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Commercial plant extracts may act as antioxidants or pro-oxidants in cosmetic emulsions based on argan oil

PAULINA MALINOWSKA, ANNA GLISZCZYŃSKA-ŚWIGŁO, and HENRYK SZYMUSIAK, *Faculty of Commodity Science, Poznań University of Economics and Business, 61-875 Poznań, Poland*

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Synopsis

Cosmetic emulsions containing plant extracts should be tested in a range of temperatures from 5°C to 40°C to be sure that they will be stable during general use by consumers and that plant extracts used as antioxidants do not accelerate oxidative degradation of their oil base. The oxidative stability of argan oil-in-water emulsions containing 1% and 5% commercial acerola, willow, and rose extracts [or 0.01% butylhydroxytoluene (BHT)], stored at 5°C and 20°C for 6 months and at 40°C for 4 weeks, was monitored by the determination of peroxide content. The antioxidant or pro-oxidant activities of extracts or BHT in emulsions were expressed as the protection factor (PF) and inhibition of peroxide formation (Ip). At the end of storage, 5% willow, 0.01% BHT, 1% willow, and 5% acerola were the most protective for emulsions stored at 5°C. At 20°C, the most effective was 0.01% BHT, 5% rose, and 5% acerola. At 40°C, inhibition of peroxide formation calculated for 1% rose, 5% acerola, and 0.01 % BHT was similar. Altogether, the results show that some plant extracts, depending on storage conditions, may act as pro-oxidants, whereas the others can be applied as natural antioxidants instead of synthetic BHT.

INTRODUCTION

Cosmetic products containing plant compounds have recently become very popular, as the consumers prefer cosmetics without synthetic preservatives, antioxidants, perfumes, and dyes. Therefore, cosmetic producers very often use plant extracts rich in polyphenols which exert multidirectional activities (1). Plant polyphenols with high antioxidant activity may prolong the stability of cosmetic products and replace synthetic antioxidants such as butylhydroxyanizole (BHA) and butylhydroxytoluene (BHT), which are suspected of causing the exanthemas and allergic contact dermatitis (2,3). Moreover, plant polyphenols influence physiology of skin demonstrating particular activities such as sealing of capillary vessels, induction or inhibition of some enzymes in the skin, antiphlogistic, antiallergic, UV-protective, antimycotic, antibacterial, antiviral, and estrogen-like properties (4–7).

In cosmetic industry, producers suggest the application of plant extracts at much higher concentrations (1–5%) than those recommended in food industry (0.001–0.1%). Food antioxidants are the most often effective at low concentrations and may show pro-oxidative

Address all correspondence to Anna Gliszczyńska-Świgło at anna.gliszczynska-swiglo@ue.poznan.pl.

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activity at higher concentrations (8,9). In contrast to low concentrations, higher concentrations of antioxidants in cosmetic emulsions show different activities, and as a consequence, they may improve the appearance of the skin.

There is limited information in literature (10–12) about application of plant extracts as antioxidants at higher concentrations (1–5%) recommended by their producers. Our previous studies concerning the effect of plant extracts with healing properties such as acerola fruit (*Malpighia punicifolia* L.), rose buds (*Rosa canina* L.), and willow bark (*Salix alba* L.) on the oxidative stability of cosmetic emulsions based on different oils (13,14) revealed that these extracts may act as antioxidants or pro-oxidants depending not only on the concentration of extract or storage conditions but also on the type of oil. Therefore, we decided to analyze these extracts in emulsion based on argan oil. Argan oil obtained from *Argania spinosa* L. seeds is eaten raw in southwest of Morocco and it is also used in traditional medicine. It is a rich source of unsaturated fatty acids and it has anti-allergic, anti-inflammatory, and UV-protective activities so it is applied in cosmetics for dry, mature, sensitive, and allergic skin. Moreover, it improves acne-prone skin condition, moisturizes, smoothes, revitalizes, and firms the skin, as well as protects it against the dryness and soothes irritations. It also strengthens hair and nails. Therefore, it is used as an ingredient in many kinds of cosmetics, e.g., massage and skin care oils, face, body and hair care cosmetics, such as creams, lotions, and milks (15).

