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INTEGRATED PROCESS TO OBTAIN ANTHOCYANIN ENRICHED PALM-FAT PARTICLES FROM ELDERBERRY JUICE

Sz. Bánvölgyi[b]*, T. Vatai[a], Zs. Molnár[b], I. Kiss[c], Ž. Knez[d], Gy. Vatai[b] and M. Škerget[d]

[a]Sprin S.p.A BIC Incubatori FVG, Via Flavia 23/1, 34148 Trieste. Italy
[b]Department of Food Engineering, Faculty of Food Science, Corvinus University of Budapest, H-1118 Budapest, Ménesi út 44. Hungary
[c]Fitomark Ltd., H-3934 Tolcsva, Arany J. u. 16/a. Hungary
[d]Laboratory for Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, SI-2000 Maribor. Slovenia

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Two novel technologies were applied in order to investigate concentration and formulation of anthocyanins for potential use in food industry. Integrated membrane process technology was applied for concentrating elderberry juice. In the first step, the juice was clarified by microfiltration, followed by a pre-concentration step with reverse osmosis. Finally, the juice was concentrated to the end concentration of 56 °Brix by osmotic distillation. The elderberry juice concentrate was formulated in a powderous form by a high-pressure process – Particles from Gas Saturated Solution (PGSS™) – using supercritical CO_2. The applied carrier material was palm fat. The products with different anthocyanin-carrier ratios were measured for their colour properties (lightness, hue angle, and saturation). Colour stability was monitored for prolonged storage at different conditions (light/dark and ambient temperature/refrigerator). The obtained powderous anthocyanin-palm fat products showed good colour stability, which gives good bases for potential applications in the future.

Keywords: elderberry juice, anthocyanin, integrated membrane process, product formulation, PGSS™, stability

The new trends in food research seek technologies that are specific, efficient, and economic, altogether. Additionally, there are growing demands from the side of consumers' for "safer and healthier food" and making legislation rules controlling the food processing and the environmental impact stricter.

The membrane processes can operate at ambient temperature and with low energy consumption. Microfiltration (MF) and ultrafiltration (UF) are used for the clarification and sterilization of fruit juices (Cassano et al., 2007; Laorko et al., 2013). Nanofiltration (NF), reverse osmosis (RO), membrane distillation (MD), and osmotic distillation (OD) are used as concentration techniques (Galaverna et al., 2008; Bánvölgyi et al., 2009; Echavarría et al., 2012; Zambra et al., 2015). There are also some limitations of these techniques, such as membrane fouling, concentration polarization, and wetting problems (Strathmann et al., 2006). Encapsulation has been used in food industry for more than 60 years in order to provide solid or liquid ingredients a protection against environmental and/or chemical interactions (Barbosa-Cánovas et al., 2005; Morais et al., 2005; Coradini et al., 2014).

The advantages of encapsulation include improved flow properties and solubility, easier handling, since they are solid instead of liquid. Stability of the encapsulated material can be improved due to protection from moisture, light, heat, metals, etc., therefore extending the

* To whom correspondence should be addressed.
Phone: +36-1-482-6111; fax: +36-1-482-6323; e-mail: szilvia.banvolgyi@uni-corvinus.hu

shelf-life of functional foods (Hedegaard & Skibsted, 2013). The disadvantages of the conventional technologies, however, include high processing temperatures, long processing times, strong mechanical impacts, and organic solvent residual problems. Using supercritical fluids (SCFs) (Knez et al., 2014) gives various advantages and easier tailoring possibilities in comparison to conventional methods (Jung & Perrut, 2001). The sensitivity of supercritical fluids to small changes in temperature and pressure in the highly compressible region offers the potential to control both particle size and morphology over a wide range, with only small adjustments of process conditions (Knez et al., 2014). Carbon dioxide (CO_2) is the most commonly used SCF in food applications (Brunner, 2005).

Our aim was to produce an anthocyanins rich concentrate and to formulate it in a proper form to provide a protection to the anthocyanin pigments and prolong their colour stability (Vatai et al., 2008). For formulation Particles from Gas Saturated Solution (PGSS™) technique was used. In this process the compressible medium is solubilised in the substance which has to be micronized (Knez & Weidner, 2003). So far, they were considered for the processing of relatively small quantities of high-value components (Lack et al., 2001).

