Heat and pH stable curcumin-based hydrophilic colorants obtained by the solid dispersion technology assisted by spray-drying

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HIGHLIGHTS

- Hydrophilic formulations containing curcumin were obtained for colorant use.
- Final product was a free flow powder readily dispersible in water.
- Chemometric analyses identified the conditions leading to the most stable products.
- Stability depended on the encapsulant material, chemicals contents and pH.

ARTICLE INFO

ABSTRACT

Natural food colorants are on demand due to food safety concerns related with some synthetic counterparts. Health-friendly alternatives can be available from plant sources, which include curcumin extracted from *Curcuma longa* L. However, its industrial use is difficult to achieve due to the low water affinity, pH and thermal instability, which is particularly challenging, e.g. for baked foods. In this work, the solid dispersion technique followed by spray-drying, an emergent approach in the context of colorants, was applied to curcumin using k-carrageenan, poly(vinyl alcohol) and polyvinylpyrrolidone, as the encapsulant materials. An orthogonal central composite design with dummy-variables was applied, and principal component analysis (PCA) and hierarchical cluster analysis (HCA) carried out to identify the experimental conditions leading to the most effective formulations. In general, particles with a wide range of pH and heat stability have been produced depending on the chosen encapsulant material, used formulation (curcumin, surfactant and polymer contents), and synthesis conditions (pH). Moreover, the used mathematical approach showed to be a valuable tool to support the development of tailor-made formulations directed to specific applications where pH and temperature are relevant processing parameters.

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1. Introduction

Curcumin is a polyphenol with remarkable properties like endogenous antioxidant (Jaiswal et al., 2016), anti-inflammatory (Aggarwal and Harikumar, 2009), and antimicrobial activities (Bajpai et al., 2015; da Silva et al., 2017), together with the ability to modulate the action of important enzymes like the ones involved in the cholinergic equilibrium (Ahmed and Gilani, 2009). Curcumin bioactivity has been subjected to extensive studies and reviewed in detail (Prasad et al., 2014), but only recently its colorant ability caught the attention of the industrial sector, particularly the food industry, which urges for alternatives to synthetic colorants. In fact, until date, it remains a challenge to substitute artificial colorants such as tartrazine, which despite being an allergen for a large part of the population, is still in use to give the yellow and orange hue to foodstuffs.

In the context of tartrazine substitution, curcumin is a very promising candidate, but its wide use has been hindered due to its unique physico-chemical properties. It exists as a keto-enol equilibrium (Siviero et al., 2015), and its color is remarkably

affected by pH, ranging from bright yellow in acidic medium, to orange in neutral to slightly basic medium, and to brown in high pH. This color instability is not desirable from an industrial point of view, namely during food processing where pH changes throughout the industrialization steps, compromising product standardization. Another constraint associated with curcumin is its poor solubility in water, which besides compromising bioavailability, and consequently its nutraceutical efficacy (Salvia-Trujillo et al., 2017; Siviero et al., 2015), hinders its efficient used as colorant in water-rich food systems.

Color instability is a major concern for natural colorants, and to curcumin in particular, with encapsulation emerging as a promising technology to overcome this constraint (Obón et al., 2009; Sousdaleff et al., 2013). Moreover, encapsulation is envisioned as a feasible technological approach to improve water compatibility of hydrophobic compounds (Ramachandraiah et al., 2018). In this context, Solid Dispersions (SD) have been extensively tested to protect and improve water compatibility, and thus the bioavailability of hydrophobic substances (Vasconcelos et al., 2007).

Although SD may be achieved by hot melt extrusion with good results (Chuah et al., 2014), the method of dissolution in a common solvent is an alternative considered easier to apply and less expensive. Nevertheless, it presents as the main constraint the solvent evaporation step, which has been reported to be efficiently conducted by spray-drying technique. Gangurde et al (2015), which compared curcumin solid dispersions obtained by spray-drying and simple evaporation, reported an improved water compatibility for the spray-dried samples. Other authors also corroborated that solid dispersions can be successfully achieved by spray-drying (Li et al., 2013; Paradkar et al., 2004). In fact, spray-drying, a technique highly implemented at industrial level, is extensively described as suitable to be used in the encapsulation of substances for the food industry. It is a flexible process in terms of encapsulant agents and active principles, besides offering an ease scale-up to semi-pilot and industrial level production.

Water affinity of curcumin solid dispersions is influenced by the used encapsulant material, and dissolution tests have demonstrated an increased water solubility when amphiphilic polymers like polyvinylpyrrolidone are used (Seo et al., 2012). Water solubility is remarkably improved if surface active encapsulants are used, however substances with no surface activity should be preferred for food formulations since they have less impact on technological properties (e.g. viscosity or foaming formation). Thus, the selection of the most suitable carrier (usually a polymer) is critical; it must take into account long term stability, and in *in vivo* and *in vitro* performance, which are expected to depend on carrier's chemical nature (Patel et al., 2015).

Solid dispersions, produced with the main focus of improving curcumin bioavailability, were already obtained with encapsulants like Poloxamer 188 and polyethylene glycol (Hu et al., 2015), methacrylate copolymers and polyvinylpyrrolidone (PVP) (Meng et al., 2015), and cellulose-based polymers (Gangurde et al., 2015; Li et al., 2013). Particularly, in the case of curcumin solid dispersions based on PVP (Meng et al., 2015; Paradkar et al., 2004), an increased curcumin water affinity was observed (Seo et al., 2012). Also, when compared to cellulose derivatives, PVP conducted to a higher curcumin concentration in the aqueous phase, thus to a better color saturation as well (Li et al., 2013), proving the feasibility of this technique to produce curcumin-based colorants, until date not explored at this level.

Despite the various tested materials, there are still some important alternatives to be evaluated as curcumin carriers in solid dispersions, namely it is important to widen the use of natural options, which can be crucial under the increasing constraints of food products legislation. Moreover, the chosen material is expected to impact the color power of the produced particulate

powders, as well as their stability upon food processing conditions (e.g. pH and heating conditions). Other important points deserving investigation is to understand the influence of processing parameters such as surfactant concentration and pH on color power and stability.

In the development of products and processes, frequently qualitative variables must be considered in the optimization, such as the polymer type used as encapsulation agent. In this sense, the data analysis technique employed should allow to represent the obtained results in terms of quantitative terms without imposing unrealistic measurement assumptions on the categorical variables. Thus, the dummy-variables can be introduced, by coding the categorical variables to be used in a standard regression estimation (Hardy, 1993). Another strategy to evaluate the possible relations between the processing variables and the responses evaluated is the use of unsupervised chemometric methods, such as Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA). They aim to evaluate whether clustering exists in a dataset without using class membership information in the calculations. Natural clustering of samples/objects is the result of understanding the measurement system used to characterize the samples and this union between statistical analysis and analytical methods aids in elucidating the physical reasons for the presence/absence of clustering in the data (Granato et al., 2018).

The objective of this work was to evaluate the color stability of curcumin solid dispersions produced with 3 polymeric carriers (PVP, polyvinyl alcohol (PVA) and k-carrageenan), concerning pH and heating conditions, to act as new natural-based colorants. To the authors' best knowledge, PVA and k-carrageenan (a natural polymer) were never used in the context of solid dispersions production, and curcumin solid dispersions itself were never used in the context of colorant agents. Solid dispersions were obtained under different experimental conditions of pH, curcumin and Tween 80 concentration, defined using a central composite design with three orthogonal blocks to evaluate the effect of the chosen encapsulant materials. In order to systematize the obtained information, data was analyzed by principal component analysis and hierarchical cluster analysis. Experimental data is therefore represented in a comprehensive way facilitating decision-making of end-users, helping to choose the best formulation for a certain application where pH and temperature are relevant processing parameters.

