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Stability of Sun Creams Formulated with Thermal Spring Waters from Ourense, Northwest Spain

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Abstract: Sun protection creams were formulated with a commercial rosemary extract and with thermal waters from different springs in the Northwest Spain. A six month stability study was carried out and microbiological and chemical stability, as well as sensorial characteristics, were evaluated. In all creams, the mesophilic count always remained low (under 10 cfu/mL) and most of them showed greater antioxidant stability than the control cream formulated with distilled water. Color was stable during storage in almost all creams. Sensory analysis showed a quite similar valoration of the creams regardless the sex of the panelists, and small differences were found between consumers aged 30–40 and >40. Formulations elaborated from Outariz and A Chavasqueira thermal waters were preferred to those prepared with distilled water as a control.

Keywords: thermal spring water; sun cream; stability; sensory analysis

1. Introduction

The use of herbal products in the cosmetics sector is increasing as a result of consumer demands for safer and less aggressive compounds and due to the recent interest on replacing synthetic chemicals by natural ingredients. Herbal products may have advantages related to skin benefits, cosmetic preservation, and marketing image. Herbs and spices are effective natural antioxidants [1,2]. Among the herbs of the Labiatae family, rosemary extracts have been extensively studied, are safe for food preservation [3,4], and were the first marketed natural antioxidants with several trade names available. Rosemary extracts are increasingly employed in the food, health, and cosmetics industries [5,6] and also confer satisfactory fragrance properties in foods and cosmetics [7]. The major bioactives are phenolic compounds (rosmarinic acid, caffeic acid, carnosic acid, and carnosol), carotenoids (β-carotene and lutein), and flavor compounds (monoterpenes hydrocarbons, oxygenated monoterpenes, and sesquiterpenes) [6,8]. Carnosic acid and rosmarinic acid received much attention, but minor constituents also contribute to the biological activities due to synergic effects [6]. Other biological activities of rosemary include antibacterial, antifungal, anticancer, antiinflammatory, antidiabetic, antiulcerogenic, antinociceptive, antidepressant, antianxiety, antithrombotic, antiadipogenic, α-glucosidase inhibitory, ACE inhibitory, and protective effects against neurological disorders [6,8].

Rosemary extracts and components show potential for dermal application, as photoprotective agents in human fibroblasts, as antiaging by preventing oxidative alteration of skin surface lipids, or by other mechanisms [5], by suppressing tumorigenesis and decreasing skin damage induced by free radicals [9], or due to membrane-rigidifying effects which may hinder diffusion of free radicals [10]. Rosemary oils, which showed bacteriostatic and fungistatic activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* [11], were proposed as a natural cosmetic preservative [12].

UV radiation is absorbed by skin molecules and causes oxidative damage to cellular components. In order to minimize the damaging effects of UV radiation, the use of sunscreens in cosmetic preparations has been increasing [13,14] and recent trends projected the use of natural products [15]. For the formulation of effective cosmetic products, it is important to choose the right active plant extracts or compounds, and to confirm of their activity, their stability, and synergistic effects [16]. Polyphenols and triterpenes are among the natural products with antiaging properties, able to scavenge free radicals from skin cells, to prevent trans-epidermal water loss, and to contribute to protect skin from wrinkles [17].

Mineral waters have intrinsic pharmacological properties. Of the numerous salts in thermal waters showing pharmacological properties, hydrogen sulfide has received greater attention [18]. Sulfurous mineral water may be useful in the treatment of gastrointestinal diseases and dermatological diseases and improved the redox state of the organism [19]. Mineral waters have potential as adjuvant treatment in dermatology, since they moisturize the skin barrier, stimulate the proliferation of fibroblasts, repair and inhibit inflammatory reactions [20], allow regulation of skin pH, offer fresh sensation and an increased cutaneous hydration [21], reinforce the immunological system, and improve atopical dermatitis and acne [22].

In the Northwest of Spain, Galicia has more than 300 points of thermal and mineral water springs. Ourense is named "Thermal town" because several hot springs have been used by the public for a long time for the characteristics of its waters with therapeutic indications for the treatment of dermatological and rheumatologic conditions [23].

At this time, international brands of the cosmetics industry use medicinal mineral and thermal spring waters in the formulation of their products because this type of water possesses anti-inflammatory and immunomodulatory properties, and may even produce an improvement in skin with atopic dermatitis or acne [22].

In the formulation of creams, water usually acts as an excipient, carrying active ingredients and determining their application and dosage, but in the case of using mineral water, this can also act as an active ingredient. The aim of this work is to prepare sun creams formulated with rosemary extracts added as a commercial natural antioxidant and thermal waters from five sources (As Burgas, Muíño da Veiga, Tinteiro, A Chavasqueira, and Outariz). The microbiological and chemical safety and stability for up to six months was evaluated as well as their acceptance by consumers.

2. Materials and Methods

2.1. Origin of the Waters

The thermal mineral waters used in the preparation of the sun creams were collected in the springs of As Burgas, Muíño da Veiga, Tinteiro, A Chavasqueira, and Outariz in Ourense city (NW of Spain) and transported refrigerated to the laboratory. The location of water springs and chemical composition are summarized in Table 1.

Table 1. Localization, temperature, and composition (mg/L) of the thermal spring waters used (from Delgado-Outeiriño et al. [23]).

Parameter	As Burgas	Muíño da Veiga	Tinteiro	A Chavasqueira	Outariz
UTM (H29) X	593,603.10	589,865.59	592,083.15	592,509.32	589,359.93
UTM (H29) X	4,687,765.28	4,689,598.92	4,689,706.57	4,689,113.66	4,689,132.74
Temperature (°C)	66.30	66.00	45.60	53.40	60.00
pH	7.56	8.10	7.76	7.90	8.43
Conductivity (μS/cm)	960	406	406	574	555
CO_3H^-	462	246	236	307	292
SO_4^{2-}	3	9	13	11	10
Cl^-	25	13	12	19	18
NO_3^-	0.30	0.50	0.50	1.30	0.50
NO_2^-	n.d.	0.10	0.10	n.d.	0.10
Na^+	102	103	101	127	129
K^+	8.2	4	3	4	5
Ca^{2+}	11	2	2	6.1	6
Mg^{2+}	0.69	1	1	0.30	1
Fe^{2+}	0.01	0.30	0.02	0.10	0.02

UTM: Universal Transversal Mercator coordinate system; n.d.: Not detected.

2.2. Sun Cream Preparation

Sun creams were prepared, in duplicate, with the thermal spring waters and a control prepared with distilled water was also used for comparative purposes. All the rest of ingredients used in the sun cream formulation appear in the list of substances permitted by European regulation [24] concerning cosmetic products. The sun creams were elaborated following the recommendations contained in the Guide to Good Practices of Manufacture of the standard UNE-EN-ISO 22716/2008 [25]. Sun Protection Factor (SPF) was determined using spectrophotometric method and Mansur formula according to Fonseca and Rafaela [26].

