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Final Report

Title of service: Designing of complex processing (Block scheme) of Aronia melanocarpa with application in cosmetics

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Designing of complex processing (Block scheme) of Aronia melanocarpa with application in cosmetics.

Introduction

There are several options of potential use of Aronia melanocarpa raw material:

- a. Dried finely milled pomace
- b. Pomace Extract
- c. Leaf Extract

In terms of effect two alternatives were considered:

- a. Anti-aging agent based on blocking enzymes that cause skin breakdown and elasticity
- b. UV protection factor based on the ability to capture free radicals

Two forms of application

- a. Protective creams
- b. Spray form.

Powder production is not patent protected. The production of the antioxidant concentrate from Aronia was patented by the patent at the Food Research Institute (VUP) but it is already after the protection period.

There are unexpectedly many patents on cosmetic products to be considered.

We will provide a detailed technological procedure for the production of pure antioxidant concentrate with a minimum content of ballast substances (especially sugars) suitable for the preparation of cosmetic products including material and energy balances and proposals of cosmetic formula recipes in case of confirmation of interest in such a solution.

Aronia melanocarpa.

Aronia melanocarpa (black chokeberry) is shown to be a good source of an bioactive compounds mainst polyphenols and food colorants mainst anthocyanin. They can be found mainly in the external layer of the pericarp (the skin). The total anthocyanins content is between 4 – 10 g/kg berries depending on climate condition and the status of maturity at the harvest time. In A. melanocarpa skins were in group anthocyanins identified cyanidin-3-O-galactoside (38.8%), cyanidin-3-O-arabinoside (6.4%), cyanidin-3-O-glucoside (3.6%), cyanidin-3-O-xyloside (0.5%), and the cyanidin aglycon (50.7%); HPLC analysis of anthocyanins reveals the ratio between the components (cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-3-O-glucoside and cyanidin-3-O-xyloside) to be almost constant

during processing. Second colorants in *Aronia melanocarpa* – carotenoids (mainst beta-carotene and beta-cryptoxantene) its content is not bigger than 50 mg per kg of berries.

In berries the major phenolic acids are cinnamic and benzoic acid derivatives. *A. melanocarpa* is a rich source of hydroxycinnamic acid derivatives. They are represented mainly by caffeic acid derivatives, chlorogenic acid and neochlorogenic acid. In *A. melanocarpa* fruit, the content of those acids reaches 301.85 mg/100 g and 290.81 mg/100 g DW (dry weight), respectively.

The phenolic compounds protect plants against adverse factors such as pathogens, physical damage, UV radiation, and other factors.

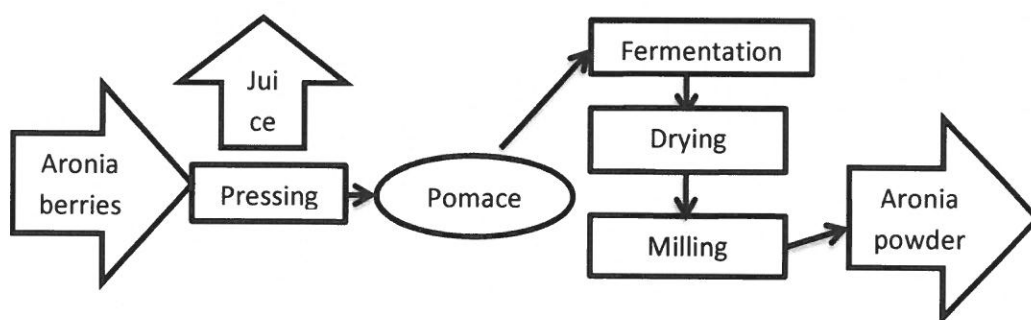
Obtaining concentrates of biologically active substances.

Whole fruits *A. melanocarpa* are for use as food additives and for cosmetic purposes unsuitable especially for the high content of accompanying ballast substances. In particular, they are reducing and alcoholic sugars and organic acids. Therefore, it is advantageous to use in particular the combined processing. Pressing to obtain juice for the beverages production and pomace extracting or drying.