1. Materials and methods

1.1. Materials

Elderberry (*Sambucus nigra* L.) juice was obtained from Fitomark Ltd. (Tolcsva, Hungary). The juice was produced by pressing the fresh Haschberg type berries, which are rich in anthocyanins (Stéger-Máté et al., 2006). In order to ease the filtration, pectolytic enzyme (Pektopol PT 400) was used before the pressing. The palm fat used as carrier material for product formulation was purchased from Degussa (Düsseldorf, Germany) and the emulsifier (Tween 40) from Fluka (Switzerland). The reagents and solvents were obtained from Merck (Darmstadt, Germany) and Reanal (Budapest, Hungary).

1.2. Integrated membrane process

The clarification was achieved by cross-flow MF in batch mode with a ceramic multi-tubular membrane module (type Schumasiv, pore size of 0.45 μm, membrane surface area 0.125 m^2). The operating parameters were 0.4 MPa transmembrane pressure (TMP), 30 °C temperature, and 400 l h^{-1} flow rate, the Re number was 3723. The clarified juice was pre-concentrated by RO in batch mode (Molnár et al., 2012) using a TRISEP flat sheet membrane (type ACM2, salt rejection 99.5 %, membrane surface area 0.18 m^2). It was carried out at 400 l h^{-1} flow rate, at 30 °C, and at 5 MPa TMP, the Re number was 1220. The TSS content of retentate and permeate was measured continually during the clarification and concentration with Atago PAL-α digital refractometer (each time three times). The RO retentate (26 °Brix) was further concentrated by OD in laboratory scale apparatus (Molnár et al., 2012). For the experiment a hydrophobic, hollow fibre membrane module (Microdyn, Germany) with 0.1 m^2 active area was used. The module contained 40 polypropylene capillaries, the average pore size was 0.2 μm. The measurements were carried out at 30 °C temperature. As brine solution, 41.4 w/w% calcium-chloride-dihydrate was used (the saturated value is 44% w/w at 30 °C). The elderberry juice circulated in the hollow fibres and the $CaCl_2$ solution circulated in the shell. During the experiments the mass of the separated water was measured with a digital laboratory scale (Sartorius PMA 7500).

1.3. Total anthocyanins (TA) content

The content of monomeric anthocyanins was measured by the pH-differential spectrophotometric method (Giusti & Wrolstad, 2001). The absorbances of each dilution were measured at 520 and 700 nm. The anthocyanins content was calculated on the basis of cyanidin-3-glycoside (MW=449.2 and ε=26 900) and the results were expressed as mg TA g^{-1} material.

1.4. Product formulation with PGSS™

The melted palm fat was mixed with the emulsifier and the elderberry concentrate (OD) using an electrical homogenizer. The emulsion was immediately put into the autoclave (NWA-Lorrach, Germany; max. pressure 40 MPa, max. temperature 400 °C) (Fig. 1) and CO_2 was introduced initially in open system to drive off the dissolved oxygen, and then pressurized using a high pressure pump until the desired pressure was achieved (~10 MPa). The autoclave was then heated up to the operating temperature, which was slightly higher than the melting point of the palm fat (~60 °C). The autoclave with its content was mixed constantly until reaching the equilibrium (approximately 2 h). The gas saturated solution was then expanded through the nozzle (Spraying systems, Germany; diameter 0.75 mm, spray angle 60°) and the compressible gas evaporated in the expanding chamber causing the micronization of the particles.

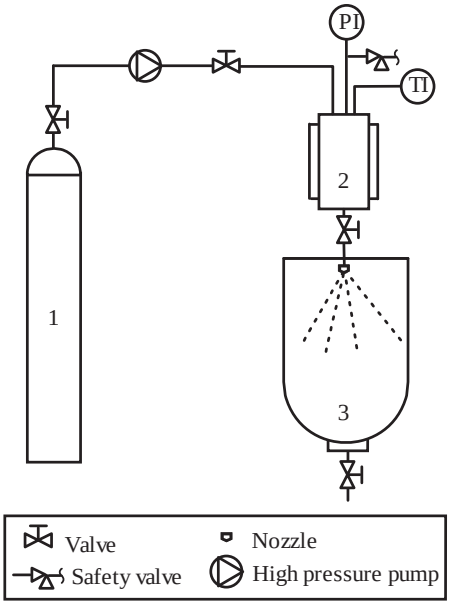

Fig. 1. Scheme of the PGSS™ apparatus: 1: gas cylinder; 2: autoclave, 3: expansion chamber

The particle size was determined by granulometer (Fritsch analysette 22 compact, Germany). The shape and surface characteristics of the particles were observed by Scanning Electron Microscopy (SEM) under high vacuum (Quanta 200 3D, SEM).