Extracts from acerola, willow, and rose are applied in many cosmetics and exhibit stronger or weaker antioxidant activity. It was reported that acerola fruit extract has antioxidant activity measured with the use of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) assays, superoxide anion radical scavenging activity, and reducing power (16,17). Ethanolic willow bark extracts were found to have antioxidant activity in DPPH (18) and β-carotene/linoleic acid bleaching (19) assays. There are less scientific reports indicating the antioxidant activity of rose bud extracts (20), literature provides more data concerning properties of rosehip (21–23) and rose leaves extracts (24). The antioxidant activities of these three extracts were confirmed in our previous study with the use of DPPH, Trolox Equivalent Antioxidant Capacity (TEAC), and Ferric Reducing Antioxidant Power (FRAP) tests (13).

Therefore, in the present study, the effect of 1% and 5% acerola, willow, and rose ready-to-use commercial extracts on the oxidative stability of oil-in-water (o/w) cosmetic emulsions based on argan oil stored in different conditions was evaluated.

MATERIALS AND METHODS

CHEMICALS

BHT was purchased from Merck (Darmstadt, Germany). Phenochem preservative (mixture of parabens in 2-phenoxyethanol) was from Custom Ingredients (New Braunfels, TX). Polyoxyethylene (20) sorbitan monolaurate (Tween 20) was from Fluka (Buchs, Switzerland).

COMMERCIAL PLANT EXTRACTS

Ready-to-use commercial plant extracts, acerola fruit (*M. punicifolia* L.) hydroglycolic extract, rose buds (*R. canina* L.) glycolic extract, and willow bark (*S. alba* L.) glycolic extract

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were obtained from cosmetic companies. They were preserved by the producers with paraben mixture. The content of parabens in acerola extract was 0.25%. For other extracts such information was unavailable. They were stored at room temperature in the dark.

COMMERCIAL PLANT OIL

Cold-pressed argan oil (*Argania Spinosa* Kernel Oil) was obtained in opaque plastic container from Statfold company (Tamworth, Great Britain). It was stored in the original container at 5°C in the dark and it was used for the preparation of cosmetic emulsions not later than 2 days after obtaining it from the producer. Acid number (AN), peroxide value (PV), anisidine value (AV), and totox value of argan oil were determined as described previously (14).

PREPARATION AND STORAGE OF ARGAN OIL-IN-WATER MODEL EMULSIONS

Oil-in-water emulsions were prepared by mixing argan oil (5% w/w), Tween 20 (4% w/w), plant extract (1 or 5% w/w) or BHT (0.01% w/w), and Phenochem preservative (0.8% w/w). Phosphate buffer (0.1 M, pH = 6) was added to 100% (w/w) and all ingredients were homogenized (SilentCrusher S, Heidolph Instruments GmbH & Co. KG, Schwabach Germany). Emulsions without extract or BHT (control samples) were also prepared. Each type of oil-in-water emulsion was prepared in duplicate and transferred to closed polypropylene containers. They were stored in incubators in different conditions: (1) 5°C ± 1°C for 6 m, (2) 20°C ± 1°C for 6 m, (3) 40°C ± 1°C for 4 w with periodic access of air and light (samples were opened for 10 s every day). All emulsions were physically and microbiologically stable.

DETERMINATION OF PEROXIDE CONTENT IN COSMETIC EMULSIONS

The oxidative stability of emulsions was monitored by the determination of the peroxide content as described in (25), with the modification involving the use of a solvent mixture of methanol and butanol instead of ethanol (14). The results were expressed as mmol O_2/ml of emulsion.

EFFECT OF PLANT EXTRACTS ON OXIDATIVE STABILITY OF ARGAN OIL–WATER COSMETIC EMULSIONS

The potential of plant extracts and BHT to protect emulsion against oxidation was expressed as protection factor (PF), calculated according to the following equation:

$$\mathrm{PF} = \frac{T_{\mathrm{sample}}}{T_{\mathrm{control}}}$$

where T_{sample} is the time necessary to increase the peroxide content to 0.4 mmol O_2/ml in sample and T_{control} is the time necessary to increase the peroxide content to 0.4 mmol O_2/ml in control sample.

