SUPERCRITICAL FLUIDS FOR FORMULATION AND STABILIZATION OF ANTHOCYANIN COLORANTS

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Abstract

Particle formation using supercritical fluid (SCF) technologies has been researched intensively in the recent years. Applications for many fields have been developed and it is likely that these technologies will gain their position in the industry. The SCF product formulation is becoming a potential for future applications in food industry as well, due to its advantages compared to conventional technologies.
This research was carried out in 3 different laboratories. The overall goal was to develop an anthocyanin based natural colorant suitable for applications in food industry. Product formulation with SCF techniques, namely Concentrated Powder Form (CPF™), Particles from Gas Saturated Solutions (PGSS™) and Supercritical Antisolvent Precipitation (SAS) were carried out. Different anthocyanin-concentrates and carrier materials were tested. The products were analyzed for their colour properties (Lightness, Hue angle and Chroma values). The stability of the colour was monitored during prolonged storage (light/dark and ambient temperature/refrigerator). The SCF formulated anthocyanin products showed extended colour stability compared to non-formulated extracts. Improving the saturation of the colour of the obtained products, these products can represent an alternative to synthetic colorants as well.

1. Introduction

Anthocyanins are group of polyphenols, responsible for the colours of yellow, orange, red, pink and blue of most of the flowers and fruits. Their role as natural colorants in food systems has been reviewed by many authors [1-4]. In USA and EU there are several anthocyanin-colorants allowed for the use in food products [5-6]. The major drawback of their use and competitiveness compared to synthetic colorants is their colour instability, which may result in degradation of colour, during processing and storage. Different possibilities of stabilization were discussed [7-11]. Formulation of the product in powderous form is one way of stabilization [12], which also facilitates the admixing into food. However, the conventional technologies often use high temperatures, high amount of organic solvents and long processing time, accelerating the degradation of the anthocyanins. Hence, novel technologies, which also have lower environmental impact, will have important role in the future food industry. Particle formation using supercritical fluids (SCFs) is one of the new technologies, which has been intensively investigated and improved in the recent years [13]. Applications for many fields have been developed and it is likely that these technologies will gain their position in the industry [14]. The interest in SCF product formulation for food industry has increased as well, due to its advantages compared to conventional technologies [15].
The overall goal of this research, carried out in 3 different laboratories, was to develop an anthocyanin based natural colorant suitable for applications in food industry. Product formulation with SCF

techniques, namely Concentrated Powder Form (CPF™), Particles from Gas Saturated Solutions (PGSS™) and Supercritical Antisolvent Precipitation (SAS) were carried out in order to obtain the product in powder form. Different anthocyanin-concentrates and several carrier materials were tested. The products were analyzed for their colour properties (Lightness, Hue angle and Chroma values) [16]. The stability of the colour was monitored during prolonged storage (light/dark and ambient temperature/refrigerator).

2. Materials

The red grape marc (GM) extract was produced in the laboratory of the University of Maribor. The elder berry (EB) and black-currant (BC) concentrates were kindly donated by the Corvinus University of Budapest (Hungary). The silica and starch carrier materials were purchased from Degussa GmbH (Düsseldorf, Germany). The palm fat was obtained from Loders Croklaan (Wormerveer, Netherlands). The reagents and solvents were obtained from Merck (Darmstadt, Germany). Carbon dioxide (CO_2) of purity 2.5 (99.5 % (v/v)) was obtained from the local suppliers.

3. Product formulation with HP technologies

3.1. Concentrated Powder Form (CPF™)

The experiments were performed at the Ruhr-University of Bochum (Germany). Extracts of red grape marc were used for the formulation experiments, in concentrations of 1 wt %, 10 wt % and 30 wt % in solvent (water or 50 wt % ethanol-water mixture). As carrier material modified silica and starch were tested. The solution was filled into an autoclave. CO_2 was dissolved in the extract solution at a pressure ranging from 10 to 18 MPa at ambient temperature. The carrier was filled into a vessel. The carrier was dozed to the spraying zone by an additional CO_2 cylinder with a pressure of 0.1 to 0.2 MPa. An inert atmosphere was provided by leading nitrogen (N_2) to the system. The solution formed in the autoclave was depressurized over a nozzle into the spray tower. Due to the rapid expansion of the gas the liquid was disintegrated into very fine droplets. Concurrently the solid, powderous carrier was added to the spraying zone. The turbulence in the tower, caused by the expanding gas led to an intensive mixing of the droplets and the solid carrier. The coarse fraction was collected at the bottom of the spray tower while the fine fraction was obtained in the cyclone.

