Augmentation of the bioactive potency of natural ingredients by fermentation yielding added benefits of prebiotics and post biotics to maintain the skin microbiome balance

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Abstract

Background

Cosmetics that contain probiotic ingredients are rapidly gaining popularity around the world as consumers started associating pre-, pro-, and postbiotic ingredients with safety, health, wellness, and sustainability. Fermentation is a sustainable way of processing biological substrates, adding valued benefits. The study focuses on the augmentation of the bioactive content of the natural extracts by fermentation.

Methods

The free radical scavenging activity was determined by DPPH assay. Total phenolic contents were estimated using the Folin-Ciocalteu reagent. The skin hydration efficacy was evaluated based on capacitance measurement of a stratum corneum, by corneometer.

Results

The study demonstrated a substantial increase in the antioxidant potential and bioactive content of the fermented substrates. The enhancement is almost two-fold in the case of carrot roots and soybeans whereas it is 30% in the case of radish roots. Rice water fermentation showed 40% improvement and onion ferment demonstrated the most outstanding result where 88% augmentation was achieved. The total phenolics were found to be increased by 75%. The ferment extracts proved to retain the skin hydration even after 120 minutes in comparison with the conventional extracts. Fermentation extraction also indicated the occurrence of prebiotics and postbiotics in the medium.

Conclusion

The study discloses a new and natural ingredient portfolio containing carrot, radish, soy, rice, and onion ferment filtrates having improved bioactive content with added skin microbiome benefits of pre and postbiotics. It also provides novel perspectives on the naturally processed ingredients for skin microbiome-conscious skin and scalp care.

Keywords: fermentation; prebiotics; postbiotics; skin microbiome

Introduction

Beauty habits have changed considerably over the years, with many consumers swapping their customary cosmetics for a simpler and more holistic regime aiming to safeguard the skin from environmental factors giving it the opportunity to restore and strengthen. The industry is witnessing the boom in skin microbiome-friendly ingredients and the interest in fermented ingredients is expanding. On the other hand, the release of cosmetic debris, and cosmetic discharges in the domestic sewage leads to serious concern about human health. Some cosmetic constituents are even considered severe environmental pollutants [1]. Thus, effective and safe alternatives for the development of future cosmetics are necessary. The food-based bioactive principles may provide a possible way to advance cosmetic research and its market. Recently, scientific reports are revealing the potential of fermented plant beverages and fermented plant extracts in cosmetic fields. Phytochemicals are known for several pharmacological and cosmeceutical applications. The fermentation process improved the quality of the active phytochemicals and also facilitates their easy absorption by the human system. Recently, several research groups are working on the cosmeceutical importance of fermented plant extracts, particularly on the anti-aging, anti-wrinkle, and whitening properties [2-5].

The human skin microbiome has recently become a focus for both the dermatological and cosmetic fields. Understanding the skin microbiota, that is the collection of vital microorganisms living on our skin, and how to maintain its delicate balance is an essential step to gaining insight into the mechanisms responsible for healthy skin and its appearance. Imbalances in the skin microbiota composition (dysbiosis) are associated with several skin conditions, either pathological such as eczema acne, allergies, or dandruff, or non-pathological such as sensitive skin, irritated skin, or dry skin [6]. Therefore, the development of approaches that preserve or restore the natural, individual balance of the microbiota represents a novel target not only for dermatologists but also for skin care applications.

Biotics are the newest ingredients in skincare based on normalizing the microbiome to achieve skin health. Prebiotic skincare products are based on the plant sugars, oils, peptides, etc. that are able to provide nutrition for bacteria on the skin surface, which constitute the microbiome. Thus, prebiotic skin care products contain botanically-based sugars and oils that provide bacterial nutrition. Postbiotics are produced during the fermentation process of probiotic bacteria. Examples of postbiotics include enzymes, peptides, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids. These are not new to dermatology as many currently marketed skin care products contain bacterial fermentation products such as lactic acid and glycerol. The therapeutic value of these ingredients is well established. There are numerous studies providing evidence of the benefits of specific probiotic strains for skin health [7-9]. In addition, the mechanisms of anti-aging suggest strains can help to regulate pH, reduce oxidative stress, protect from photoaging, and improve the skin barrier function [10].