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The time necessary to increase the peroxide content is the time in which the oxidative changes in oil are undetectable or very low (9). In the case of argan oil emulsions, this level was determined as 0.4 mmol O_2/ml. This value enables the comparison of oxidative changes occurring in all applied temperature conditions.

The PF value higher than 1.0 indicates antioxidant properties and the protection of emulsion from oxidation. The PF value equal to 1.0 means the lack of protective effect of antioxidant and the PF value lower than 1 indicates that antioxidant acts as pro-oxidant (9).

The long-term ability of plant extracts and BHT to inhibit the peroxide formation (Ip) was calculated at the end of storage in all temperature conditions (percentage of peroxide content in relation to control sample).

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation of three independent determinations for each emulsion prepared in duplicate (n = 6). All data were analyzed by SPSS Statistics 14.0. software program using analysis of variance (ANOVA). Games Howell post hoc test was applied. Differences were considered to be statistically significant at $\alpha = 0.05$.

RESULTS AND DISCUSSION

CHARACTERISTICS OF ARGAN OIL

Argan oil is a rich source of unsaturated fatty acids (Table I), thus it is susceptible to oxidation. Before preparation of oil-in-water emulsions, the quality of argan oil was assessed by the determination of AN, PV, AV, and totox value (Table I).

The AN, PV, and AV of argan oil met the quality requirements for cold-pressed oils. According to the Codex Alimentarius (27), AN cannot be higher than 4 mg KOH/g of oil whereas PV cannot exceed the level of 15 milliequivalents of active oxygen/kg of oil. The maximum AV is not included in the requirements of the European regulations for

Table I
Quality Characteristics of Cold-Pressed Argan (*Argania Spinosa* kernel) Oil (26)

Fatty acids (%)	
16:0	13.0
18:0	5.0
18:1	45.0
18:2	36.0
18:3	0.5
AN (mg KOH/g)	0.3
PV (meq O_2/kg)	1.4
AV	0.4
Totox	3.2

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cold-pressed oils, but according to the Polish standard PN-EN ISO 6885:2001 (28), AV cannot exceed the level of 8.0. Argan oil did not exceed this value.

EFFECT OF ACEROLA, WILLOW, AND ROSE EXTRACTS ON OXIDATIVE STABILITY OF ARGAN OIL–WATER COSMETIC EMULSIONS

A major cause of quality deterioration of emulsions is the susceptibility of their lipids to oxidation. Temperature is one of the most important factors influencing the stability of unsaturated fats, thus in the present study the effect of two concentrations of three plant extracts on the oxidative stability of emulsions based on argan oil was investigated. Emulsions were stored at 5°C, 20°C, and 40°C and the PFs of extracts as well as their long-term ability to inhibit the peroxide formation (Ip) in cosmetic emulsions were calculated. All extracts were characterized by good antioxidant activities. Their TEAC and FRAP values were, respectively, 40.4 and 41.3 μmol/g (for rose extract), 44.4 and 46.8 μmol/g (for willow extract), and 55.3 and 141.8 μmol/g (for acerola extract). In the DPPH assay, their AA_{DPPH} values, calculated as $1/EC_{50}$, were 12.5 for rose extract, 16.7 for willow extract, and 50.0 for acerola extract. The polyphenol contents were 5.5 mg/g for rose extract, 6.2 mg/g for willow extract, and 11.8 mg/g for acerola extract (13,14).

Figure 1 presents the effect of plant extracts and BHT on the peroxide contents in tested cosmetic emulsions stored in different conditions. Potential of extracts and BHT to protect emulsions from oxidation, expressed as the PF values, is shown in Figure 2.

