Incorporating strawberry leaf extract from waste biomass in prototype topical gel and cream formulations

Incorporación de extracto de hoja de fresa procedente de biomasa residual en prototipos de formulaciones tópicas de gel y crema

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Keywords

Leaves; natural extracts; polyphenols; strawberry.

Abstract

Berry fruits are an important source of polyphenols with beneficial antioxidant properties for human health. However, higher bioactive phytochemical content has been reported in berry leaves, which are usually discarded or underutilized. In this study, a hydroalcoholic extract with relevant polyphenol content obtained from strawberry leaves (*Fragaria x ananassa* cv. Festival), an agro-industrial waste material, was incorporated in two commercial topical formulations (aqueous gel and oil/water cream), in three concentrations of active ingredient (0.02, 0.1 and 1% strawberry leaf extract). The physicochemical properties of the formulations were monitored for 45 days at different temperatures. Particle size and stability analysis showed that the cream-based formulations were the most optimal for the development of a topical prototype with potential bioactive activities.

Palabras clave

Extractos naturales; hojas; polifenoles; fresa.

Resumen

Las bayas son una fuente importante de polifenoles con propiedades antioxidantes beneficiosas para la salud humana. Sin embargo, se ha reportado mayor contenido de fitoquímicos bioactivos en las hojas, las cuales generalmente se desechan o se subutilizan. En este estudio, se incorporó un extracto hidroalcohólico con contenido relevante de polifenoles obtenido de hojas de fresa (*Fragaria x ananassa* cv. Festival), material de desecho agroindustrial, en dos formulaciones tópicas comerciales (gel acuoso y crema aceite/agua), en tres concentraciones de ingrediente activo (0.02, 0.1 y 1% extracto de hoja de fresa). Las propiedades fisicoquímicas de las formulaciones se monitorearon durante 45 días a diferentes temperaturas. El análisis de estabilidad y tamaño de partícula mostró que las formulaciones a base de crema fueron las óptimas para el desarrollo de un prototipo tópico con potenciales actividades bioactivas.

Introduction

Strawberry leaves have been identified as a potential source of apparently abundant biomass with relevant bioactive components, particularly antioxidant polyphenols [1], [2], [3], [4]. Previous studies have reported possible antioxidant, photoprotective, and anti-aging activities in cosmetic and pharmaceutical topical products containing strawberry components [5], [6], [7], [8], [9].

Bioactive phytochemicals could replace some of the environmentally contaminant chemicals in cosmetics, while potentially providing added health benefits, particularly in skin-care products. Moreover, such bioactive phytochemicals are available in industrial and agricultural waste, which are often unexploited and could be incorporated into environmentally friendlier product design.

The purpose of this study was to evaluate the physicochemical properties and general stability of gel and cream prototype formulations containing a strawberry (*Fragaria x ananassa* cv. Festival) leaf extract (SLE) rich in polyphenols [1]. A similar gel formulation developed by Auliya *et al.*

[10] containing 1% strawberry leaf extract showed an SPF barrier protection efficacy of 59.94 ± 0.260. Meanwhile, Silva *et al.* [11] proposed that industrial-scale production of a gel-cream with 17.5% strawberry leaf extract could gain relevant profitability in the short term.

Methodology

Aqueous gel (water, carbopol, *Aloe barbadensis* extract, glycerin, sodium hydroxide 0.001%, and phenoxyethanol) and oil-in-water cream (water, glyceryl stearate, ceteareth-20, ceteareth-12, cetearyl alcohol, cetyl palmitate, sodium polyacrylate, *Aloe barbadensis* extract, glycerin, and phenoxyethanol) base formulations were provided by Samira Dreams S.R.L. (Costa Rica). The hydroalcoholic strawberry leaf extract (SLE) was prepared from strawberry leaves (*Fragaria x ananassa* cv. Festival; Llano Grande, Cartago, Costa Rica; permit R-CM-ITCR-001-2022-OT-CONAGEBIO) as reported before [1].

