation of E162, spray-dried beetroot juice and betanin in a monodisperse food-grade water-in-oil-in-water (W/O/W) emulsions by using microchannel emulsification. We compare the tinctorial strength of the encapsulated pigments and investigate the effect of temperature, storage period and pigment concentration on emulsion stability and color. Betanin increases the overall stability of the W/O/W emulsion, reduce the oil droplet size and improve size distribution when compared to the negative control without pigment and to emulsions containing betanin from other sources.

Keywords – beetroots, betalain, betanin, monodisperse emulsion, pigment stabilization, microchannel emulsification.

1. INTRODUCTION

Consumers prefer foods and beverages containing raw natural materials to artificial substances (Oplatowska-Stachowiak & Elliott, 2017; Román, Sánchez-Siles, & Siegrist, 2017). Products without (*or with reduced amount of*) artificial substances are classified as *clean label* and have been associated to putative health benefits and lower environmental impact (Asioli et al., 2017). Betanin (betanidin 5-*O*-glucoside, EEC #E162), the major pigment of the red beetroot, is a water-soluble nontoxic natural pigment belonging to the class of betalains that was approved by the FDA and has been used in foods since 1967 (Herbach, Stintzing, & Carle, 2006). Betalains show higher tinctorial strength compared to anthocyanins, and keep their color in low-acid food (within the pH 3 to 7 range), where the color of anthocyanins is loss due to hemiketal formation (Quina & Bastos, 2018). However, betanin is sensitive to thermal and photochemical decomposition and subject to acid- and base-catalyzed hydrolysis that may cause color alterations that limit its application as a food additive (Attoe & von Elbe, 1981; Attoe & von Elbe, 1984; Bastos & Gonçalves, 2017; Esteves et al., 2018; Goncalves, Da Silva, DeRose, Ando, & Bastos, 2013; Herbach, Stintzing, & Carle, 2004; Khan, 2016).

The encapsulation of betalains in complex matrices increases its persistence in food products (Khan, 2016), and may be useful to expand the application of natural and artificial derivatives as food colorants (Fernandes et al., 2016; Goncalves, Da Silva, et al., 2013; Goncalves, Tonelli, et al., 2013; Pavliuk et al., 2017; Rodrigues et al., 2018). There are several techniques for microencapsulation, which include emulsification followed by solvent removal, spray drying and milling. These techniques usually produce polydisperse material, *i.e.*, non-uniform particle sizes, and heat that may not be adequate for temperature-sensitive samples, such as betalains. Water-in-oil (W/O)

emulsions, where oil refers to any water-insoluble liquid, are suitable for encapsulating polar substances. However, multiple water-in-oil-in-water (W/O/W) emulsions allow the encapsulation of hydrophilic substances in water with a protecting layer of oil (Dias, Ferreira, & Barreiro, 2015). W/O/W emulsions have been used as delivery systems for plant bioactives, in preventing the exposure of sensitive substances to light oxygen and heat, and for the manufacture of food products with improved sensorial characteristics (Lamba, Sathish, & Sabikhi, 2015; Muschiolik & Dickinson, 2017). Homogenization of water and oil mixtures using a rotor-stator, ultrasound or high rotation result in polydisperse W/O/W emulsions. However, microchannel emulsification (MCE) produces monodisperse emulsions with improved physical stability (Khalid et al., 2014; Kobayashi & Nakajima, 2006; Souilem et al., 2013).

Aqueous beet extract was encapsulated in W/O/W type double-layer emulsion to study intestinal digestion *in vitro*, but the emulsion obtained is polydisperse and unstable, producing cream after storage at room temperature (Kaimainen, Marze, Järvenpää, Anton, & Huopalahti, 2015). Here we describe the encapsulation of three different sources of betanin in monodisperse W/O/W emulsions prepared by MCE of water, soybean oil and food-grade surfactants. The emulsion formulation was optimized by varying the volume fraction of the internal aqueous phase, the concentration of the hydrophobic and hydrophilic emulsifiers, and the flux of the dispersed phase. The tinctorial strength and color variability of encapsulated E162, powdered beetroot juice and betanin as well as the physical stability of the emulsions kept at 4 °C, 25 °C and 60 °C were monitored over 7 days and compared.

2. EXPERIMENTAL DETAILS

2.1 General

All chemicals were purchased from commercial sources with the highest purity available and were used without further purification. Refined soybean oil, D(+)-glucose, and polyoxyethylene (*n* = 20) sorbitan monolaurate (Tween 20) were purchased from Wako Pure Chemical Industries. Tetraglycerin monolaurate condensed ricinoleic acid ester (CR-310) was obtained from Sakamoto Yakuhin Kogyo Co. Ltd. Aqueous solutions were prepared with deionized water (conductivity 18.2 MΩ cm at 25 ºC, Milli-Q, Millipore).

2.2 Sources of betanin

Three sources of betanin were used: E162, (mixture of beetroot extract and maltodextrin; 0.4% w/w betanin, TCI/ABCr, sample A), betanin (sample B) and spray dried beetroot juice (sample C).

2.2.1 Betanin, sample B

Betanin was purified as described elsewhere (Gonçalves, Di Genova, Dörr, Pinto, & Bastos, 2013). Briefly, red beetroots (*Beta vulgaris*, *subsp.* vulgaris) were washed, peeled and processed in a centrifugal juice extractor. The beetroot juice was centrifuged (10 min, 5 ºC, 7,000 × *g*) and the supernatant was filtered through a silica gel plug (2 × 10 cm). The betanin/isobetanin mixture was purified from beetroot juice by reversed-phase column chromatography (silica gel 90 C18, conditioned and eluted with water at a flow rate of 0.3 mL min^{-1}). Magenta-colored fractions were collected, analyzed by HPLC and lyophilized; yield: 200 mg of betanin L^{-1} of juice (ca. 3.0 kg of beetroots).