2. Material and methods

2.1. Materials

Curcumin (80% purity) was acquired from Sigma-Aldrich. Polyvinyl alcohol (PVA, Celanese Chemicals), polyvinylpyrrolidone (PVP, Sigma-Aldrich) and k-carrageenan (Acros Organics) were used as synthetic (PVA and PVP) and natural (k-carrageenan) encapsulant materials. They were chosen because their chemical structure would favor the formation of the solid dispersion. Tween 80 (Alfa Aesar) and absolute ethanol (Honeywell, 99.8%) were used as surfactant and solvent, respectively. Citric acid (PanReac 99.5%) and sodium citrate (PanReac 99.0%) were used to prepare the buffer to control pH during particles production.

2.2. Curcumin solid dispersions preparation

Particles were obtained using the solid dispersion technique as described by da Silva et al. (2017) with minor modifications (Fig. 1). By this method, encapsulant and encapsulating substance are dissolved in a common solvent is and particle formation occurs by chemical interactions between them (Li et al., 2013). Briefly, the
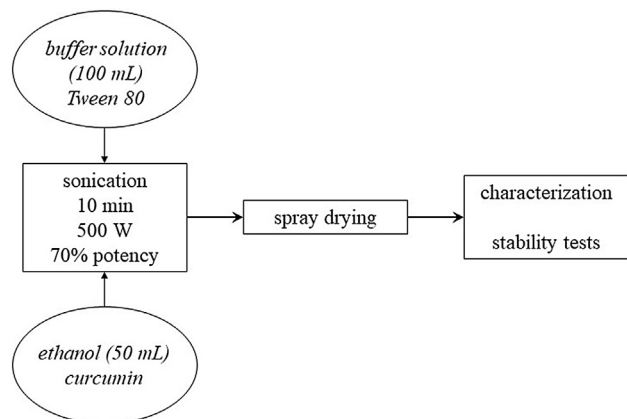
Fig. 1. Schematic diagram of the particles production.

encapsulant polymer (0.4 g) together with Tween 80 (amount according to Table 2) were solubilized in citric acid/sodium citrate aqueous buffer solution with the required pH (100 mL), whereas curcumin (amount according to Table 2) was solubilized in ethanol (50 mL). The ethanol solution was poured into the aqueous phase and the mixture sonicated (Qsonica model Q500, 500 W0.) at 70% potency for 10 min (30 s on and 10 s off to avoid overheating). Right after sonication, the dispersion was spray dried under nitrogen flux (667 L h^{-1}) using the following conditions: inlet at 140 °C, outlet at 70 °C, solution volumetric flowrate of 11 mL min^{-1}, aspiration rate of 35 m^3 h^{-1}. The obtained particles were then stored at 10 °C protected from light until characterization.

2.3. Experimental design

For the observed responses a quadratic model with dummy-variables was adjusted according to Eq. (1) (Bona et al., 2002).

$$\hat{y}(\mathbf{x}, \mathbf{z}) = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_3 x_3 + \beta_{33} x_3^2$$
$$+ \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \gamma_1 z_1 + \gamma_2 z_2 \qquad (1)$$

where x_i represent the quantitative factors ((x_1 - curcumin content (%w/w, polymer-basis), x_2 – pH, x_3 – Tween 80 content (%w/w, polymer-basis)); z_i the dummy-variables assigning the encapsulant type (PVA, k-carrageenan and PVP); β the coefficients for quantitative factors, and γ the coefficients for dummy-variables. The quantitative factors and dummy-variables coding are presented in Table 1.

The implemented central composite experimental design with three orthogonal blocks is described in Table 2.

Regression analysis was carried out using scripts developed by the authors in MATLAB R2008b (MathWorks Inc., Natick, MA, USA). In the ANOVA table, the source of variation "regression" was unfolded in the effect of the quantitative variables (x_i) and

the effect of the dummy variables (z_i) in order to evaluate the influence of the encapsulant type on the dependent variables.

2.4. Curcumin solid dispersions characterization

The obtained curcumin solid dispersions were characterized in what concerns particle size distribution, and color parameters.

Particle size distributions were determined by Laser Diffraction (Malvern, Mastersizer 3000) using distilled water as the dispersant medium. The evaluated parameters were D_{10}, D_{50} and D_{90}, in number, which indicated the particle size corresponding to 10, 50 e 90% of the total number of the particles in the sample.

Particle color parameters (CIE L*, a* and b*) were determined using a colorimeter (CR-400 model, Konica Minolta Sensing Inc., Japan). Chroma (C*) and Hue angle (H°) were calculated using Eqs. (2) and (3), respectively. This quantitative color system was chosen in order to give insights on color as perceived and not only based in specific wavelengths.

$$C^* = \sqrt[2]{a^{*2} + b^{*2}} \qquad (2)$$

$$\theta = \left[\tan^{-1} \left(\frac{b*}{a*} \right) / 6.2832 \right] \times 360 \qquad (3)$$

With:

a* > 0 and b ≥ 0 then H° = θ
a* < 0 and b ≥ 0 then H° = 180 + θ
a* < 0 and b < 0 then H° = 180 + θ
a* > 0 and b < 0 then H° = 360 + θ

2.5. Color stability to pH and temperature

For color stability evaluation, the particles were dispersed in water and the resulting solutions subjected to pH changes according to the procedure described by Sousdaleff et al. (2013) with minor modifications. Briefly, for each experimental condition, an amount of the produced solid dispersions, corresponding to approximately 5 mg of curcumin, was dispersed in water (1 mL) and protected from light. pH was then adjusted to 1, 3, 5, 7, 9 e 11 using NaOH or HCl 0.1 mol L^{-1}. Absorbance was immediately measured (Varian Cary 50Scan) using a wavelength range from 300 to 800 nm. The acquired spectra were normalized [0,1] after baseline correction using a polynomial offset based on regions free of peaks (Brereton, 2018).

Color stability was studied by placing samples of approximately 1 g in an oven at 180 °C and by monitoring color variation with time (30, 60 and 120 min). This temperature was chosen since it is the most common temperature used in bakery products, a relevant application for the new colorants produced in this work. Color variation, relative to the sample before heating, was determined by Eq. (4) (Bermejo-Prada and Otero, 2016; Mcguire, 1992). Δa^*, Δb^* and ΔL^* represent the variation of each color parameter, as

Table 1
Quantitative factors and dummy-variables, and respective levels.

	Level				
	(−1,633)	(−1)	(0)	(+1)	(+1,633)
x_1 (curcumin content, %w/w, polymer-basis)	5	8.876	15	21.124	25
x_2 (pH)	3	3.581	4.5	5.419	6.0
x_3 (Tween80 content, %w/w, polymer-basis)	0	5.814	15	24.186	30
Dummy-variable coding					
	PVA		k-carrageenan		PVP
z_1	1		0		0
z_2	0		1		0

Table 2
Uncoded matrix of the orthogonal central composite experimental design.