Peroxide content in control sample stored at 5°C for 6 months increased from 0.22 to 0.80 (Figure 1A). The contents of peroxides in control emulsions stored at 20°C for 6 m and at 40°C for 4 w were 1.15 (Figure 1B) and 1.20 mmol O_2/ml (Figure 1C), respectively. Taking into account the same time of storage (about 31 d), it was observed that the peroxide content in control samples increased to 0.41 mmol O_2/ml at 5°C, to 0.63 mmol O_2/ml at 20°C, and to 1.20 mmol O_2/ml at 40°C (Figure 1). These observations confirmed that the temperature of storage affected the rate of emulsion oxidation. Faster formation of hydroperoxides in oil-in-water emulsions stored at higher temperatures has already been reported (14).

EFFECT OF ACEROLA, WILLOW, AND ROSE EXTRACTS ON OXIDATIVE STABILITY OF ARGAN OIL–WATER COSMETIC EMULSIONS STORED AT 5°C FOR 6 MONTHS

It was observed that 1% acerola in emulsion stored at 5°C showed neither antioxidant nor pro-oxidant activities (PF = 1.0) up to 60 d but after this time it started to act as pro-oxidant (Figures 1A and 2). The most effective was 5% willow extract (PF = 6.6; Figure 2) with moderate phenolic content and antioxidant activity expressed as the TEAC, FRAP, and AA_{DPPH} values. Its protective activity was similar to that of 0.01% BHT (PF = 5.6). The long-term abilities of 5% willow extract and BHT to inhibit the peroxide formation (Ip), calculated at the end of storage, were also the highest among all tested antioxidants and amounted to 48.8% and 47.5%, respectively (Figure 3). No significant difference was observed for 1% willow and 5% acerola extracts (PFs were 2.2. and 2.0, respectively, $p > 0.05$), but their ability to inhibit the peroxide formation (Ip) calculated at the end of storage was 31.3% for 5% acerola and 25% for 1% willow. The calculated

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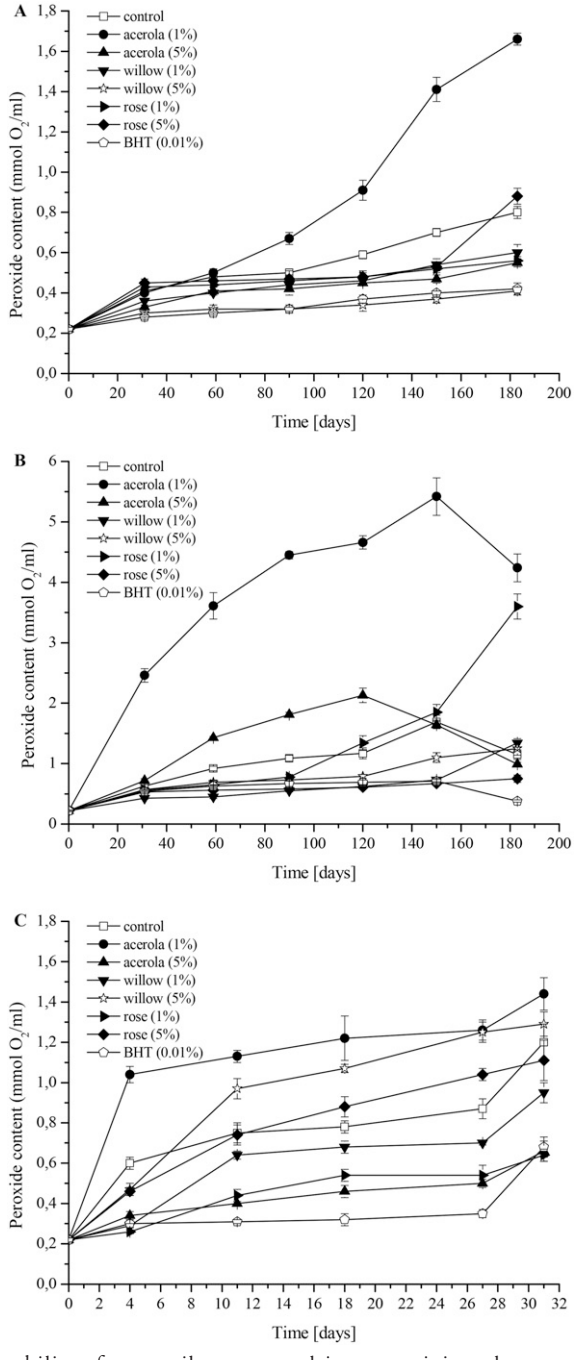

Figure 1. Oxidative stability of argan oil–water emulsions containing plant extracts or BHT stored at (A) 5°C for 6 months, (B) 20°C for 6 months, (C) and 40°C for 4 weeks.