2.2.2 Spray dried beetroot juice, sample C

Fresh beetroot juice (1 L) was spray dried without any encapsulating agent using a Mini-Spray Dryer (Büchi, inlet air temperature: 140 °C, outlet air temperature: 73 °C, liquid feed rate: 4.5 mL min^{-1}); yield: 200 g L^{-1} of juice.

2.3 Emulsion preparation

2.3.1 Preparation of W/O emulsions

Soybean oil solution containing 2 to 8% w/w of the emulsifier CR-310 was used as the oil phase. Phosphate buffer solution pH 6.2 (100 mmol L^{-1}) containing betanin (0.1-1.0% w/w) and D-glucose (1% w/w) was used as the inner aqueous phase. The W/O mixture (100 mL) with a volume fraction (ϕ_{Ai}) of the inner aqueous phase of 10-30% was emulsified with a rotor-stator homogenizer (PolytronPT-3000; KinematicaAG, 10,000 rpm, 5 min, ice bath). The resultant W/O emulsions had a whitish color with smooth consistency.

2.3.2 Formulation of W/O/W emulsions

W/O/W emulsions were prepared by MCE as described elsewhere (Kuroiwa et al., 2016; Yamanaka et al., 2017). The MCE apparatus consists of an asymmetric straight-through MC array plate (WMS11-1, Hitachi, 24 mm × 24 mm), a liquid chamber to feed the continuous aqueous phase and a syringe pump (Model 11; Harvard Apparatus. Inc.) to feed the dispersed phase (W/O emulsion) all assembled on a stainless-steel module. Droplet generation occurred when the dispersed phase was forced through the microchannel at a controlled flux (from 5 to 100 L m^{-2} h^{-1}) and was monitored using an inverted optical microscope (MS-511B, Seiwa Kougaku Sesakusho Ltd.) (Figure 1). Before assembling the MCE module, the MC array plate was degassed in the presence of the outer aqueous phase (water with 1-3% w/w Tween 20) by ultrasonic vibration (VS-

100III, As One Co., 100 kHz, 20 min). The W/O emulsion containing E162 (up to 1.0% w/w) and D-glucose (1% w/w) was prepared using a rotor-stator homogenizer with phosphate buffer pH 6.2, soybean oil, and the CR-310 emulsifier. D-glucose was used to reduce water activity and stabilize betanin (Herbach et al., 2006). Food-grade emulsifiers were selected for their low toxicities and according to their HLB values (Griffin, 1949, 1954). Tween 20 has HLB = 16.7 and is suitable for stabilize W/O emulsions while CR-310, HLB < 1, stabilizes O/W emulsions (Scheme S1).

< Figure 1 >

2.3.3 Average droplet diameter ($d_{3,2}$) and droplet size distribution

The average droplet diameter (Sauter mean diameter, $d_{3,2}$) and droplet size distribution of the W/O/W emulsions were determined by dynamic light scattering using a laser diffraction particle size analyzer (measuring range: 0.04 μm to 2,000 μm, LS13320, Beckman Coulter Inc.). The width of the droplet size distribution was reported as the *Relative Span Factor* (RSF), defined as:

$$RSF = \frac{d_{90} - d_{10}}{d_{50}} \qquad \text{Eq. 1}$$

where d_{90}, d_{10} and d_{50} represent the droplets diameter as a function of the accumulated percentage volume. The surface weighted mean droplet diameter (*viz.*, Sauter mean diameter, $d_{3,2}$) is defined as the diameter of the sphere having the same volume/surface area ratio as the particle of interest:

$$d_{3,2} = \frac{\sum_i (n_i \times d_i^3)}{\sum_i (n_i \times d_i^2)} \qquad \text{Eq. 2}$$

where n_i is the percentage of particles with an equivalent diameter d_i, respectively.

The densities of dispersed and continuous phases were measured at 25 ºC using a density meter (DA-130N, Kyoto Electronics Manufacturing Co.). The viscosities of dispersed and continuous phases were measured at 25 ºC with a vibro viscometer (SV-10, A&D Co.). The static interfacial tension between the oil and the inner or outer aqueous phases was measured using the pendant drop method with a fully automatic interfacial tensiometer (PD-W, Kyowa Interface Sciences Co.).

2.4 Physical stability and color of W/O/W emulsions containing betanin

W/O/W emulsions were kept at different temperatures (4 °C, 25 °C and 60 °C) for 7 days and monitored by optical microscopy and digital imaging. Emulsions were placed in front of a black background with a light bulb positioned to illuminate half of the flask. Pictures were taken using a digital camera with manual settings to prevent change in brightness and contrast. Images were cropped to include the shadow region and the illuminated region of each sample and the mean color was measured considering the color space CIELa*b* using the ColorGraber software. Color analysis was carried out using the program ColorHexa (http://www.colorhexa.com).

2.5 Data analysis

All experiments were performed in triplicate and results are reported as mean ± standard deviation. Mathematical and statistical data analyses were performed using Origin (v.2016, OriginLab) and Prism7 (v.7.0c, GraphPad Software) software. Bean plots were generated using the BoxPlotR program (http://shiny.chemgrid.org/).

3. RESULTS AND DISCUSSION

3.1 Formulation of W/O/W emulsions loaded with betanin

E162 (sample A) was encapsulated in a W/O/W emulsion formulated using a W/O emulsion as the dispensed phase and an aqueous solution of Tween 20 as the continuous phase. The effect of formulation conditions on droplet size distribution, average droplet diameter ($d_{3,2}$) and degree of emulsion dispersion was investigated. Initial experimental conditions were defined as follows: volume fraction of the internal aqueous phase (ϕ_{Ai}): 30% v/v, concentration of CR-310 ($C_{CR\text{-}310}$): 4% w/w, concentration of Tween 20 ($C_{Tween20}$): 2% w/w, concentration of betanin (C_{Bn}): 0.5% w/w and dispersed phase flux ($f_{W/O}$): 10 L m^{-2} h^{-1}. Among these independent variables, only the flow rate of the dispersed phase affects the average droplet diameter, *viz.*, when the flux is increased from 5.0 to 100 L m^{-2} h^{-1} the value of $d_{3,2}$ increases ca. of 10 μm (Figure 2). A subtle increase in the value of $d_{3,2}$ is also caused by increasing the concentration of CR-310. The degree of monodispersity, as measured by the relative span factor (RSF), decreases at the maximum value of flux used, resulting in a polydispersed emulsion, *i.e.*, RSF value > 0.5. All other variables do not seem to affect the value of RSF; however, lowering the concentration of CR-310 to 2% w/w cause emulsion breaking and coalescence (Figure S1).