Experimental run	Block	Curcumin content (%w/w, polymer-basis)	pH	Tween80 content (%w/w, polymer-basis)	Polymer
1	1	21.12	3.58	24.19	PVA
2	1	15.00	4.50	15.00	PVA
3	1	15.00	4.50	15.00	PVA
4	1	21.12	5.42	5.81	PVA
5	1	8.88	5.42	24.19	PVA
6	1	8.88	3.58	5.81	PVA
7	2	15.00	4.50	15.00	k-carrageenan
8	2	21.12	5.42	24.19	k-carrageenan
9	2	8.88	3.58	24.19	k-carrageenan
10	2	15.00	4.50	15.00	k-carrageenan
11	2	8.88	5.42	5.81	k-carrageenan
12	2	21.12	3.58	5.81	k-carrageenan
13	3	25.00	4.50	15.00	PVP
14	3	15.00	4.50	0.00	PVP
15	3	15.00	4.50	15.00	PVP
16	3	5.00	4.50	15.00	PVP
17	3	15.00	4.50	15.00	PVP
18	3	15.00	6.00	15.00	PVP
19	3	15.00	4.50	30.00	PVP
20	3	15.00	3.00	15.00	PVP

described in Section 2.4. The used equipment was a colorimeter CR-400 model (Konica Minolta Sensing Inc., Japan). For comparison purposes, additionally to the produced solid dispersions, pure curcumin was used as reference.

$$\Delta E^* = \sqrt[2]{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (4)$$

2.6. Principal component analysis and hierarchical analysis

For the principal component analysis (PCA) and hierarchical cluster analysis (HCA), MATLAB R2008b (MathWorks Inc., Natick, MA, USA) was used with the obtained results placed in columns and the experimental runs in lines. Before analysis, the data matrix was column scaled by its mean and standard deviation. This variance-covariance matrix was transformed using the singular value decomposition (SVD) algorithm. The first principal components with an accumulated variance of 95% were retained to evaluate the distribution of the samples in the new projection space (Brereton, 2018). PCA was applied to evaluate particles and color stability after heating treatment while HCA was applied to investigate color stability under different pH.

3. Results and discussion

3.1. Experimental design

The results of particle size, particle color parameters, and color variation after heating treatment, for each run of the defined experimental design, are presented in Table 3. ANOVA results and overall goodness of fit for each model are presented in Table 4.

From the results listed in Table 3, it can be observed that the solid dispersion produced particles with D_{50} values of around 500 nm, meaning that the diameter of 50% of the particles are below this size. The formation of this range of nanosized particles is described in the literature as occurring when the chemical structure of the polymeric encapsulant and curcumin allow the development of molecular interactions such as hydrogen bonding. Thus, in the particular case under study, a network may be formed due to the existence of two hydroxyl groups in curcumin leading to crosslinking with the hydroxyls and carbonyls of the polymeric encapsulant, which induces the precipitation in the form of nanoparticles (Yen et al., 2010). During the spray drying process, these nanoparticles may agglomerate forming macroscopic entities

(results not showed). Nevertheless, it is expected that upon contact with water these macroscopic entities disrupt into the former nanometric-sized particles. This is corroborated by the achievement of a D_{90} diameter of around 750 nm.

Concerning color parameters, the only significant models were obtained for L* and a* meaning that the predictive capacity of the models is unsatisfactory for the other parameters (b*, C* and H°) as denoted by the R^2_{adj} values. Moreover, the effect of the used polymeric encapsulant was only significant for luminosity ($p_{dummy-variables} < 0.05$). It is worth noting that the objective was to use the produced particles as a yellow coloring agent, and for this reason b* and Chroma (C*) parameters are of major importance. For this reason, the obtained data was evaluated using principal components analysis (PCA) and hierarchical cluster analysis (HCA) in order qualitatively deduce how experimental parameters influence color and particle size. In addition, a multivariate analysis may allow a simultaneous comparison of all particle features.

3.2. Principal components analysis (PCA)

Five principal components were chosen for this analysis since they were able to describe 95.85% of the total variation found within the experiments. Loadings size (Table 5) were used to select the principal components to be used for each parameter since the size of the loadings for each PC is a measure of feature's importance to the PC model. Loadings close to the origin of the coordinate system represent less important features (Sena et al., 2002). The correlation between the variables was determined and is presented in Table S1 (Supplementary Material).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ces.2019.04.044.

Parameters b* and Chroma (C*) were chosen to evaluate color attributes due to their major importance for the yellow color. The combination of PC 1 and PC 4 was selected for these parameters (loadings magnitude for b* were 0.404 and −0.425, and for C* 0.421 and −0.396, respectively for PC 1 and PC 4). PC 1 and PC 2 were chosen for particle size parameter because D_{10}, D_{50} and D_{90} present the highest loadings in these projections as can be inferred by the analysis of the data included in Table 5. For color variation after a 30 min heating period the combination of PC1 and PC4 was used, while the combination of PC4 and PC5 describes more adequately the variations after 60 and 120 min. In the subsequent sections, the influence of each experimental condition is discussed separately for each response. It is important to highlight that low

Table 3
Values of particle diameter corresponding to 10%, 50% and 90% of the number of particles (D_{10}, D_{50} e D_{90}), particle color parameters (L^*, a^*, b^*, C^*, $H°$) and color variation (ΔE) after heating treatment at 180 °C during 30, 60 e 120 min for each experimental run.

Experimental run	Particle size (μm)			Color parameters					Color variation (ΔE) after heating		
	D_{10}	D_{50}	D_{90}	L^*	a^*	b^*	C^*	$H°$	ΔE^* 30 min	ΔE^* 60 min	ΔE^* 120 min
1	0.371	0.483	0.704	84.103	15.060	95.750	96.927	81.061	45.388	40.670	58.558
2	0.368	0.478	0.686	87.707	8.623	91.857	92.261	84.637	36.677	32.242	54.892
3	0.373	0.486	0.714	87.227	9.043	88.777	89.236	84.183	29.805	29.835	33.537
4	0.360	0.459	0.638	88.010	8.060	83.080	83.470	84.459	22.574	30.077	28.881
5	0.369	0.481	0.693	91.300	0.743	91.227	91.230	89.533	39.218	41.646	38.581
6	0.366	0.474	0.670	89.110	4.070	90.593	90.685	87.427	35.258	35.727	35.295
7	0.369	0.480	0.678	88.653	5.490	81.423	81.608	86.142	20.687	33.706	31.464
8	0.366	0.473	0.662	87.827	7.197	73.220	73.573	84.386	17.812	37.993	23.977
9	0.373	0.486	0.682	91.063	−1.443	75.873	75.887	91.090	30.741	46.817	42.304
10	0.383	0.497	0.722	87.243	4.383	85.630	85.742	87.069	30.048	48.743	35.573
11	0.373	0.487	0.707	90.793	1.317	63.217	63.230	88.807	17.627	23.071	35.658
12	0.366	0.473	0.667	86.810	9.977	65.983	66.733	81.402	24.875	32.305	22.256
13	0.364	0.469	0.656	85.153	0.090	78.970	78.970	89.934	26.158	41.517	48.741
14	0.405	0.516	0.754	87.547	−2.523	85.623	85.661	91.688	27.274	39.320	43.510
15	0.376	0.491	0.709	85.047	2.947	78.160	78.216	87.841	29.374	30.327	53.771
16	0.367	0.474	0.664	88.843	−8.767	76.700	77.199	96.521	28.872	34.411	58.678
17	0.372	0.486	0.701	84.863	2.557	73.180	73.225	87.999	17.520	30.376	47.219
18	0.374	0.488	0.701	83.340	2.840	74.700	74.754	87.823	11.268	8.373	9.843
19	0.379	0.496	0.715	86.137	0.587	79.243	79.246	89.576	23.663	34.342	44.800
20	0.378	0.493	0.707	73.507	25.690	72.503	76.920	70.489	33.840	32.171	52.703

Table 4
ANOVA results and overall goodness of fit for each model.