Sun creams were prepared with an oil phase and a water phase containing the components shown in Table 2 [27] and purchased from Guinama (Valencia, Spain). The melted oils were mixed in a water bath. Commercial rosemary extract (Monteloeder S.L., Elche, Spain) and Fenonip were added as antioxidant and antimicrobial agents respectively, and then the oil phase was homogenized. Carbopol and propylene were mixed with water, sonicated, and neutralized with triethanolamine to form a gel. The oily and aqueous phases were mixed under constant stirring.

Table 2. Composition of the sun cream (SPF 8).

Oil Phase		Water Phase	
Compound	**Content (g)**	**Compound**	**Content (g)**
Cream basis (o/w) [1]	7.75	Propylene	1.83
Dimethicone 350	2.56	Carbopol ultrez 10	0.46
Avocado oil	1.28	Triethanolamine	0.46
Sunscreen [2]	3.42	Demineralized or thermal water	180
Titanium dioxide	7.78	Rosemary extract	0.15
Fenonip	0.15	Tetramer cyclomethicone	0.18

[1] Mixture of fatty alcohols, glycerids, and fatty acids esters; [2] 4-methylbenzylidene camphor, benzophenone-3.

The prepared creams were packaged into 50 mL glass flasks with screw caps, and analyzed microbiologically and chemically after their elaboration and after three to six months, keeping them at all times at room temperature and in darkness. Glass was selected since the emulsions stored in plastic containers could alter the sunscreen concentrations during storage although they could show physico-chemical stability [13].

2.3. Microbiological Control

Waters were analyzed using membrane filtration method prior to use for detection of coliforms and aerobic microorganisms, and specific detection of *Escherichia coli*, as well as microorganisms that have special importance in the safety of cosmetic products, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. The initial microbial loads of thermal waters from the selected springs and the distilled water used in this work were analyzed to assess their suitability according to Real Decreto 140/2003 [28], regulating the sanitary aspects in waters for human consumption.

The creams were analyzed following protocols standardized for the detection of specific and non-specific microorganisms [29]: aerobic mesophilic bacteria, molds, and yeasts using Plate Count Agar and Saboureaud Dextrose Agar (ASD) medium supplemented with 5% chloramphenicol, respectively; *E. coli* onto Agar Levine Eosine and Methylene Blue; *P. aeruginosa* onto Cetrimide Agar; *S. aureus* onto Baird Parker Agar; and *C. albicans* using ASD whith chloramphenicol. The results were expressed in colony forming units per milliliter (cfu/mL) or colony forming units per gram of cream (cfu/g).

2.4. Physico-Chemical Control

2.4.1. pH

The evolution of pH of different emulsions over time was recorded in a pH meter GLP 21 (Crison S.A., Barcelona, Spain).

2.4.2. Oxidation

Oxidation occurs in two stages, the first leading to formation of peroxides, and the second, yields a complex mixture, including aldehydes, ketones, and other volatiles, which cause undesirable rancid odors. The primary oxidation of lipids was analyzed through the index of peroxides (PV) [30] and secondary oxidation (PA) by *p*-anisidine value according to the AOAC procedure [31]. Total oxidation of the creams was calculated by the TOTOX index, calculated as PA + 2 PV [32] and defined the overall oxidation state.

2.4.3. Color

The color of different creams and at different times has been measured using the CIELab system with a colorimeter CR-4000 (Konica Minolta, Osaka, Japan), which specifies the color three-dimensionally by the coordinates L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness). Chroma or chromaticity (C) was calculated as $C = \sqrt{\left[(a)^2 + (b)^2\right]}$, hue angle (H°) was calculated by the formula: H° = 180° + arctg(b^*/a^*), and the color difference value (ΔE) with the equation: $\Delta E = \sqrt{\left[(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2\right]}$ (L_0, a_0, b_0 represented the coordinate values of the sun cream elaborated with distilled water).

2.5. Sensory Analysis

The analysis was based on a descriptive sensory analysis [33] where the volunteer could evaluate six different emulsions coded with three-digit random numbers. Cellulose tissues were available for cleaning between samples. Among the 36 volunteers who participated in the analysis were 12 men and 24 women aged between 18 and 55 years. Testers evaluated different descriptors which allude both to the external appearance, odor, and color at the time of opening the jar, and tactile and olfactory sensations when extending different creams on skin. The intensity of each attribute was evaluated in a scale of 0 to 10, on the basis of the terms "I dislike" (0) and "I like" (10). Finally, the assessors expressed their overall acceptance.

2.6. Statistical Analysis

Physico-chemical data from the sun creams were subjected to an analysis of variance (ANOVA) with a confidence interval of 95% to evaluate the influence of the water employed in their formulation or the influence of the storage-time (0, 3, 6 months). Means were compared by the least squares difference (LSD) test, using the computer program Statistica 8.0 for Windows (Statsoft Inc., Tulsa, OK, USA). Principal Component Analysis (PCA) was performed using original data of the mean intensities of each sensory attribute and overall impression described by the panelists.

3. Results and Discussion

In this work, six sun creams were elaborated in duplicate, from five thermal waters and distilled water as control. Microbiological and physico-chemical analysis were carried out at 0, 3, and 6 months to evaluate the stability of emulsions. Sensory analysis was also performed to determine the preference of the potential consumers.

The thermal spring waters used in this study have been traditionally used for the treatment of different diseases. Thus, As Burgas, Tinteiro, and A Chavasqueira were used for dermatological and rheumatological purposes, and Muíño da Veiga and Outariz also for the locomotor system [34]. Rosemary extract can also provide different therapeutic benefits [9], aroma, and certain yellow to brownish powder. Extracts from rosemary leaves were used, since they are more effective at retarding oxidation in oil-in-water emulsion than the extracts from other parts of the plant [35].

3.1. Microbiological Control

Microbial contamination constitutes a threat to consumer safety and to the marketing image of cosmetics. It is well known that informed consumers prefer cosmetics with natural ingredients. Several authors have proposed the use of plant extracts and essential oils as natural preservative agents for cosmetic preparations, alone or in combination with other preservatives. However, in the present work, a commercial preservative was added.

3.1.1. Thermal Spring Waters

Bacteriological analysis provides information on the microbial density and safety, which could limit the proposed uses of waters and are particularly useful in thermal spring waters since the high emerging temperature could promote the growth of pathogenic microorganisms [36]. Table 3 shows the average values for colony forming units counts found in each of the selected thermal spring waters, referred both to aerobic mesophilic organisms incubated at 37 °C, and to psychrophilic aerobic microorganisms incubated at 22 °C during 24–48 h.

Table 3. Microbiological analysis (cfu/mL *) of the thermal spring waters used in this study and distilled water (DW) used as control.

