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Beetroot juices as colorant in plant-based minced meat analogues: Color, betalain composition and antioxidant activity as affected by juice type

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ABSTRACT

The aim of this work was to study the suitability of the application of beetroot juices (obtained from fresh beetroot, cooked beetroot, beetroot powder and commercial beetroot juice) as colorants in the development of meat analogues, as well as their antioxidant properties. Fresh beetroot juice showed the highest betalain content (119.53 mg betanin/100 ml and 48.27 mg vulgasanthin I/100 ml) and commercial juice the lowest (49.40 mg betanin/100 ml and 7.21 mg vulgasanthin I/100 ml). Juices from fresh and cooked beetroot showed ABTS, FRAP and FIC values higher than values obtained for commercial juice (ascorbic acid added). Textured soy protein with cooked beetroot juice or with commercial juice showed the same appearance that of minced beef and pork meat, respectively, which is in accordance with their hue values and reflectance spectra. The attractive red color of betalains and their stability at the pH value of meat analogues make beetroot juices ideal for their application as colorants in meat analogues.

1. Introduction

The boom of plant-based meat products sector (the global plant-based meat market size will hit $12.32 billion by 2027, at a CAGR of 18.3%) (Kbvresearch, 2022), together with the great importance of food color in determining the purchase decision of consumers (Singh, 2017), are causing an incessant search of ways to mimic the typical red color of meat in meat analogues. Currently, the predilection for the use of natural colorants as opposed to artificial ones, and the fact that these products are intended for a public aimed at vegan trends, both reasons are limiting this search toward red colorants from vegetable origin.

In view that the color of the meat analogue matrix (textured vegetables such as textured pea, or textured vegetable proteins, such as textured soy proteins) is far different (yellow-brown or beige color) from the well accepted and mostly expected red color of raw minced meat, or the brown color of cooked meat, the addition of colorants to the ingredient mix is highlight needed.

Assuming all these aspects, red colorants from vegetable origin including beetroot (*Beta vulgaris* L.) and radish (*Raphanus sativus* L.) extracts or concentrates, seem to be the most suitable option to obtain the desired coloring effects. Red varieties of radish obtain their red color from the anthocyanin pelargonidin pigment which has shown a high lability to pH and temperature (changing to non desired colors) (Rodriguez-Saona et al., 2006), which is an important technical obstacle that must be solved before its use. On the contrary, the compounds responsible for the red color in beetroots are betalains, which are composed of two main groups: the red-violet betacyanins (e.g. betanin and isobetanin) and the yellow betaxanthins (e.g. vulgaxanthin I and II) (Bangar et al., 2022). The betacyanins (betanin and isobetanin) are water-soluble immonium conjugates of betalamic acid with 3,4-dihydroxyphenylalanine (cyclo-DOPA), which may be glucosylated (Bangar et al., 2022; Vieira Texeira da Silva et al., 2019). In beetroot, the most abundant betacyanin is the betanin (betanidin 5-O-β-D-glucoside) which is a betanidin 5-O-β-glucoside, containing a phenolic and a cyclic amine group, both shown to be very good electron donors, acting as antioxidants (Kanner et al., 2001).

The red color of these compounds shows high storage stability at the pH value of the meat analogue (5–7; at this pH they retain their

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tinctorial strength and color shade) and degrades toward brown color upon heating (Herbach et al., 2006a), imitating the color changes of meat during cooking (browning or decoloring its initial red color due to myoglobin denaturation) (Hollenbeck et al., 2019). Betanins show a high stability under freezing conditions (longer than 9 months at −30 °C), but it decreases under refrigeration (20 days at 4 °C) depending on several factors such as pH, temperature and the presence or absence of oxygen (Vieira Texeira da Silva et al., 2019). This stability has been established for purified betanin, but it could be improved into the food matrix due to the protective effect of some natural compounds (with antioxidant properties) on the chemical structure of betanins (Herbach et al., 2006b).

Han et al. (1998) reported that betanin degradation results in the formation of betalamic acid and cyclo-DOPA-5-O-glycoside, being a reversible reaction, which means that it can be degraded and regenerated continuously during storage, therefore the final betanin concentration remains stable. In addition, several authors have reported antioxidant properties for betacyanins (Esatbeyoglu et al., 2015; Fernández-López et al., 2018), which reinforce the technological interest in the food applications of beetroots not only as colorant (the main objective) but also as antioxidant. Even in the case of its application for the development of meat analogues, these potential antioxidant properties would be very interesting because of the control or inhibition in lipid oxidation. Lipid oxidation is considered one of the main reasons for food deterioration responsible for color, taste and odor changes perceived as unpleasant by consumers (Fernández et al., 1997; Wu et al., 2022).

This excellent technical behavior together with its safety and low cost have contributed to its wide use as natural colorant in the global food industry and recently it was shown to also have a great potential for the plant-based meat industry. In addition, its use as food colorant (red beetroot) has been approved in both, the European Union, under code EEC E−162 (EFSA, 2015), and the United States, under Section 73.40 in Title 21 of the Code of Federal Regulations (CFR) stipulated by the Food and Drug Administration (FDA, 2009).

In addition, potential health benefits of betalains have also been suggested mainly attributed to their antioxidant and anti-inflammatory properties (Bangar et al., 2022; Gliszczy'nska-Swigło et al., 2006; Vieira Texeira da Silva et al., 2019). Therefore, betalains are regarded as bioactive pigments and their inclusion in the dietary intake may be an alternative to prevent certain diseases (Fernández-López et al., 2018; Martínez-Rodríguez et al., 2022; Polturak & Aharoni, 2018).

Although initially the use of beetroot extracts as source of betalains in the food industry was only intended for their colorant effect, recent research about their technological and functional properties have opened interest as functional ingredient. Nowadays, for the development of meat analogues, it seems to be more interesting to apply it not as beetroot extract, but as beetroot juice because it also covers the hydration function on the textured ingredients used as basic structure of the meat analogue. In addition, it is in line with the clean label concept, highly demanded by today's consumers, mainly based on the use of less ingredients, more natural and obtained under sustainable practices (Asioli et al., 2017).