	Particle size (μm)			Color parameters					Color variation (ΔE) after heating		
	D_{10}	D_{50}	D_{90}	L^*	a^*	b^*	C^*	$H°$	ΔE^* 30 min	ΔE^* 60 min	ΔE^* 120 min
$p_{regression}$	0.344	0.159	0.154	$<10^{-3}$	0.035	0.088	0.087	0.055	0.107	0.265	0.125
$p_{dummy-variables}$	0.217	0.138	0.314	$<10^{-3}$	0.236	0.180	0.192	0.410	0.173	0.213	0.158
$p_{lack of fit}$	0.247	0.351	0.573	0.053	0.001	0.078	0.078	0.002	0.704	0.346	0.498
R^2	0.703	0.786	0.789	0.904	0.876	0.829	0.829	0.855	0.816	0.766	0.805
R^2_{adj}	0.194	0.420	0.427	0.835	0.664	0.535	0.536	0.607	0.500	0.284	0.472
Standard error of the estimate (SEE)	0.009	0.009	0.021	1.553	4.157	5.986	5.933	3.282	5.889	7.350	9.459

Table 5
Loadings for each principal component (PC) and explained variance (in parenthesis).

	PC 1 (31.31%)	PC 2 (25.24%)	PC 3 (24.24%)	PC 4 (9.24%)	PC 5 (5.83%)
D_{10}	0.279	0.489	0.040	−0.041	0.149
D_{50}	0.279	0.509	−0.002	−0.018	0.071
D_{90}	0.310	0.465	−0.031	−0.124	−0.031
L^*	−0.095	−0.102	0.524	−0.183	0.286
a^*	0.116	−0.139	−0.565	−0.085	0.193
b^*	0.404	−0.253	0.137	−0.425	−0.145
C^*	0.421	−0.259	0.080	−0.396	−0.146
$H°$	−0.078	0.108	0.579	0.049	−0.245
ΔE^* 30 min	0.434	−0.281	−0.005	0.142	0.018
ΔE^* 60 min	0.289	−0.169	0.205	0.396	0.713
ΔE^* 120 min	0.327	−0.079	0.041	0.652	−0.491

correlation coefficients were found between particle size and color parameters.

3.2.1. Analysis of particle's color parameters

Fig. 2 presents the biplot of PC1 *versus* PC4, chosen to evaluate the particle's color parameters.

Parameter b^* showed a strong positive correlation ($r = 0.994$) with color saturation (C^*), which was already expected due to the strong yellow color shown by all samples (positive b^* values). The used type of encapsulant polymer (PVP, k-carrageenan and PVA) was the main grouping factor. For instance, samples produced with PVA as the encapsulant material (experiments 1 to 6) are grouped in the same quadrant of vectors representing b^* and C^*, with the exception of experiment 4. It is also possible to observe a cluster composed by samples produced with k-carrageenan as

the encapsulating material (experiments 7, 8, 11 and 12); only experiment 10 is positioned in a different quadrant. These two clusters include samples corresponding to formulations using different pH, and contents of curcumin and Tween 80. A third cluster was formed by samples produced with PVP as the encapsulating agent and different curcumin amounts (13, 16 and 17), but with the same pH (4.5) and Tween 80 content (15%). This observation could be motivated by the achieved high color saturation in PVP samples, even when a low curcumin content is used. The Tween 80 content, used at low levels in all experiments, presented low influence on color parameters. On the other hand, the use of higher pH during the particles production (experiments 8, 11 and 18), decreases color saturation on the yellow range (b^* and C^*). Also, acidic pH (experiments 1 and 6) led to particles characterized by a stronger yellow color.
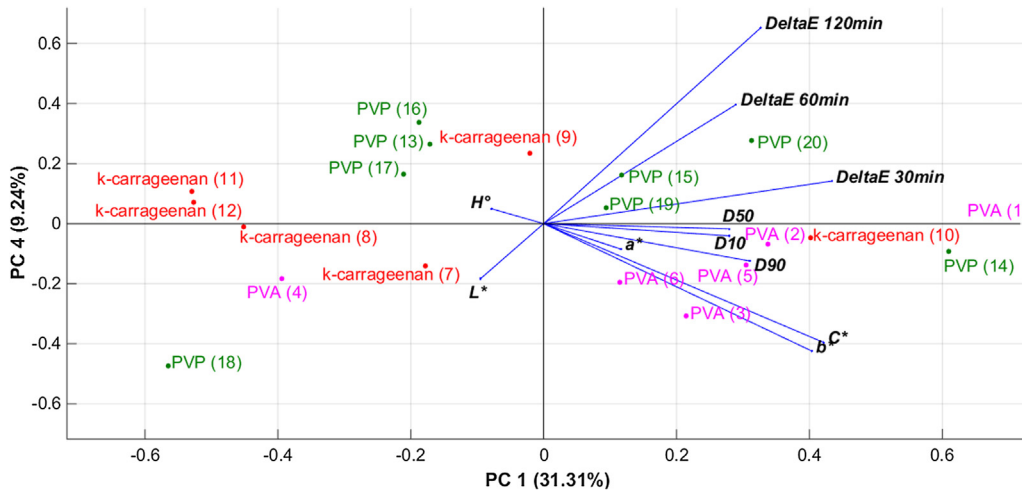
Fig. 2. Biplots of PC1 *versus* PC4 used to evaluate particle's color parameters (a*, b*, L*, C* and H°).

3.2.2. Analysis of particle size

The first two principal components (PC1 and PC 2) were able to describe the behavior of the defined particle size parameters (D_{10}, D_{50} and D_{90}) and their biplot combination is presented in Fig. 3.

PCA showed that the particle size parameters, D_{10}, D_{50} and D_{90}, are highly correlated since their vectors are aligned (Sena et al., 2002) and present high correlation coefficients (Table S1, Supplementary Material). Experiments 4, 8, 12 and 13, those with higher
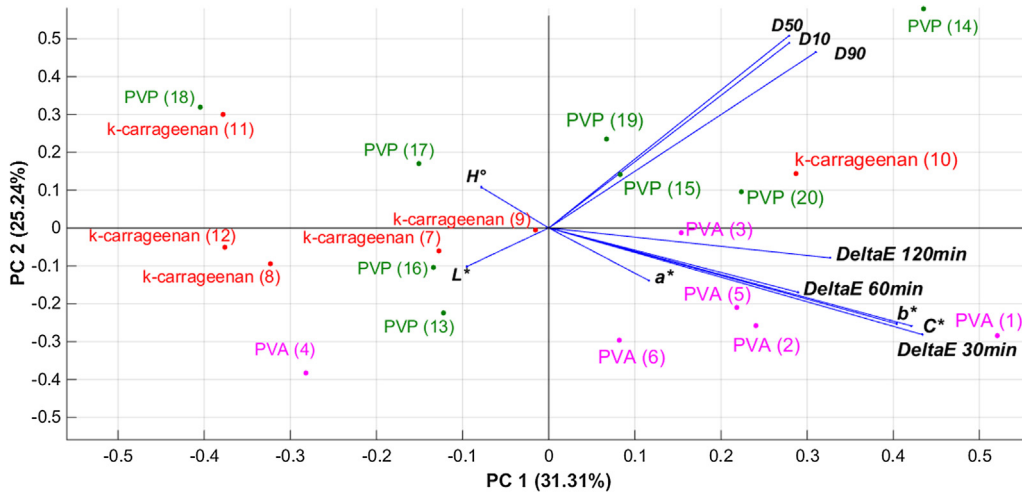
Fig. 3. Biplots of PC2 *versus* PC1 used to evaluate particle size parameters (D_{10}, D_{50} and D_{90}).
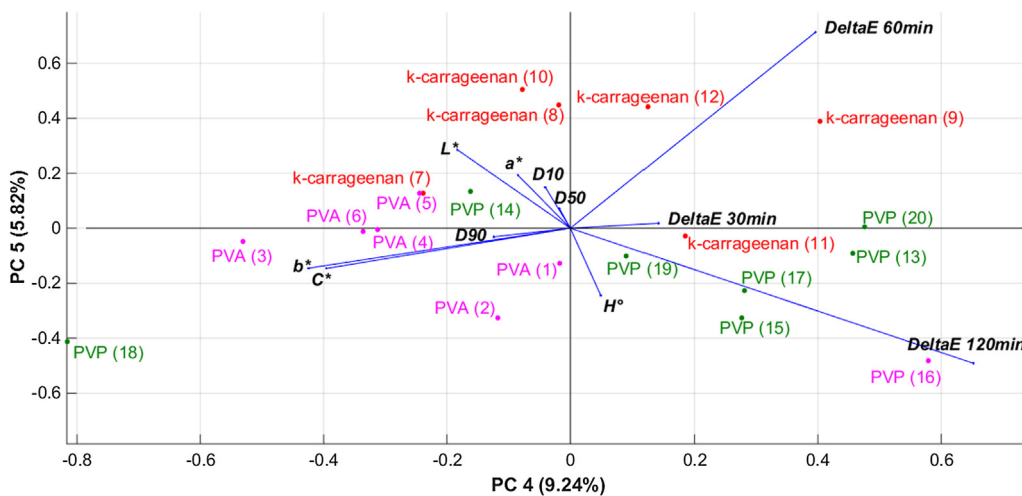
Fig. 4. Biplots of PC4 *versus* PC5 used to evaluate color change after 60 and 120 min heating at 180 °C.