< Figure 2 >

The droplet size distribution measurements were made on a laser diffraction analyzer (Figure S2). When the dispersed phase flux is 10 L m^{-2} h^{-1}, the use of an intermediate concentration of CR-310 (4% w/w) produce a monodisperse emulsion with smaller full width at half maximum (FWHM = 7.7 μm). However, if the flux is increased above 80 L m^{-2} h^{-1}, the emulsion becomes polydisperse, as inferred from the analysis of RSF values (Figure S3). These results are confirmed by visualization of droplet

formation in the microchannel system (Figure 3, Video S1). The increase of the flux from 5 to 20 L m^{-2} h^{-1} increases the number of droplets without significantly affecting their size. However, when the flux is carried out above 100 L m^{-2} h^{-1} it is observed that the droplet size increases and the emulsion becomes polydisperse.

< Figure 3 >

In MCE the formation of droplets is a spontaneous process that depends on the interfacial tension between liquids and viscosity, being defined by the capillary number (C_a):

$$C_a = \frac{\eta U}{\gamma} \qquad \text{Eq. 3}$$

whre η is the viscosity of the dispersed phase (if it is the most viscous phase), U is the flux of the dispersed phase and γ is the interfacial tension. Values of $C_a \gg 1$ are associated with high viscosity or high flow rate and result in irregular deformation of the droplets. As a result, the volume of the droplets increases, the area of contact with the slits decrease and emulsion becomes polydisperse. In the case of low C_a (< 1) the interfacial tension is the most important term, and its increase implies in the decrease of the interfacial area, leading to the formation of spherical droplets and monodisperse emulsions (Kobayashi, Nakajima, & Mukataka, 2003; Sugiura, Nakajima, Kumazawa, Iwamoto, & Seki, 2002). Under these conditions the formation of droplets occurs because the pressure exerted by the dispersed phase on the continuous phase exceeds the so-called drop breakthrough pressure (P_{bt}), defined as:

$$P_{bt} = \frac{4\gamma cos\theta}{d} \qquad \text{Eq. 4}$$

where γ is the interfacial tension between dispersed and continuous phases, θ is the contact angle between the droplet and the microchannel surface e *d* is the microchannel diameter (Sugiura, Nakajima, Tong, Nabetani, & Seki, 2000). In the experiments conducted with a flow rate below 80 L m^{-2} h^{-1}, the passage of the W/O emulsion through the slit results in the formation of monodisperse emulsions with droplets of ca. 50 µm (Figure S3). In this condition, when the contact angle θ reaches a critical value the detachment of the droplets occurs and its size does not depend on the flux of the disperse phase.

Considering these results, the following experimental conditions were used to investigate the other two sources of betanin: ϕ_{Ai} in 30%, $C_{CR\text{-}310}$: 4% w/w, $C_{Tween20}$: 1% w/w, C_{Bn}: 0.5% w/w and $f_{W/O}$: 10 L m^{-2} h^{-1}. The concentration of the betanin source (C_{Bn}) was fixed at 0.5% w/w_{dp}, *i.e*., 75 mg of sample in 15 g of dispersed phase, to allow the comparison of the tinctorial strenght of samples A, B and C, as well as to determine the effects of each sample on the properties of the emulsion. The final molar concentration of betanin in each sample was estimated by UV/vis absorption spectrophotometry in aqueous solutions considering a molar absorption coefficient of 65,000 L mol^{-1} cm^{-1} at 536 nm: (Schwartz & von Elbe, 1980) 1.5×10^{-5} mol L^{-1} (sample A), 1.7×10^{-3} mol L^{-1} (sample B) and 1.4×10^{-4} mol L^{-1} (sample C).

In the optimized experimental conditions with sample A, the emulsion formulated with sample B is also monodisperse, but the emulsion formulated with powdered beetroot (sample C) is polydisperse (Figures S4 and S5). This result can be explained by the presence of enzymes and proteins that are not found in samples A and B and may affect surface tension and emulsion characteristics (Damodaran, 2006; Hailing & Walstra, 1981). Considering the superior tinctorial strength of pure betanin compared to

the other samples, the effect of the dispersed phase flux variation on the emulsion containing sample B was analyzed and the results are presented in Figure S6.

3.2 Effect of temperature and storage period

W/O/W emulsions of samples A, B and C were kept at 4 °C, 25 °C and 60 °C and monitored for seven days. An W/O/W emulsion without betanin was used as a negative control. All samples were kept in the dark between the analyzes. Figure 4 shows the variation of the average droplet diameter ($d_{3,2}$) (a) and RSF (b) of the emulsions in the absence of betanin and in the presence of betanin on the first and last day of monitoring. The white horizontal lines represent the values of $d_{3,2}$ and RSF in each temperature studied and the black horizontal lines represent the average value obtained, considering all the temperatures. The negative control without betanin and the emulsion of sample A show a similar variation in the average diameter of the droplets over the course of a week, whereas the sample C presents a larger range, suggesting that components of the raw beetroot powder compromise the effect of the emulsifiers agents present in the emulsion. Sample B presents a subtle variation in the mean diameter of the droplets, which is lower than that observed in the control emulsion. This fact suggests that betanin is stabilizing the emulsion. The RSF values show that over the course of one week the control emulsion is polydisperse, while the emulsion of samples A and B are monodisperse. The emulsion of the sample C is polydisperse from the start of the stability test, since polydispersity is already observed in its emulsification process.