3.2. Particles from Gas Saturated Solutions (PGSS™)

The experiments were performed at the University of Maribor (Slovenia). For this formulation experiments, EB concentrate was used. The carrier material was palm fat. The melted palm fat was mixed with the emulsifier and the EB concentrate using an electrical homogenizer. The emulsion was immediately filled into the autoclave and CO_2 was introduced 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). Simultaneously the pressure reached the operating value. 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 and the compressible gas evaporated in the expanding chamber causing the micronization of the particles.

3.3. Supercritical Antisolvent micronization (SAS)

At the University of Trieste (Italy) the possibility to produce micro particles from a black-currant concentrate (BC) was explored using the SAS technique. The solution of BC was pumped to the precipitator at constant flow rate by a high pressure pump. The solution was sprayed into the thermostatic precipitator through a nozzle. Liquid CO_2 was cooled down and pumped to the precipitator by a volumetric pump. Before the inlet of the precipitator the CO_2 was led through a

thermostatic water bath to obtain the temperature of the reactor. Both the solution and CO_2 were added to the cell from the top, resulting in co-current flow. The outlet flow was filtered to prevent the precipitate to leave the precipitator. The regulation was done with a heated valve in order to prevent freezing. The precipitator was furthermore equipped with a safety valve. The temperature inside the reactor was controlled by a thermometer and the pressure was measured with a pressure transducer. The obtained product was collected and analysed.

4. Characterization of the product

4.1. Particle size and morphology

The particle size was determined by Fritsch Particle Size analyser. The shape and surface characteristics of the particles were observed by Scanning Electron Microscopy (SEM).

4.2. Colour evaluation

The colour was measured on the principles of CIEL·a·b method (International Commission on Illumination, Vienna), using a colorimeter (Chromameter CR-400, Konica Minolta Sensing, INC). The colorimeter measures *L* which is a measure of 'lightness' and two coordinates *a* and *b*. Positive values of *a* are in the direction of 'redness' and negative values in the direction of the complement 'green'. Positive values of *b* are in the direction of the vector for 'yellowness', and negative for 'blueness'. For better descriptions of colour changes *L*, *a* and *b* are converted to the L·C·H system where the L=lightness (L) with 100=absolute white and 0=absolute black. Chroma (C) is a measure of intensity (or saturation) and calculated as $(a+b)^{1/2}$. Hue angle (H) is determined as arc tan (b/a). Hue angle is expressed on a 360° grid where 0°=red, 90°=yellow, 180°=green and 270°=blue. This system avoids the use of negative numbers and differences in hue angle of 1° are readily discernible by the human eye. The HP formulated products were stored at 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 several months.

5. Results and discussion

Powders with different morphologies were obtained. The SEM pictures showed a very porous material, mostly spherical shaped, in case of PGSS™ and CPF™ methods. The mean particle size of CPF™ products, where the carrier was silica, was ~9 μm. For the PGSS™ products with palm fat and EB, the particle size varied between 10 and 18 μm, depending on the ratio of liquid anthocyanin-concentrate to the solid carrier material. The lower was the liquid content the higher was the mean particle size. Furthermore, as the liquid was more concentrated the particle size increased, as well. The initial colour characteristics (L, C and H) of some obtained GM and EB powders are shown in Fig. 1. The sample codes, the anthocyanin-concentrate/carrier ratios and the total anthocyanin (TA) content of the products (mg TA/kg total) are presented in Table 1. The colorimetric values are compared to synthetic colorants, erythrosine and allura red, which are still permitted in the food industry (however, some EU countries limited their use).