1.5. Colour evaluation

Colour characteristics were measured using the CIE L*a*b* colourimetric method (WROLSTAD et al., 2005). The obtained PGSS™ samples were stored under different conditions. They were divided into two parts. One part was put to dark into refrigerator and the other part was stored at ambient temperature and light. The colour stability was monitored periodically for 34 weeks using a colorimeter (Chromameter CR-400, Konica Minolta Sensing, INC).

2. Results and discussion

2.1. Clarification and concentration of elderberry juice by membrane processes

During the clarification step (MF) the permeate flux decreased along the processing from 6.5 l m^{-2} h^{-1} to 3 l m^{-2} h^{-1}. The main factors that contribute to the flux decay are the concentration polarisation and the pore blocking. The main disadvantage of microfiltration of pulpy juice is the fouling of the membrane pores, which results in the flux decline. The microfiltration resulted a clear elderberry juice. The TSS content in the permeate was about 10.5 °Brix during the whole process.

A decrease in permeate flux was observed during the RO pre-concentration due to the concentration polarization and the increasing juice viscosity (Fig. 2), which results an increase in concentration factor. The initial value of TSS was 9.3 °Brix, till the end of the measurement it increased up to 26.1 °Brix. When the total soluble solid content of the retentate reached the 26 °Brix, its content in the permeate began to increase from 0 up to 1.2 °Brix. For this reason the concentration by RO to obtain a juice concentrate with high valuable component content is applicable till 25 °Brix without TSS losses. When the juice is concentrated, and assuming a perfect retention (100%) of all solids, a twofold concentration to 25 ºBrix results an increasing in osmotic pressure, therefore the driving force is reduced. This causes that the flux will come to zero when the fruit juice has been concentrated by 2.5 times, or to about 25–30 ºBrix (KOZÁK et al., 2008). The reached volumetric reduction ratio (VRR) was 3.2.

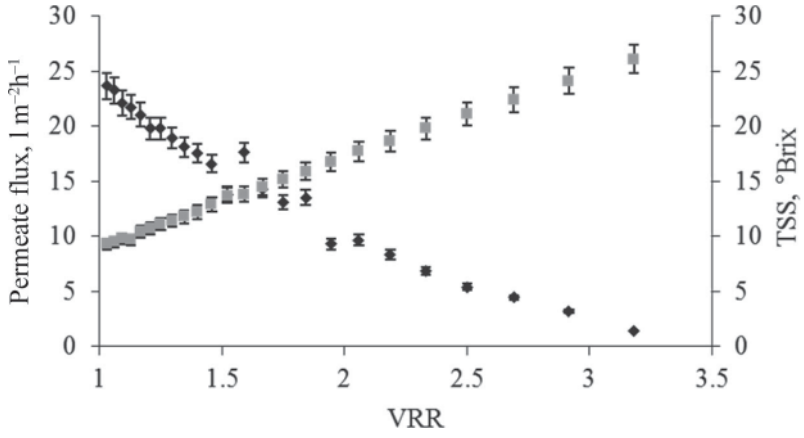

Fig. 2. Permeate flux and TSS content in elderberry juice during RO concentration at 30 °C, 400 l h^{-1}, 5 MPa. ◆: Flux; ■: TSS

The 26 °Brix RO retentate was finally concentrated by OD to end concentration of 56 °Brix. The osmotic agent was diluted to 31%, w/w. The experiment was repeated three times, and it was reproducible. In all cases the same trend was observed in the flux decreasing and TSS increasing (Fig. 3). The standard deviation of the measured points was 1–3%. The initial flux was 0.8 kg m^{-2} h^{-1}, which decreased to 0.46 kg m^{-2} h^{-1} during the 7.2 hours long process. The decrease in the distillate flux is primarily the result of the decrease in the driving force (the concentration difference between the two solutions), because the osmotic agent is diluted during the concentration. A second effect could be an increase in the viscosity in the boundary layer. The TSS of the elderberry juice increased during the process. The reached volumetric reduction ratio was 2.5. The results of concentration by RO and OD (flux values, concentration factors, reached TSS content) are in accordance with other publications and with our previous results (Kozák et al., 2008; Cassano et al., 2011).