< Figure 4 >

Droplet diameter measurements (Table S1) and optical microscopy images (Figure 5) were performed on the first and last day to evaluate possible changes in the size and shape of the droplets and to evaluate the stability of the emulsion. Optical microscopy shows that the droplets in all samples do not change size or morphology when the

W/O/W emulsion is maintained at 4 °C for one week (Figure 5). The droplets present in the emulsions containing samples A and B have similar diameters, whereas sample C results in 25 μm larger droplets. At 25 ºC the emulsion of sample A undergoes a subtle reduction in size in one week. Sample B, in addition to the decrease in droplets in the period, shows greater homogeneity inside the droplet, which suggests that the W/O/W emulsion has been transformed into an O/W emulsion. A similar behavior is observed for the droplets containing the sample C. The droplets of the emulsions of samples A and C kept at 60 °C become smaller over time, *i.e.*, diameter is smaller than 3 μm on the seventh day. Sample B showed a small decrease in droplet size. In addition, on the first day of the stability test, the interior of the droplets begins to show phase separation, which suggests the beginning of the coalescence process of the emulsion droplets. After one week at 60 °C, the emulsion of sample B is flocculated, confirming the occurrence of coalescence.

< Figure 5 >

In order to verify the influence of the different betanin samples on the volume contraction rate of the emulsions, the volume of the droplets of the emulsions kept at 25 °C on days 1 and 7 was calculated from the average droplet diameter data ($d_{3,2}$) of the droplets (Table S1) and can be visualized in Figure 5. Calculations were performed considering an ideal system behavior. The control emulsion shows a contraction of 70% in its volume, whereas in samples A and B this rate is 50% and 65%, respectively. Sample C has a 95% volume contraction rate. These results corroborate with the behavior observed in optical microscopy and with the hypothesis that betanin stabilizes the emulsion, because samples A and B presented a lower contraction rate of volume than the control emulsion. This result may be related to the increase of the repulsion forces between the double layers resulting from the presence of negative charges in

betanin at pH > 3.5 (Nilsson, 1970), a hypothesis supported by the colloid stability theory of Deryaguin, Landau, Verwey and Overbeek (DLVO theory).(Israelachvili & McGuiggan, 1988; Matsumoto, 1985) In beetroot juice powder (sample C), the presence of high concentrations of salt and other biomolecules affect interfacial tension, compromising the stability of the emulsion (Table S2). Sample A, on the other hand, does not seem to affect the characteristics of the droplets when compared to the control, since it contains less amount of betanin and large amount of dextrin, a neutral polysaccharide.

The decrease in the droplet size of the emulsions maintained at 60 °C (Figure 5) can be explained by the decrease in dispersed phase viscosity, which leads, according to Eq. 3, to a decrease in the value of C_a. Consequently, the breakup of the droplets is also facilitated at higher temperatures, as well as the appearance of new droplets of reduced size. The effect of temperature on the viscosity of the medium also explains a lower average diameter of the droplets at 25 °C over the W/O/W emulsions maintained at 4 °C.

3.3 Emulsion color

A convenient method based on image analysis was developed to measure the relative amount of betalain in the emulsion. Glass vials were half-filled with emulsions containing betanin and illuminated in such a way to create a shadow zone. Next, photographs of each emulsion were taken with a black background, under the same illumination conditions (Figure S7). The color at the interface between light and shadow was sampled and classified according to the CIELa*b* color space (Figure S8). The luminance (L) increases with decreasing amount of pigment and is higher in W/O emulsions than in W/O/W emulsions. The values of parameter a* indicate a color scale ranging from red (+) to green (–), while b* varies from yellow (+) to blue (–). In the W/O emulsions, the highest tinctorial strenght of sample B (pure betanin), evidenced by the

highest and most positive value of a* and the most negative of b* (more red and blue, resulting in magenta) is evident compared to the other samples (Table S3). The same effect is observed in W/O/W emulsions; however, all samples become more opaque, resulting in a decrease in saturation (values of a* and b* closer to zero).

The color of the W/O/W emulsions was monitored for 7 days. Samples were kept at 4 °C, 25 °C and 60 °C in the dark and photographs were taken every two days in order to observe the color change of the emulsions. Betanin from different sources encapsulated in W/O/W emulsion showed to be temperature sensitive. In all W/O emulsions the values of a* decrease with increasing temperature, as inferred from the loss of red color (Figure 6 and Figure S9). At the same time an increase in the values of b* is observed, indicating a shift towards the yellow region, probably due to the formation of betalamic acid, decarboxylated derivatives or oxidization products (Esteves et al., 2018). Similar behavior is observed in the W/O/W emulsions and control samples. It is still possible to observe that W/O and W/O/W emulsions containing pure betanin have higher values of a*, *i.e.*, more intense coloration because the concentration of betanin in this emulsion is higher compared to emulsions containing commercial betanin in maltodextrin and powdered beetroot juice.

< Figure 6 >

4. CONCLUSIONS

Betanin was encapsulated in W/O/W emulsions using the microchannel emulsification (MCE) technique. The droplets formed have a size of 46 ± 10 μm and are monodisperse. Emulsifying agents suitable for use in food and the use of soybean oil resulted in a W/O/W emulsion that can be used in the preparation of functional foods kept under refrigeration for up to 7 days. The emulsification conditions were optimized and the dispersed phase flux was the most important factor influencing the capillary number and,

therefore, the degree of droplet dispersion. The use of spray dried beetroot juice as a source of betanin resulted in polydisperse emulsions with larger droplets. However, pure betanin tends to produce monodisperse droplets with a uniform size distribution when compared to the control without betalains, spray dried beetroot juice and E162, probably due to the increase in the electrostatic repulsion between the droplets and increase in the potential barrier for coalescence and flocculation.

Author Contributions

APEP, NK, IK and MN performed the experiments. IK, MN, MAN, ELB contributed with reagents, materials, analysis costs and technology. APEP and ELB prepared the manuscript. All authors analyzed the data and revised the manuscript.