During fermentation, the microbial metabolism enhances the macromolecules' digestibility and improves the bioavailability of macro/ micronutrients and phytochemicals. For the removal of antinutrients, allergens and toxins, fermentation is considered as one of the most effective processing methods [11]. Fermentation also improves the mineral bioavailability by producing a phytase enzyme that degrades the phytic acids in plant foods. Such a phytic acid reduction may enhance the level of calcium, iron, and zinc several-fold [12]. Fermentation is used to improve the bioavailability of minerals that could be further utilized to enhance the level of micronutrients in plant-based extracts. The ferment filtrates better known as 'Bioferments' are augmented extracts with improved bioactive delivery, oxygen uptake, moisturization, and reduced skin irritation. The process of fermentation can phenomenally increase the potency and bioavailability of the original bio substrates. The study portfolio includes *Lactobacillus*/Carrot root ferment filtrate, *Lactobacillus*/Radish root ferment filtrate, *Bacillus*/Soybean ferment filtrate, *Bacillus*/ Rice ferment filtrate, *Saccharomyces*/Coconut oil/Onion bulb ferment filtrate which are branded as Kopcarotol, Kopraditol, Kopsoyatol, Kopryza, and Kopalleum respectively. The research facts and figures substantiate the augmentation of the bioactive content by the process of fermentation.

Material and Methods

Fermentation

The solid substrates were grated sliced or soaked before sterilization to facilitate maximum surface area for the microorganisms to act upon. An optimized size of inoculum was then transferred to the sterile substrates and was fermented in solid state for 15-20 days. Further the fermentation mixture was subject to filtration separating the spent biomass. The ferment filtrate obtained was sampled for the augmentation studies. The microorganisms were screened from the wild and the selected strains were identified by 16SrRNA sequencing.

DPPH radical scavenging assay

The free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Blois [13] and Desmarchelier et al. [14]. The hydrogen atom donating ability of the test samples was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/ purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of sample in methanol at different concentrations (12.5–150 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as a reference. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = (A0 - A1)/A0 - \times 100$$

where A0 is the absorbance of the control, and A1 is the absorbance of the extracts/standard. Then % of inhibition was plotted against concentration.

Determination of total phenolics

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method described by Wolfe et al. [15]. An aliquot of the extract was mixed with 2 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 2 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 20 min at 25 °C for color development. Absorbance was then measured at a 760 nm UV-spectrophotometer. Salicylic acid was used as the reference standard.

Skin Moisturisation

The moisturization measurements were carried out by Corneometer CM 825. The measurements were based on capacitance measurement of a dielectric medium, here the stratum corneum, the uppermost layer of the skin. With increasing hydration, its dielectric properties change. The measurement was with respect to the fact that water has a higher dielectric constant (81) than most other substances (mainly < 7).

Gold tracks on top of the probe head, separated from the skin by a glass lamina, build up an electric field between the tracks with alternating attraction. One track builds up a surplus of electrons (minus charge) the other a lack of electrons (plus charge). When put on the skin surface, the scatter field penetrates the very first layer of the skin.

The Corneometer® CM 825 measures the change in the dielectric constant due to skin surface hydration changing the capacitance of a precision capacitor. The measurement can detect even the slightest changes in the hydration level. Unlike the impedance measurement, no galvanic relation between the device and the measuring object and no polarization effects exists.

The test subjects' sites were marked as 5x5 cm squares as a study area where the probes were positioned for the readings which got recorded on the computer. The readings were recorded at 10 minutes and 120 minutes after the application of the test samples.

Results

All the substrates in the portfolio were subject to the radical scavenging activity assay with and without fermentation to evaluate the enhancement in the bioactive content. The carrot roots, radish roots, and soybeans were fermented with *Lactobacilli* and *Bacilli* and the radical scavenging assay results are as below:

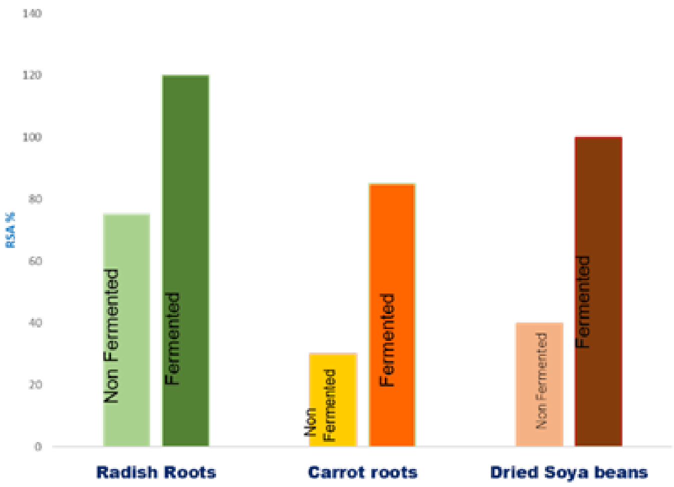

Fig 1. The radical scavenging activity of fermented and nonfermented substrates.