SLE contained 108.83 ± 4.65 mg gallic acid equivalents g^{-1} dry weight (DW) total polyphenols; 10.25 ± 0.50 mg quercetin equivalents g^{-1} DW total flavonoids; 57.53 ± 36.84 µg mg^{-1} DW gallic acid; 3.62 ± 4.97 µg mg^{-1} DW caffeic acid; 3.70 ± 2.68 µg mg^{-1} DW p-coumaric acid; 7.14 ± 3.40 µg mg^{-1} DW rutin; 21.90 ± 39.72 µg mg^{-1} DW ellagic acid; 19.44 ± 3.98 µg mg^{-1} DW quercetin; and 2918 ± 281 µmol trollox equivalents g^{-1} DW oxygen radical absorbance capacity (ORAC) as reported before [1]. SLE was added to gel and cream in three concentrations (0.02, 0.1 and 1%) in triplicates, and they were monitored macroscopically for 45 days at 4 °C, room temperature (~25 °C), or 37 °C.

Rheology measurements were performed at 23-24 °C in a rotational rheometer (Ares G2, WatersTM, Germany) at Centro de Investigación en Ciencias e Ingeniería de Materiales (CICIMA, Universidad de Costa Rica). The pH was measured in reflection photometer (Quantofix ® Relax, Macherey-Nagel GmbH & Co., Germany). The hydrodynamic diameter, polydispersity index (pdI) and ζ-potential of the particles from cream and gel samples dissolved in water (10 mg mL-1) were measured in a Zetasizer Nano (Nano ZS, Malvern-Panalytical, UK) at Centro de Investigación y de Servicios Químicos y Microbiológicos (CEQUIATEC, Instituto Tecnológico de Costa Rica) as reported before [12]. Total polyphenol content was determined by the Folin-Ciocalteu method as described previously [1].

Microbial content was determined in a random sample by standard methods (PT-MIC-35 Petrifilm, AOAC 997.02; PT-MIC-37 Petrifilm; PT-MIC-27 Petrifilm, AOAC 991.14, AOAC 998.08; and *Bacillus* sp. selective medium) at CEQUIATEC.

Results and discussion

In this study, topical gel and cream formulations were supplemented with 0.02, 0.1 and 1% strawberry (*Fragaria x ananassa* cv. Festival) leaf extract (SLE), and their general physicochemical properties and stability were evaluated in freshly prepared samples and after 45 days in different temperatures [13].

Immediately after preparation, the gel and cream topical formulations with 0.02, 0.1 and 1% strawberry leaf extract (SLE) were centrifuged at 3000 rpm for 30 min at room temperature, showing no observable changes (no precipitates, no phase separation). No contaminating microorganisms (filamentous fungi and yeast, *Staphylococcus aureus*, faecal coliforms, *Escherichia coli* and *Bacillus* sp.) were detected in a random sample of the formulations.

The gel formulation showed lower apparent viscosity (presented on $\log_{10}$ for better appreciation) compared to the cream system (Figure 1). In general, for both systems, a reduction in the apparent viscosity with the increase of the shear rate can be observed. This is characteristic of a non-

Newtonian behaviour and indicates that these systems have sheared thinning characteristics. Additionally, the apparent viscosity decreased with the increase of the strawberry leaf extract concentration in both systems (Figure 1). This viscosity reduction behaviour has been reported before [14], and it is probably due to the reduction of the capacity of water-binding with the polymer/micelles in the system caused by the addition of the strawberry leaf concentrate, consequently reducing the regeneration of more complex structures.