The aim of this article was to study the suitability of the application of different beetroot juices (obtained from fresh beetroot, cooked beetroot, beetroot powder and commercial beetroot juice) as natural colorants in the development of minced meat analogues as well as their potential antioxidant properties.

2. Materials and methods

2.1. Chemical reagents and materials

ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6- sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Ethylenediaminetetraacetic, acetic acid and acetonitrile were purchased from Sigma Aldrich CO. (St. Louis, MO, USA). Besides, all the other reagents of analytical grade were obtained from Panreac Appli Chem (Barcelona, Spain).

Four types of beetroot juices were used: (1) Commercial beetroot juice (CBJ) from Sonatural (El Alentejo, Portugal) 100% natural, obtained by cold pressure and underwent to a high pressure treatment; (2) Fresh beetroot juice (FBJ) obtained by liquefying fresh beetroots purchased in a local supermarket (Orihuela, Alicante, Spain), using a commercial juice extraction blender Juice & Fresh 400 (Cecotec, Valencia, Spain) working at 20.000 rpm; (3) Cooked beetroot juice (CKBJ) obtained by liquefying commercial cooked beetroots (Naturally Organic S.L., Murcia, Spain) under the same conditions than those reported for FBJ; (4) Reconstituted beetroot juice (RBJ) obtained by mixing commercial beetroot powder (Idda herbal, Sofia, Bulgaria) with tap water (1:9).

Textured soy protein (>90 g/100 g proteins) was obtained from Suministros River S.L.U (Alicante, Spain), and textured pea (55 g/100 g carbohydrates, 24 g/100 g protein and 8.5 g/100 g dietary fiber) from Esgir S.L. (Algemesí, Valencia, Spain). Fresh beef and pork minced meat was purchased from a local supermarket (Orihuela, Alicante, Spain) and kept refrigerated until analysis.

2.2. Beetroot juice analysis

The four types of beetroot juice were subjected to the following analysis: physicochemical properties (pH, total soluble solid content, density and color), betalain content and antioxidant properties.

2.2.1. Physicochemical properties

Juice pH was determined using an SensIOT^{Tm} pH-meter (Hach Company, Iowa, USA).

Total soluble solid content (TSSC) in juices was determined by the refraction index, using a MA871 refractometer (Milwaukee Electric Tool Corporation, Wisconsin, USA) and it is referred as degrees Brix.

Juice density was determined by the pycnometer method using water as working liquid with well-known density depending on temperature.

The color of juices were determined using a Minolta CM-700 spectrophotocolorimeter (Minolta Camera Co., Osaka, Japan) with the following settings (illuminant D65, SCI mode and, observation angle 10°). CIELAB color coordinates (Lightness (L*), redness (a*) and yellowness (b*)) were obtained. From color coordinates, psychophysical magnitudes, hue (h*) and chroma (C*). The reflectance spectrum between 360 and 740 nm (at every 10 nm) were also obtained. All determinations were performed in triplicate.

2.2.2. Determination of betalain content

Red beetroot juices were pre-diluted and filtered (through a 0.45 μm nylon filter) prior to betalain analysis. Betalain pigments were quantified as previously reported (Fernández-López et al., 2002). The visible spectra (380–780 nm) of the extracts were recorded using an Agilent 8453 UV–visible spectrophotometer (Waldbronn, Baden-Württemberg, Germany). Betalain content was assessed as the sum of the concentrations of betacyanins and betaxanthins (Skalicky et al., 2020). Betacyanins were expressed as betanin (molar mass M = 550.5 g/mol) using a molar absorption coefficient (ε) of 60,000 l/(mol·cm) at 535 nm. Betaxanthins were expressed as vulgaxanthin I (molar mass M = 339.3 g/mol), applying a molar absorption coefficient $\varepsilon = 48{,}000$ l/(mol·cm) (Wruss et al., 2015). Individual pigment analysis was performed by HPLC with a Spherisorb ODS-2 column (4.6 mm × 250 mm, 5 μm) (Waters, Wilmslow, United Kingdom) an elution gradient between 175 mM acetic acid in water and 175 mM acetic acid in acetonitrile, and diode array detection (Fernández-López et al., 2002).

2.2.3. Antioxidant properties

The antioxidant properties of beetroot juices were evaluated using three different antioxidant assays. The 2,2′-Azinobis-(3-

Ethylbenzothiazoline-6-Sulfonic acid) assay (ABTS assay) was performed following the methodology described by Gullón et al. (2015). The Ferric Reducing Antioxidant Power (FRAP assay) was determined according to Oyaizu (1986). Lastly, the method described by Mahdavi et al. (2017) was utilized to determined ferrous ion-chelating capacity (FIC assay). The results were expressed as µg Trolox Equivalents/ml of juice in the case ABTS, and FRAP assays. In the case of FIC assay, the results were expressed as µg Ethylenediaminetetraacetic Equivalents (EDTAE)/ml of juice.

2.3. Preparation of minced meat analogues

Textured soy protein (TPSOY) and textured pea (TPEA) were used as the basic ingredient for minced meat analogues. Both ingredients were hydrated with several dilutions (1:1, 1:2, 1:3 and 1:4) of the four beetroot juices trying to mimic the color of the original minced meats (pork minced meat and beef minced meat) that were used as controls. For each dilution, 8 samples (meat analogues) were obtained: textured soy protein hydrated with commercial beetroot juice (CBR + TPSOY), cooked beetroot juice (CKBJ + TPSOY), fresh beetroot juice (FBJ + TPSOY), and rehydrated beetroot juice (RBJ + TPSOY); textured pea hydrated with commercial beetroot juice (CBR + TPEA), cooked beetroot juice (CKBJ + TPEA), fresh beetroot juice (FBJ + TPEA), and rehydrated beetroot juice (RBJ + TPEA).