amounts of curcumin, are located in the quadrant opposite to the one of D_{10}, D_{50} and D_{90} vectors, meaning that curcumin content played an important role in the obtained particle size, independent of the used polymer type. Nevertheless, particle size and color parameters are not correlated each other (Table S1, Supplementary Material). This could be due to the particle's formation mechanism,
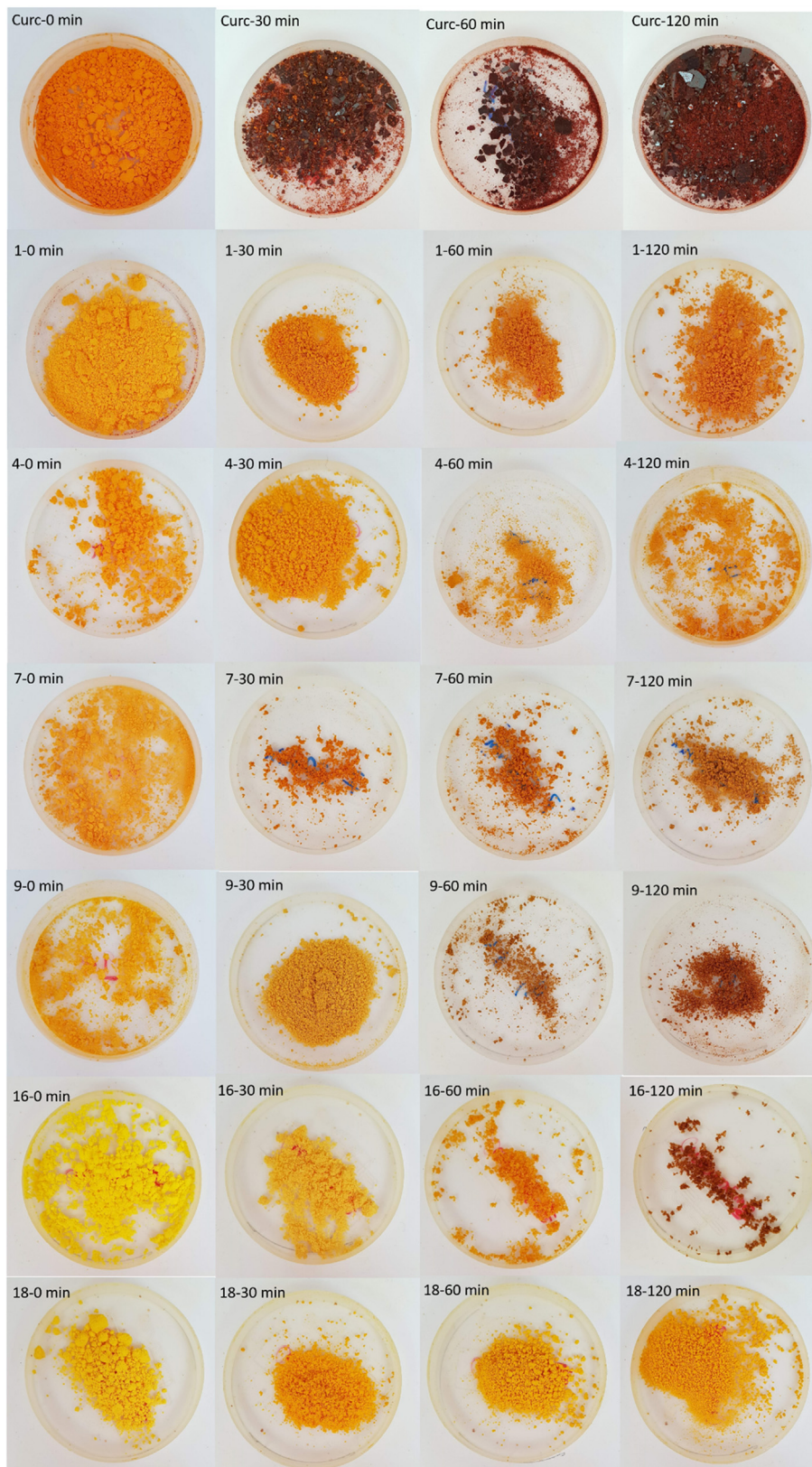

Fig. 5. Images of curcumin (Curc), and particle samples using PVA (Experiments 1 and 4), k-carrageenan (Experiments 7 and 9) and PVP (Experiment 16 and 18), before and after heating treatment at 180 °C during 30, 60 and 120 min.

which involves hydrogen bonding between curcumin hydroxyls and hydroxyl and carbonyl groups in the polymer structure (Almeida et al., 2017; Silva et al., 2017). This does not happen with Experiment 1, which also uses a high curcumin content. In fact, this experiment is located in the negative quadrants of PC2, together with all other experiments using PVA. A positive correlation was found between the type of encapsulant material and the particle size. PVA particles (experiments 1 to 6) were grouped in the negative quadrants of PC2 and oppositely to the one of the D_{10}, D_{50} and D_{90} vectors, indicating that smaller particles were obtained when PVA was the used.

3.2.3. Analysis of the color stability after heating treatment

The PC1 and PC2 combination (Fig. 3) was able to describe the color variation when samples were subjected to the 30-minutes treatment. For longer times (60 and 120 min), PC4 and PC5 were found more adequate and are represented in Fig. 4. In Fig. 5 images of samples, chosen as representative experimental runs together with curcumin itself (used for comparison purposes), before and after the heating treatment, are presented.

