COSMETIC FORMULATIONS CONTAINING BLUEBERRY EXTRACTS (*VACCINIUM MYRTILLUS* L.)

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Abstract: The blueberry is a fruit originally native to North America. Consumption has increased globally, mainly due to its reputation for boosting health and longevity. Currently, the cosmetic products market is focused on formulations containing substances with antioxidant activity. The biological properties of the blueberry have already been linked to their polyphenolic content. The aim of this study was to develop and evaluate cosmetic formulations containing Brazilian freeze-dried blueberry extract. Extracts of ripe blueberry fruits were optimized varying the parameters time of extraction and heat, and temperature. The extract with higher polyphenolic content was lyophilised and added to a non-ionic cream in concentrations of 4% and 8%. Rheological behaviour, pH, spreadability, sensorial characteristics and free antiradical activity were tested. Preliminary results suggest that the formulation developed has potential as an antioxidant cosmetic product, though more analysis will be required before the product can go to market.

Key words: blueberry, cosmetic, antioxidant, formulations.

INTRODUCTION

The blueberry was introduced into Brazil in the 1980's by the Brazilian Agricultural Research Corporation (Embrapa/ CPACT, Pelotas/RS), with the main target cultivation area being in the south of the country due to the favorable climate conditions for its growth. The plant is described as a small bush bearing bittersweet berry fruits of a dark blue-purple coloration when ripe. Among those fruits studied having antioxidant potential, the blueberry shows a greater polyphenol concentration, both in the pulp and peel. High levels of anthocyanidins are found mostly in the water-soluble purple pigment of the peel. These molecules promote collagen synthesis, this being one of the main structural components of dermal connective tissue, providing benefits to the skin and also supporting the vascular system. Additionally, these antioxidant molecules are able to prevent the deleterious effects of oxidation, inhibiting the onset of lipid peroxidation, sequestering free radicals and protecting aerobic organisms from oxidative stress, which is defined as an increase in the formation of reactive oxygen species (ROS)(Colleti, 2009 ; Lüdke, 2007 ; H. Rodrigues & al., 2003 ; S. Rodrigues, Gularte, Pereira, Borges, & Vendruscolo, 2007; Silveira, Vargas, & Rosa, 2007).

Polyphenols are a secondary product of plant metabolism, constituting a complex phytochemical group of more than 8000 known structures. These substances are divided into: anthocyanins, flavans, flavanones, flavones, flavonols and isoflavonoids. The flavonoids are among the most important phenolic compounds found in the blueberry and present the greater therapeutic activity. From the age of 20 onwards, almost imperceptibly, the human skin begins to lose some properties of strength and self-regeneration. It is a slow and irreversible process, which varies according to skin type. This progression is dependent on several endogenous (chronological aging) and exogenous (photoaging) factors, with signs appearing from an age range as early as the thirties or more subtly being perceived by the sixties. The causes of skin aging are related to age, genetic tendencies, environmental factors and lifestyle (Buchli, 2002; Dalcin, Schaffazick, & Guterres, 2003 ; Krambeck, 2009). It is suggested by some researchers that imbalances in the body's antioxidant defense mechanism can be one of the main reasons for the aging process, with increases in ROS production leading to oxidative stress (Lima & ABDALLA, 2001). Thus, there is a constant preoccupation in cosmetology to prevent and mitigate skin aging by means of research and study of effective antioxidants agents. This study aimed to produce and evaluate potential antioxidant cosmetic formulations containing lyophilized (freeze-dried) Brazilian blueberry extract.

MATERIALS AND METHODS

Materials

Blueberry fruits were obtained from the city of Arvorezinha in Rio Grande do Sul, Brazil. Harvesting of the berries took place when they had reached the stage of maturity at which they would be sent to market, and only fruits showing no signs of damage, disease or pest attack were chosen. Subsequently, the fruits were refrigerated at -5±2ºC, before being crushed to obtain the extract. Analysis of the collected extract was performed three times.

Extraction optimization

Different parameters were investigated in order to achieve a blueberry extract rich in polyphenols for use in a cosmetic formulation. A hydroalcoholic solvent (10% v/v) was used, acidified to pH 3 with tartaric acid at a ratio of 1:2 (drug/solvent). The extraction parameters evaluated were: temperature, heating and maceration times (Table 1) (Magri & Heberlé, 2009) .