2.4. Color properties of minced meat analogues

Color properties of minced meat (controls and analogues) were determined using the same equipment and conditions that described in point 2.2.1 for beetroot juices.

2.5. Statistical analysis

All determinations were performed in triplicate and results are shown as mean ± standard deviation. Comparison was conducted using the one-way Analysis of Variance (ANOVA) and when significant ($P < 0.05$), Tukey's mean comparison test was applied to determine which juices presented significant differences at a confidence level of 95%. Prior to that, the normality assumption of the variables was checked (Shapiro-Wilk test) at $P < 0.05$. Pearson correlation between betalains content and antioxidant activity of beetroot juices was also calculated ($P < 0.05$). These analyses were carried out with the statistical program SPSS for Windows v. 27.0 (SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Physicochemical properties of beetroot juices

The yield of juice extraction from fresh beetroots was 55% and that of cooked beetroots was 33%. Fig. S1 (Supplementary Material) shows the four studied beetroot juices and Table 1 their physicochemical properties.

pH values of all juices were significantly different ($P<0.05$) (FBJ> RBJ > CKBR > CBJ) although they were within the range of pH values described for beetroots (4.8–6.2) (Gómez & Duque-Cifuentes, 2018) except CBJ that showed more acid values (3.8). It could be due to the incorporation of some acidulant (ascorbic acid) to improve its stability during commercialization.

The measurement of °Brix in beetroot juices is an indication of the soluble solids content, mainly sucrose, as the main sugar in beetroots. It has been reported that the sugar content of fresh beetroot is around 6% (Ceclu & Nistor, 2020); taking into account that most of it is extracted with the juice and that the juice extraction yield was higher in FBJ than in CKBJ ($P<0.05$), it would be expected that FBJ would have higher °Brix than CKBJ (10.2 for FBJ *vs* 9.3 for CKBJ). CBJ showed the highest Brix degrees and RBJ the lowest ($P<0.05$). The different juice processing conditions could be responsible for the higher or lower extraction of soluble solids and so for the °Brix differences. In any case, all these values are into the range of Brix values reported for vegetable juices (between 5 and 12) (Kosters & Waldron, 2009).

Table 1
Physicochemical properties of beetroot juices.

	FBJ	CKBJ	CBJ	RBJ
pH	6.13 ± 0.03^{a}	5.52 ± 0.04^{c}	3.83 ± 0.02^{d}	5.98 ± 0.03^{b}
°Brix	10.20 ± 0.00^{b}	9.33 ± 0.12^{c}	12.53 ± 0.12^{a}	7.5 ± 0.10^{d}
Density (kg/m^3)	1014 ± 13.45^{a}	991 ± 25.24^{c}	1013 ± 3.51^{a}	1004 ± 16.54^{b}
Color Parameters				
Lightness (L*)	24.51 ± 0.08^{b}	26.22 ± 0.08^{a}	26.50 ± 0.17^{a}	24.95 ± 0.12^{b}
Red/Green (a*)	1.19 ± 0.01^{b}	8.00 ± 0.11^{a}	7.52 ± 0.09^{a}	1.78 ± 0.08^{b}
Yellow/Blue (b*)	−0.56 ± 0.11^{c}	0.73 ± 0.03^{b}	−1.49 ± 0.02^{a}	−0.22 ± 0.01^{d}
Chroma (C*)	1.32 ± 0.01^{b}	8.03 ± 0.04^{a}	7.66 ± 0.02^{a}	1.79 ± 0.08^{b}
Hue (h*)	334.7 ± 1.31^{c}	5.16 ± 0.01^{d}	348.75 ± 1.22^{b}	352.93 ± 0.71^{a}

FBJ: juice from raw beetroot; CKBJ: juice from cooked beetroot; RBJ: juice rehydrated from beetroot powder; CBJ: commercial beetroot juice. $^{a\text{-}d}$: Different superscript letters in each row indicate significant differences according to Tukey's HSD post-hoc test ($P<0.05$).

The density of the four juices was in the range of 991–1014 kg/m^3, showing RBJ and CBJ the highest values ($P<0.05$) without differences between them, corresponding with the highest °Brix values. The relationship between °Brix and density in a sucrose solution is well established. In this case, the relationship is not so direct, because the presence of other components in beetroot juices (not only sucrose) should be affecting this relation. Similar behavior between density and °Brix values has been reported in fruits and vegetal juices and even empirical equations modeling this relation have been proposed (Garza & Ibarz, 2010; Kosters & Waldron, 2009).

Regarding color parameters, although significant differences ($P<0.05$) were detected in L* and b* values between all the juices, these differences were less than 2 units, which could not have practical importance. The differences found for the a* coordinate (red/green) and chroma (C*) values seem to be more significant, following a similar pattern: the highest values were found for juice obtained from cooked beetroot (CKBJ) and commercial juice (CBJ), and the lowest values for juice obtained from fresh beetroot (FBJ) and reconstituted juice (RBJ) ($P<0.05$). This would indicate that the CKBJ and CBJ juices present higher compounds that contribute to the red color component and that they are more saturated (pure) colors than those of the other juices. It can also be observed that the juices with the highest a* values (CKBJ and CBJ) are those with the lowest pH values ($r = -0.72$). Although, contrary to what happens in the case of anthocyanins, the color of betacyanins does not change as much as a function of pH (into the pH range of these juices), variations in the shades depending on the pH of the medium have been described (Esatbeyoglu et al., 2015; Gasztonyi et al., 2001). Betacyanins are easily hydrolyzed in an alkaline medium, showing a maximum stability between pH 4 and 5 (Flores-Mancha et al., 2019).