It is worth noting that color change after heating treatments during 30, 60 and 120 min were not correlated to each other

(Table S1, Supplementary Material). Moreover, the samples located in the opposite direction of color change, for the three evaluated times (experiments 4, 5, 8, 11 and 18), were obtained under nearly neutral pH and presented less color changes. This improvement of resistance to heating can be also corroborated by the analysis of the images included in Fig. 4. The encapsulants PVP (experiments 17 and 18) and k-carrageenan (experiments 8 and 11) were the more effective in protecting curcumin against heating. In fact, PVA particles that presented a more intense color after production, suffered higher color changes, indicating that this encapsulant was less effective towards heating processing. The achieved findings suggest that PVA-curcumin particles are colorant systems more suitable to be incorporated in foods where no heating treatment is needed. On the other hand, PVP and k-carrageenan would be useful systems to be applied in the case of foodstuffs subjected to heating stages.

Another important finding is that PVA was more effective in preserving curcumin color after 30 min heating, but this scenario changes when higher heating times are used (60 and 120 min). k-carrageenan was able to protect curcumin color until 60 min of heating while PVP was the most efficient in preventing color changes for longer heating periods (120 min). This behavior is well
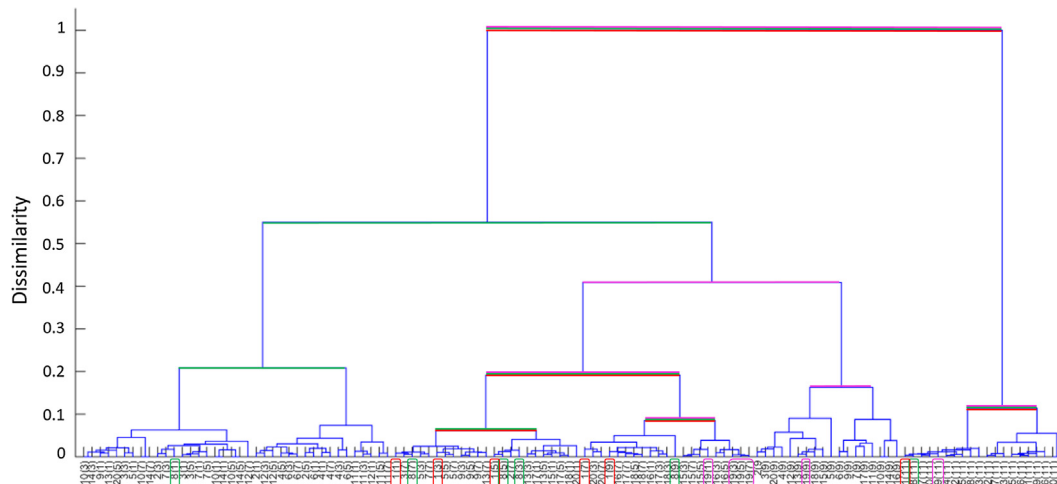

Fig. 6. Hierarchical cluster analysis (HCA) of the UV–Vis spectra of the water dispersions containing the particles under different pH (first number is the experimental run and the number in parenthesis the pH). Experiments 1, 8 and 19 are highlighted in red, green and magenta, respectively.
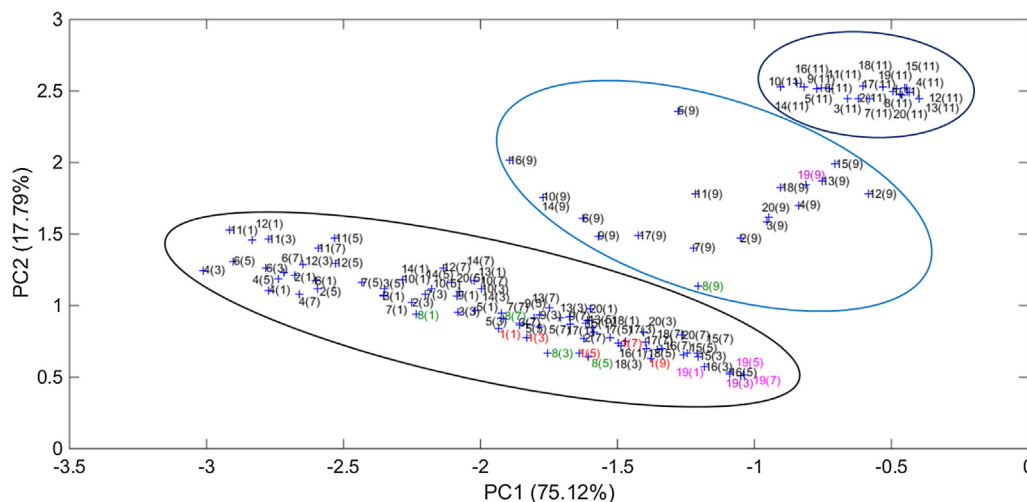

Fig. 7. Principal component analysis (PCA) of the UV–Vis spectra of the water dispersions containing the particles under different pH (first number is the experimental run and the number in parenthesis the pH). Experiments 1, 8 and 19 are highlighted in red, green and magenta, respectively.

represented by Experiments 4 (PVA as encapsulant), and 18 (PVP). Among all the tested experimental conditions, the ones of experiment 18 were the most effective for protecting curcumin against long heating periods at 180 °C.

It is also important to observe that curcumin itself, i.e. curcumin in its non-encapsulated form, could not stand heating treatments

(Fig. 4, first line) being fully discolored after only 30 min at 180 °C. Although the determination of color after 120 min of heating treatment is important to understand how long color could be maintained, this heating time, for most oven baked food products, is oversized since less than 60 min are enough for a wide variety of products. In this context, results demonstrated that encapsulation
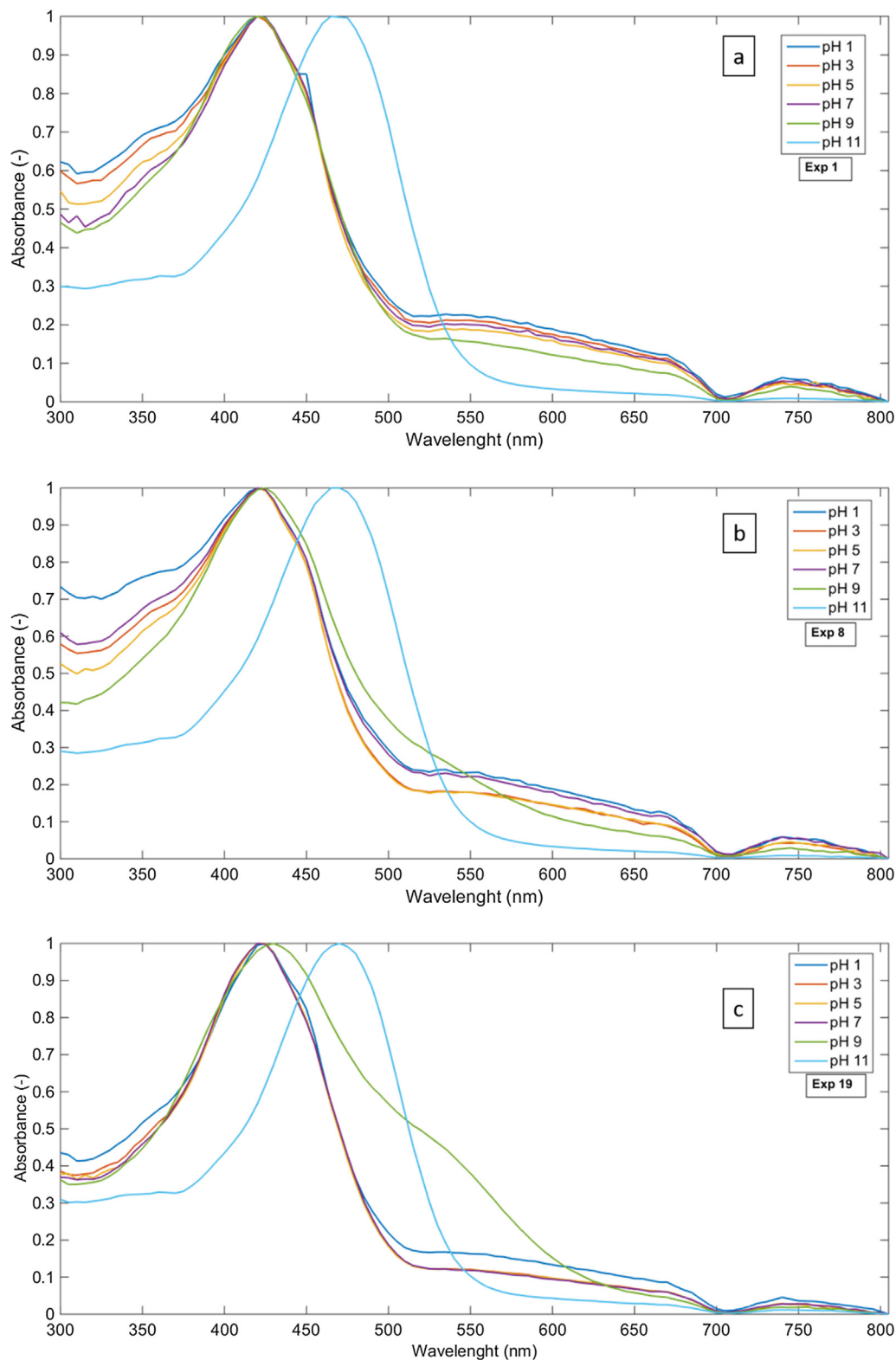