Table 1: CPF™ and PGSS™ products. GM 1-3: grape marc with silica carrier and EB 1-6: elder berry with palm fat carrier.

METHOD	**CPF**			**PGSS**					
SAMPLES	**GM1**	**GM2**	**GM3**	**EB1**	**EB2**	**EB3**	**EB4**	**EB5**	**EB6**
liquid/carrier ratio (wt)	60/40	56/44	44/56	5/95	10/90	20/80	30/70	41/59	50/50
mg TA[#]/kg total	41	38	90	333	666	1332	1998	2737	3350

[#] Total anthocyanins (measured by pH-differential method) [17]

The formulation of the product with CPF™ resulted in darker powders compared to the PGSS™ products. This is mainly due to the different anthocyanin material, since the grape marc, obtained during the processing of the grapes has brownish-red hue, while elder berry concentrate reddish-pink. Secondly, the silica carrier is more transparent than palm fat, which enables the higher appearance of the colour. As expected, by increasing the anthocyanin concentration the L values decrease (samples become darker), as well as the C values increase (the colour is more intense). By acidifying the anthocyanin-concentrate (prior to formulation) the colour-shift toward red hues can be achieved (for ex. GM 1 and GM 2). The 1:1 ratio of palm fat and elder berry showed similar L and H values as the synthetic colorants, however big difference is observed in the saturation of the colour. Compared to conventional formulation techniques, SAS is a more mild method for formulating the sensitive anthocyanin compounds. Co-precipitation with different materials is possible, as well.