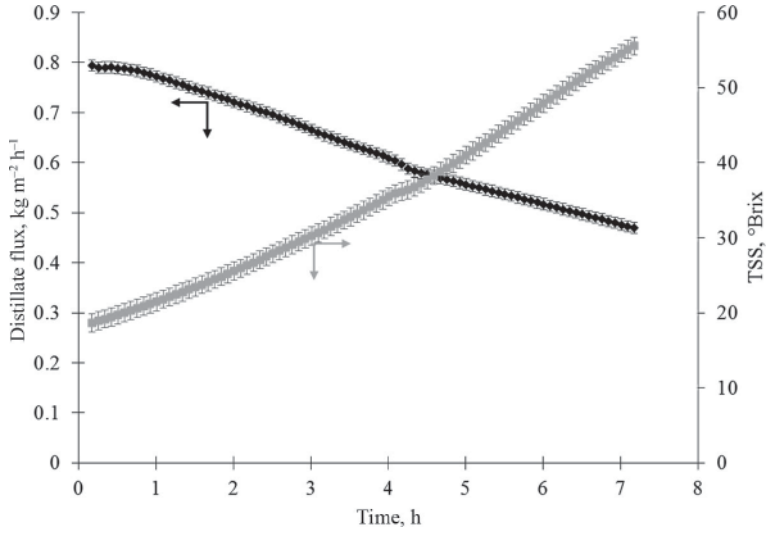

Fig. 3. Distillate flux and TSS content in elderberry juice during OD concentration at 30 °C. ◆: Flux; ■: TSS

2.2. Formulation of product in powderous form by PGSS™

The products obtained by PGSS™ process were homogeneous free flowing powders with colours from light pink to violet, as observed by the naked eye. However, the more precise colour indications are shown by the CIE parameters (Table 1).

Table 1. The CIE colour properties of the PGSS™ samples

Sample code	Liquid/carrier w/w ratio	mg TA per kg carrier	Lightness	Hue angle	Saturation
M1	5/95	333	93.9	83.2	6.0
M2	10/90	666	90.2	64.2	6.7
M3	20/80	1332	83.2	52.1	8.2
M4	30/70	1998	74.8	40.7	11.2
M5	41/59	2378	72.6	40.9	11.1
M6	50/50	2737	66.9	51.8	13.4

Six different samples are presented, where the amount of the liquid anthocyanins-concentrate on the powderous carrier was varied. The liquid concentrations in the powders were 5, 10, 20, 30, 40, and 50%, w/w. The lightness values of the samples decreased from 94 to 67. Parallelly, the colour saturation of the samples increased, showing values from 6 to 13. The hue angle values do not follow that trend. By the samples of 5, 10, 20, and 30% w/w liquid concentrations, with the increasing amount of anthocyanins, the hue angle values decrease (shift from yellow angle towards red). By the samples of 41 and 50%, w/w liquid content, the hue angle values slightly increase again, showing values of 41 and 52, respectively. However, the colour parameters represent the samples with all three values; the change in the lightness causes change in the saturation of the colour, which also modifies the hue angle and vice versa. For example, synthetic colorants often used as food colorants for achieving pink-red colour as erythrosine and allura red, have the lightness values of ~70 and hue angle values of 39 and 25, respectively (Giusti & Wrolstad, 2003). These values are comparable with the values of our PGSS™ products; however, the significant difference is by the values of chroma, which are ~70 for the synthetic colorants. The arrears of the natural colorants could be due to the carrier material as well, since the palm fat used for the experiments originally has a white colour, which softens the bright colour of anthocyanins.

