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Research article

Methods for assessing the stability of phytocosmetics

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Keywords: Cosmetic Stability, Phytotherapy, Plant Extracts, Shelf life, Date of Validity of Products.

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Abstract

Plant-based cosmetics are currently an important focus of product research and development. The formulation of phytocosmetics is challenging, since the function of its components must be kept stable and conserved. For this, stability tests in formulations are essential to guarantee physical and microbiological quality standards under different storage conditions. Since the parameters can be defined by the researcher, the objective was to propose a sequence of stability tests to be used for a creamy phytocosmetic formulation, using green banana peel extract as an active ingredient. Organoleptic, physical-chemical and microbiological tests have been proposed for preliminary, accelerated and shelf stability studies. The developed formulation remained microbiologically stable during the stability studies. Having its organoleptic and physical-chemical characteristics also preserved during the 6 months of the shelf test, it is possible to determine the minimum validity period of the formulation of 180 days. The proposed test sequence establishes a protocol to evaluate the stability of phytocosmetics and consists of physical-chemical tests compatible with the structure of pharmacies, allowing to determine the shelf life of the products handled.

Introduction

The search for products from natural sources is growing worldwide, and plant extracts are the most used forms for incorporation in cosmetics [1]. The formulation of phytocosmetics is challenging and the consumer wants effective, safe and attractive products. For this, the formulation must be produced in order to maintain the stability and function of its components, reducing the possible chemical reactions that would promote interactions between compounds [2]. Thus, the validity of a product is dependent on heat, humidity, mechanical stresses, exposure to light and microbial contamination [3].

Stability tests in formulations, especially emulsions, are essential to guarantee physical and microbiological quality standards under different storage conditions [4].

The inspection of chemical, physical and microbiological aspects ensures safety and efficacy of the product throughout its validity [5].

The parameters to be evaluated in the stability tests are determined by the researcher and depend both on the characteristics of the product and on the formulation components. They can be classified into organoleptics, physicochemical and microbiological [6, 7]. In view of the above and with the parameters being able to be defined by the researcher, the objective was to propose a sequence of stability tests, involving physical-chemical and microbiological analyzes to be used for a developed phytocosmetic, using the extract of the green banana peel as an active ingredient.

Material and methods

Preparation of the extract and formulation

The peels of the green banana (*Musa sapientum*) were dried in heating chambers and their powder was macerated with a glycolic mixture for 7 days. After filtration, the extract was incorporated into a non-ionic cream, whose formulation followed composition (except for the preservative) and preparation technique described in the Brazilian Pharmacopoeia National Form [8]. The testimony material of *Musa sapientum* was deposited in the herbarium of the Sapucaí Vale University, in the form of exsiccate, under number UNIVAS-003.

Preliminary stability test

Also known as a screening or short-term test, it was performed to adjust the formulation. Extreme temperature conditions were used, accelerating possible reactions between its components.

The organoleptic characteristics (appearance, color and odor), physical-chemical characteristics (pH and density) and microbiological characteristics (total count of viable bacteria, total count of fungi and yeasts and research of specific pathogens) were evaluated.

The samples containing 30g were filled in opaque white high density polyethylene pots and, in triplicate, stored in a refrigerator at 5 ± 2ºC, at room temperature (27 ± 2ºC), in a heating chamber at 45 ± 2ºC and in a refrigerator at -5 ± 2ºC. The preparations were submitted to macroscopic evaluation (organoleptic characteristics) and pH, for 15 consecutive days. Density tests were performed on days 0 for the sample stored at room temperature (27 ± 2ºC) and on days 7 and 15 for all samples.

Samples were subjected to extreme temperature conditions, in the so-called cycles, totaling six cycles during the test. The freeze and thaw cycles alternated 24 hours at high temperatures and 24 hours at low temperatures, with the assemblies shown in Figure 1, with a variation of ± 2ºC in temperature [7]. The determination of organoleptic characters, density and pH were performed at the beginning and end of the six cycles (12 days) [9].

Determination of pH

The pH was determined by potentiometry, using a digital pH measurement device (pH meter). The pH meter was duly calibrated with pH 4.0 and 7.0 buffer solutions at a temperature of 25 ºC. The determination of pH was carried out in a 10% aqueous dispersion (weight by weight) of the samples in purified water [6]. The electrode was inserted directly into the aqueous dispersion under agitation, resulting in the pH of the sample [10].