The nonfermented extracts of carrots and soybeans in the market were also compared to the fermented filtrates for the antioxidant content estimated as Ascorbic acid equivalents which are tabulated as below:

Table 1. Bioactive content of various market extracts in comparison with Ferment filtrates

No.	Extracts	Ascorbic acid equivalents (µg/ml)
1	*Daucus carota*, Carrot liquid extract	79.2
2	*Daucus carota*, Carrot liquid extract Glycerin Base	35.2
3	*Daucus carota*, Carrot liquid extract[100% PG]	122.0
4	Soya liquid extract	38.0
5	Soya liquid extract Glycerin Base	88.0
6	Soya liquid extract [100% PG]	71.2
7	Radish liquid extract	60.0
8	Carrot liquid extract	36.8
9	Carrot ferment filtrate	376.8
10	Soybean ferment filtrate	473.3

Rice water also was compared between the fermented and the nonfermented as represented in Fig. 2. The black Kavuni rice, a rare variety of rice in South India was chosen for fermentation.

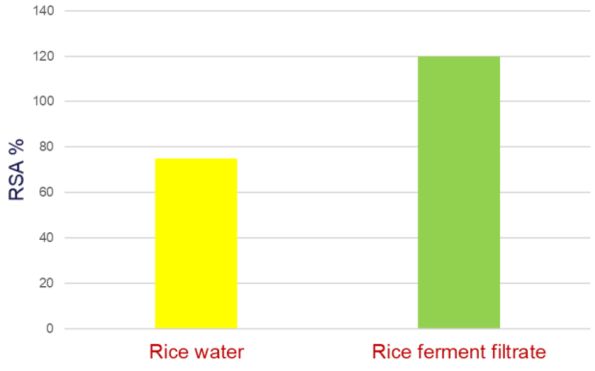

Fig 2.. The radical scavenging activity of fermented and Rice water

Red onions are a good substrate for fermentation augmentation but the high sulfur content generates a very pungent smell. To minimize the odor coconut oil was used as the medium for fermentation using *Saccharomyces* and the results obtained is as below: (Fig.3., Fig.4.)

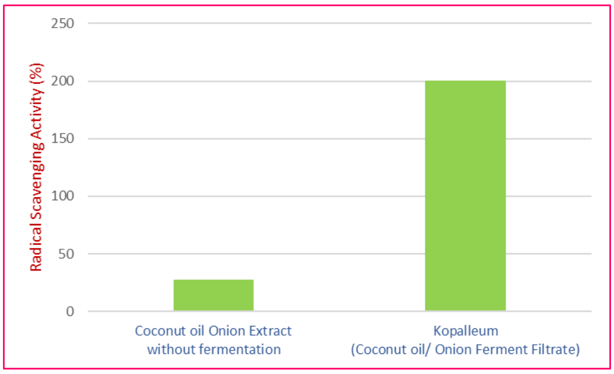

Fig.3 The radical scavenging activity of fermented and non-fermented onions in coconut oil

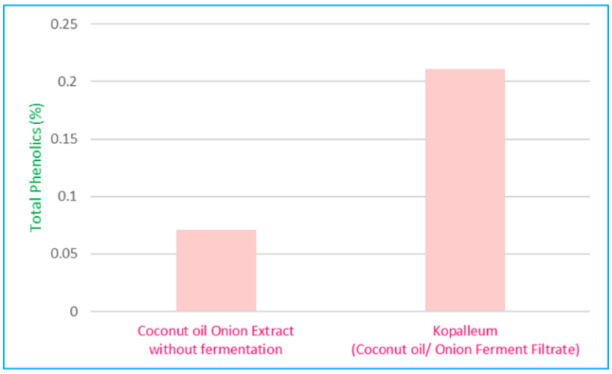

Fig.4. The total phenolics in onion extract with and without fermentation

The skin hydration readings of ferment filtrates in comparison with the nonfermented extracts are represented in the graphs Fig.5., Fig.6.