PFs for 1% and 5% rose (1.0) indicated neither antioxidant nor pro-oxidant effect of these extracts, although the Ip value for 1% rose at the end of storage was 30% and for 5% rose was negative, indicating pro-oxidant activity (Figure 3).

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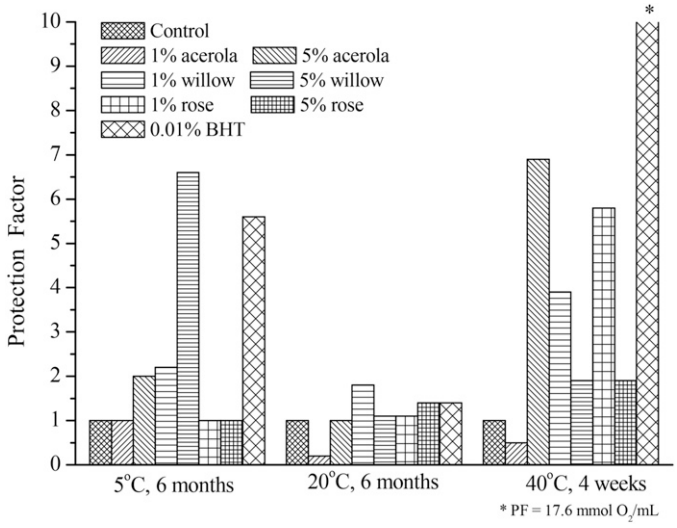

Figure 2. The PF of acerola, willow, rose extracts and BHT in argan oil–water emulsions stored in different conditions.

EFFECT OF ACEROLA, WILLOW, AND ROSE EXTRACTS ON OXIDATIVE STABILITY OF ARGAN OIL–WATER COSMETIC EMULSIONS STORED AT 20°C FOR 6 MONTHS

The most effective antioxidants for emulsions stored at 20°C were 1% willow (PF = 1.8), 5% rose (PF = 1.4), and 0.01% BHT (PF = 1.4), although only 5% rose and BHT inhibited peroxide formation at the end of storage at the levels of 34.8% and 67%, respectively. Willow (5%) and rose (1%) extracts showed low protective activity (PF = 1.1). Moreover, 1% rose was protective up to about 120 d, and after this time it started to act as pro-oxidant (Figure 1B). Willow at 5% also exhibited pro-oxidant activity at the end

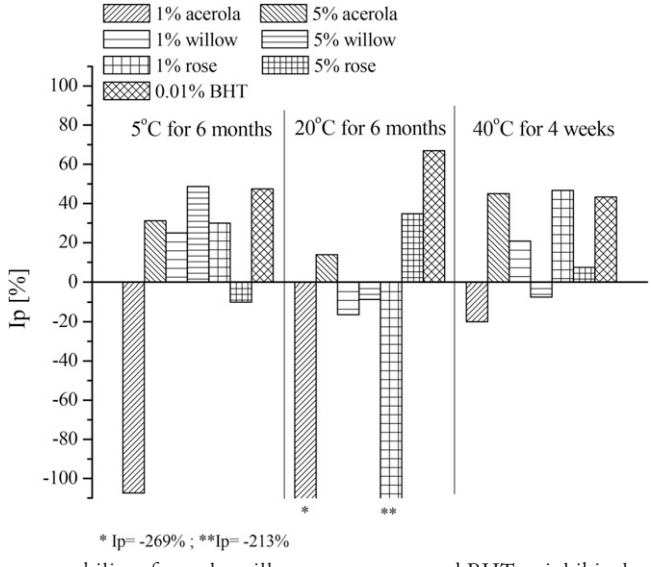

Figure 3. The long-term ability of acerola, willow, rose extracts and BHT to inhibit the peroxide formation (Ip) in argan oil–water emulsions stored in different conditions.