Fig. 8. UV–Vis spectra of the particles dispersed in water at pH 1, 3, 5, 7, 9 and 11 for (a) Experiment 1 (PVA as encapsulant), (b) Experiment 8 (k-carrageenan) and (c) Experiment 19 (PVP).

was effective in promoting protection of curcumin colorant power mainly during heating periods not exceeding 60 min.

3.3. Hierarchical cluster analysis (HCA) to evaluate color stability against pH

Particles were dispersed in water with pH ranging from 1 to 11, and each experimental condition analyzed by UV–Vis. The obtained spectra were evaluated using hierarchical cluster analysis (HCA) and the results are presented in Fig. 6. The scores for PC1 and PC2 obtained by PCA are shown in Fig. 7. Spectra obtained for experiments 1 (PVA as encapsulant) 8 (k-carrageenan) and 19 (PVP) are presented in Fig. 8.

It is to be noted that the evaluation of pure curcumin was not performed due to its highly hydrophobic character and consequent insolubility in water. Also, it is worth noting that curcumin color is very sensitive to pH changes, varying from bright yellow in acid pH to brown in alkaline pH. Thus, it is important from a technological point of view to assure that encapsulation stabilizes curcumin color leading to a less pH-dependent behavior. In Fig. 6 one may observe color dissimilarity of the particles water dispersions under pH 1, 3, 5, 7 and 9 for experiment 1 (PVA as encapsulant material). This dissimilarity was evaluated as 20%, being this formulation the one leading to the least dissimilar results along the used pH conditions. When k-carrageenan was the encapsulant material, dissimilarity was also 20% in experiment 8 for pH 3, 5, 7 and 9, and 50% for pH 1. PVP particles (experiment 18) presented the smallest dissimilarity (10%) in pH 1, 3, 5 and 7 but increasing to 50% for pH 1. For experiment 19 (PVP as encapsulant) dissimilarity was 10% for pH 1, 3, 5 and 7 while for pH 9 it increased to 40%. For all cases, spectra obtained at pH 11 were grouped and showed a dissimilarity of 50% in comparison with the other pH values. Since the majority of foodstuffs present pHs in the range of 3 to 9 the produced particles of experiments 1, 8 and 19 can be applied since they result in minor color variations.

PCA analysis (Fig. 7) showed spectra grouped in three regions: spectra obtained at pH 11 (grouped in the upper-right region of the graphic) differ from the ones using other pH conditions confirming the hierarchical plot results shown in Fig. 6; spectra obtained at pH 9 were grouped in the central region of the graphic; and spectra obtained at pH 1, 3, 5 and 7 were also grouped in the lower-left region of the graphic. Moreover, experiments 1, 8 and 19 presented less scattered points meaning that spectra presented high level of similarity.

Spectra obtained at pH 11 shifted to higher wavelengths when compared to the ones obtained under lower pH (Fig. 8), which was followed by a color change from yellow to intense orange. For pH 9, a shoulder was formed in the region 500–600 nm, which was more prominent in some samples (see Fig. 8c), even the wavelength corresponding to the maximum of absorbance did not significantly change. These facts highlight the importance to evaluate this data using the entire spectra by chemometrics tools and not just the maximum absorbance point (Sousdaleff et al., 2013).

4. Conclusion

In this work, solid dispersions technology was applied aiming at producing curcumin-based colorants with improved resistance to heat and to pH variation aqueous medium. An experimental design was implemented in order to evaluate the effect of curcumin and surfactant (Tween 80) concentration, pH and type of encapsulant polymer (PVA, PVP and k-carrageenan) on particle size and color parameters. Data generated by the experimental design were qualitatively evaluated by principal component analysis (PCA) and hierarchical cluster analysis (HCA). Smaller particles were achieved

using PVA as encapsulant, and the use of higher curcumin levels led to the formation of smaller particles. The formation of particles with more intense color (higher b* and saturation C* values) were favored by using PVA and acidic pH conditions during the synthesis.

The produced particles, and curcumin itself (used as control), were treated at 180 °C and color changes evaluated. Non-encapsulated curcumin lost color after 30 min under heating, whereas the encapsulated forms were able to maintain the color for longer times, emerging as promising colorant formulations to be used in bakery products. Tween 80 did not impact on particle's color stability, but it was relevant to confer heating protection.

Finally, color stability in aqueous medium under different pH was evaluated using HCA, which demonstrated to be an efficient methodology. HCA identified the experimental conditions that led to the lowest UV–Vis spectra variation, helping to simulate pH variations during foodstuffs processing. The use of PVA as the encapsulant material guaranteed a similarity of 80% among the spectra obtained in the pH range of 1 to 9. PVP was efficient at pH values from 1 to 7 (similarity of 90%), whereas and k-carrageenan resulted in a similarity of 80% between pH 3 and 9.

In general, one may conclude that particles with a wide range of technological properties, in what concerns stability to heat and pH variations, may be obtained by using the proposed curcumin encapsulation technique. These properties will depend on the chosen encapsulant material, used formulation (curcumin, surfactant and polymer contents), and synthesis conditions (e.g. pH). PCA and HCA were able to identify the experimental conditions that favor the obtainment of particles with suitable technological characteristics to be used as effective curcumin-based colorant systems.

Conflicts of interest

None.

Acknowledgements

References

Aggarwal, B.B., Harikumar, K.B., 2009. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int. J. Biochem. Cell Biol. 41, 40–59. https://doi.org/10.1016/j.biocel.2008.06.010.

Ahmed, T., Gilani, A.H., 2009. Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. Pharmacol. Biochem. Behav. 91, 554–559. https://doi.org/10.1016/j.pbb.2008.09.010.

Almeida, M., da Rocha, B.A., Francisco, C.R.L., Miranda, C.G., Santos, P.D.de F., Araújo, P., Sayer, C., Leimann, F.V., Gonçalves, O.H., Bersani-Amado, C.C.A., 2017. Evaluation of the in vivo acute antiinflammatory response of curcumin-loaded nanoparticles. Food Funct., 440–449 https://doi.org/10.1039/C7FO01616F.