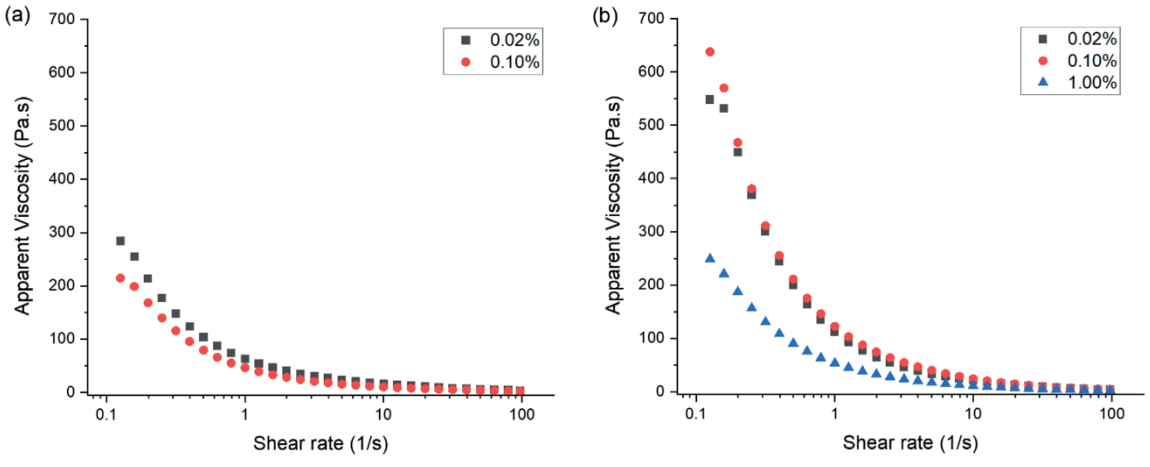

Figure 1. Apparent viscosity versus shear rate (log_{10}) on (a) gel and (b) cream formulate samples with different strawberry (*Fragaria x ananassa*, cv. Festival) leaf extract (SLE; percentage active ingredient) concentrations.

Particle size, polydispersity index and ζ-potential may help predict the transport of the formulations through biological membranes [12]. Smaller particles usually improve particle/skin adhesion and absorption [15]. Liu *et al.* [16] report that particles larger than 600 nm are not able to penetrate to deeper skin layers, while particles of less than 300 nm can achieve higher skin deposition. However, Xiang *et al.* [17] suggest that higher precipitation of larger particles could increase topical permeation. The degree of penetration also depends on surface charge; however, there is no consensus on which type of charge produces better skin absorption [15]. The routes of skin permeation must also be considered, from more superficial passive skin penetration to deeper infiltration through the hair follicles [17]. Particle size in our formulations was between 450 to 775 nm, with no significant size difference between formulations, except for the 0.02% SLE cream, which had significantly larger particles than the rest (Table 1).

The type of gel/cream formulation significantly influenced the particle size, their size homogeneity (polydispersion), and the surface charge (Table 1). These variations in the properties of formulations are induced by the interaction of active ingredients and its excipients [18]. Particle size in the gel formulations was inversely proportional to the incremental addition of SLE, showing smaller and more homogeneous size distribution, but exhibiting at the same time a decrease in their surface charge with higher SLE concentrations. This suggests that gel formulations with more SLE may precipitate easier when applied (sample < -30mV) [19], [20]. The opposite effect occurred in the cream formulations (Table 1), where the incremental SLE concentrations increased particle size and polydispersity index, while becoming more stable in solution due to more negative charges and are therefore less likely to precipitate. Particles with more negative surface charges tend to react more spontaneously and consistently with oppositely charged biomolecules, such as amino groups in proteins at acidic pH and polycations [21]. Conversely, considering interactions with negatively charged biological membranes, it is possible that

negatively charged particles will not be able to interact or enter easily through the cell membrane by passive transport, and other transport mechanisms will be required to internalize them [22], [23], [24].

Table 1. Particle size, polydispersity index (PDI), and ζ-potential from gel and cream formulations with different strawberry (*Fragaria x ananassa*, cv. Festival) leaf extract (SLE; percentage active ingredient) concentrations.