Density determination

The mass of the empty pycnometer and the mass of its content with water at 20°C were determined. The sample was transferred to the pycnometer. Excess substance was removed and weighed. The weight of the sample was obtained through the difference in mass of the filled and empty pycnometer. The relative density was calculated, determining the ratio between the mass of the sample and the mass of the water, both at 20°C. Relative density was used to calculate the mass density (r), which is the ratio of its mass to its volume at 20ºC [11].

The parameters of each sample were recorded in a specific form, containing the evaluation of the organoleptic characteristics, as well as the values for the physical-chemical aspects (Figure 2). Figure 3 shows the schematic representation of the preliminary stability tests.

Accelerated stability

Also known as exploratory, the accelerated stability was performed to verify the stability of the final product. The samples were subjected to stress conditions for 90 days. The parameters evaluated were the organoleptic characteristics (appearance, color and odor), physical-chemical characteristics (pH and density), microbiological characteristics (total count of viable bacteria; total count of fungi and yeasts and search for specific pathogens).

To perform the accelerated stability test, the tests (Organoleptic characteristics, pH, density) were performed at intervals of 0, 1, 7, 15, 30, 60 and 90 days of analysis, using the same temperature conditions as previous test, as well as the same tests described above.

Figure 4 shows the schematic representation of the accelerated stability tests.

Shelf test

Also known as long-term stability or shelf life, the shelf test validated the product's stability limits. In this study, representative samples of the product were stored at room temperature. The same organoleptic and physical-chemical tests were performed at 0, 3 and 6 months after preparation of the formulation (Figure 5).

In order to evaluate the microbiological parameters, specific tests were carried out for non-sterile finished product of vegetal origin for topical use, as determined by the Brazilian Pharmacopeia [11]. Tests of Total Count of Viable Bacteria, Total Count of Fungi and Yeasts, search of *Staphylococcus aureus*, search of *Pseudomonas aeruginosa*, search of *Escherichia coli* and search of *Salmonella* spp.

These tests were performed on samples subjected to ambient temperature conditions at the times (in days): 0, 30, 60, 90 and 180, covering the periods of preliminary, accelerated and shelf stability tests.

1st Cycle (0 hour)	2nd Cycle (24 hours)	3rd Cycle (48 hours)	4th Cycle (72 hours)	5th Cycle (96 hours)	6th Cycle (120 hours)
40°C ± 2°C	-5°C± 2°C	45°C ± 2°C	-5°C± 2°C	50°C ± 2°C	-5°C± 2°C

Figure 1. Temperatures used in freezing and thawing cycles during the preliminary stability test Source: Adapted from Isaac *et al.*, 2008.

ORGANOLEPTIC CHARACTERISTICS	
Aspect	
Normal, no change	( )
Slightly separated, precipitated or cloudy	( )
Separated, precipitated or cloudy	( )
Color	
Normal, no change	( )
Slightly modified	( )
Modified	( )
Intensely modified	( )
Odor	
Normal, no change	( )
Slightly modified	( )
Modified	( )
Intensely modified	( )
pH	
Density	

Figure 2. Sample data collection form during stability tests.

AMBIENCES	REHEARSAL TIME (DAYS)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Refrigerator (3 samples)	–	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density	org. e pH	org. e pH	org. e pH	org. . e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density
Freezer (3 samples)	–	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density
Room (3 samples)	organoleptics, pH, density,, microbiological	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density
Heating chamber (3 samples)	–	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	org. e pH	organoleptics, pH, density
Cycles (6 cycles) (3 samples)	–	-	-	-	-	-	-	-	-	-	-	--	organoleptics, pH, density	–	–	–

Figure 3. Schematic model proposed for the preliminary stability tests of the phytocosmetic formulation. Org = Organoleptics.

AMBIENCES	REHEARSAL TIME (DAYS)						
	0	1	7	15	30	60	90
Refrigerator (3 samples)	–	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density
Freezer (3 samples)	–	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density
Room (3 samples)	organoleptics, pH, density, microbiological	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics pH, density, microbiological	organoleptics, pH, density, microbiological	organoleptics, pH, density, microbiological
Heating chamber (3 samples)	–	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density	organoleptics, pH, density

Figure 4. Schematic model proposed for the accelerated stability tests of the phytocosmetic formulation.