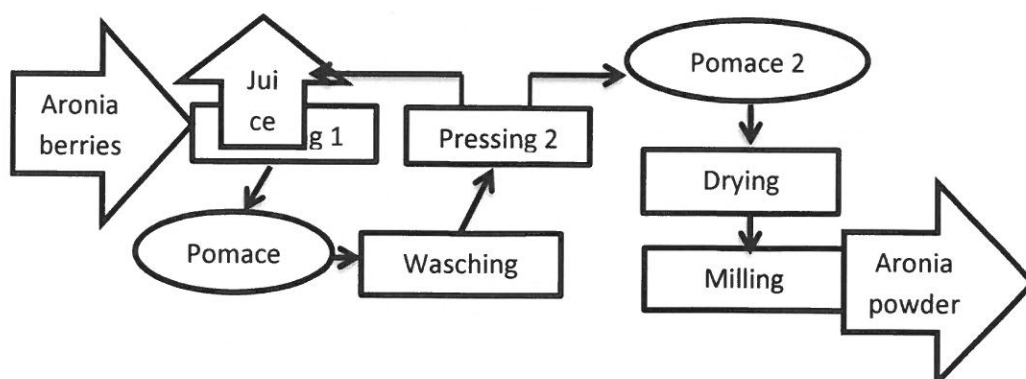
Drying of pomace.

Dried *Aronia* pomace are used in tea blends. But there is also a potential space for applying finely ground pomace to cosmetic creams. A simple 3-step process is offered.

1. Fermentation removal of reducing sugars - mixed semi-dry fermentation after inoculation with a mixture of lactobacilli and yeast
2. Drying - vacuum or in a controlled atmosphere
3. Fine Grinding - The use of modern high-energy grinding for several micrometre particles



The fermentation can be replaced by faster process of washing the pomace with cold water.



The sugar and acid-free material thus obtained will retain antioxidant and antiradical activity and, in cream application has a partial abrasive effect.

Extraction of *Aronia melanocarpa* pomace.

The concentrates of biologically active substances from the fruit marc of *A. melanocarpa* are for the purpose of preparing food additives and cosmetics prepared by extraction. Water, water-alcoholic solutions of C₁ to C₃ alcohols, but also CO₂ in supercritical state, acetone, ethyl acetate, butylene glycol, propylene glycol, dichloromethane and hexane, and mixtures thereof are used as extraction agents. Most commonly, water and 40-80% aqueous ethanol are used.

Extraction times range from a few minutes to several hours and temperatures from 15 ° C to 140 ° C for high pressure extraction into water.

To increase the yields and the extraction rate, the extracted material is milled into smaller particles, intensive mixing, ultrasound, microwave heating or enzymes are used to disrupt plant tissue integrity.

The resulting composition of the extracts strongly depends on the polarity of the extraction agent and the extraction temperature.

For example, a comparison of the composition and antioxidant activity of extracts of *A. Melanocarpa* extracts obtained by extraction into water

- a. SCW - Extraction under supercritical conditions (140 ° C)
- b. UAE - Ultrasonic-assisted extraction
- c. MAE - Microwave-assisted extraction

The results showed that there was a significant difference between the SCW extracts and all other extracts. Namely, total extracted compounds from aronia stems by using this extraction technique was 37.58% which is more than twice higher yield than in case of UAE (15.32%), and 1.6-fold higher than in case of MAE (23%).

Even more significant is the difference in the concentration of total phenolic substances and flavonoids in the extracts obtained, which is shown in the following figure.