Water	Aerobic Mesophilic Microbes	Aerobic Psychrophilic Microbes	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	Use
As Burgas	8.0	<1	-	-	-	-	Suitable
Muiño da Veiga	60	30	-	-	-	-	Suitable
Tinteiro	20	<1	-	-	-	-	Suitable
A Chavasqueira	10	10	-	-	-	-	Suitable
Outariz	10	10	-	-	-	-	Suitable
DW	10	<1	-	-	-	-	Suitable

* cfu/mL: colony forming units per milliliter of sun cream; -: absence.

Since the average counts in the thermal spring waters from Ourense were under 100 cfu/mL, and pathogenic organisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* were not detected, the selected waters were directly used for the formulation of sun creams. These results were expected according to the public use declaration as mineral and thermal interest since XIX century and they periodical testing. In case the microbial population exceeded the established limits, the thermal spring waters could be used after a filtration step to reduce the microbial load in the creams, which could be regarded as sterilized cosmetics, similarly to other products, such as those from Avène [37]. In other springs with heterotrophic counts under this value, the total number of microorganisms living in these waters as natural habitat could be higher (10^5 cfu/mL), but they are metabolically inactive, they do not replicate and, therefore, the cultured microorganisms would also be low [38–40].

3.1.2. Sun Creams

According to the Scientific Committee on Consumer Safety (SCCS), the Spanish Ministry of Health and Consumption, and the Spanish Agency of Drugs and Sanitary Products (AEMPS), the recommended limits are 10^2 cfu/g of product for cosmetics intended for children under three years of age, or those for use around the eyes or mucous membranes; 10^3 cfu/g of product for the rest of cosmetic products, and absence of pathogens to analyze one gram of the product.

All creams prepared met the limits established for human consumption. Table 4 summarizes the average values obtained during microbiological analysis. In the cream prepared with distilled water, some microbial growth was observed during storage, but it was under the established limits and could be suited for topical use in adults. Similar behavior was observed in the cream prepared with As Burgas spring water.

Table 4. Microbiological analysis (cfu/g *) of sun creams prepared with thermal spring waters from Ourense and distilled water (DW) used as control.

Sun Cream	Aerobic Mesophilic Bacteria (cfu/g) *			Fungi (cfu/g)			*E. coli*			*P. aeruginosa*			*S. aureus*			*C. albicans*			*Use*
Time (months)	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	
As Burgas	<10	60	5.2×10^2	<10	14	1.4×10^2	-	-	-	-	-	-	-	-	-	-	-	-	Suitable
Muiño da Veiga	70	5.5×10^2	4.2×10^2	37	2.4×10^2	55	-	-	-	-	-	-	-	-	-	-	-	-	Suitable
Tinteiro	18	8.0	1.9×10^2	8.0	<10	40	-	-	-	-	-	-	-	-	-	-	-	-	Suitable
Chavasqueira	5.0	3.5×10^2	12	8.0	2.7×10^2	6.2×10^2	-	-	-	-	-	-	-	-	-	-	-	-	Suitable
Outariz	3.0	23	15	3.0	5.0	3.0	-	-	-	-	-	-	-	-	-	-	-	-	Suitable
DW	5.0	40	98	15	73	1.0×10^2	-	-	-	-	-	-	-	-	-	-	-	-	Suitable

* cfu/g: colony forming units per gram of sun cream; -: absence.

The microbial growth pattern observed in creams prepared with Muíño da Veiga water showed an initial increase in the first three months and stable values at more prolonged times and those prepared with Tinteiro water remained low in the first three months and then increased 2 log units after six months. In the creams prepared with A Chavasqueira waters, the aerobic mesophilic bacteria increased 2 log units in three months and then decreased after six months. The fungal population also increased in 2 log units and then remained constant. The cream formulated with Outariz spring water remained stable during the studied period and could be considered suited both for topical use in adults and in the area around the eyes and mucoses. It is also suited for children under three years old, since the cfu count was under 10^2 per gram of cream, and no pathogenic microorganisms were detected. The protection of children against the adverse effects of UV radiation is of pivotal importance since excessive UV radiation is a main risk factor in the etiology of melanoma and other skin cancers in adults. The development of a photoprotector for children requires particular care regarding the ingredients, preservatives, sunscreens, or the absence of perfume or microbiological specifications [41].

In any of the sun creams prepared, the initial microbial population decreased during storage. The opposite was reported on cosmetics formulated with vegetal extracts by Kunicka-Styczynska et al. [42], who observed that the microbial population decreased within 14 days with no increase up to the 28th day. It is probable that increased concentration of natural preservatives could be beneficial either alone or in combination with synthetic agents.

The results of the microbiological analysis established that all creams are safe during the studied period, since in all cases the values found are within the legal limits. The load of bacterial aerobic mesophilic and psychrophilic in all waters collected was around or less than 10 cfu/mL and when used in sun creams, the values remained low and although they increased 1 log unit at six months, the values were under the limits and no pathogens were detected.

3.2. Physico-Chemical Control

3.2.1. pH

The pH values of the sun creams remained stable between 6.16 and 6.75 after six months (Figure 1). No significant differences ($p < 0.05$) were observed among the creams formulated with different thermal waters or among each product along the studied time. The value in the control cream remained almost constant during the studied storage period, with values of 6.66–6.67. However, the creams formulated with thermal waters showed an increase in the pH value after three months of storage. The highest, but non-significant, change was observed in creams prepared with Tinteiro and A Chavasqueira waters, which showed a 10% increase of pH values in this period. Except for Burgas after three months and Tinteiro after six months, all creams elaborated from mineral waters presented a lower pH than distilled water.

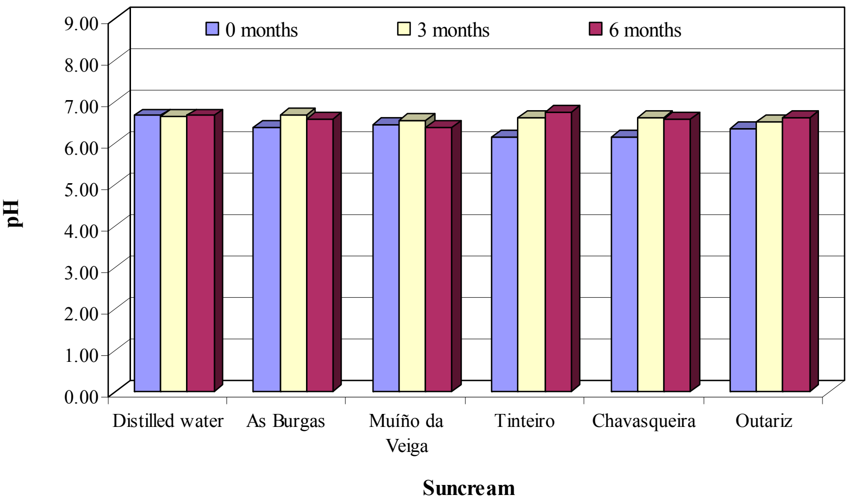


Figure 1. Evolution of the pH of sun creams with storage-time.