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of storage (Figure 3). Acerola extract, at the concentration of 5%, showed neither antioxidant nor pro-oxidant activity up to about the 30th day of storage (PF = 1.0), but after this time, it reversed its activity to pro-oxidative up to about 150 d (Figure 1B). At the end of storage, its activity was again antioxidative with Ip value about 14% (Figure 3). Pro-oxidant effect during the whole storage time was observed for 1% acerola extract (PF = 0.2 in Figure 2, negative IP value in Figure 3).

Peschel *et al.* (29) described a good antioxidative effect of 1% golden rot and artichoke extracts in oil-in-water emulsion containing evening primrose oil, stored at room temperature for 5 months. The authors observed higher activities of golden rot and artichoke extracts (PV < 0.30 for both) than 0.01% BHT, and pro-oxidant activity of apple extract (PV = 2.75).

EFFECT OF ACEROLA, WILLOW, AND ROSE EXTRACTS ON OXIDATIVE STABILITY OF ARGAN OIL–WATER COSMETIC EMULSIONS STORED AT 40°C FOR 4 WEEKS

At 40°C, pro-oxidant effect was observed for 1% acerola (PF = 0.5) and it was maintained to the end of storage (negative Ip; Figure 3). Antioxidant activity was shown by 5% acerola (PF = 6.9), 1% rose (PF = 5.8), and 1% willow (PF = 3.9), but their PFs were much lower than those calculated for 0.01% BHT (PF = 17.6). The long-term abilities of 5% acerola and 1% rose to inhibit the peroxide formation, calculated at the end of storage, were also the highest among all extracts tested and were 45.0% and 46.7%, respectively (Figure 3). Their activities after 4 w were comparable to the activity of BHT (Ip = 43.3%). Periodic pro-oxidant activity was exhibited by 5% rose and willow extracts (Figure 1C), although their PFs were 1.9, indicating antioxidant activities at the beginning of storage. The highest effectiveness of BHT (expressed as the PF) to protect emulsions stored at 40°C was also observed in the study conducted for emulsions based on evening primrose oil (13) and wheat germ oil (14). Peschel *et al*. (29) found better antioxidant activities of golden rot, apple, and artichoke extracts in comparison with BHT and tocopherol derivatives applied in evening primrose oil-in-water emulsions stored at 40°C for 5 weeks. They reported that 0.1% apple, 1% golden rot, 0.1% and 1% artichoke extracts were better antioxidants than both 0.15% tocopherol derivatives and 0.01% BHT.

COMPARISON OF ACEROLA, WILLOW, AND ROSE EXTRACTS' ANTIOXIDANT ACTIVITIES IN ARGAN OIL–WATER COSMETIC EMULSIONS

In our previous study concerning the effect of plant extracts on the oxidative stability of emulsions based on evening primrose (13) and wheat germ (14) oils, it was found that willow extract at 1% and 5% inhibited the peroxide formation in emulsions stored at 5°C, 20°C, and 40°C. The PF of 5% willow extract in wheat germ oil emulsions stored at 5°C and 20°C was even higher than observed for 0.01% BHT (14). Taking into account the PFs of willow extract in argan oil (Figure 2) and other emulsions, this extract could be recommended in cosmetic creams as effective antioxidant irrespectively of oil used for emulsion preparation.

Acerola extract, exhibiting the highest antioxidant activities in the DPPH, FRAP, and TEAC tests, effectively inhibited the peroxide formation in emulsions stored in all applied conditions but only at the concentration of 5% (Figures 2 and 3). Acerola extract at 1% concentration generally acted as pro-oxidant. Similar effect has already been observed

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for acerola extract in emulsions based on evening primrose (13) and wheat germ (14) oils. All our studies confirmed that preferably 5% acerola extract, not 1% extract, might be applied as an antioxidant in cosmetic emulsions. Some of well-known antioxidants, e.g., ascorbic acid, gallic acid (30), quercetin, rutin, or carnosine, (31) have been reported to exhibit pro-oxidant activity at low concentrations in contrast to antioxidant activity at higher concentrations. Acerola is rich in ascorbic acid, which at lower concentrations may induce lipid hydroperoxide decomposition to free radicals and aldehydes responsible for oxidative degradation of lipids, proteins, and vitamins (32). It may also enhance the catalytic effect of iron and copper acting as pro-oxidants (14,33). In this way, ascorbic acid may contribute to the pro-oxidant activity of acerola extract.