Acknowledgements

We thank the São Paulo Research Foundation – FAPESP (ELB, 2014/22136-4, 2016/21445-9), the Brazilian National Council for Scientific and Technological Development – CNPq, and the University of Tsukuba for financial and fellowship support.

Conflict of interest

The authors declare no competing financial interest.

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FIGURE CAPTIONS

Figure 1. Microchannel emulsification (MCE) system. a) Transversal section of the microchannel, composed of a cylindrical microhole (200 μm × 10 μm, H × W) connected to a microgroove (10 μm × 80 μm × 40 μm, H × W × D). b) Bottom view of the multichannel system, as observed by the optical microscope connected to the system. c) Simplified scheme of the multichannel system. The silicon microchannel plate containing ca. 14,000 microchannels in an active area of 10 mm^2 is assembled between two glass plates in a stainless-steel module. The system is fed with the continuous and dispersed phases from opposite sides of the microplate in order to produce droplets. The generation of droplets depends on the interfacial tension between the immiscible liquids, the microchannel geometry and the spacing between the various microchannels (Kobayashi & Nakajima, 2006; Sugiura, Nakajima, Iwamoto, & Seki, 2001).

Figure 2. Modified radar graphs that indicate the effect of the experimental conditions (minimum, intermediate and maximum) of each independent variable on the average droplet diameter ($d_{3,2}$) and *Relative Span Factor*, RSF. The data are presented in logarithmic scale. [a] Percentage in weight of the disperse phase (W/O emulsion); [b] Percentage in weight in the W/O/W emulsion.

Figure 3. Micrograph of the generation of droplets by microchannels by varying the flux of the dispersed phase. a) 5 L m^{-2} h^{-1}, b) 10 L m^{-2} h^{-1}, c) 20 L m^{-2} h^{-1}, d) 40 L m^{-2} h^{-1}, e) >100 L m^{-2} h^{-1}. All images are in the same scale; bar = 80 μm.

Figure 4. Split bean plots for the effect of temperature and storage time on (a) the average droplet diameter ($d_{3,2}$) and (b) the RSF of the emulsions formulated in the absence of betanin and in the presence of three different pigment sources. The graphs were prepared with data obtained at 4 °C, 25 °C and 60 °C (horizontal white lines, raw

data in Table S1). Horizontal dotted lines indicate the mean value measured, while the red dotted line at (b) indicates the limit of a monodisperse emulsion, RSF = 0.5.

Figure 5. Optical microscopy images of W/O/W emulsions containing E162, purified betanin and powdered beetroot juice at 4 °C, 25 °C and 60 °C on the first and last (7^{th}) days of the stability test. Images on the right are the magnification of a drops in the W/O/W emulsions; bar = 50 μm.

Figure 6. Average sampled color of W/O and W/O/W emulsions containing E162, beetroot juice or betanin kept at 4 ºC, 25 ºC and 60 ºC for seven days. Original pictures and raw data are given in Figure S9 and Table S3.