Table 1. Parameters for the blueberry extraction optimization.

Extracts	Maceration time (days)	Temperature (°C)	Heating time (min)
E 1	3	40°C	240
E 2	3	70°C	240
E 3	7	70°C	180
E 4	7	70°C	240
E5	7	70°C	300
E6	14	70°C	180
E7	14	70°C	240
E8	14	70°C	300
E9	21	70°C	180
E 10	21	70°C	240
E 11	21	70°C	300

Determination of phenolic compounds using the Folin-Ciocalteu method.

The total phenolic compound of extracts was determined using the Folin-Ciocalteu reagent, following the method described by Singleton and Rossi (Singleton & Rossi, 1965). Sample readings were made using a spectrophotometer (Cary 100 Bio, Varian) at 765 nm. The total phenolic compound content was obtained through building a calibration curve, using gallic acid in concentrations of 50 to 500 $\mu g.mL^{-1}$, as the standard substance with results being expressed in mg of EGA (equivalent to gallic acid) per L of extract.

Extract Lyophilization

The tested extract with the biggest total polyphenol content was freeze-dried (Lyophilized Liotop Model: L202) and frozen at -30°C.

Emulsion Preparation

A non-ionic base cream as described in the National Formulary was chosen as the base for the formulation. The aqueous phase was heated to 80ºC in a glass beaker using a magnetic stirrer with heater (Model MA-085, Marconi), and the oil phase melted in a porcelain mortar using a hot water bath (Model MA-156, Marconi) at 75ºC. The aqueous phase was then poured over the oil phase and manually stirred until cool (Brasil, 2005) , with the lyophilized blueberry extract being subsequently incorporated. Two preparations were produced with each containing concentrations of 4% and 8% blueberry extract, respectively.

Organoleptic characteristics

Each preparation was evaluated for appearance and color. A visual assessment was made by adding a sample portion to a glass plate, placed over a white background, and comparing it to the original non-ionic base cream (Brasil, 2004).

pH Determination

The pH of each preparation was determined using a sample diluted by purified water (1:10 p/v) obtained by reverse osmosis (Marconi), and using a previously calibrated pH meter (DM-20, Digimed) (Amaral & Vilela, 2003; Brasil, 2010 ; Gil, Matias, & Serrano, 2005).

Assessment of Spreadability

The sample was applied to a glass support plate (20cm x 20cm) positioned over a sheet of graph paper and centralized using a circular plate with a central hole. This plate was subsequently removed and replaced at one minute intervals with plates of predetermined weights, with the diameter of the spread of the sample being measured. The spreadability (Ei), at 25° C is calculated by the equation: (Isaac *et al.*, 2008 ; Spellmeier & Heberlé, 2007; Zanin, Miguel, Chimelli, & Dalmaz, 2001).

$$Ei = d2 \times \pi / 4$$

Where: Ei: spreadability of sample to weight i (mm^2); d: mean diameter (mm).

Viscosity Determination

A study of the rheological behavior of the formulations was made with a rotational viscometer (DV-I+, RV series, Brookfield) using the spindle SC4-29, inserted on a sample without air bubbles and with a stable temperature (Brasil, 2004). This research evaluated viscosities from $0.1s^{-1}$ to $500s^{-1}$, and from $500s^{-1}$ to $0.1s^{-1}$, with a 1 minute delay between measurements.

Activity of free antiradicals

This analysis was made through use of the stable free radical DPPH (2.2-diphenyl-1-picryl-hydrazyl-hydrate) (Elmastas *et al.*, 2006). A DPPH methanolic solution of $50.0\mu g.mL^{-1}$ was prepared, with 1.0mL then being added to a 3.0mL methanolic solution of the formulation samples, at concentrations of $1.0\mu g.mL^{-1}$, $5.0\mu g.mL^{-1}$, $20.0\mu g.mL^{-1}$, $40.0\mu g.mL^{-1}$, $60.0\mu g.mL^{-1}$ and $100.0\mu g.mL^{-1}$. The mixtures were vigorously stirred and kept in the dark at room temperature for 30 minutes. After this time, the absorbance of the samples (n=3) and of a control (1.0mL solution of DPPH $50.0\mu g.mL^{-1}$ with 3.0mL of methanol) were read by a spectrophotometer (Cary 100–Bio, Varian) at a wavelength of 517 nm, which corresponds to the

maximum absorption for the free radical being used. Methanol was used as blank solution (Lange, Heberlé, & Milão, 2009). The ability of the extracts to reduce the free radicals is calculated according to the equation:

% inhibition of DPPH = [(A0 – A1) / A0 x 100]
Where:
A0 = absorbance of the control reaction
A1 = absorbance of the samples.