Formulation	SLE (%)	Size (nm)	PDI	ζ -Potential
Gel	0.02	2426.33 ± 494.95^{a}	1.00 ± 0.00^{a}	-49.27 ± 3.96^{bc}
	0.1	775.8 ± 230.55^{b}	0.74 ± 0.07^{b}	-46.07 ± 1.91^{b}
	1	580.87 ± 60.41^{b}	0.47 ± 0.04^{c}	-27.30 ± 6.09^{a}
Cream	0.02	449.13 ± 75.72^{b}	0.65 ± 0.04^{b}	-53.20 ± 1.51^{bc}
	0.1	683.70 ± 46.94^{b}	0.61 ± 0.08^{bc}	-53.50 ± 2.71^{bc}
	1	746.9 ± 49.29^{b}	0.73 ± 0.07^{b}	-58.10 ± 2.95^{c}

Statistical differences between gel vs. cream formulations within each concentration were determined by a General Lineal Model and Tukey´s multiple comparison test, and they are indicated by superscript letters ($p < 0.05$). Equal variances were determined by Levene´s test ($p > 0.05$) (Minitab v.19.1.1., USA).

In terms of appearance, the 1% SLE formulations experienced observable loss of consistency and intense browning after 45 days at room temperature (~25 °C), while the 0.02% and 0.1% formulations remained stable to the touch, with no detectable changes in odour, and with only mild browning relative to the original yellow-like coloration (Figure 2). However, in the samples kept at 37 °C, both gel and cream formulations showed intense browning and extreme dehydration, to the point that only dry flakes remained of the 0.02% and 0.1% gel (Figure 2). Thus, our SLE-containing gel and cream formulations should be stored in opaque airtight containers, and the preservative component of the formulations should be improved to prevent further oxidation.

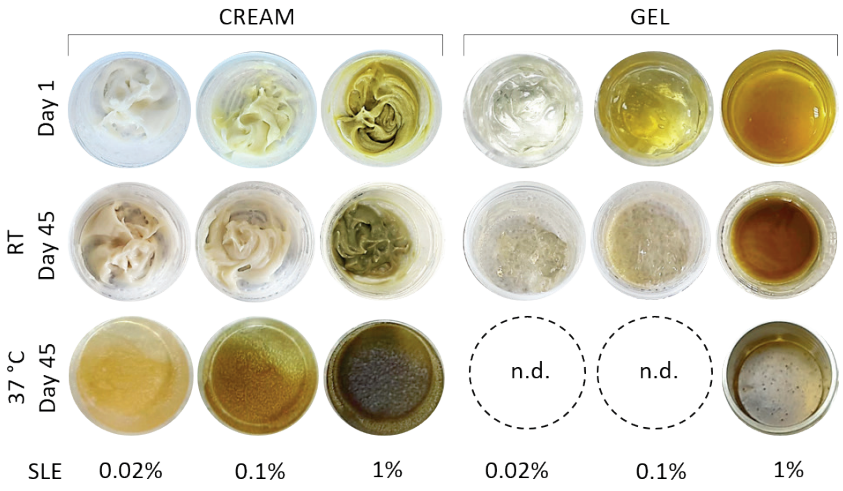

Figure 2. Colour changes in gel and cream topical formulations containing strawberry (*Fragaria x ananassa*, cv. Festival) leaf extract (SLE, percentage active ingredient) after 45 days at room temperature (RT, ~25 °C) or at 37 °C in a translucent container. Gel samples at 37°C that were completely dry and crystalized after 45 days are reported as “not detected” (n.d.).

The increasing SLE concentrations did not seem to relevantly affect the pH values of the gel and cream formulations (Table 2); however, a larger sample size is required to statistically confirm this observation. On the other hand, the total average pH of all formulations was significantly ($p < 0.05$, unpaired t test; GraphPad Prism v. 10.1.1., USA) more acidic after 45 days incubation (5.43 ± 0.90, mean ± SD) relative to the average pH values observed immediately after gel/cream preparation (6.35 ± 0.58, mean ± SD). Nonetheless, all formulations remained close to the recommended range of pH from 4 to 6 for optimal topical applications [25] and, if anything, more acidification could eventually be necessary, particularly in the cream formulations.