These values are in the range 6.1–6.7, optimal for dermal application. In different studies on sun protection and photostability [43] or rheological behavior [44] of sun creams, the evolution of pH was not reported.

3.2.2. Oxidative Stability

The influence of the addition of different thermal spring waters on the oxidation of creams, expressed as TOTOX index, during storage for six months in darkness is shown in Figure 2. Peroxide values (data not shown) were kept at low values (between 0.009 and 0.039) and no significant differences among the tested samples were observed at each time or between the added waters; only sun cream elaborated with A Chavasqueira water was significantly highest in the sixth month. Creams formulated with As Burgas, A Chavasqueira, and Muíño da Veiga waters showed primary oxidation levels lower than those found with distilled water. However, the high *p*-anisidine values reveal that secondary oxidation, leading to products responsible for the flavor, the rancid and undesirable odor had already occurred in the tested interval at the assayed dose, in similar extent as with the synthetic antioxidant. Other studies considered the addition of higher doses of natural antioxidants [45] and the oxidative susceptibility in emulsion is also higher than in bulk oils, i.e. in emulsions, carnosol and carnosic acid were markedly less effective antioxidants than in bulk oils [46]. Almost all samples showed lower values than the control, except creams formulated with Tinteiro and A Chavasqueira waters in the sixth month. Significant differences in the oxidation values were found in all sun creams. Sun creams elaborated with As Burgas, Tinteiro, and A Chavasqueira progressively increased this parameter with the storage-time, and with Muíño da Veiga remained stable for the first three months and then increased. The major contribution in the TOTOX values corresponded to PA values, which showed similar trends.

Oxidative changes also involve minor components and in some studies the extracts performed better in stripped substrates as different components may interfere [47]. Also, the presence of some metals in mineral waters could act as catalysts in emulsions. Transition metals promote oxidation by decomposing lipid hydroperoxides located at the droplet surface into free radicals and ferrous (Fe^{2+}) is a stronger pro-oxidant than ferric (Fe^{3+}) [48], no correlation with the ferrous content was observed.

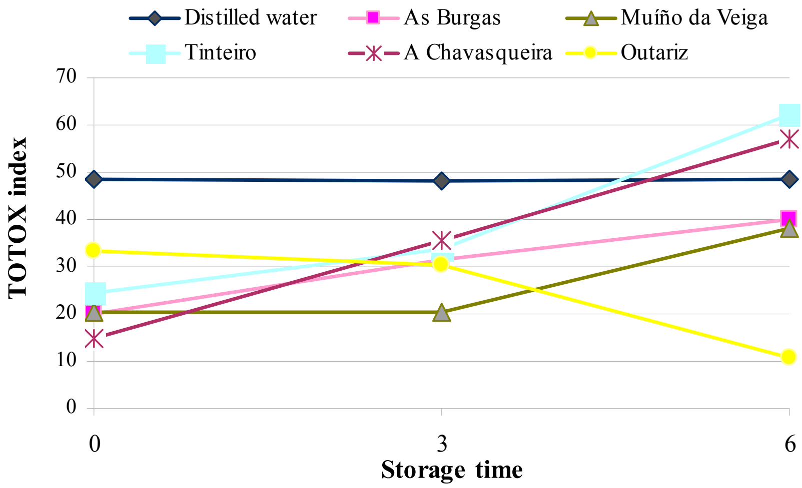


Figure 2. Influence of storage time (months) on TOTOX values of the sun creams formulated with distilled water (control) and with thermal spring waters.

3.2.3. Color

The cream color was analyzed according to CIELab values, *L**, *a** and *b**, and the chromaticity, hue angle, saturation, and the color difference with respect to control samples were calculated.

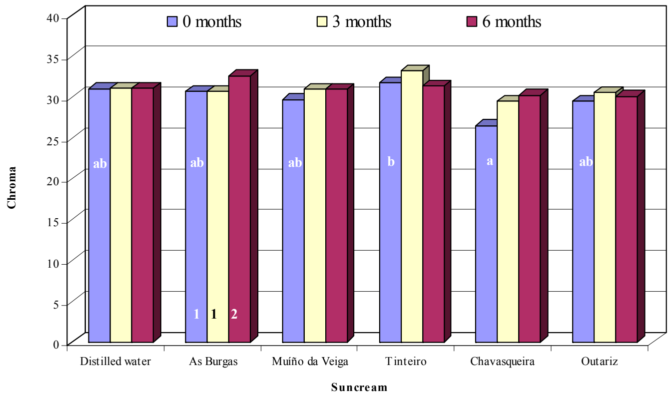
Chromaticity values of the creams, shown in Figure 3a, were non-significantly different ($p < 0.05$) for the different thermal waters at three and six months. The highest average values at these times corresponded to the cream formulated with Tinteiro spring water (33.22 and 31.26, respectively); on the contrary, the lowest values were found in creams formulated with A Chavasqueira spring water at three months (29.50) and with Outariz thermal water at six months (30.07). The chromaticity of the sun creams significantly differed between them because the sample elaborated with A Chavasqueira showed the lowest value (26.48) and when Tinteiro water was employed, this colorimetric parameter showed the highest value (31.70). With respect to the storage time, non-significant differences were found in all samples, except for the cream prepared with As Burgas water, which has similar values at the moment of preparation and after three months (30.64 and 30.65, respectively) and increased significantly, up to 32.58, after six months.

The values ranged between 26 and 32 and the creams presented an orange color, between red ($a > 0$) and yellow ($b > 0$), and showed a similar color to those of sunscreen lotions elaborated with ethanolic extract from dried rhizomes of *Curcuma longa* [43].

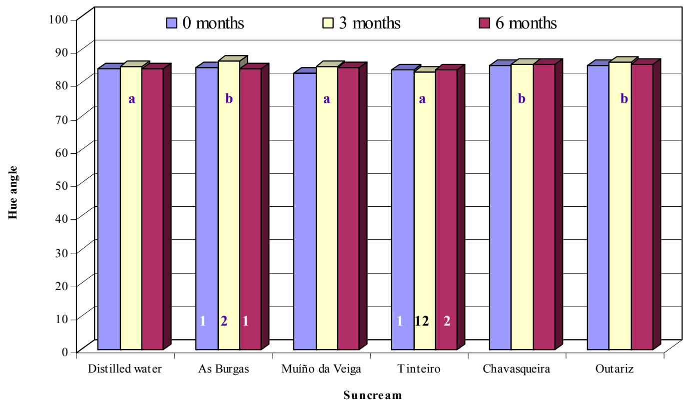
The evolution of the hue angle is shown in Figure 3b and the values ranged from 83.02 to 86.66. The creams formulated with mineral waters showed increased tone values after three months, although this change was only significant with As Burgas and Tinteiro waters. After six months the tone decreased, except in creams with water from Tinteiro, which showed a low but significant ($p < 0.05$) increase, probably due to the presence of oxidation products, measured by *p*-anisidine and TOTOX.