Herbach et al. (2006a) reported that heat treatment can modify the color of betacyanins. In this case, the thermal treatment of beetroot cooking and that applied during the industrial processing of commercial juice would be contributing to modify the values of the a* coordinate, with respect to those obtained for fresh beetroot juice. During thermal processes, betalains can be degraded by isomerization, deglycosylation, hydrolysis, dehydrogenation, and decarboxylation (Ruiz-Gutiérrez et al., 2014). The occurrence of browning leads to a gradual reduction in color from the characteristic red of these pigments to light brown. This color modification is very interesting for the development of meat analogues because it mimics the color changes of meat during the cooking process (Maillard reactions, denaturation and aggregation of proteins, evaporation of water, melting of fat crystals, etc.) which changes from pink to brown (Zhou et al., 2022).

All the juices presented hue values between magenta (330°) and red (30°). According to the classification of the Spanish National Institute of Rationalization (IRANOR, 1981), the shades of the juices analyzed were: magenta-purple for FBJ, purple-reddish for CKBJ, magenta-purple for CBJ and purple-magenta for RBJ.

For the preservation of the color of beetroot juices, it is very important to protect them from light and contact with oxygen, because they oxidize easily and this can cause irreversible loss of color (Flores-Mancha et al., 2019).

Fig. 1 shows the reflectance spectrum of the 4 beetroot juices. Spectrally, there were no differences ($P > 0.05$) between FBJ and RBJ samples for all wavelengths. On the contrary, CKBJ and CBJ showed spectra that are similar to each other in shape, but with different percentages of reflectance, especially at wavelengths between 320 and 490 nm, and between 580 and 740 nm, corresponding to magenta and red colors, respectively. CBJ showed higher reflectance percentages than CKBJ in the magenta color region, while in the red color region it is CKBJ that shows higher values ($P<0.05$). At intermediate wavelengths (490–580 nm) all the juices are isobestic, i.e. they have similar reflectance values ($P > 0.05$), which suggests that they all have a compound, or mixture of them, with very similar spectral behavior (Hapke, 2017). In addition, CKBJ and CBJ has other isobestic point at 620 nm.

3.2. Betalain content in beetroot juices

Betalain content varied considerably depending on the juice type (Table 2). In these juices, red pigment content (betacyanins) ranged from 49.40 to 119.53 mg/100 ml, while contents of yellow pigments (betaxanthins) varied from 7.21 to 48.27 mg/100 ml. This agrees with the betalain content reported for most of the beetroot varieties, with higher concentrations of betacyanins and relatively low levels of betaxanthins (Gasztonyi et al., 2001). The highest content of red pigments was found in fresh beetroot juice (FBJ), being substantially higher than in the rest of the samples, which were quite similar. Manipulation of natural extracts alters their original color and decreases their pigment level (Esatbeyoglu et al., 2015; Skalicky et al., 2020). Betanin and isobetanin were the main pigments in the four red beet juices analyzed. Fig. 2 confirms that the ratio between the two main betacyanins in the juices (betanin and isobetanin) determines a clear influence on their colorant properties and the profile of the visible spectra. The stability of these metabolites is a function of their structure, and various studies confirmed the lower lability of betacyanins compared to betaxanthins, which stability was further increased by glycosylation. Degradation of betalains in beetroots with a subsequent change of color could be a result of pH values (out of the optimal range) or exposure to high temperatures, where first order kinetic changes are involved (Skalicky et al., 2020). In addition, they are also sensitive to water content, light, oxygen, and the presence of certain metals (Esquivel, 2016; Fernández-López et al., 2018). Considering that pigment content is a determining factor in foods (Nieto-Sandoval et al., 1999) and in order to preserve the highest food quality, natural extracts should be handled as little as possible to ensure that they retain their original characteristics.

Table 2
Betalain content in beetroot juices.

Juice	Betacyanins	Betaxanthins
FBJ	119.63 ± 12.47^{a} (mg betanin/100 ml) BETANIN—76% ISOBETANIN – 23%	48.27 ± 7.48^{a} (mg vulgaxanthin I/100 ml)
CKBJ	52.47 ± 4.37^{b} (mg betanin/100 ml) BETANIN – 70% ISOBETANIN – 29%	9.19 ± 2.02^{c} (mg vulgaxanthin I/100 ml)
CBJ	49.40 ± 6.44^{b} (mg betanin/100 ml) BETANIN – 83% ISOBETANIN – 16%	7.21 ± 2.36^{c} (mg vulgaxanthin I/100 ml)
RBJ	54.67 ± 8.25^{b} (mg betanin/100 ml) BETANIN – 62% ISOBETANIN – 37%	24.42 ± 3.99^{b} (mg vulgaxanthin I/100 ml)

FBJ: juice from raw beetroot; CKBJ: juice from cooked beetroot; RBJ: juice rehydrated from beetroot powder; CBJ: commercial beetroot juice. Data are presented as mean ± standard deviation. Different superscript letters in each column indicate significant differences according to Tukey's HSD post-hoc test ($P<0.05$).

3.3. Antioxidant properties of beetroot juices

As it is well known that the antioxidants can exert their action by very diverse mechanisms (suppressing the generation of the first radicals that initiate oxidative damage, scavenging free radicals, chelating