AMBIENCE	REHEARSAL TIME (MONTHS)		
	0	3	6
Room (3 samples)	Matches the accelerated time 0	Matches the accelerated time 90	organoleptics, pH, density, microbiological

Figure 5. Schematic model proposed for the shelf stability tests of the phytocosmetic formulation.

Statistical analysis

For the analysis, were used Minitab version 18.1 and Statistical Package for the Social Sciences, inc. (SPSS) Chicago, USA, version 22.0. To analyze the results, analysis of variance was applied using the Levene test. The level of significance used as an acceptance or rejection criterion in the statistical tests was 5% ($p < 0.05$).

Results and discussion

The selection of the solvents used for extraction was directed to the compounds present in the extract. Knowing that most of the active phenolic compounds are not found in the free state in nature, but in the form of esters or heterosides, water and polar solvent (propylene glycol) were used as extracting vehicles [12] Polyphenolic compounds such as flavonoids and most other bioactive compounds are said to be more soluble in this solvent mixture [13].

The incorporation of plant extracts in bases for cosmetic purposes is a widespread practice. It is necessary to choose the base to which the topical active ingredients will be incorporated, thus ensuring the absorption of its assets and the stability of the formulation [14].

The stability tests accelerate the possible reactions that can occur under different storage conditions, providing data on the behavior of the formulation during its use [6]. These tests are used to determine the shelf life of cosmetic products, ensuring functionality and safety [4].

The assessment of appearance, color and odor is an important tool to assess stability [15]. Emulsion stability directly affects the appearance of products and most instabilities can be seen with the naked eye [16]. During the preliminary stability study, the organoleptic characteristics remained stable in all storage conditions, without any changes.

Changes in pH values may be related to undesirable chemical reactions or component degradation [17]. The pH of the formulation remained stable between 5 and 6. The stability of this physical-chemical parameter, even with temperature changes, may indicate that there was no degradation of compounds [15]. The ideal skin pH is between 4 and 7 and topical formulations must meet this parameter [18]. Table 1 shows the average pH values of the samples in triplicate. Without significant variations ($p > 0.05$), it reveals the stability of the formulation during the preliminary tests and the compatibility with the skin.

The presence of air can change color, odor and destabilize the emulsion. With the density it is possible to detect the incorporation of air in the product, that is, if the density decreases it means that there was incorporation of the air [19]. In the case of liquids or semi-solids, this parameter

may also indicate loss of volatile ingredients [6]. Not showing significant changes ($p>0.05$) in the different storage conditions, as shown in Table 2, the formulation continued to study the accelerated stability.

Emulsions are thermodynamically unstable. Thus, the accelerated tests using high temperatures, allows to understand the behavior of emulsions throughout their validity period, since the stability must be maintained throughout the period [20]. An emulsion is stable when there is no evidence of phase separation due to the resistance of the droplets dispersed in water to coalescence [21]. Thus, the samples stored in the freezer, refrigerator, heating chamber and room temperature remained stable and had their physical-chemical characteristics (pH and density) preserved during the accelerated study (Table 3; $p>0.05$).

Organoleptic aspects were also preserved during the accelerated studies on samples stored in freezer, refrigerator and room temperature. In the heating chamber, the samples showed changes in organoleptic aspects from the 30th day. These observations are in accordance with a stability study cited in the literature, where the action of heat and the storage time provide changes in the organoleptic characteristics of the formulations [22]. The color change may be due to changes or deformation of the phytocomposites present in the formulas [23]. However, the samples stored in the heating chamber did not show significant variations in the physical-chemical aspects, demonstrating stability of the formulation.

In shelf stability studies, samples remained stable for 6 months. For at least 180 days, the organoleptic characteristics were maintained and the pH, density (Table 4) without significant variations.

During product development, it is necessary to ensure the microbial preservation of cosmetics, ensuring consumer safety and maintaining product quality [24]. Most cosmetics contain large amounts of water and components that are substrates for microorganisms [25]. The microbiological quality of cosmetic formulations has been an issue seriously addressed by the pharmaceutical industries, since microbial contamination leads to changes in the stability of formulations, causing changes in color, odor, consistency and phase separation [26]. Therefore, even in non-sterile products, the qualitative and quantitative standards of microorganisms present in the formulation must be defined, ensuring that the microbial load is within the specified limits, which will allow harmlessness and therapeutic efficacy to the user [27].