The saturation is shown in Figure 3c. Only in the creams prepared with Tinteiro and A Chavasqueira waters the chromatic saturation show significant variations ($p < 0.05$) with the storage time. This parameter, also named purity, refers to the intensity and it could be observed that the cream formulated with Tinteiro water lost intensity and that formulated with A Chavasqueira water, increased intensity. Saturation values are similar for all creams, except for that prepared with Tinteiro water, which showed the highest saturation at the three times evaluated.

The color difference was measured in relation to the control cream (Figure 3d) and all of them showed more color than control (1.5–9 units), particularly the cream prepared with Outariz water, which during storage lowered up to three times. The cream prepared with Tinteiro water showed the most stable color (3.12–4.58).



(**a**)



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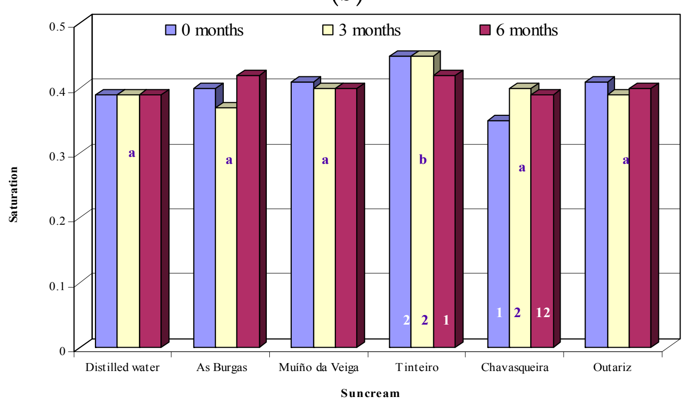
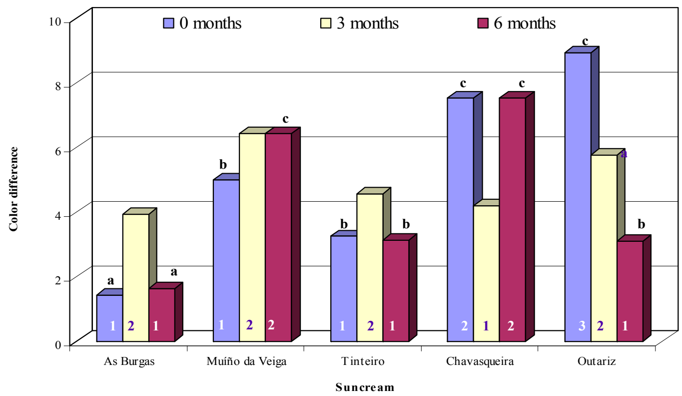


(**c**)

Figure 3. *Cont.*



(**d**)

Figure 3. Evolution of (**a**) chromaticity; (**b**) hue angle; (**c**) saturation; and (**d**) difference of color during storage for sun creams formulated with thermal spring waters. Significance (95%): a–b–c indicate, for the same month, that the creams elaborated with different waters were significantly different; 1–2–3 indicate, for the same water, that the values were significantly different with the storage-time.

3.3. Sensory Analysis

The use of sensory analysis in the quality control of a cosmetic allows the evaluation of relationships between the properties of the product and the information that the juries or consumers reported on their perception about the aroma, texture, sensation, appearance, consistence, etc. [49–51]. Therefore, sensory analysis has turned in an indispensable technique to help the formulator to evaluate the quality of new products, their sensorial characteristics and acceptability by the consumer and their stability [52].

Hedonic judgements concerning five appearance attributes (gloss, consistency, color, odor intensity, and odor preference) and eight skin parameters (spreadability, penetration, softness, texture preference, skin gloss, skin feel, skin odor intensity, and skin odor persistence) and global preference tested for each sun cream were also collected.

The sensory analysis was evaluated with volunteers aged from 18 to 55 of both sexes, although other studies were exclusively based on the opinion of females [53]. Some of the panelists (14%) knew some of the techniques, although the evaluation by untrained panelists has also been used [54]. This evaluation was proposed as a consumer test for the five mineral waters used and the cream with distilled water was used as control.

Figure 4 shows the scores corresponding to color, odor, and texture preferences, as well as the overall score of the sun creams according to the sex and the age of panelists. No significant differences in scores according to sex were observed (Figure 4a), except that females ranked higher than males (one point) regarding the color valoration. Panelists older than 40 provided higher scores to the different aspects, except odor, but panelists between 30 and 40 provided lower scores (1–2 points) to the different attributes (Figure 4b). The most valued descriptors were smoothness and spreadability and the least valued were olfactory were in relation to the odor intensity both in the glass container and in the skin. The highest scores were given to cream texture, which contributed more than color or odor.

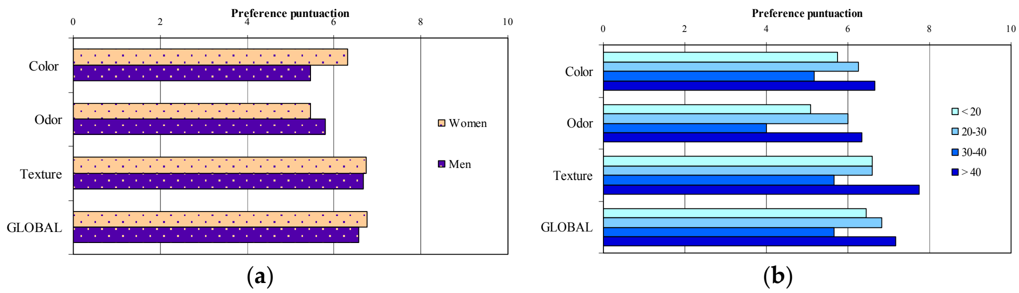


Figure 4. Score-preferences about the sensory attributes of the sun creams according to sex (**a**); and age (**b**).

The Principal Component Analysis (PCA) of the sensory attributes accounted for 84.45% of the variability. The main variance is explained by Factor 1 (66.81%) and Factor 2 (17.64%). The loading plot of the sensory variables presented in Figure 5 showed that the overall preference of the sun creams was significantly related to the textural characteristics (spreadability, penetration, softness, and skin feel) [55] and to the persistence of the odor in the skin [56].

With respect to the thermal water employed in the elaboration of the sun creams (Figure 6), the evaluation of volunteers of different sex and age showed that there were no large differences between the scores given by men compared to those granted by women (Figure 6a), the creams formulated with Muíño da Veiga water showing more disparity of opinion. Men scored better emulsions prepared with the waters of Outariz, A Chavasqueira, and As Burgas. Volunteers between 30 and 40 provided a more accurate distinction among the creams, with more than two points of difference between sun creams elaborated with Muíño da Veiga and Outariz waters (Figure 6b).