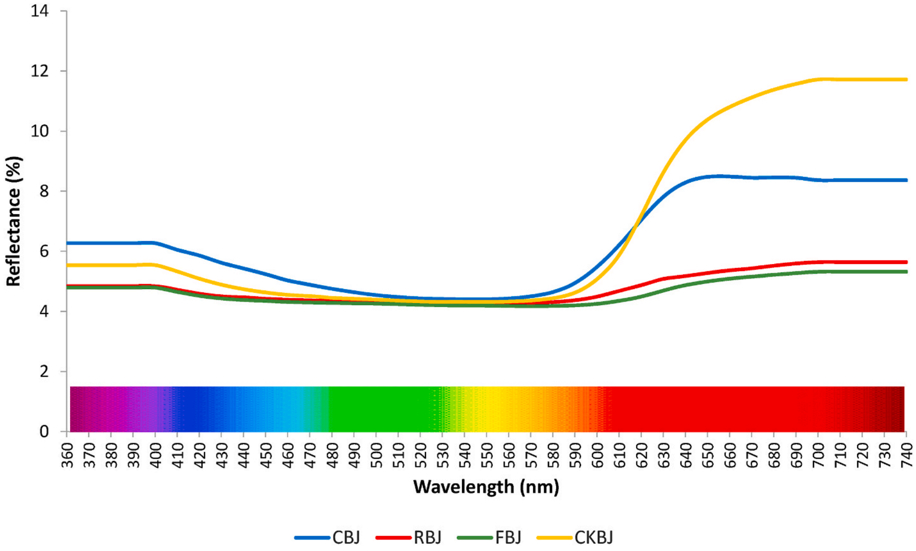

Fig. 1. Reflectance spectra (360–740 nm) of beetroot juices.

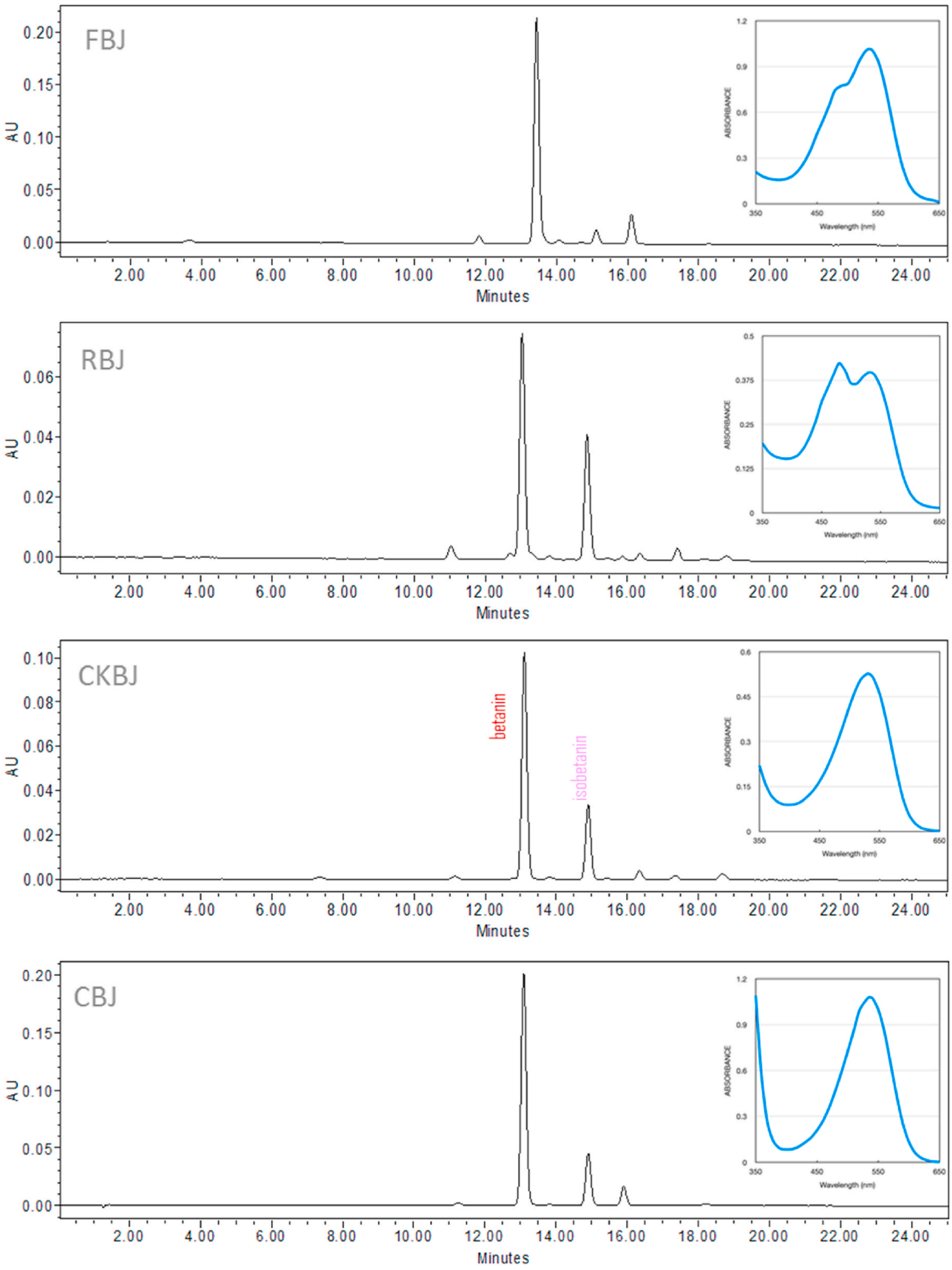

Fig. 2. HPLC patterns (535 nm) and visible spectra (350–650 nm) of different red beet juices analyzed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

metals, chelating initiate oxidative damage, form complexes, reduce certain compounds, induce the activity of biological antioxidant systems, etc.) (Munteanu & Apetrei, 2021; Viuda-Martos et al., 2010) and in the juices there may be mixtures of different antioxidant compounds (Bangar et al., 2022) with different mechanisms of action, between which, in addition, synergistic reactions can be established, different analyses will be necessary to consider the possible mechanisms of action of all the antioxidants present in the juices. Table 3 shows the results obtained in the antioxidant capacity assays for the four beetroot juices. The high antioxidant activity of CBJ is surprisingly considering that it

Table 3
Antioxidant activity of beetroot juices.