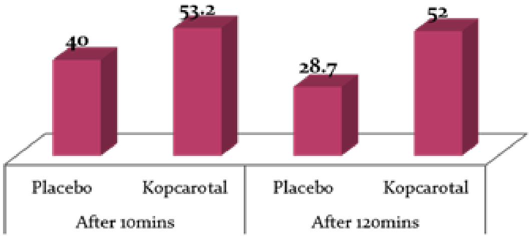

Fig.5 Skin hydration after the application of Carrot ferment filtrate (Kopcarotol) cream

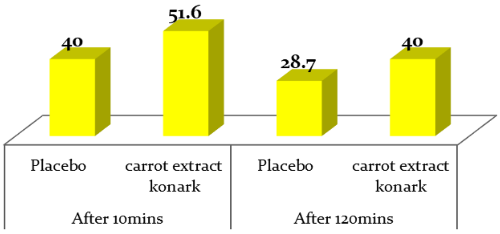

Fig.6. Skin hydration after application of Carrot extract cream (nonfermented)

Discussion

Human skin is naked and is constantly and directly exposed to the air, solar radiation, other environmental pollutants, or other mechanical and chemical insults, which are capable of inducing the generation of free radicals as well as reactive oxygen species (ROS) of our own metabolism. A free radical can be defined as a chemical species possessing an unpaired electron [16]. It can also be considered as a fragment of a molecule. Free radicals, important for living organisms, include hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$), nitric oxide ($NO^{\cdot}$), thyl ($RS^{\cdot}$), and peroxyl ($RO_2^{\cdot}$). Peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and ozone (O_3) are not free radicals but can easily lead to free radical reactions in living organisms. The term reactive oxygen species (ROS) is often used to include not only free radicals but also the nonradicals ($^1O_2$, $ONOO^-$, H_2O_2, and O_3). Reactive oxygen species are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function or excessive quantities, state called oxidative stress. Oxidative stress is the term referring to the imbalance between generating of ROS and the activity of the antioxidant defence [17]. Severe oxidative stress can cause cell damage and death. Antioxidants function by slowing or preventing the oxidation of the molecules by donating hydrogen to prevent the free radical formation or by neutralizing existing ones.

Radish, carrot, and soybeans are rich sources of natural antioxidants which are well proven for skin protection and nourishment. Like many other colored vegetables, carrots is a gold mine of antioxidants. The presence of a high concentration of antioxidant carotenoids especially β-carotene may account for the biological and medicinal properties of carrots [18]. Soy isoflavone exhibits biological activities similar to synthetic estrogen because it is a heterocyclic phenolic compound. The disadvantage of most topical ingredients based on isoflavone is that they contain biologically inactive glycoside forms, which must be converted to a readily absorbed aglycone for the topical application. Fermented soybean extract is reported to enhance skin absorption with proven effects compared to estrogen application [19]. Further, controlled fermentations are being used for ages to enhance the nutritional value of various products. This fermentation idea was tried on radish roots, carrot roots, and soya beans, and antioxidant activity was studied as a measure of increased skin nourishment potential of these natural antioxidants.

The antioxidant activity or the radicle scavenging activity of the fermented and nonfermented substrates as presented in **Fig.1**. substantiates the augmentation in the activity by virtue of fermentation. The enhancement is almost two-fold in the case of carrot roots and soybeans whereas it is 30% in the case of radish roots. The mobilization of the hardcore active contents released during fermentation is because of the action of a variety of enzymes produced during fermentation.

Active fermentation is another indication that the prebiotics and postbiotics have also been added to the soup during the process. The human skin is one of the largest organs of the body corresponding to a surface of 2 m^2 which extends to approximately 25 m^2 considering the plethora of hair follicles and sweat ducts [20,21]. This huge surface area is heterogeneous across the body, and it is continuously exposed to the external environment and has many vital functions. Skin acts as a physical, chemical, immunological, radiation and free radical barrier. Its main function is to maintain homeostasis by preventing water and extracellular fluid loss (permeability barrier), by keeping a constant body temperature through the perspiration process and by protecting the body from infection and toxic substances [22,23]. In addition, the skin harbors immune cells and is inhabited by billions of resident commensal microorganisms which constitute the so-called skin microbiome. Therefore, the development of approaches that preserve or restore the natural, individual balance of the microbiota represents a novel target not only for dermatologists but also for skin care applications. The increased bioactive content and the pre and postbiotics in the filtrate make it a superpower extract compared to the convention extracts available in the market from the same substrates. The tabulated data in **Table 1**. corroborates the hypothesis.