Statistical Analysis

Anova and Tukey's test was used to analyze the obtained results with a degree of confidence of 95% (p=0.05), through the Statistical Support System, SAE program (Ahlert, 2005).

RESULTS AND DISCUSSION

Extraction optimization

Overall, the extraction parameters affect the quality of the extraction solutions. According to Figure 1 the extract E2 showed the greatest polyphenol content (1420.45mg/L ± 9.39).

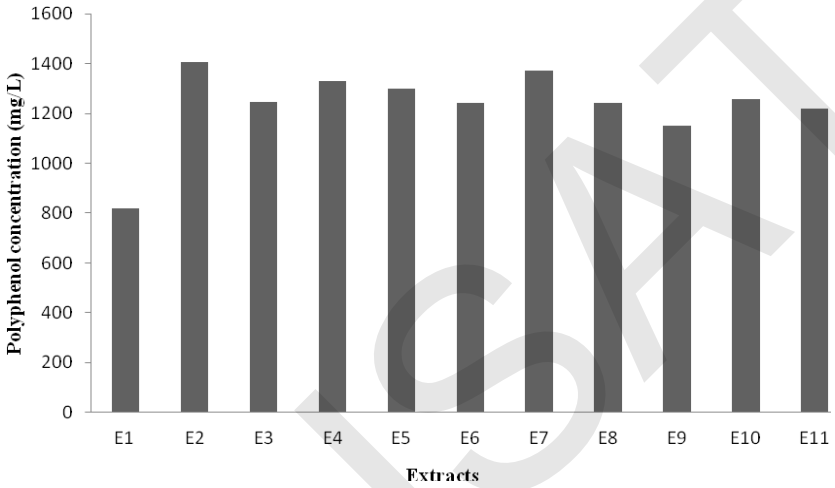

Figure 1: Total polyphenol content of extracts tested.

Extract lyophilization

The lyophilized extract maintained the purple color of the liquid extract, presented a pH 3.72 ± 0.02 and had a yield of 3%.

Organoleptic characteristics

The product containing 4% extract presented a pink color and glossy appearance, with this color becoming more intense in the product containing an 8% concentration of extract.

pH determination

The formulation containing 4% extract of lyophilized blueberry had a pH of 3.32 (±0.006), whilst that containing 8% extract had a pH of 3.20 (±0.01). There was no statistically significant difference between the pH values of the formulations, however, these values are not suitable for dermatologic use as products remaining on the skin for prolonged periods must have a pH of between 5.5 and 6.5, compatible with the pH of the human skin (Isaac, *et al.*, 2008). Therefore, an adjustment was made to the pH of the formulations with the addition of 10µL and 20µL of AMP-95 to the products containing 4% and 8% extract, giving pH values of 5.52 (±0.08) and 5.60 (±0.07), respectively.

Determination of viscosity and spreadability

Rheology is the study of the flow or deformation of a material when subjected to a tension. It is important for quality control of the intermediate or final product as well as determining the shelf life and product acceptability to the consumer, and includes the analysis of viscosity and spreadability parameters. The viscosity of a fluid is given as its resistance to flow or movement. The higher the viscosity, the slower the speed at which the fluid moves, having a lower spreadability. The formulations showed non-Newtonian behavior (Figure 2) as the curve does not pass through the origin but intersects the shear stress axis, called the transfer value, and characteristic of plastic material where the flow does not begin until its transfer value is reached. The viscosity of a plastic decreases with increasing shear rate and this behavior makes the formulations suitable for topical use, making it easier to use and requiring the application of pressure to start the flow, thus preventing container leakage (Mariott, 2005, Sinko, 2008).