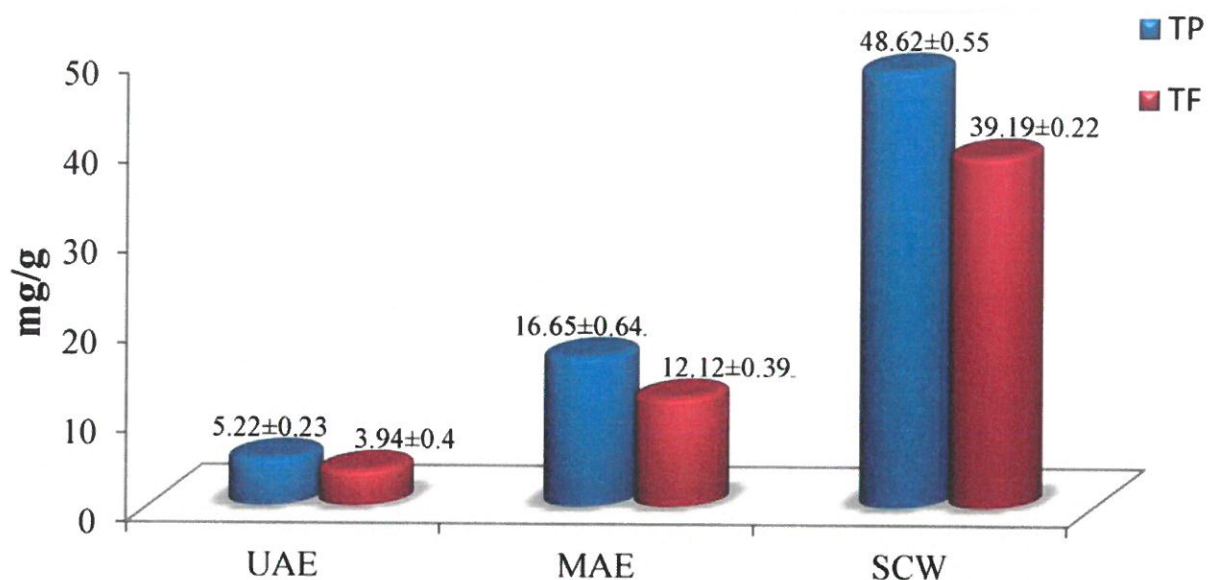


Fig. 1. Total phenolic content (TPC) and total Flavonoid content (TFC) of aronia stems extracts obtained by different extraction techniques. Results are mean values \pm SD from three replicates.

Similar differences exist in the content of individual phenolic compounds as shown in the following table.

Table 1 Phenolic components in aronia stem extracts obtained by different extraction techniques (mg per g of dry extracts).

No	Components	MAE	UAE	SCW
1	Protocatehin acid	nd	nd	nd
2	p-Hydroxybenzoic acid	nd	nd	0.392
3	Caffeic acid	nd	0,001	0,012
4	Vanillic acid	nd	nd	nd
5	Chlorogenic acid	nd	0,007	0,066
6	Syringic acid	nd	0,047	0.223
7	p-coumaric acid	0.079	0.016	0.147
8	Ferulic acid	0.351	0.029	0.200
9	Synapic acid	0.560	0.127	0.617
10	Rutin	4.552	0.738	7.335
11	Luteonin-gly	nd	nd	0.254
12	Apigenin-gly	0.433	nd	nd
13	Rosmarinic acid	0.430	nd	nd
14	Quercetin	1.962	0.021	0.789
15	Luteonin	0.164	0.046	0.368
16	Nidringenin	0.292	0.013	0.168
17	Kaempferol	0.276	0.051	0.286
18	Apigenin	0.278	0.064	0.358

However, minor differences were found in the antioxidant and antiradical activity of the extracts. All three extracts have less peroxiradical inhibitory activity as commonly used synthetic antioxidants. On the contrary, they have comparable antiradical activity as commonly used synthetic antioxidants (Gallic acid, Ascorbic acid, BHT and alfa-tokoferol).

Table 2. Antioxidant capacity of extracts obtained by different extraction techniques and comparison with standard antioxidant compounds. (IC50 (µg/mL))