The morphology of PGSS™ particles shows a very porous structure (Fig. 4). The shapes are not defined, dominating by particle agglomeration. According to the colour of the powders, the anthocyanin pigments adsorbed on the surface of the palm fat. Particle size varied from 7 to 18 μm. The higher the liquid content was on the carrier, the smaller the particle sizes were. The unprocessed palm fat particle size was 384 μm, while by the palm fat samples sprayed with the same operating conditions but without anthocyanins, 11.6 μm particle size was measured. Figure 5 shows the probability density (μm^{-1}) vs. particle size (μm) of some of the obtained powders (5, 20, and 42%, w/w liquid on the carrier). Relatively narrow particle size distribution was observed.

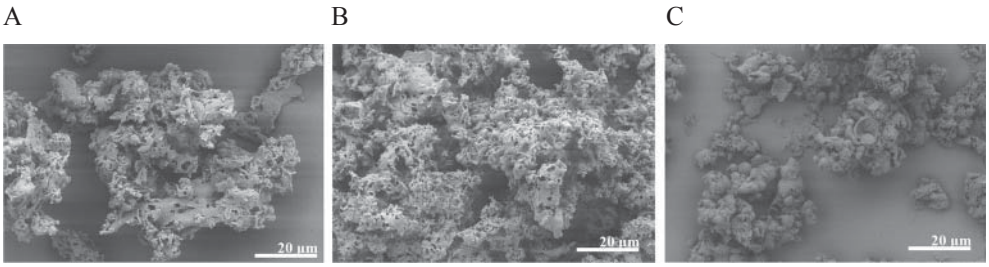

Fig. 4. Morphology and particle size of three different PGSS™ products: anthocyanin concentrates and palm fat with 5, 10, and 42% w/w liquid/carrier ratios
A: Liquid/carrier ratio (w/w): 5/95, particle size: 15.6 μm; B: Liquid/carrier ratio (w/w): 20/80, particle size: 9.5 μm; C: Liquid/carrier ratio (w/w): 42/58, particle size: 7.7 μm

2.3. Colour stability of the product

For the PGSS™ products of elderberry concentrate and palm fat, no significant changes in the colour values were observed during prolonged storage (Fig. 6). Similar results were obtained in a previous work, where it was shown that formulation of product in powderous form by using another SCF micronization technique, i.e. Concentrated Powder Form

(CPF™), improves the colour stability of anthocyanins (VATAI et al., 2008). There, grape marc extract was sprayed by using SC CO_2 on silica carrier. It was also shown, that by the nonformulated extracts, deterioration of the colour properties occurred during storage, faster at the samples stored in light at ambient temperature, slower at the samples stored in dark and cold.

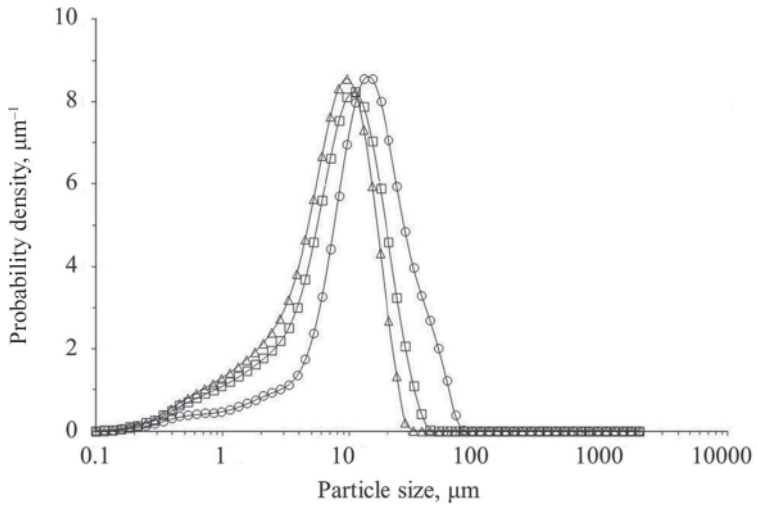

Fig. 5. Particle size distribution: Probability density of three PGSS™ products; 5, 20, and 42% w/w liquid on the carrier. Liquid/carrier ratio (w/w) ○: 5/95; □: 20/80; △:42/58

3. Conclusions

In this work, two environmentally benign technologies were applied for separation and formulation of natural compounds, namely anthocyanin pigments. Integrated membrane process technology was used for concentration of elderberry juice, where the initial juice of 10.5 °Brix was concentrated to 56 °Brix. The obtained dense anthocyanins-concentrate was formulated by high pressure process PGSS™ with palm fat as a carrier, resulting in a homogeneously coloured free flowing fine powder. The obtained powder showed colour stability during prolonged storage in dark and in light as well. No significant changes in lightness, hue angle, and chroma values during 56 weeks were observed. The obtained powderous product of the palm fat in combination with natural pigments of anthocyanins gives a good perspective for applications in the food industry.