Juice	ABTS	FIC	FRAP
FBJ	2909.20 ± 22.59^b	366.22 ± 0.97^a	202.51 ± 3.99^a
CKBJ	3090.22 ± 37.65^a	33.44 ± 0.19^c	188.41 ± 2.27^b
RBJ	1764.47 ± 30.12^d	44.07 ± 0.16^b	146.10 ± 1.96^d
CBJ	2275.61 ± 15.06^c	26.49 ± 0.13^d	168.67 ± 2.74^c

FBJ: juice from raw beetroot; CKBJ: juice from cooked beetroot; RBJ: juice rehydrated from beetroot powder; CBJ: commercial beetroot juice. Values for ABTS and FRAP are expressed as μg Trolox Equivalents/ml of juice. FIC values are expressed as μg EDTA Equivalents/ml of juice. Values followed by the same superscript letter in the same column were significantly different according to Tukey's HSD post-hoc test ($P < 0.05$).

has the lowest betalains content (Table 2), but the explanation lies in the addition of ascorbic acid as an antioxidant, which is reflected on the product label. So, these values (CBJ) are not further considered in this discussion about antioxidant properties.

FBJ and CKBJ showed the highest ABTS and FRAP values, and RBJ the lowest ($P<0.05$). The concordance between the results obtained for these two methods could be expected taking into account that both methods evaluate the same antioxidant action mechanism based on the free radical scavenging activity by lipid oxidation chain-breaking carried out by Single Electron Transfer (SET) (Apak et al., 2016). Regarding FIC values, based on metal chelating effects, FBJ showed the highest values ($P<0.05$) that were about 10 times higher than those of the other juices. These results seem to be well correlated with betalain content in beetroot juices because FBJ that showed the highest content (Table 2) also had, the highest antioxidant activity ($r = 0.459$, $r = 0.994$ and $r = 0.739$ for ABTS, FIC and FRAP, respectively). It is important to note the high relevance of the pH of the medium upon the charge alteration of betanin and isobetanin and so upon their antioxidant activity, but between the pH range ($3.5 < pH < 7$) of the four juices under study (Table 1), both compounds appear at the same form (dianion with deprotonated C2-, C15- and C17–COOH groups) (Frank et al., 2005). The bioavailability of betalains is at least as high as flavonoids, which are well-accepted natural antioxidants. Betalains, as natural antioxidants, may provide protection against oxidative stress-related disorders (Fernández-López et al., 2018; Polturak & Aharoni, 2018; Tesoriere et al., 2005).

3.4. *Minced meat analogues: color properties and reflectance spectra*

Color is one of the most influential visual appearance traits in meat and meat products, and therefore of their analogues, because it is usually the decision-making parameter for consumers when selecting them at the point of purchase (Tomasevic et al., 2021). The use of objective criteria in the measurement of food color, such as color coordinates and other parameters obtained from spectral measurements, is one of the main tools frequently used to evaluate the impact of the incorporation of ingredients and/or additives on the meat product appearance, and even more so if these additives are used with a coloring effect.

Color coordinates of meat analogues hydrated with beetroot juices (diluted with water at 4 proportions: 1:1, 1:2, 1:3 and 1:4) are shown in Fig. 3. Regarding lightness, it was observed that is depended on the type of juice ($P<0.05$) but not on the type of textured base ($P > 0.05$). For all dilutions, L* values of minced meat analogues were higher when CBJ was used, followed by CKBJ, RBJ and the lowest L* values for FBJ ($P<0.05$) without differences between the two texturized bases used (soy protein or pea). As can be expected, L* values increased ($P<0.05$) with the dilution levels (Simon et al., 2017) but all dilutions showed the same behavior in reference to the type of juice and textured base used. As the degree of juice dilution increases, the concentration of pigments on the surface of the textured base decreases, thereby increasing light reflection and hence lightness, coinciding with a higher free water on the product surface (Simon et al., 2017).

Minced pork meat showed L* values similar than that obtained for hydrated meat analogues (dilution 1:1), with CBJ (CBJ + TPSOY and CBJ + TPEA), and minced beef meat with the analogues hydrated with CKBJ (CKBJ + TPSOY and CKBJ + TPEA). As L* values in meat