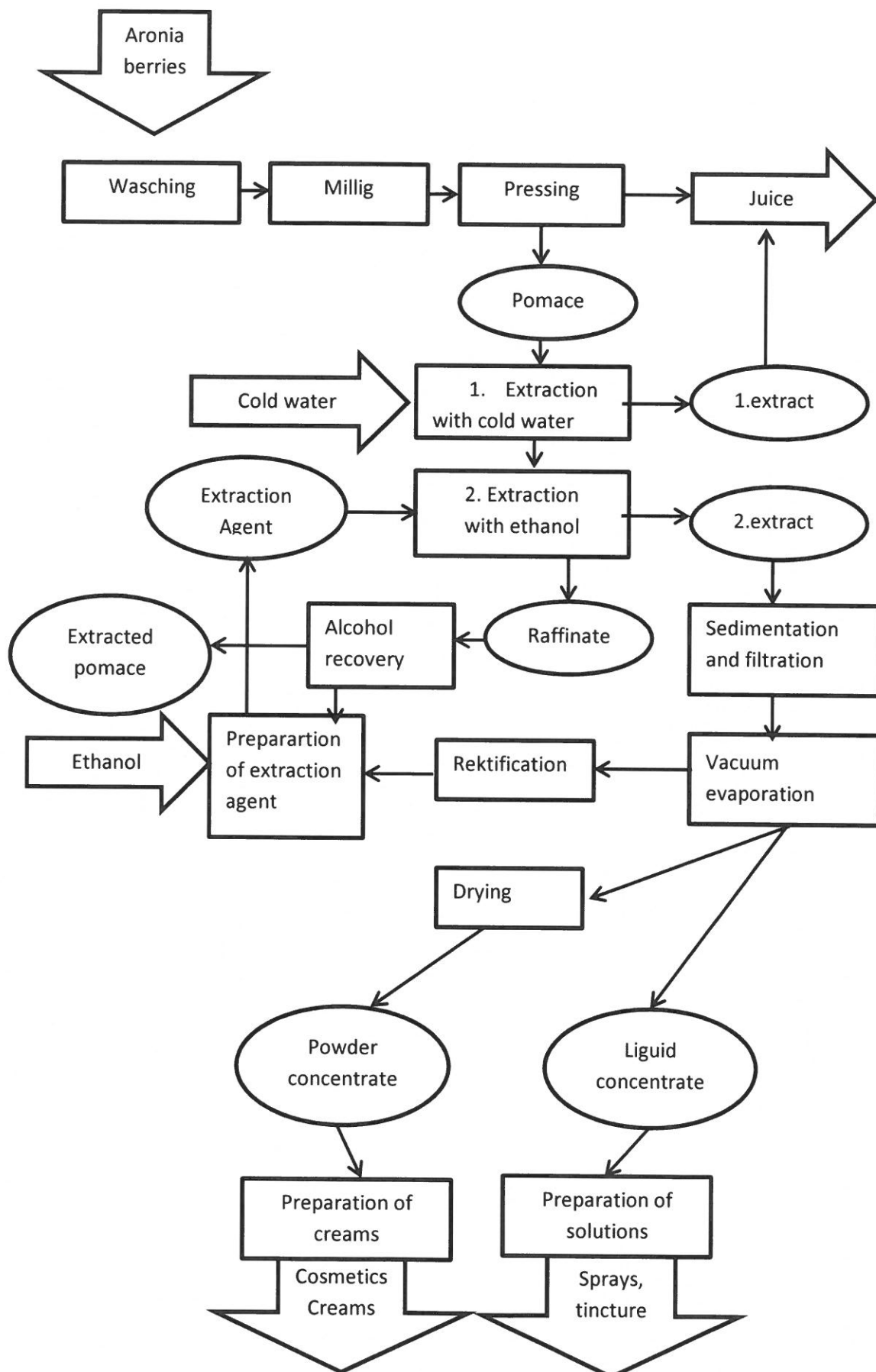
Samples	Inhib. activity lipid peroxid.	Hydroxyl radical scaveng. activity	ABTS radical scavenging assay
UAE	34.57	41.56	10.09
MAE	33.73	40.83	8.44
MAE	30.10	38.61	6.79
Gallic acid	255.43	59.14	1.96
Ascorbic acid	>1000	160.55	10.98
BHT	1.00	33.92	7.23
α-Tocopherol	0.48	-	-

The extracts obtained by conventional methods contain relatively large amounts of accompanying substances and the concentration of anthocyanins on a dry matter basis is only 8-15%. Therefore, combined technologies, using membrane, fermentation and adsorption processes are used to obtain more concentrated extracts. But these are significantly more economical and energy intensive.

A better solution is to rinse the pomace with cold water to remove a large portion of the water soluble reducing and alcohol sugars and low molecular weight organic acids. (Patent Document 282 152, Silhár, S., Kintlerova, A., Fiala, L., Method of Production of High Quality Anthocyanin Concentrates from Pomace of Dark Coloured Fruits.