The thixotropy can be evaluated by the area of hysteresis on the rheogram (Figure 2), where the descending curve appears shifted to the left of the ascending curve. This parameter indicates the ability and time it takes for the formulation to return to its structure after the removal of applied tension. Achieving topical formulations with a thixotropic character is very desirable as they become more fluid during application, making it easier to spread but recovering the initial viscosity as soon as application has ended, thus avoiding product leakage. However, it is important that the thixotropic value is not too high as the product will run off the skin after application due to very slow recovery of its structure (Gaspar & Maia Campos, 2003; Mariott, 2005; Sinko, 2008). The formulations analyzed presented similar rheological characteristics with no significant differences between the base values of maximum viscosity.

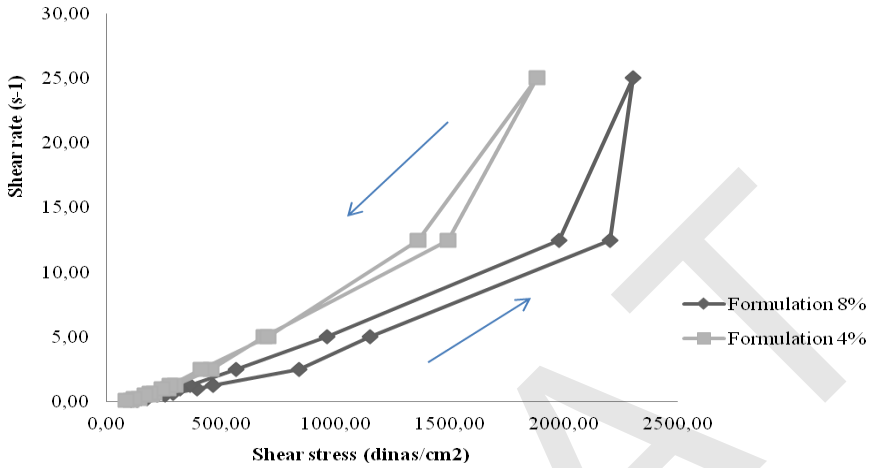

Figure 2: Rheogram of the produced formulations with 4% and 8% lyophilized blueberry extract.

Figure 3 shows the spreadability of formulations with both showing similar behavior and no significant difference between the base values of maximum spreadability.

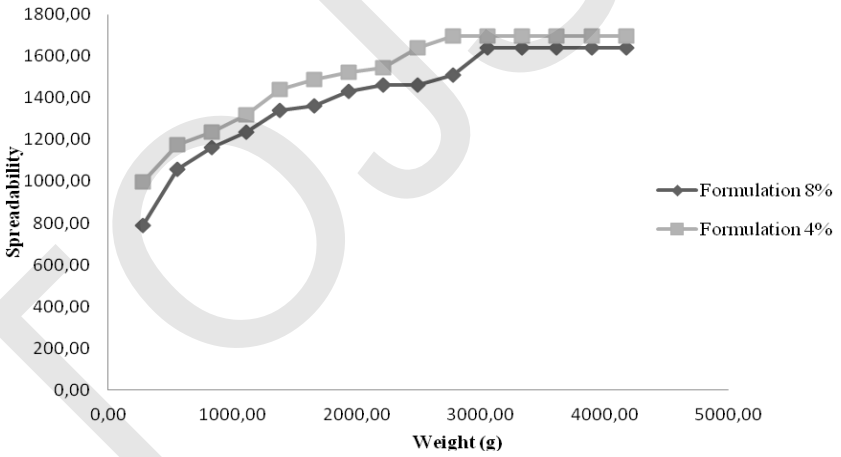

Figure 3. Spreadability of produced formulations with 4% and 8% lyophilized blueberry extract.

Determination of antioxidant activity

The evaluation model for antioxidant activity uses DPPH based on the ability of the stable free radical 2.2-diphenyl-1-picryl-hydrazyl-hydrate to react with substances that donate hydrogen, including compounds. (Mensor *et al.*, 2001). The antioxidant activity of lyophilized blueberry extract was compared with the standard natural and synthetic quercetin and BHT antioxidants (Figure 4). At the highest concentration, the extract demonstrated DPPH sequestering ability (radical scavenging activity) similar to the standard, however statistically significant differences were observed for all tested concentrations (Figure 4).