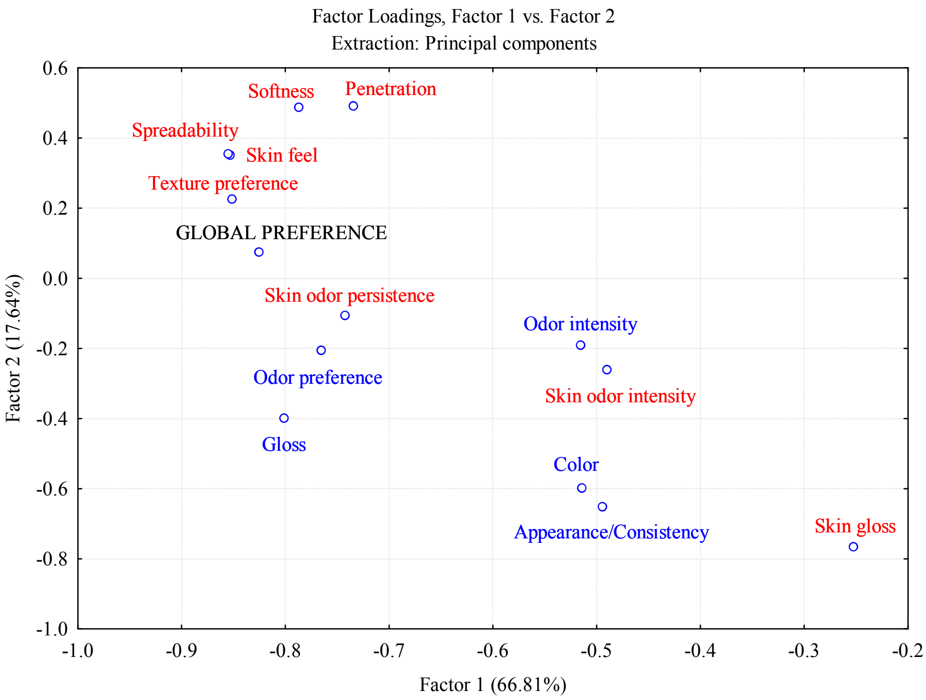
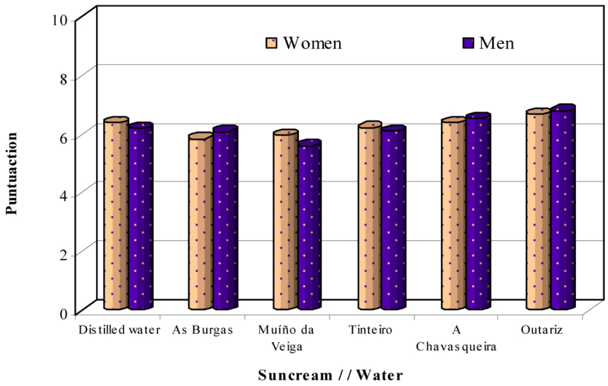
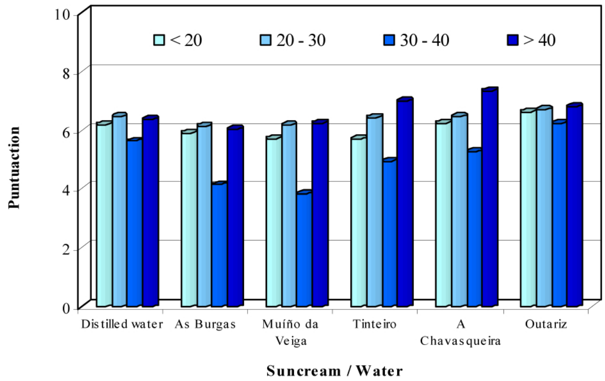


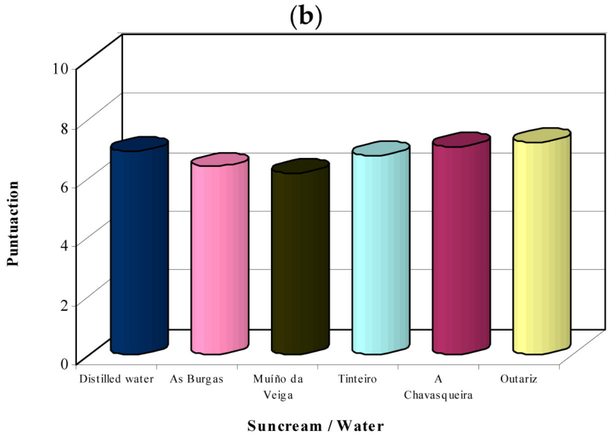
Figure 5. Principal Component Analysis (PCA) of sensory attributes of the sun creams elaborated with thermal waters.



(**a**)



(**b**)



(**c**)

Figure 6. Valuation of sun creams according to sex (**a**); age (**b**); and overall acceptance (**c**) of the creams on the basis of the thermal spring water used.

The valoration of the creams as a whole, taking into account all the attributes, shown in Figure 6c, shown that the favorite emulsion was the prepared with Outariz water, followed by those prepared with A Chavasqueira and with distilled water that served as a control. The cream with the lowest score was prepared with Muíño da Veiga water, which showed the lowest color and odor ranking. However, the scores ranged from 6.1 to 7.2 and therefore, all creams would be well accepted by consumers.

4. Conclusions

In this work, the valorization of thermal mineral water from different hot springs through the development of sun creams with rosemary was proposed. All of them, As Burgas, Muíño da Veiga, Tinteiro, A Chavasqueira, and Outariz are located at the city of Ourense, in the Northwest of Spain.

Using distilled water as a control and the different hot springs waters, six sun creams—not excessively oily, and with orange tonality and pleasant rosemary odor—were elaborated. The sun creams were stable microbiologically and chemically until at least six months, and they are suitable for personal use from a legislative point of view. In addition, no alterations in pH, solar factor protection (SFP), smell, and color have been detected. All the sun creams were well accepted by consumers, but the best were those formulated with waters of the hot springs in Outariz and A Chavasqueira.