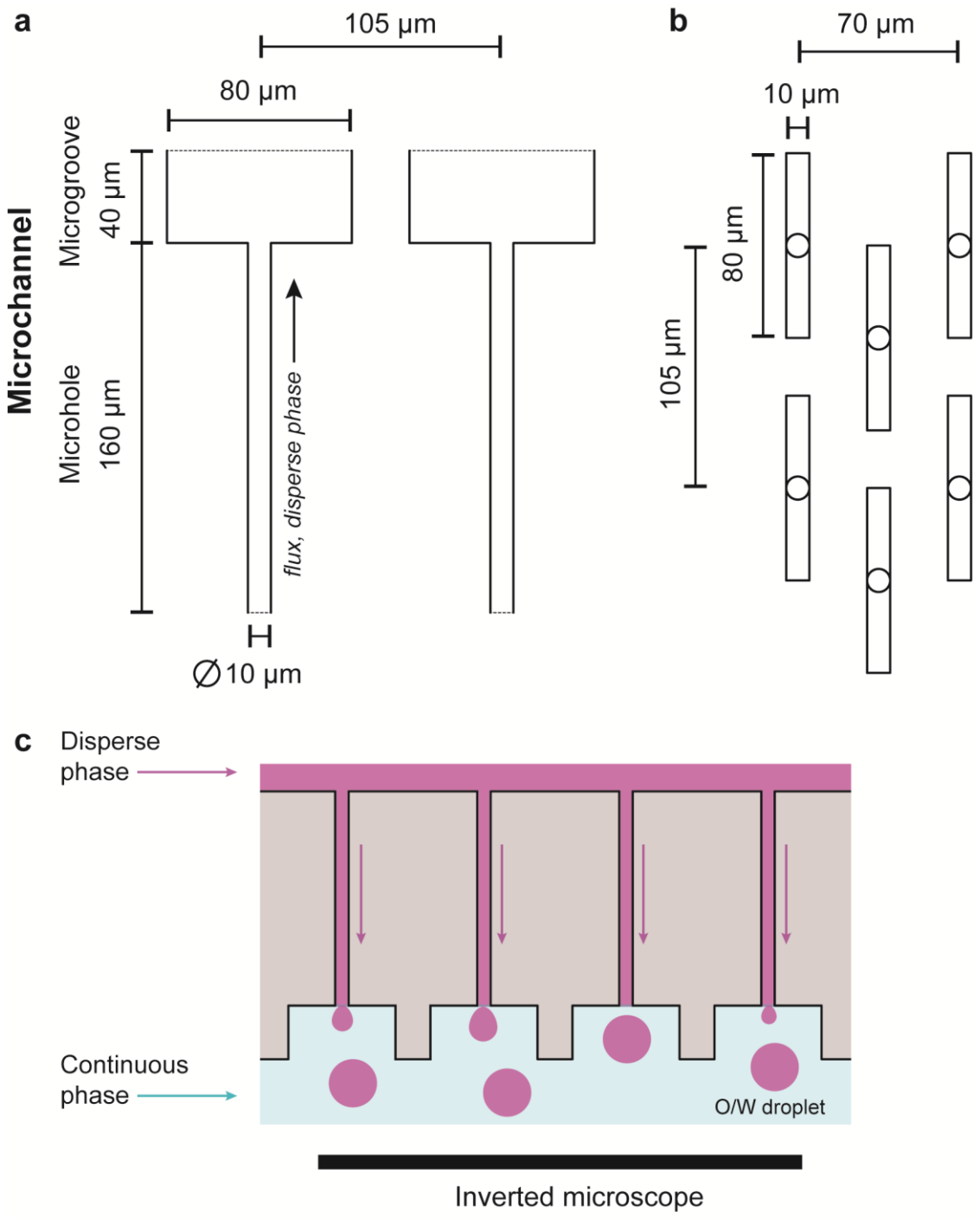
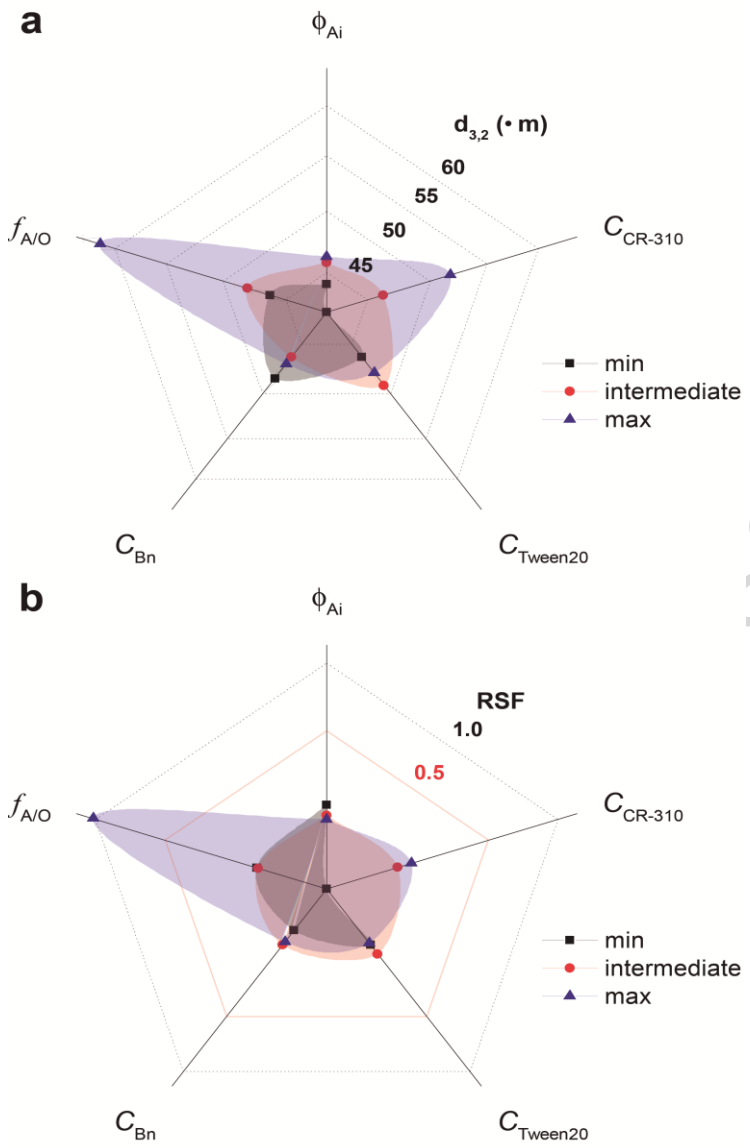


Figure 1



Variable	Condition		
	Min	Interm	Max
Φ_{Ai}	0.1	0.2	0.3
C_{CR-310} (% w/w)[a]	2.0	4.0	8.0
$C_{Tween20}$ (% w/w)[b]	1.0	2.0	3.0
C_{Bn} (% w/w)[a]	0.1	0.5	1.0
$f_{W/O}$ (L m^{-2} h^{-1})	5	20	100