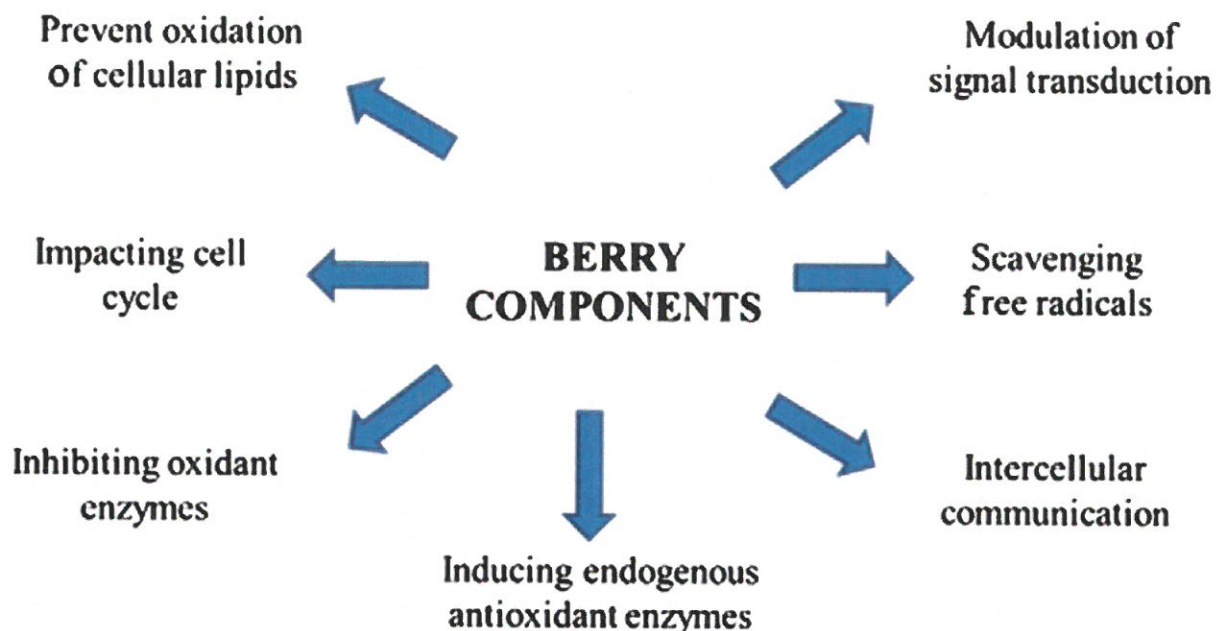
In this way it is possible to prepare concentrates with an anthocyanins content in the range of 40-60% calculated on the dry matter. Such extracts are fully suitable for use in cosmetics.

A block diagram for obtaining Aronia extract suitable for use in cosmetics.



Aronia melanocarpa - health - cosmetics.

The cosmetic effects, in particular the effect on the skin, are based on the antioxidant, antiradical and enzyme blocking effect, especially polyphenolic compounds found in the mature fruits.



It is established that anthocyanins isolated from fruits of *Aronia melanocarpa* markedly inhibited the mutagenic activity of benzo(a)pyrene and 2-amino fluorene in the Ames test. In the Sister Chromatid Exchanges (SCEs) test with human blood-derived lymphocytes cultured in vitro, a significant decrease of SCEs frequency induced by benzo(a)pyrene was observed in the presence of anthocyanins. In the case of mitomycin C the effect of anthocyanins on SCEs frequency was smaller but still noticeable. Anthocyanins markedly inhibited the generation and release of superoxide radicals by human granulocytes. The antimutagenic influence of anthocyanins is exerted mainly by their free-radicals scavenging action as well as by the inhibition of enzymes activating promutagens and converting mutagens to the DNA-reacting derivatives. These preliminary data seem to be important in the aspect of a possible antimutagenic and anticarcinogenic potency of anthocyanins commonly present in fruits and vegetables.