Rice water can undergo spontaneous fermentation and is an age-old practice in Asian countries for health as well as skin and hair benefits. The skin healing benefits of rice have been known for centuries. Rice (*Oryza sativa*) water is a food processing waste that can potentially be incorporated into cosmetic formulations. Rice water was previously evaluated in terms of physicochemical composition and in terms of in vitro biological antioxidant activity and elastase inhibitory effect. Rice water was incorporated into a hydrogel and the developed formulation was subjected to pharmacy technical tests such as pH and viscosity. Biological and sensory effects were evaluated on a panel of 12 volunteers for 28 days. The safety evaluation study was performed on rice water gel, using the Human Repeat Insult Patch test protocol. Rice water presented in vitro biological antioxidant activity and elastase inhibitory effect. The gel formulation containing 96% rice water was biocompatible with the human skin and presented suitable cosmetic properties [24]. Rice water should be thus considered as an anti-aging ingredient to be used as raw material for skincare applications. In the present study, controlled fermentation with specific bacteria improved the potency of rice water as substantiated in **Fig. 2** where a 40% improvement in the radicle scavenging activity has been demonstrated.

Onion (*Allium cepa*) recognized with healing qualities include their antibacterial, cleansing, stimulating, and nourishing powers. Onions contain a number of important minerals and vitamins, such as vitamins C and B6, calcium, magnesium, potassium, and germanium. Onion also has high sulphur content. Sulphur is a mineral present in every cell in our body, with its greatest concentration in hair, skin and nails. It has often been called the “beauty mineral” and the “healing mineral” because of its ability to promote circulation and decrease inflammation. These qualities also lend to the theory that adequate amounts of sulphur can jump-start hair growth in people with deficiencies. High amounts of sulphur in onions make them particularly effective in regenerating hair follicles and stimulating hair regrowth. In addition, naturally-concentrated sulphur compounds have been proven to show additional hair-restoring [25]. Onion is a tough substrate to ferment with high content of phenolics and sulfur. Hence coconut oil was chosen as the medium to ferment and the microorganism was yeast, unlike the other substrates where bacteria was facilitating the fermentation. The results as presented in Fig.3. are outstanding achieving almost 88% augmentation in fermentation. In addition to the antioxidant activity, the total phenolics was also estimated post-fermentation and was found to be increased by 75% as presented in **Fig.4.**

Skin hydration is another attribute which improves by the process of fermentation. There is an interesting relationship between the skin and the fermentation of lactic acid bacteria . Supernatants of these bacteria contain lactate and amino acids, which contribute to the hydration of the skin. Many cosmetic ingredients have been developed using milk fermented by *Streptococcus thermophilus* has skin hydration, antioxidative, and pH control effects. Moreover, the cell protective effect of this ingredient has been proven in recent research. Aloe vera fermented by *Lactobacillus plantarum*, which was selected from 119 strains of lactic acid bacteria, possesses a fourfold greater skin hydration effect than nonfermented aloe vera juice. Soybean milk fermented by *Bifidobacterium breve* has the potential to enhance hyaluronic acid production in the three-dimensional culture of human cells. *S. thermophilus* YIT 2084 was proven able to produce hyaluronic acid [26]. It is believed that the technology introduced here will be useful for the development of next-generation cosmetic ingredients. The results in the present study also confirm these findings as the ferment extracts are proved to retain the skin hydration even after 120 minutes of skin hydration in comparison with the conventional extracts as depicted in **Fig. 5 and Fig.6.**

Conclusion

The cosmetic industry is always very dynamic and vibrant with novel ingredients and formulations continuously being introduced to reciprocate the changing consumer interests and demands. Modern consumers are pickier of their cosmetic stuff and are eager about the ingredients in them to secure them from the harmful and the toxic. So, cosmetic scientists worldwide are in an urge to screen more and more natural ingredients with potent and proven benefits. It has been scientifically proved that the fermentation process improves the phytochemical content and its effective absorption has been authenticated. Thus, Bioferments, the ferment filtrates of natural substrates are most likely to fuel a mega trend in the cosmetic arena and could be the possible hope for the sustainable development of cosmetic products for skin microbiome and eco-conscious consumers.

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Conflict of Interest

All authors declare that there is no conflict of interest.

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