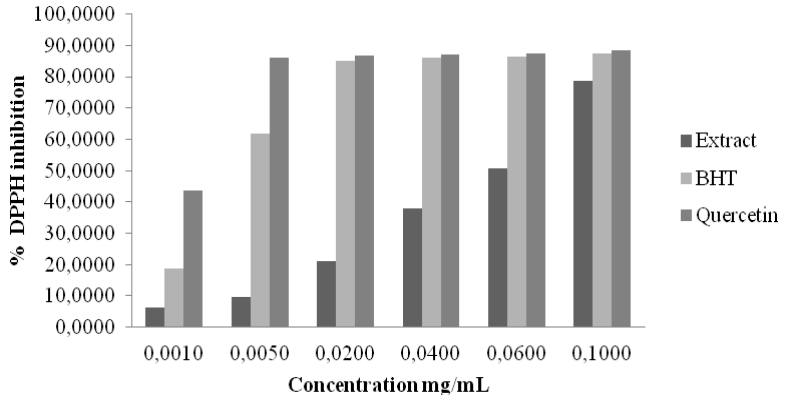

Figure 4. Antioxidant activity of blueberry extract, BHT and quercetina.

The results for the formulations with 4% extract, including the form alkalinized with AMP-95, showed a significant difference only at a concentration of 0.0200 mg/mL (Figure 5). No significant difference for all concentrations was observed in the formulations containing 8% extract. These results demonstrate that adjusting the pH did not alter the antioxidant activity.

When comparing the antioxidant activity of the prepared formulations with two anti-aging skin products, one national and one imported, they produced the same inhibition of free radicals in comparison to the imported cosmetic product for the two lower concentrations, as shown in Figure 5. As regards the national cosmetic product, the formulations also showed no significant difference for the highest concentration, and for the other tested concentrations showed a higher antioxidant activity than both the national and imported cosmetic products.

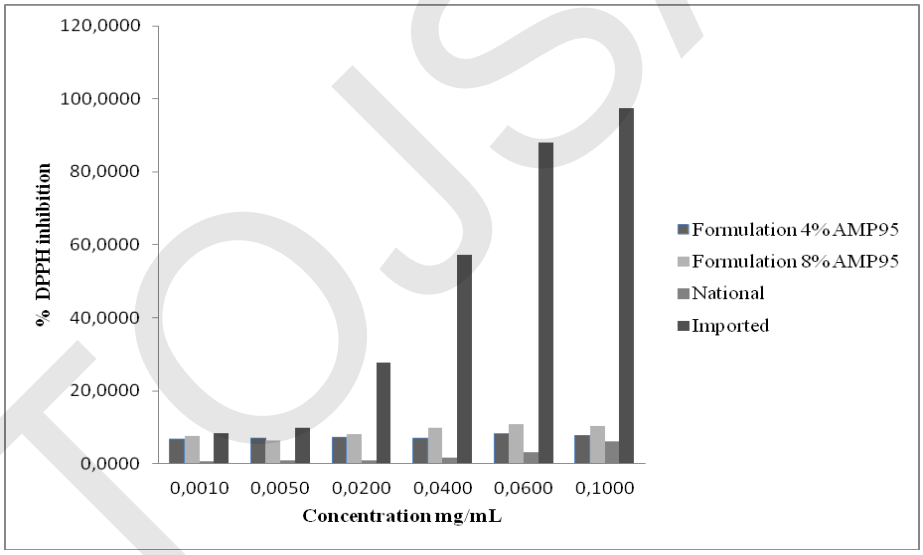

Figure 5. Antioxidant activity for the alkalinized formulations and the national and imported cosmetic product.

CONCLUSIONS

It can be concluded from the tests that the formulations containing 4% and 8% freeze-dried blueberry extract showed no significant difference in relation to spreadability and viscosity. In terms of antioxidant activity there was a difference only at the concentration of 0.06mg/mL. Adjusting the pH of the formulations did not alter their antioxidant activity, and they presented a superior antioxidant activity than the national cosmetic product analyzed. Based on these results it would appear that the formulations produced for this study show potential for development as antioxidant cosmetic products, though additional research is needed to continue the process of their development as an anti-aging product, such as those found on the market.

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