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References

1. Yanishlieva, N.V.; Marinova, E.M. Stabilisation of edible oils with natural antioxidants. *Eur. J. Lipid Sci. Technol.* **2001**, *103*, 752–767. [CrossRef]
2. Yanishlieva, N.V.; Marinova, E.; Pokorný, J. Natural antioxidants from herbs and spices. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 776–793. [CrossRef]
3. European Commission Directive 2010/67/EC. Directive 2008/84/EC Laying down Specific Purity Criteria on Food Additives Other than Colours and Sweeteners. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010L0067 (accessed on 14 December 2016).
4. European Commission Directive 2010/69/EC. Annexes of Directive 95/2/EC on Food Additives Other than Colours and Sweeteners. Available online: https://publications.europa.eu/en/publication-detail/-/publication/856f3f44-c877-43d2-b73f-32d280b5f9fb/language-en (accessed on 14 December 2016).
5. Birtić, S.; Dussort, P.; Pierre, F.X.; Bily, A.C.; Roller, M. Carnosic acid. *Phytochemistry* **2015**, *115*, 9–19. [CrossRef] [PubMed]
6. Ribeiro-Santos, R.; Carvalho-Costa, D.; Cavaleiro, C.; Costa, H.S.; Albuquerque, T.G.; Castilho, M.C.; Ramos, F.; Melo, N.R.; Sanches-Silva, A. A novel insight on an ancient aromatic plant: The rosemary (*Rosmarinus officinalis* L.). *Trends Food Sci. Technol.* **2015**, *45*, 355–368. [CrossRef]
7. Ayşegül Koluman, N.M.; Süzgeç-Selçuk, S. Pyhtocosmetics in pharmacy. *Marmara Pharm. J.* **2016**, *20*, 7–20.
8. Al-Sereiti, M.R.; Abu-Amer, K.M.; Sen, P. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. *Indian J. Exp. Biol.* **1999**, *37*, 124–130. [PubMed]
9. Baumann, L.S. Less-known botanical cosmeceuticals. *Dermatol. Ther.* **2007**, *20*, 330–342. [CrossRef] [PubMed]
10. Pérez-Fons, L.; Aranda, F.J.; Guillén, J.; Villalaín, J.; Micol, V. Rosemary (*Rosmarinus officinalis*) diterpenes affect lipid polymorphism and fluidity in phospholipid membranes. *Arch. Biochem. Biophys.* **2006**, *453*, 224–236. [CrossRef] [PubMed]
11. Packer, J.F.; Da Luz, M.M.S. Evaluation and research method for natural products inhibitory activity. *Braz. J. Pharmacogn.* **2007**, *17*, 102–107. [CrossRef]
12. Muyima, N.Y.O.; Zulu, G.; Bhengu, T.; Popplewell, D. The potential application of some novel essential oils as natural cosmetic preservatives in a aqueous cream formulation. *Flavour Fragr. J.* **2002**, *17*, 258–266. [CrossRef]
13. Santoro, M.I.; Da Costa, E.; Oliveira, D.A.; Kedor-Hackmann, E.R.; Singh, A.K. The effect of packaging materials on the stability of sunscreen emulsions. *Int. J. Pharm.* **2005**, *297*, 197–203. [CrossRef] [PubMed]
14. Nohynek, G.J.; Antignac, E.; Re, T.; Toutain, H. Safety assessment of personal care products/cosmetics and their ingredients. *Toxicol. Appl. Pharmacol.* **2010**, *243*, 239–259. [CrossRef] [PubMed]
15. Mukherjee, P.K.; Maity, N.; Nema, N.K.; Sarkar, B.K. Bioactive compounds from natural resources against skin aging. *Phytomedicine* **2011**, *19*, 64–73. [CrossRef] [PubMed]
16. Kusumawati, I.; Indrayanto, G. Natural antioxidants in cosmetics. *Stud. Nat. Prod. Chem.* **2013**, *40*, 485–505.
17. Tundis, R.; Loizzo, M.R.; Bonesi, M.; Menichini, F. Potential role of natural compounds against skin aging. *Curr. Med. Chem.* **2015**, *22*, 1515–1538. [CrossRef] [PubMed]
18. Carubbi, C.; Gobbi, G.; Bucci, G.; Gesi, M.; Vitale, M.; Mirandola, P. Skin, inflammation and sulfurous waters: What is known, what is believed. *Eur. J. Inflamm.* **2013**, *11*, 591–599.

19. Costantino, M.; Giampaolo, C.; Filippelli, A. Effects of drinking SPA therapy on oxidative stress. *Clin. Ther.* **2013**, *163*, e13–e17.
20. Nunes, S.; Tamura, B.M. Biochemical and toxicological assessment of a Brazilian mineral water and its effects on the skin. *Surg. Cosmet. Dermatol.* **2011**, *3*, 197–202.
21. Elkhyat, A.; Courderot-Masuyer, C.; Mac-Mary, S.; Courau, S.; Gharbi, T.; Humbert, P. Assessment of spray application of Saint GERVAIS® water effects on skin wettability by contact angle measurement comparison with bidistilled water. *Skin Res. Technol.* **2004**, *10*, 283–286. [CrossRef] [PubMed]
22. Merial-Kieny, C.; Castex-Rizzi, N.; Selas, B.; Mery, S.; Guerrero, D. Avène thermal spring water: An active component with specific properties. *J. Eur. Acad. Dermatol. Venereol.* **2011**, *25*, 2–5. [CrossRef] [PubMed]
23. Delgado-Outeiriño, I.; Araujo-Nespereira, P.; Cid-Fernández, J.A.; Mejuto, J.C.; Martínez-Carballo, E.; Simal-Gándara, J. Behaviour of thermal waters through granite rocks based on residence time and inorganic pattern. *J. Hydrol.* **2009**, *373*, 329–336. [CrossRef]
24. European Regulation (EC) 1223/2009, on 30 November 2009, on Cosmetic Products. Available online: http://ec.europa.eu/health//sites/health/files/endocrine_disruptors/docs/cosmetic_1223_2009_regulation_en.pdf (accessed on 14 December 2016).
25. UNE-EN ISO 22716/2008. Cosmetics—Good Manufacturing Practices (GMP). Guidelines on Good Manufacturing Practices. Available online: http://www.aenor.es/aenor/normas/normas/fichanorma.asp?tipo=N&codigo=N0041130#.WE6MP-bhCUk (accessed on 14 December 2016).
26. Fonseca, A.P.; Rafaela, N. Determination of sun protection factor by UV-Vis spectrophotometry. *Health Care Curr. Rev.* **2013**, *1*, 1–4.
27. Balboa, E.; Soto, M.L.; Nogueira, D.; González-López, N.; Conde, E.; Moure, A.; Vinardell, M.P.; Mitjans, M.; Domínguez, H. Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics. *Ind. Crops Prod.* **2014**, *58*, 104–110. [CrossRef]
28. Real Decreto 140/2003. De 7 de *Febrero, por el que se Establecen los Criterios Sanitarios de Calidad del Agua de Consumo Humano*. Available online: https://www.boe.es/buscar/act.php?id=BOE-A-2003-3596 (accessed on 14 December 2016).
29. UNE-EN ISO 18415/2012. Cosmetics. Microbiology. Detection of Specific and Non-Specific Microorganisms. Available online: http://www.aenor.es/aenor/normas/normas/fichanorma.asp?tipo=N&codigo=N0048756#.WE6QzObhCUk (accessed on 14 December 2016).
30. Díaz, M.; Dunn, C.M.; McClements, D.J.; Decker, E.A. Use of caseinophosphopeptides as natural antioxidants in oil-in-water emulsions. *J. Agric. Food Chem.* **2003**, *51*, 2365–2370. [CrossRef] [PubMed]
31. AOAC (Association of Official Analytical Chemists). *International Official Method of Analysis, Cd 18–90. p-Anisidine Value*; AOAC: Gaithersbrug, MD, USA, 1997.
32. Wang, Z.; Hwang, S.H.; Lim, S.S. Lipophilization of phenolic acids with phytosterols by chemoenzymatic method to improve their antioxidant activities. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1–12. [CrossRef]
33. Isaac, V.; Chiari, B.G.; Magnani, C.; Corrêa, M.A. Análise sensorial como ferramenta útil no desenvolvimento de cosméticos. *Rev. Ciênc. Farm. Básica Apl.* **2012**, *33*, 479–488. (In Portuguese)
34. Ourense Thermal. Available online: http://termalismo.ourense.es/en/ (accessed on 27 July 2016).
35. Gallego, M.G.; Gordon, M.H.; Segovia, F.J.; Skowyra, M.; Almajano, M.P. Antioxidant properties of three aromatic herbs (rosemary, thyme and lavender) in oil-in-water emulsions. *J. Am. Oil Chem. Soc.* **2013**, *90*, 1559–1568. [CrossRef]
36. Rodríguez, L.A. Microbiología de las aguas. In *Ourense Termal*; Deputación de Ourense: Ourense, Spain, 2001; pp. 23–30. (In Spanish)
37. Eau Thermale Avéne. Available online: http://www.eau-thermale-avene.fr/bebe/soins-de-bebe/creme-hydratante-cosmetique-sterile (accessed on 27 July 2016).
38. Mosso, M.A.; Sánchez, M.C.; Rodríguez, C.; de la Rosa, M.C. Microbiología de los manantiales mineromedicinales del Balneario Cervantes. *Anal. Real Acad. Nac. Farm.* **2006**, *72*, 285–304.
39. De la Rosa, M.C.; Mosso, M.C. Diversidad microbiana de las aguas minerales termales. In *Panorama Actual de las Aguas Minerales y Minero-Medicinales en España*; Instituto Geológico Minero Español (IGME): Madrid, Spain, 2000; pp. 153–158.
40. De La Rosa, M.C.; Pintado, C.; Rodríguez, C.; Mosso, M.A. Estudio IV: Análisis microbiológico de las aguas. In *Balnearios de España Aguas Minerales y mineromedicinales Monografía XXXII El Raposo*; Real Academia Nacional de Farmacia: Madrid, Spain, 2013; pp. 65–85.