The useful life of the products is conditioned to the action of the preservative system and consequent microbial preservation [28]. According to Table 5, the samples maintained the microbial limits within the specifications for at least 180 days.

This formulation remained microbiologically stable during the preliminary, accelerated and shelf studies. Having its organoleptic and physical-chemical characteristics also preserved during the 6 months of the shelf test. Therefore, it is possible to determine the minimum validity period of the formulation of 180 days.

Table 1. pH of phytocosmetic samples during preliminary stability study at room temperature, heating chamber, freezer, refrigerator and cycles.

Days	Room	Heating chamber	Freezer	Refrigerator	Cycles
0	5.43	5.43	5.43	5.43	5.43
1	5.42	5.49	5.43	5.49	-
2	5.38	5.47	5.44	5.47	-
3	5.36	5.45	5.43	5.45	--
4	5.40	5.45	5.42	5.45	-
5	5.41	5.46	5.42	5.46	-
6	5.40	5.44	5.45	5.44	-
7	5.39	5.43	5.47	5.43	-
8	5.38	5.37	5.42	5.37	-
9	5.44	5.45	5.44	5.45	-
10	5.42	5.43	5.43	5.43	-
11	5.43	5.43	5.44	5.43	-
12	5.41	5.44	5.44	5.44	5.44
13	5.40	5.45	5.43	5.45	-
14	5.42	5.44	5.44	5.44	-
15	5.42	5.44	5.43	5.44	-

Table 2. Phytocosmetic density (g/mL) during preliminary stability study at room temperature, heating chamber, freezer, refrigerator and cycles.

Days	Room	Heating chamber	Freezer	Refrigerator	Cycles
0	1.009	1.009	1.009	1.009	1.009
7	1.007	1.075	1.022	1.008	-
12	-	-	-	-	1.008
15	1.007	1.070	1.022	1.008	-

The values represent the average of 3 samples of the phytocosmetic.

Table 3. pH and density (g/mL) of the phytocosmetic during an accelerated stability study at room temperature, heating chamber, freezer and refrigerator.

	Room		Heating chamber		Freezer		Refrigerator	
Days	pH	Density	pH	Density	pH	Density	pH	Density
0	5.43	1.009	5.43	1.009	5.43	1.009	5.43	1.009
7	5.39	1.007	5.43	1.075	5.47	1.022	5.54	1.008
15	5.42	1.007	5.44	1.070	5.43	1.022	5.53	1.008
30	5.44	1.008	5.46	1.000	5.44	1.021	5.52	1.009
60	5.44	1.008	5.49	1.000	5.43	1.021	5.52	1.010
90	5.43	1.008	5.46	0.990	5.43	1.022	5.53	1.010

The values represent the average of 3 samples of the phytocosmetic.

Table 4. pH and density values (g / mL) of phytocosmetic samples during the shelf study at room temperature.

Months	pH	Density
0	5.43	1.009
3	5.43	1.008
6	5.44	1.008

The values represent the average of 3 samples of the phytocosmetic.

Table 5. Microbiological tests during stability studies.

Microbiological Assay	ATCC	0	30	60	90	180
Viable bacteria (CFU/g)	-	< 01	< 01	< 01	< 01	< 01
Fungi and yeasts (CFU/g)	-	< 01	< 01	< 01	< 01	< 01
Escherichia coli	8739	Ø	Ø	Ø	Ø	Ø
Pseudomonas aeruginosa	9027	Ø	Ø	Ø	Ø	Ø
Salmonella spp	14028	Ø	Ø	Ø	Ø	Ø
Staphylococcus aureus	6538p	Ø	Ø	Ø	Ø	Ø

CFU= Colony Forming Units
Ø = Absent
ATCC = American Type Culture Collection

Conclusion

The proposed test sequence establishes a protocol to evaluate the stability of phytocosmetics and consists of physical-chemical tests compatible with the structure of pharmacies, allowing to determine the shelf life of the products. The formulation developed was stable throughout the stability study, ensuring acceptability by the consumer.

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Author contributions

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no competing conflicts of interest.

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