Rose extract, showing the lowest antioxidant activities in the DPPH, FRAP, and TEAC tests, at both concentrations had no protective effect at the beginning of emulsion storage at 5°C (PF = 1.0; Figure 2), but after about 30 d, 1% rose effectively inhibited the peroxide formation in tested emulsion (Figure 1A). At the end of storage, the Ip value calculated for 1% rose was 30%, whereas 5% rose was pro-oxidant (negative Ip value; Figure 3). At the concentration of 5%, at 20°C, it had the same protective factor as BHT, and at the end of storage it was even better antioxidant than 5% willow, and 5% acerola extracts (Ip value was 35%, whereas for 5% acerola—14%, and for 5% willow—negative Ip value indicating pro-oxidant activity). At the concentration of 1%, it started to be pro-oxidant in emulsions stored longer than 120 d (negative Ip value at the end of storage). At 40°C, 5% rose extract exhibited periodic pro-oxidant activity, but at the end of storage, its Ip value was positive (7.5%). Therefore, 1% rose is preferably recommended than 5% one for emulsions stored at higher temperatures. Different results were obtained by the authors in lipid oxidation studies in emulsions based on evening primrose oil (13) and wheat germ oil (14), in which rose extract turned out to be an effective antioxidant at both concentrations.

Synthetic antioxidant BHT inhibited the peroxides formation in all temperature conditions. Its PF value in emulsion stored at 5°C was comparable to PF of 5% willow extract, and lower than 1% willow extract in emulsions stored at 20°C. At the end of storage, its protective activity in emulsion stored at 5°C, measured as Ip value, was similar to 5% willow. It was the most effective antioxidant for long-term stored emulsions at 20°C (Ip = 67%). At 40°C, its Ip value was comparable to those calculated for 5% acerola and 1% rose (Figure 3).

It is also worth to notice that the rate of oxidation of various oils used in our previous (13,14) and present studies was different and was related to the contribution of unsaturated fatty acids in oil. Emulsions based on evening primrose oil, containing about 75.3% of linoleic acid (13), were more susceptible to oxidation than those based on wheat germ (55% of linoleic acid) and argan oil (36% of linoleic acid). Taking into account the increase in the content of peroxides in control samples based on evening primrose oil (about 9.4-fold at 5°C, 30-fold at 20°C, and 75-fold at 40°C), wheat germ oil (about 4-fold at 5°C, 16-fold at 20°C, and 18-fold at 40°C), and argan oil (about 1.9-fold at 5°C, 2.9-fold at 20°C, and 5.5-fold at 40°C) stored for similar time (29–31 d), it was found that the stability of argan oil is much higher than the stability of wheat germ and evening primrose oils.

CONCLUSIONS

Altogether, the results of the present study have revealed that willow and rose extracts could maintain or prolong the oxidative stability of cosmetic emulsions based on argan

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oil, but acerola extract, especially at the concentration of 1%, should not be recommended for emulsions based on this kind of oil.

The antioxidant activity is an important property of commercial plant extracts, which may influence the oxidative stability of cosmetic formulations. Cosmetic emulsions should be tested in a range of temperatures from 5°C to 40°C to be sure that they will be stable during general use by the consumers and that antioxidants do not accelerate oxidative degradation of their oil base. Moreover, plant extracts intended as antioxidant additives in cosmetic emulsions should be tested in emulsions based on different oils.

Taking into account the results of our previous (13,14) and present studies, it can be concluded that some plant extracts may act as pro-oxidants in long-term stored cosmetics. Selection of proper plant antioxidants for cosmetic emulsions is an essential element of cosmetic production but this selection should be related to the oil used as the base of emulsion.

This article does not contain any studies with human or animal subjects.

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