Bajpai, S.K., Chand, N., Ahuja, S., 2015. Investigation of curcumin release from chitosan/cellulose micro crystals (CMC) antimicrobial films. Int. J. Biol. Macromol. 79, 440–448. https://doi.org/10.1016/j.ijbiomac.2015.05.012.

Bermejo-Prada, A., Otero, L., 2016. Effect of hyperbaric storage at room temperature on color degradation of strawberry juice. J. Food Eng. 169, 141–148. https://doi.org/10.1016/j.jfoodeng.2015.08.030.

Bona, E., Borsato, D., Sérgio, R., 2002. Planejamento e otimização de sistemas mistos controlados por variáveis qualitativas e quantitativas. Acta Sci. 24, 1843–1850.

Brereton, R.G., 2018. Chemometrics: Data Driven Extraction for Science. John Wiley & Sons.

Chuah, A.M., Jacob, B., Jie, Z., Ramesh, S., Mandal, S., Puthan, J.K., Deshpande, P., Vaidyanathan, V.V., Gelling, R.W., Patel, G., Das, T., Shreeram, S., 2014. Enhanced

bioavailability and bioeficacy of an an amorphous solid dispersion of curcumin. Food Chem. 156, 227–233.

Gangurde, A.B., Kundaikar, H.S., Javeer, S.D., Jaiswar, D.R., Degani, M.S., Amin, P.D., 2015. Enhanced solubility and dissolution of curcumin by hydrophilic polymer solid dispersion and its insilico molecular modeling studies. J. Drug Deliv. Sci. Technol. 29, 226–237.

Granato, D., Santos, J.S., Escher, G.B., Ferreira, B.L., Maggio, R.M., 2018. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. Trends Food Sci. Technol. 72, 83–90. https://doi.org/10.1016/j.tifs.2017.12.006.

Hardy, M.A., 1993. Regression with dummy variables. Sage Publications, Newbury Park, CA.

Hu, L., Shi, Y., Li, J.H., Gao, N., Ji, J., Niu, F., Chen, Q., Yang, X., Wang, S., 2015. Enhancement of oral bioavailability of curcumin by a novel solid dispersion system. AAPS PharmSciTech 16, 1327–1334. https://doi.org/10.1208/s12249-014-0254-0.

Jaiswal, S.K., Sharma, A., Gupta, V.K., Singh, R.K., Sharma, B., 2016. Curcumin mediated attenuation of carbofuran induced oxidative stress in rat brain. Biochem. Res. Int. 2016, 1–7. https://doi.org/10.1155/2016/7637931.

Li, B., Konecke, S., Wegiel, L.A., Taylor, L.S., Edgar, K.J., 2013. Both solubility and chemical stability of curcumin are enhanced by solid dispersion in cellulose derivative matrices. Carbohydr. Polym. 98, 1108–1116.

Mcguire, R.G., 1992. Reporting of objective color measurements. HortScience 27, 1254–1255.

Meng, F., Trivino, A., Prasad, D., Chauhan, H., 2015. Investigation and correlation of drug polymer miscibility and molecular interactions by various approaches for the preparation of amorphous solid dispersions. Eur. J. Pharm. Sci. 71, 12–24. https://doi.org/10.1016/j.ejps.2015.02.003.

Obón, J.M., Castellar, M.R., Alacid, M., Fernández-López, J.A., 2009. Production of a red-purple food colorant from Opuntia stricta fruits by spray drying and its application in food model systems. J. Food Eng. 90, 471–479. https://doi.org/10.1016/j.jfoodeng.2008.07.013.

Paradkar, A., Ambike, A.A., Jadhav, B.K., Mahadik, K.R., 2004. Characterization of curcumin–PVP solid dispersion obtained by spray drying. Int. J. Pharm. 271, 281–286. https://doi.org/10.1016/j.ijpharm.2003.11.014.

Patel, B.B., Patel, J.K., Chakraborty, S., Shukla, D., 2015. Revealing facts behind spray dried solid dispersion technology used for solubility enhancement. Saudi Pharm. J. 23, 352–365. https://doi.org/10.1016/j.jsps.2013.12.013.

Prasad, S., Gupta, S.C., Tyagi, A.K., Aggarwal, B.B., 2014. Curcumin, a component of golden spice: From bedside to bench and back. Biotechnol. Adv. 32, 1053–1064. https://doi.org/10.1016/j.biotechadv.2014.04.004.

Ramachandraiah, K., Choi, M.J., Hong, G.P., 2018. Micro- and nano-scaled materials for strategy-based applications in innovative livestock products: A review. Trends Food Sci. Technol. 71, 25–35. https://doi.org/10.1016/j.tifs.2017.10.017.

Salvia-Trujillo, L., Verkempinck, S.H.E., Sun, L., Van Loey, A.M., Grauwet, T., Hendrickx, M.E., 2017. Lipid digestion, micelle formation and carotenoid bioaccessibility kinetics: Influence of emulsion droplet size. Food Chem. 229, 653–662. https://doi.org/10.1016/j.foodchem.2017.02.146.

Sena, M., Frighetto, R.T., Valarini, P., Tokeshi, H., Poppi, R., 2002. Discrimination of management effects on soil parameters by using principal component analysis: a multivariate analysis case study. Soil Tillage Res. 67, 171–181. https://doi.org/10.1016/S0167-1987(02)00063-6.

Seo, S.W., Han, H.K., Chun, M.K., Choi, H.K., 2012. Preparation and pharmacokinetic evaluation of curcumin solid dispersion using Solutol ® HS15 as a carrier. Int. J. Pharm. 424, 18–25. https://doi.org/10.1016/j.ijpharm.2011.12.051.

da Silva, A.C., Santos, P.D. de F., Palazzi, N.C., Leimann, F.V., Fuchs, R.H.B., Bracht, L., Gonçalves, O.H., 2017. Production and characterization of curcumin microcrystals and evaluation of the antimicrobial and sensory aspects in minimally processed carrots. Food Funct. https://doi.org/10.1039/C7FO00452D.

Siviero, A., Gallo, E., Maggini, V., Gori, L., Mugelli, A., Firenzuoli, F., Vannacci, A., 2015. Curcumin, a golden spice with a low bioavailability. J. Herb. Med. 5, 57–70. https://doi.org/10.1016/j.hermed.2015.03.001.

Sousdaleff, M., Baesso, M.L., Neto, A.M., Nogueira, A.C., Marcolino, V.A., Matioli, G., 2013. Microencapsulation by freeze-drying of potassium norbixinate and curcumin with maltodextrin: stability, solubility, and food application. J. Agric. Food Chem. 61, 955–965. https://doi.org/10.1021/jf304047g.

Silva, J.T.P., Geiss, J.M.T., Oliveira, S.M., da Brum, E., da, S., Sagae, S.C., Becker, D., Leimann, F.V., Ineu, R.P., Guerra, G.P., Gonçalves, O.H., 2017. Nanoencapsulation of lutein and its effect on mice's declarative memory. Mater. Sci. Eng. C 76, 1005–1011. https://doi.org/10.1016/j.msec.2017.03.212.

Vasconcelos, T., Sarmento, B., Costa, P., 2007. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov. Today 12, 1068–1075. https://doi.org/10.1016/j.drudis.2007.09.005.

Yen, F.-L., Wu, T.-H., Tzeng, C.-W., Lin, L.-T., Lin, C.-C., 2010. Curcumin nanoparticles improve the physicochemical properties of curcumin and effectively enhance its antioxidant and antihepatoma activities. J. Agric. Food Chem. 58, 7376–7382. https://doi.org/10.1021/jf100135h.