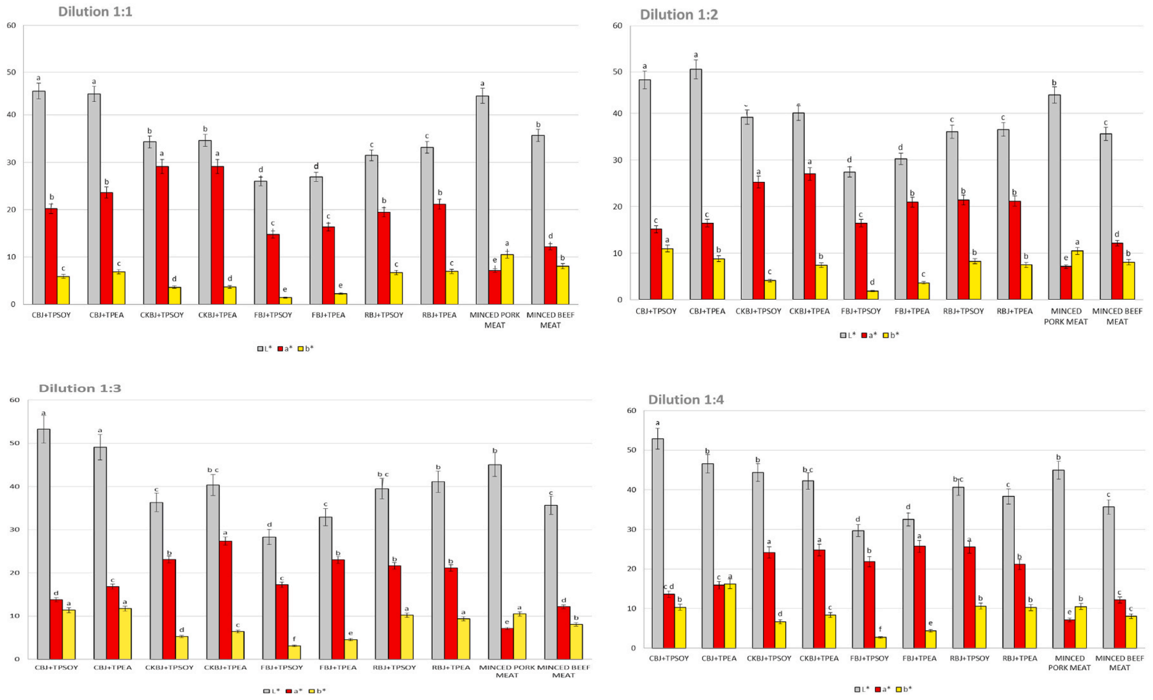

Fig. 3. Color coordinates (L*: lightness, a*: red/green coordinate, b*: yellow/blue coordinate) of minced meat analogues (at several dilution of beetroot juices) and of minced meats (pork and beef). $^{a-f}$ For the same color coordinate, different letters indicate significant differences between samples according to Tukey's HSD post-hoc test ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

analogues increased with juice dilution level but they do not change in minced pork or beef meat, L* in analogues hydrated (at the highest dilution level) with CKBJ (CKBJ + TPSOY and CKBJ + TPEA), and with RBJ (RBJ + TPSOY and RBJ + TPEA) was similar ($P > 0.05$) than L* in pork and beef meat, respectively. Lightness describes the brightness or darkness of the color and so, several meat analogues obtained using different juices at different dilution levels could have similar L* values than minced meat.

In reference to a* values the behavior was different. At dilution 1:1, the highest a* values ($P<0.05$) were shown in analogues hydrated with CKBJ (CKBJ + TPSOY and CKBJ + TPEA) followed by CBJ and RBF (without differences between them) and the lowest a* values ($P<0.05$) were found in analogues hydrated with FBJ (FBJ + TPSOY and FBJ + TPEA). Also in this case, a* differences between samples were more dependent on the type of juice than on the textured base used. However, changes in a* values due to dilution levels did not follow the same pattern in all analogues and it was dependent on the type of juice. The a* values in analogues hydrated with CBJ, CKBJ and RBJ decreased or were not modified with increasing dilution levels, in contrast with the behavior of redness in analogues hydrated with FBJ, increasing ($P<0.05$) at higher dilution levels. All meat analogues showed higher a* values than minced pork or beef meat ($P<0.05$). Only a* values of analogues hydrated with CBJ (CBJ + TPSOY and CBJ + TPEA) at the highest dilution level (1:4) were similar than a* values in beef meat ($P<0.05$) but higher than corresponding to pork meat ($P<0.05$). Redness (a*) is one of the most important quality criteria for this type of product, as it is associated not only with the pigment content but also with the pigments state (AMSA, 2012; Hernández et al., 2016). It is therefore important that the colorants used in the development of meat analogues can achieve similar a* values to those of the products they try to mimic.

The behavior of b* values in meat analogues was also mainly dependent on the type of juice. The highest b* values were obtained in analogues hydrated with CBJ and RBJ (without differences between them) followed by CKBJ and the lowest b* values ($P<0.05$) in analogues hydrated with FBJ (FBJ + TPSOY and FBJ + TPEA). This pattern was observed at all dilution levels although with increasing b* values at higher dilution levels. Yellowness of minced meat was similar to that obtained in meat analogues hydrated with RBJ and CKBJ with increasing dilution levels.

Regarding chroma values (Table S1, Supplementary Material), significant differences ($P<0.05$) were observed depending on the type of juice, dilution level and on the type of textured base used. In reference to the type of juice, the highest C* values were observed when CKBJ was used (CKBJ + TPSOY and CKBJ + TPEA) and the lowest for FBJ (FBJ + TPSOY and FBJ + TPEA) ($P<0.05$), while analogues hydrated with CBJ or RBJ showed medium saturation values without differences between them. As saturation refers to how vivid or dull the color is (the greater the chroma the more vivid the color) (Wrolstad & Smith, 2017), it could be said that CKBJ contributes to more vivid colors in the meat analogues. The level of dilution did not show the same effect in sample saturation: samples hydrated with CBJ or CKBJ showed lower C* values at higher dilution level, while it was contrary when FBJ and RBJ was used. Saturation values of meat analogues were dependent on the type of textured base, showing the samples with textured pea, higher C* values than samples with textured soy protein, although not in all cases these differences were significant. As it was expected, the color properties of meat analogues were mostly depending on the type of juice (due to its colorant effect) than on the vegetable material (pea or soy) which are colorless or with a slight sandy color.

Meat analogues hydrated with CBJ and RBJ showed the highest h* values (Table S1, Supplementary Material) and those hydrated with FBJ and CKBJ the lowest ($P<0.05$). In all cases, hue values increased with the level of dilution ($P<0.05$) being this increase the highest in samples hydrated with CBJ (>50%). There was not clear effect of the type of textured base in the hue values of samples. However, samples with the nearest h* values to minced meats were CKBJ + TPSOY at 1:3 dilution in the case of beef meat (33.5°) and CBJ + TPSOY at 1:4 dilution for pork meat (55.8°). Both samples showed an appearance very similar to that of their respective minced meats, as can be seen in Fig. 4. It is in accordance with the fact that hue correlates with the color description in common language (red, yellow, green, blue, etc.) and is developed by the specific wavelengths reflected from a food surface back to the detector (AMSA, 2012; Wrolstad & Smith, 2017).

Fig. 5 shows the reflectance spectra of all samples at different dilution levels and also of the controls (pork and beef minced meats). These spectra have been obtained from 360 to 740 nm, which is the range of the visual spectrum for humans. In this narrow range of the electromagnetic spectrum, our eyes have the ability and the brain the capacity to separate wavelengths into color groups. For instance, red color (meat characteristic) is associated with light of approx. 650–700 nm wavelengths (AMSA, 2012).