41. Denis, A. What are the laboratories purposes for the development of a sunscreen for children? *Nouv. Dermatol.* **2001**, *20*, 303–305.
42. Kunicka-Styczynska, A.; Smigielski, K.; Prusinowska, R.; Rajkowskal, K.; Kusmider, B.; Sikora, M. Preservative activity of lavender hydrosols in moisturizing body gels. *Lett. Appl. Microbiol.* **2014**, *60*, 27–32. [CrossRef] [PubMed]
43. Rasheed, A.; Shama, S.N.; Mohanalakshmi, S.; Ravichandran, V. Formulation, characterization and in vitro evaluation of herbal sunscreen lotion. *Oriental Pharm. Exp. Med.* **2012**, *12*, 241–246. [CrossRef]
44. Gaspar, L.R.; Campos, P.M.B.G.M. Rheological behavior and the SPF of sunscreens. *Int. J. Pharmac.* **2003**, *250*, 35–44. [CrossRef]
45. Madsen, H.L.; Sørensen, B.; Skibsted, L.H.; Bertelsen, G. The antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in dressing stored exposed to light or in darkness. *Food Chem.* **1998**, *63*, 173–180. [CrossRef]
46. Hopia, A.I.; Huang, S.W.; Schwarz, K.; German, J.B.; Frankel, E.N. Effect of different lipid systems on antioxidant activity of rosemary constituents carnosol and carnosic acid with and without α-tocopherol. *J. Agric. Food Chem.* **1996**, *44*, 2030–2036. [CrossRef]
47. Samotyja, U.; Małecka, M. Effects of blackcurrant seeds and rosemary extracts on oxidative stability of bulk and emulsified lipid substrates. *Food Chem.* **2007**, *104*, 317–323. [CrossRef]
48. Waraho, T.; McClements, D.J.; Decker, E.A. Mechanisms of lipid oxidation in food dispersions. *Trends Food Sci. Technol.* **2011**, *22*, 3–13. [CrossRef]
49. Lee, I.S.; Yang, H.M.; Kim, J.W.; Maeng, Y.J.; Lee, C.W.; Kang, Y.S.; Rang, M.J.; Kim, H.Y. Terminology development and panel training for sensory evaluation of skin care products including aqua cream. *J. Sens. Stud.* **2005**, *20*, 421–433. [CrossRef]
50. Almeida, I.F.; Gaio, A.R.; Bahia, M.F. Hedonic and descriptive skinfeel analysis of two oleogels: Comparison with other topical formulations. *J. Sens. Stud.* **2008**, *23*, 92–113. [CrossRef]
51. Chiari, B.G.; Almeida, M.G.J.; Corrêa, M.A.; Isaac, V.L.B. Cosmetics' quality control. In *Latest Research into Quality Control*; Akyar, I., Ed.; INTECH Open Science: Rijeka, Croatia, 2012; Chapter 16; pp. 337–364.
52. Aust, L.B.; Oddo, P.; Wild, J.E.; Mills, O.H.; Deupree, J.S. The descriptive analysis of skin care products by a trained panel of judges. *J. Soc. Cosmet. Chem.* **1987**, *38*, 443–448.
53. Worch, T.; Crine, A.; Gruel, A.; Lê, S. Analysis and validation of the Ideal Profile Method: Application to a skin cream study. *Food Qual. Preference* **2014**, *32*, 132–144. [CrossRef]
54. Külkamp-Guerreiro, I.C.; Berlitz, S.J.; Contri, R.V.; Alves, L.R.; Henrique, E.G.; Barreiros, V.R.M.; Guterres, S.S. Influence of nanoencapsulation on the sensory properties of cosmetic formulations containing lipoic acid. *Int. J. Cosmet. Sci.* **2013**, *35*, 105–111. [CrossRef] [PubMed]
55. Gilbert, L.; Savary, G.; Grisel, M.; Picard, C. Predicting sensory texture properties of cosmetic emulsions by physical measurements. *Chemometr. Intell. Lab.* **2013**, *124*, 21–31. [CrossRef]
56. Gonçalves, G.M.S.; Srebernich, S.M.; Vercelino, B.G.; Zampieri, B.M. Influence of the presence and type of fragrance on the sensory perception of cosmetic formulations. *Braz. Arch. Biol. Technol.* **2013**, *56*, 203–212. [CrossRef]