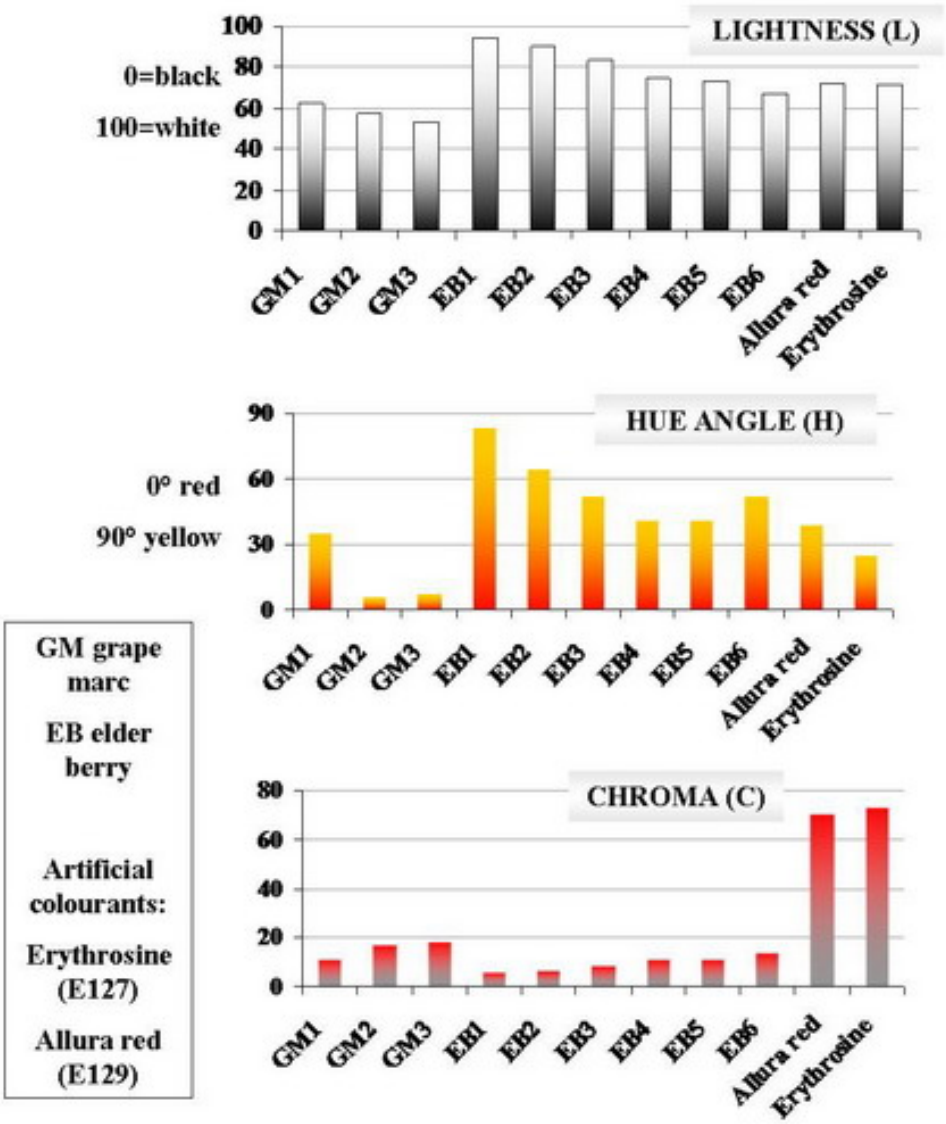

Figure 1: The comparison of colour characteristics of some GM, EB powders and synthetic colorants. For sample codes see Table 1.

The colour stability of the obtained products was monitored periodically for several months. Additionally, non-formulated anthocyanin extracts were subjected to same storage conditions for comparison. The product formulation with SCF technologies improved the colour stability. The non-formulated samples showed degradation indicated by each colorimetric value, while the formulated ones remained stable in L, C and H parameters. As an example, hue values of some products, which were stored at light and ambient temperature and monitored for several months, are shown in Fig. 2.

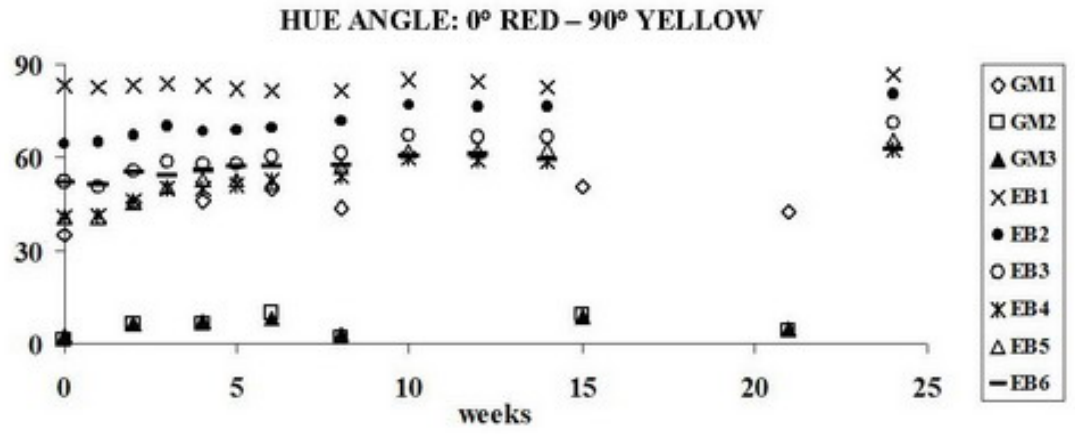

Figure 2: Hue values of some CPF™ and PGSS™ products. Samples stored at ambient temperature and at light, monitored periodically.

6. Conclusions

The tendency to reduce the use of artificial colorants in food applications is not only due to the strictening legislation but to consumers' demands, as well. There are already many products of natural colorants on the market, mostly produced by conventional technologies. In this work, formulation of anthocyanin colorants in powderous form was achieved with supercritical fluid technology, namely CPF™, PGSS™ and SAS methods. Using CO_2 as SCF for the process provides mild processing conditions for natural products and is environmentally friendly. The L and H colorimetric parameters of the obtained products showed similar values to artificial colorants, while the colour saturation of the synthetic colorants is still somewhat higher. However, the most important result is, that the stability of anthocyanins colour in the formulated product was generally improved, compared to non-formulated products.

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