The SLE used in this study was previously reported to contain 108.83 ± 4.65 total polyphenols (mg gallic acid equivalents g^{-1} dry weight) and 2918 ± 281 oxygen radical absorbance capacity (ORAC, µmol trollox equivalents g^{-1} dry weight) [1]. The addition of SLE did not relevantly increase the total polyphenol content (TPC) of the gel and cream formulations (~200 mg GAE g^{-1} DW), and TPC was reduced after 45 days both at 4 °C and 37°C, although SLE seems to have aided in maintaining higher TPC values over time (Table 3). However, as mentioned before, a larger sample size is required to statistically confirm this observation. Nonetheless, as active ingredient, the SLE concentrations used in this study might not be sufficient to provide added antioxidant properties *in vivo*, which must be tested in future experiments.

Table 2. pH measurements (mean ± SD) of gel and cream formulations (10 mg mL^{-1}) containing strawberry (*Fragaria x ananassa*, cv. Festival) leaf extract (SLE; percentage active ingredient) after 45 days at different temperatures.

Formulation	SLE (%)	Day 1	Day 45		
			4 °C	25 °C	37 °C
Gel	0	6.43 ± 0.25	n.d.	5.53 ± 0.23	n.d.
	0.02	6.0 ± 0.44	4.75 ± 0.07	4.97 ± 0.59	5.15 ± 0.21
	0.1	5.67 ± 0.57	4.70 ± 0.14	5.00 ± 1.04	5.05 ± 0.35
	1	5.70 ± 0.26	4.80 ± 0.14	5.00 ± 0.56	4.85 ± 0.35
Cream	0	6.13 ± 0.81	n.d.	6.60 ± 0.72	n.d.
	0.02	6.83 ± 0.49	4.70 ± 0.28	5.80 ± 0.53	4.75 ± 0.07
	0.1	7.27 ± 0.42	6.95 ± 2.19	7.50 ± 0.26	5.70 ± 0.28
	1	6.80 ± 0.44	4.50 ± 0.00	7.13 ± 1.69	5.20 ± 0.28

$n = 3$, except samples at 4 and 37 °C with $n = 2$. n.d.: not determined.

Table 3. Total polyphenol concentrations (mean ± SD; mg GAE g^{-1} DW) of gel and cream formulations (10 mg mL^{-1}) containing strawberry (*Fragaria x ananassa*, cv. Festival) leaf extract (SLE; percentage active ingredient) after 45 days at different temperatures.

Formulation	SLE (%)	Day 1	Day 45	
			4 °C	37 °C
Gel	0	244.17 ± 5.97	-	-
	0.02	241.83 ± 16.30	72.15 ± 4.27	78.19 ± 28.95
	0.1	240.67 ± 8.78	80.87 ± 19.46	110.40 ± 4.27
	1	261.50 ± 21.75	120.13 ± 4.75	170.81 ± 86.37
Cream	0	217	-	-
	0.02	215.17 ± 1.15	100.50 ± 20.64	40.60 ± 1.90
	0.1	231.17 ± 4.04	140.44 ± 64.30	15.60 ± 4.51
	1	243.75 ± 13.08	257.05 ± 270.05	163.53 ± 82.34

GAE, gallic acid equivalents; DW, dry weight. $n = 3$ for day 1 (except 0% SLE cream), $n = 2$ for day 45.

Conclusions

Supplementation of gel and cream topical formulations with 0.02 to 1% strawberry (*Fragaria x ananassa cv*. Festival) leaf extract (SLE) rich in polyphenols resulted in physically stable formulations, but only the cream formulations retained its general characteristics over 45 days, showing also more favorable particle behavior, and are better suited for continuing the development of a topical product with SLE.

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Author contributions

K.S.A., D.H.P., F.V.R. and R.C.C. conducted the experiments. L.A.C.C. and F.V.R. wrote the first draft, and all authors approved the final manuscript. F.V.R. is responsible for the final content.

Declarations

D.H.P. from Samira Dreams S.R.L. provided the base gel and cream formulations. The authors declare no conflict of interest. This article does not contain any studies with human or animal subjects. Datasets are available from the corresponding author on reasonable request.

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