Figure 2

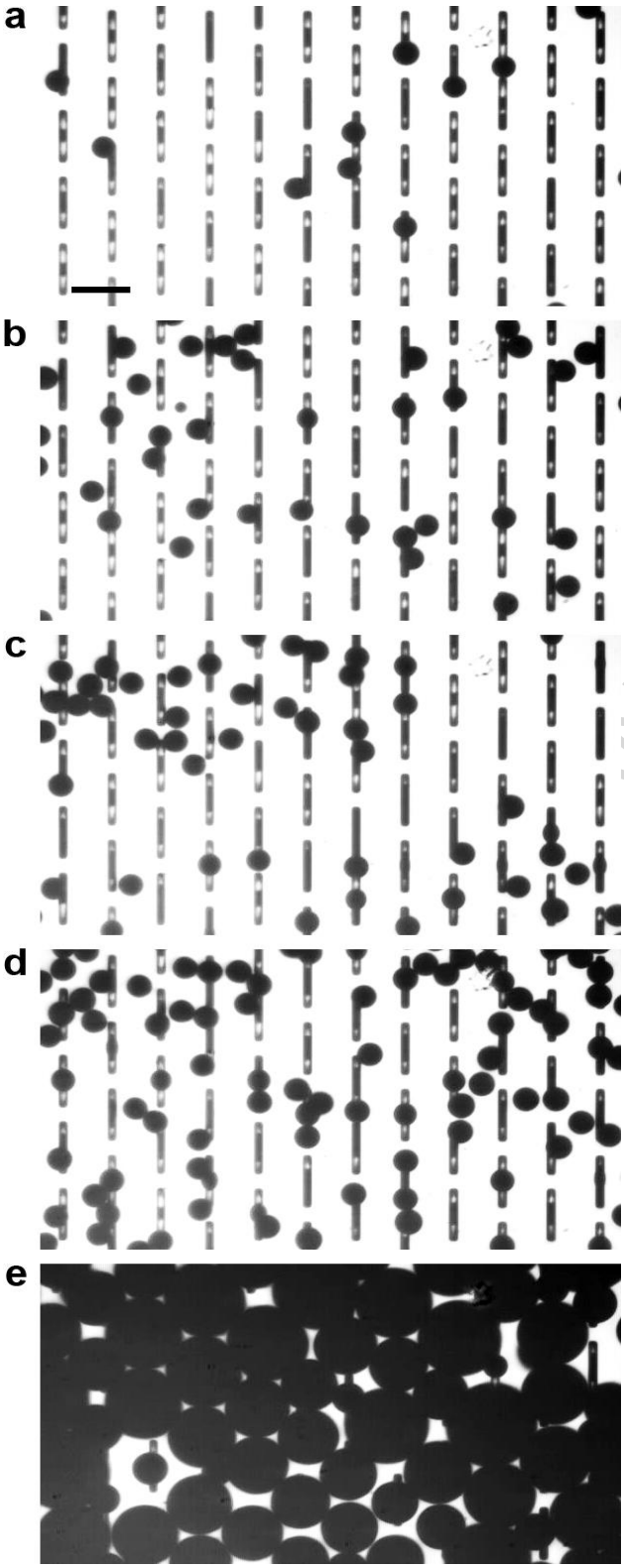


Figure 3

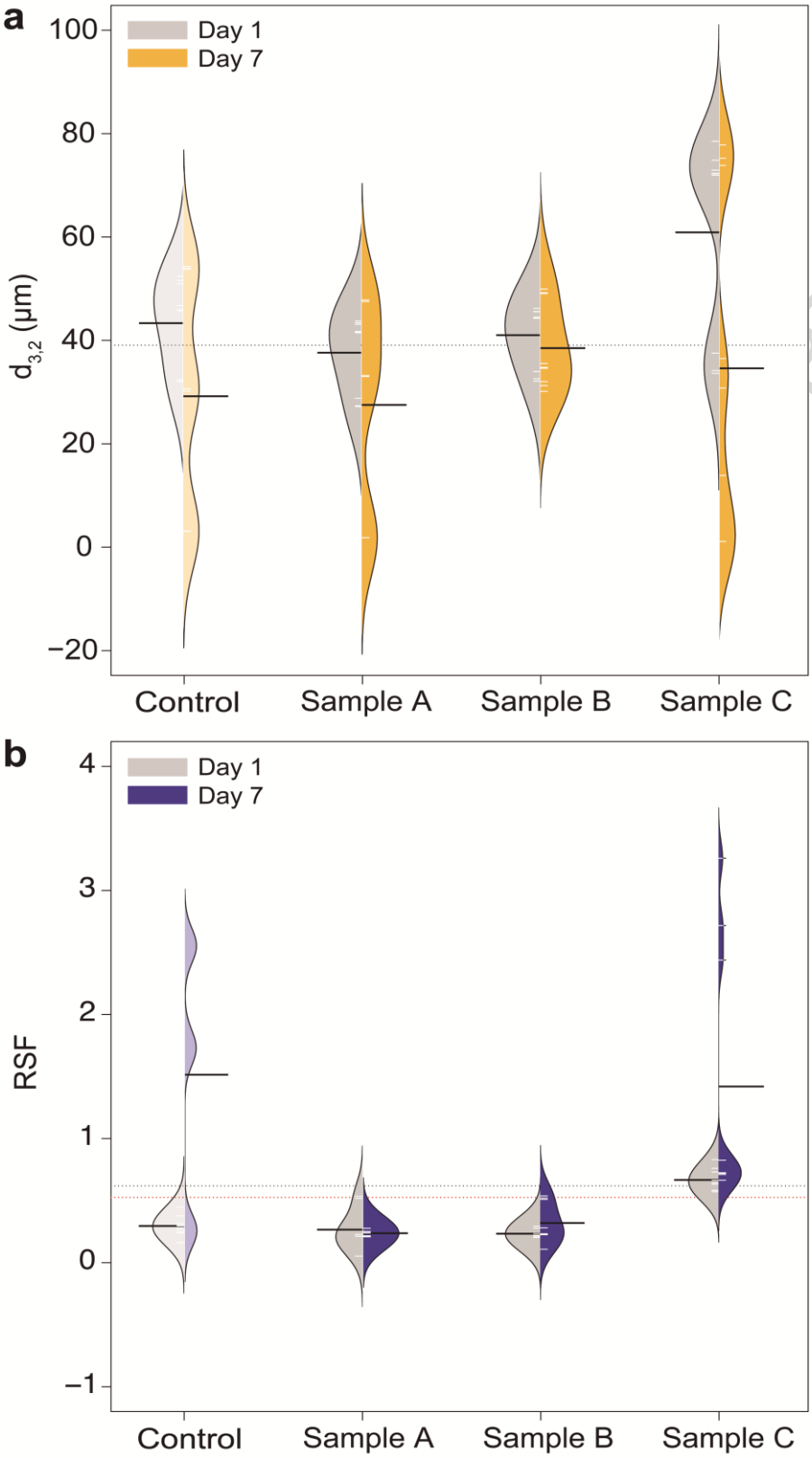


Figure 4

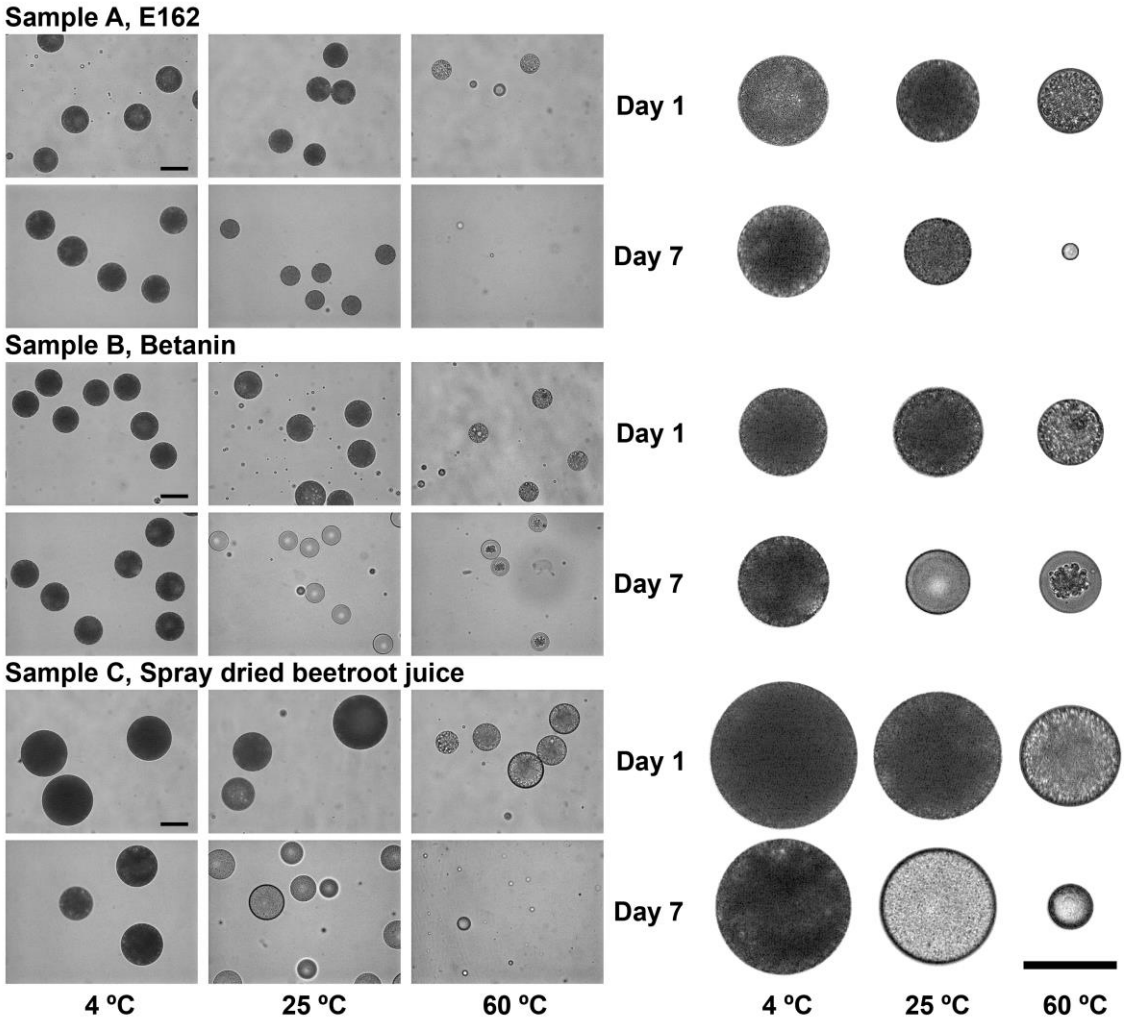


Figure 5

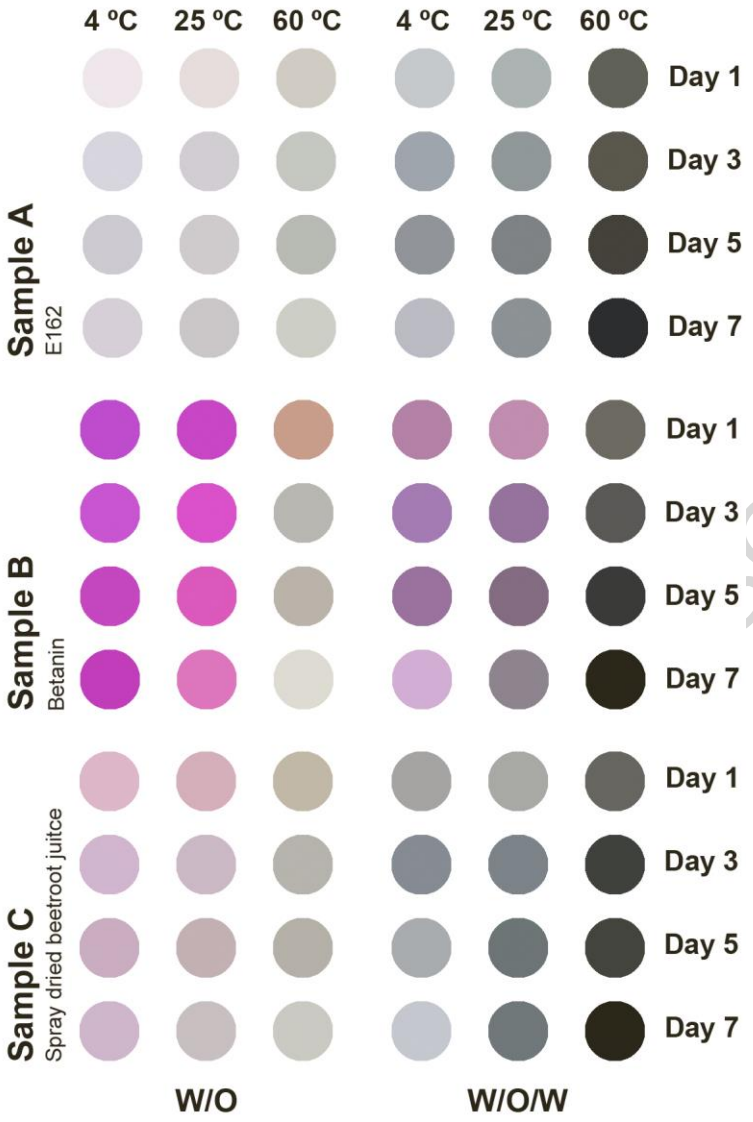


Figure 6

Graphical abstract

HIGHLIGHTS

- E162 and betanin were encapsulated in W/O/W emulsions.
- Microchannel emulsification of betanin produce monodisperse emulsions.
- Betanin stabilizes the W/O/W emulsion.