Aronia Melanocarpa extracts are capable of inhibiting one or more extracellular proteases selected from the group of: matrix metalloprotease-1 (MMP-1), matrix metalloprotease-2 (MMP-2), matrix Extract enhanced tyrosinase activity inhibition effect as compared to conventional bleaching component and suppress generation of melanin excellent melanocytes effect metalloprotease-3 (MMP-3), matrix metalloprotease-9 (MMP-9) and human leukocyte elastase (HLE).

Extract is suitable for incorporation into the dermatological formulations. suitable for the treatment or prevention of various dermatological conditions, including the skin antioxidant, collagen synthesis effects, wrinkling or sagging of the skin, irradiation induced skin and/or hair damage, have excellent cell toxicity relaxation effect , deepening of skin lines, elastotic changes in the skin, as well as for the routine care of the skin, hair and/or nails

Further, Aronia Melanocarpa extract provides the acne bacteria having antibiotic activity effective spots, freckles, and skin as well as acne dye causes the expressed anti-whitening effect, such as materials of melanin production inhibition effect of the deposition effect.

Content of Chokeberry extract is preferably based on the total cosmetic composition is a dry weight of 0.001 to 30.0% by weight. When the content of the extract is less than 0.001% by weight, almost no effect of improving the skin or more and 30.0% by weight, because the degree of increasing the effect of the content to increase is minimal and ineffective. Conventionally used in the variety is a 0.01% by weight to 20% by weight based on the total weight of crude hydrous product.

Application forms of the cosmetics are: solutions, suspensions, emulsions, pastes, gels, creams, lotions, powders, soaps, surfactant-containing cleansing, oil, powder foundation, an emulsion, but may be formulated as a foundation, a wax foundation and spray, and similar. In more detail, may be made of flexible, lotion, nutrition lotion, nutrition cream, massage cream, essence, eye cream, cleansing cream, cleansing foam, cleansing water, pack, spray or powder formulation.

When the formulation of a paste, cream or gel, the animal oils, vegetable oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silica, talc or zinc oxide and the like be used as the support component can.

When the formulations is powder or spray, lactose, talc, silica, as the support component of aluminum hydroxide, calcium silicate, or and the polyamide powders may be used, particularly if a spray-in, hydrocarbons in addition chlorofluorocarbons, propane / it may comprise propellants such as butane or dimethyl ether.

When the formulations is a solution or emulsion, the solvent, dissolving agents or emulsifying agent is used as the support component, such as water, ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1, a 3-butyl glycol oil, glycerol aliphatic ester, polyethylene glycol or sorbitan fatty acid ester.

Final Product Recipe Example:

Aronia extract 1 part by weight; and

5 parts by weight of propylene glycol;

8 parts by weight Stearyl alcohol;

2 parts by weight Stearic acid;

4 parts by weight of squalene;

6 parts by weight 2-octyldodecyl alcohol,

3 parts by weight of a polyoxyethylene alcohol esters; and
2 parts by weight Glycerol monostearate;

SLIGHTLY ACIDIC ARONIA NATURAL SOAP

However, the soap on the market existing are the most use of the surfactants in the synthetic petroleum-based. Surfactants with the ability to churn adsorbed at the interface well with bubble and fall when the discarded excessive eliminate to Fiji and moisturizing factor remains on the surface of the skin and pores to help skin health makes dry skin to promote skin aging and, it's combined with other chemicals, synthetic carcinogens nitrosamines which may have a significant effect on the human body. As such surfactant may hold the possibility to act as a factor that leads to cell death and other diseases.

On the other hand, acne is mainly formed by young men and women face and chest, a chronic disease of the mother sebaceous glands that occurs in the neck, cotton (comedone), papular (papule), pustules (pustule), cysts or nodules, such as puberty age If severe a disease that can leave a scar. Severe skin damage caused by acne, thereby jugido mental damage such as depression or interpersonal avoided.

A method that is generally used for the acne treatment method using the drug, by using the salicylic acid is retinoic acid (retinoic acid) (salicylic acid) or vitamin A derivatives agents suppressing the formation of the pores and dead skin, remove them smoothly secretion by the treatment of acne, or benzoyl peroxide (benzoyl peroxide), tree Black mountain (triclosan), sulphur (sulfur), or Tea tree Oil use (tea tree oil) formulation removes skin keratin and acne through antibacterial this method has been used to alleviate.