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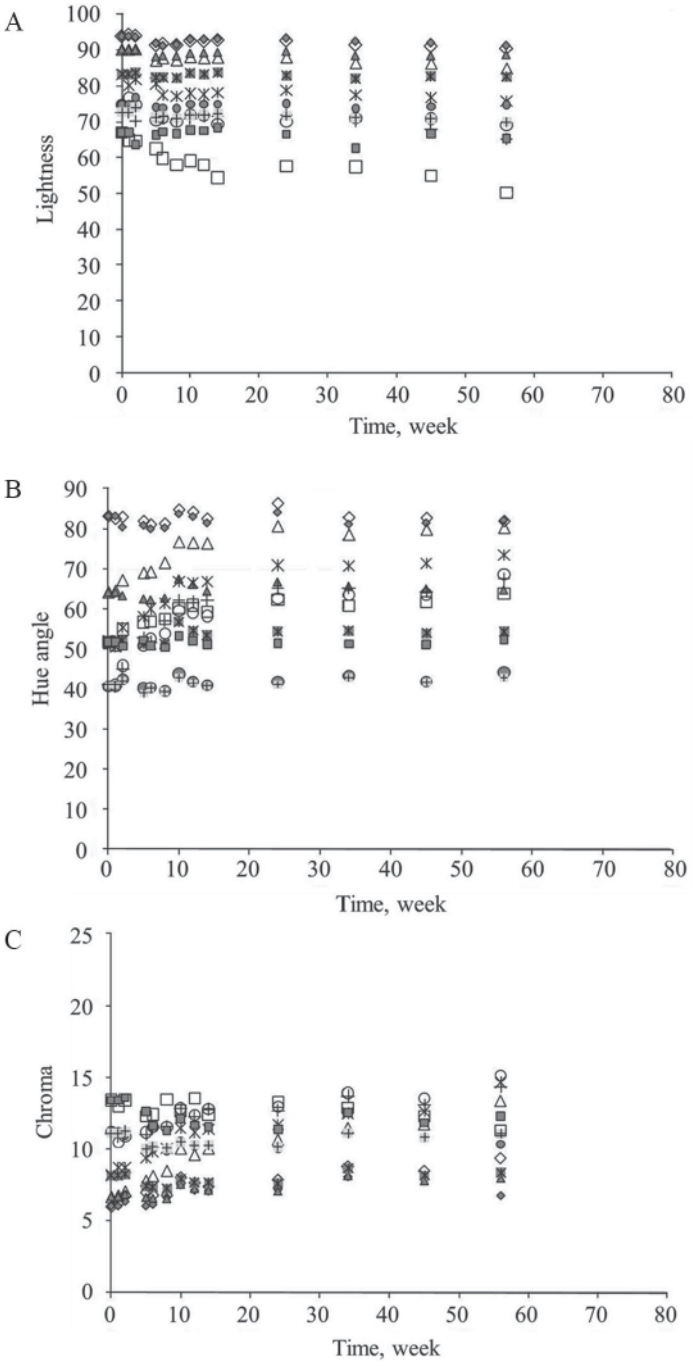

Fig. 6. Colour values of the PGSS™ formulated products stored for 56 weeks at light-ambient temperature and in dark-refrigerator. ◇: M1 light; ◇: M1 dark; △: M2 light; ▲: M2 dark; ✳: M3 light; ✳: M3 dark; ○: M4 light; ●: M4 dark; +: M5 light; ⧺: M5 dark; □: M6 light; ■: M6 dark
a) Lightness: 100=absolute white, 0=absolute black; b) Hue angle: 0° red; +90° yellow; –90° blue; c) Chroma (Saturation)

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