Reflectance values represent the light (at each wavelength) that is reflected from the food surface (after its interaction upon the food surface (ultra and macrostructure) and not the wavelengths absorbed by the surface. In this case, it seems evident that samples with higher colorant compound content at the surface should show lower reflectance values (Hernández et al., 2016). Spectra from samples hydrated with FBJ (FBJ + TSOY and FBJ + TPEA) that had the highest betalain content (Table 2) showed the lowest reflectance values. On the contrary, samples hydrated with CBJ (CBJ + TSOY and CBJ + TPEA) that had the lowest betalain content (Table 2) showed the highest reflectance values. The shape of the spectrum zone between 540 and 580 nm is characteristic for the fresh meat spectrum (corresponding to the oxymyoglobin pigment) and the percentage of reflectance vary depending on the myoglobin content (lower myoglobin content higher reflectance percentage) (AMSA, 2012). For this reason, the spectrum of pork minced meat showed higher reflectance values in this wavelength range than beef minced meat.

The spectra shape at wavelengths higher than 650 nm (red colors) is similar in all the samples although with differences in the reflectance percentage. Samples with the highest reflectance values correspond to meat analogues with CKBJ and CBJ and the lowest to meat analogues with RBJ and FBJ, following the same pattern showed for the juices spectra (Fig. 1).

4. Conclusions

The attractive red color of beetroot juices due to their main pigment (betalains) and their stability at the pH value of meat analogues make them a valuable alternative to the use of artificial colorants or from animal origin in the development of plant-based meat products. Their direct use as juices and not as betalain extracts or concentrates saves the technological processes for their extraction and contributes to the necessary hydration of the texturized vegetable ingredients used in the formulation of these meat analogues. Juices processing determines the betalain content and therefore its antioxidant and coloring properties. Juices obtained from raw beetroots showed the highest content of red pigments and the highest antioxidant activity. Textured soy protein hydrated with cooked beetroot juice (dilution 1:3) or with commercial juice (dilution 1:4) show similar appearance to that of minced beef and pork Due to the instability of the red pigments in beetroot juices, an understanding of the factors responsible for their degradation is essential for maximizing the stability of the desired color in the minced meat analogues under different storage conditions (type of packaging, storage temperature, exposed or not to light, etc.) in view of their potential commercialization.

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Fig. 4. Appearance of minced meat analogues in comparison with minced meat (pork and meat).

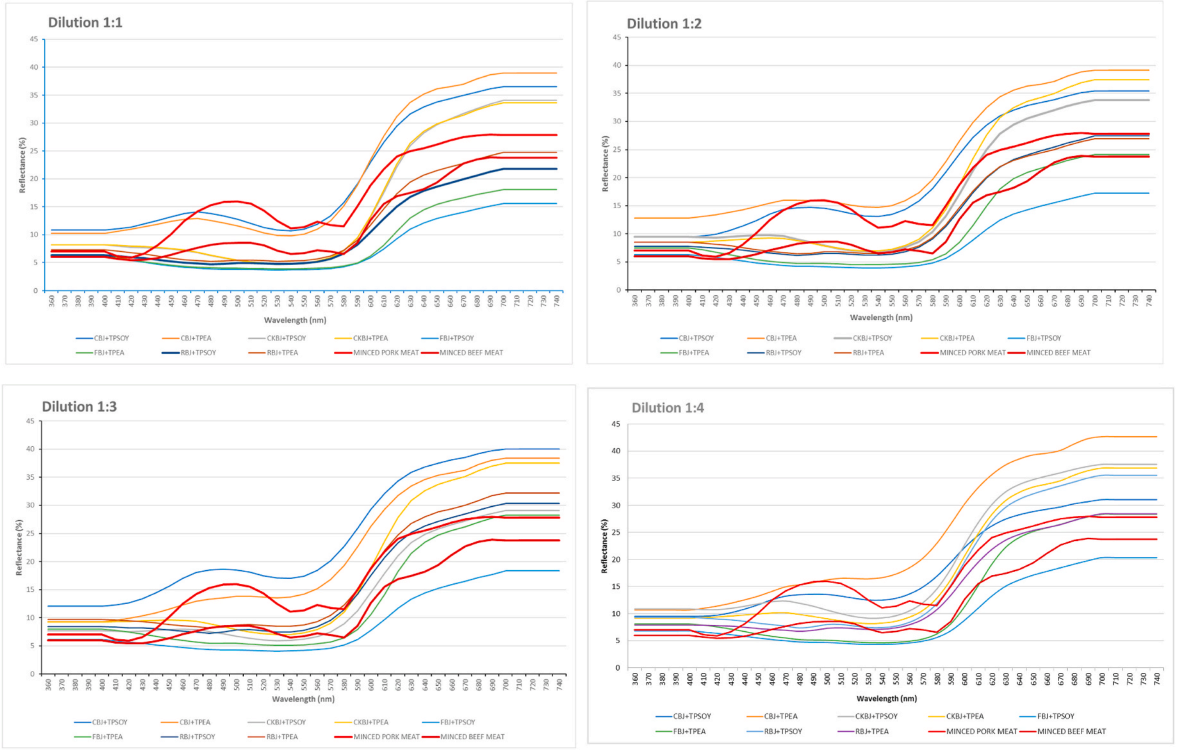

Fig. 5. Reflectance spectra (360–740 nm) of minced meat analogues (at several dilution of beetroot juices) and of minced meats (pork and beef).

Authors' contribution

Juana Fernández-López: Conceptualization, supervision, Writing-original draft preparation and Writing-review & editing, Angel J. Ponce-Martínez: Methodology and investigation, Judith Rodríguez-Párraga: Methodology and investigation, Ana M. Solivella-Poveda: Methodology and investigation, Jose A. Fernández-López: Methodology, data curation, and Writing-review & editing, Manuel Viuda-Martos: Conceptualization, formal analysis, visualization, and Writing-review & editing, José Angel Pérez-Alvarez: Conceptualization, Resources Supervision, and Writing-review & editing.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.103156.

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