Production method of a natural soap comprising the following steps:

(1) a juice Chokeberry fruit to prepare a Chokeberry juice mixture;

Juice can be used an ordinary juice, it may preferably have a juice by squeezing. Juice solution can be kept refrigerated until used.

(2) Preparing a hot-water extract from a sprout Chokeberry 2 Chokeberry sprouts;

Chokeberry available 3 ~ 5cm size sprouting from the trees, and prepare the sprouts harvested and washed several times with clean water. Hot-water extract may be prepared by heating by putting the normal Chokeberry sprout by weight of water of between 5 and 10 times.

(3) mixing the soap liquid and juice Chokeberry, Chokeberry sprout hot water extract to the base;

Soap base, Chokeberry juice solution and Chokeberry sprout mixing amount of the hot-water extract may be with respect to 100 parts by weight of the soap base Chokeberry juice mixture 5-15 parts by weight of hot-water extract Chokeberry sprouts 1 to 5 parts by weight. In a preferred embodiment, the conventional soap blends of Chokeberry juice solution and 180ml

Chokeberry sprout extract 60ml hot water to the base 2kg. Soap base means a pure fatty acid soda produced by the reaction of maintenance and caustic soda. The soap base may be used in powder form using as lump or pulverized.

(4) removing the water by stirring and heating; And

In a preferred embodiment, it is possible to stir and heated to remove water for 3 to 5 hours at 70 ~ 100 °C.

Necessary step in the process is to be accompanied by the stirring continued for heating. The agitation may be used with a mechanical stirrer (stirrer), it is also possible to heat while slowly stirring manually.

(5) injecting a mold for molding and molding the guthyeoseo soap at room temperature.

The step 5 is a step to harden the mixture of the liquid obtained in step (4) at room temperature by injecting a predetermined amount in a specific forming mold to complete the production of natural soap. Usually circular, and using a mold, such as squares, triangles, polygons, volume specified is 80 ~ 120g are preferred.

The natural soap is shown a conventional low pH 6 ~ 7 than pH 8 ~ 9 with an acidity of natural soap has a hypoallergenic properties to the skin. Natural soap of the present invention is suitable to be expressed as well as the skin problems improved, pore shrinkage, and whitening effect as weak acid used as a cosmetic soap.



Cosmetic composition, for treating human hair.

Extract from A. Melanocarpa to improve the finishing agent, to improve gloss, to improve the moisture balance, as well as for protection from the destructuring in particular by UV radiation and especially to protect against oxidative damage and to maintain growth of keratin fibers, in particular human hair should be provided in particular that can be used in combination with oxidizing agents. It is therefore an object of the present invention to reduce the side effects of environmental influences described above and oxidative hair treatments preferably already during the oxidative hair treatment but also after the oxidative treatment of hair, without the efficiency of the oxidative cosmetic, in particular in terms of color intensity, color fastness, lightening or Well deteriorating effect.

The extract is preferably used in a cosmetic carrier. The cosmetic carrier can be in particular aqueous or aqueous-alcoholic. An aqueous cosmetic carrier comprises at least 50 wt .-% water. Aqueous-alcoholic cosmetic carriers aqueous solutions comprising 3 to 70 wt .-% of a C₁-C₆ alcohol, The compositions may additionally contain further organic solvents, such as methoxybutanol, benzyl alcohol, ethyl diglycol or 1,2-propylene glycol. All water-soluble organic solvents are preferred.

The cosmetic compositions contain extract preferably in an amount of 0.05 to 2.5 wt .-%, each based on the weight of the ready agent.

Furthermore, it has been shown that the effect of the extract can be considerably increased if further comprises a vitamin of the B series is used together with an extract in a cosmetic carrier. (pantothenic acid, panthenol and pantolactone of 0.1-5 wt .-% are preferred)

The compositions comprise with preference other ingredients. These are, for example, with particular preference Esteröle (esters of C₆-C₃₀ fatty acids with C₂-C₃₀ fatty alcohols, monoester of the fatty acids with alcohols having from 2 to 24 carbon atoms)