Valorization of fruits and vegetables coproducts to produce natural organic active waters for cosmetic formulation

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Abstract

Waterless formulas become more and more attractive to consumers who see water as a useless ingredient, without activity on the skin. Formulators are thus looking for sustainable alternatives. Solid cosmetics become popular because they do not contain water. But, when considering the energy used to dry all the powders, the environmental imprint is not that good. On the other hand, food waste is a global issue, causing hunger in poor countries while 1/3 of total food grown globally is wasted, representing 1.3 billion tons per year. In addition, 8 to 10 % of global greenhouse gas emissions out of a total of 24 % emitted by agri-food activities on a global scale would be caused by uneaten food. There is an emergency in valorizing this food waste to decrease the global carbon imprint.

To answer those two topics, thanks to its patented enzymatic extraction eco-process, Biolie has developed vegetable waters, natural aqueous active extracts from organic fruits and vegetable coproducts to substitute water in cosmetic formulations.

Biolie has applied its patented enzymatic extraction eco-process to organic fruits and vegetables coproduct. Vegan, food-grade, non-GMO enzymes digest the grinded plants, open the cells and release the water and molecules of interest under controlled temperature.

A first extraction study has been performed to compare extraction with or without enzymes. In this study, fruits or vegetables are grinded and poured into a reactor with or without enzymes. The mixture is stirred for 4 hours at 40-50 °C. After extraction, the content of the reactor is centrifuged and the aqueous fraction is filtered and analyzed. Then, activity studies have been performed on vegetable waters to prove their antioxidant activity and microbiota friendliness.

First, vegetable waters have been compared to Vitamin C with DPPH method to evaluate their antioxidant properties. Then, vegetable waters have been tested on face skin microbiota. Skin microbiota of 44 healthy participants with normal skin has been analyzed to identify the most representative species of bacteria on the face skin. On this basis, a model of microbiota composed of 3 genera (*Cutibacterium, Statphylococcus* and *Corynebacterium*) and 15 species was developed, allowing to predict the impact of a compound on the bacterial populations present on the skin. In this study, each bacteria type has been cultivated individually with different concentrations of vegetable waters and compared to control (cultivated in water). After 24, 48 and 72 h, the effect of vegetable waters on bacteria is compared to control to confirm if the ingredient has:

- A positive effect on the bacteria population (increasing its number)
- No effect on the bacteria population
- A negative effect on the bacteria population (decreasing its number)

The extraction study showed that the addition of enzymes decreases the amount of solid residue (by 55 % for zucchini extraction), increases the amount of aqueous extract and improves the quantity of active molecules in the aqueous fraction (increase in dry matter, amount of polyphenols and sugars, from 30 to 50 % for zucchini extraction). DPPH test showed similar activities between vegetable waters and Vitamin C. At a dose of 25 %, pear extract inhibits 86 % of free radicals, protecting the skin from environmental pollution. After 24 h, at a dose of 20 to 40 %, cucumber and zucchini extracts had a positive or no effect on the bacteria populations, showing a prebiotic or microbiota friendly activity for face skin.

Fruits and vegetables waters are extracted from food and/or agriculture coproducts, valorizing those raw materials, improving farmer's income and decreasing food waste and carbon imprint. With enzymatic extraction, bound water from inside the cells is released with all the natural surrounding nutrients. All hydrophilic molecules are extracted together at the same time, in only one step, saving time and energy and increasing the extraction yield to valorize as much raw material as possible and improve coproduct valorization. Extraction solid residues are valorized into in-house compost to avoid carbon imprint on transporting and transforming those "wastes".

In a biorefinery concept, Biolie is valorizing agriculture and food coproducts to produce cosmetic ingredients. Vegetable waters are multifunctional ingredients that can be used from 20 to 80 % in formulations to replace water and bring activities. Vegetable waters are organic certified sustainable ingredients in the trend of upcycling and clean beauty.

Keywords

Vegetable waters; enzymatic extraction; microbiota friendly; upcycling

Highlights

- Yields of the aqueous fraction are increased by enzymatic hydrolysis compared to soaking
- Vegetable waters offers profile of promising activities for skin- and microbiota-care
- Environmental impacts of their production are consistent and in compliance with sustainability

1. Introduction

Because of climate change and resource shortage, industries must adapt their production models to this new paradigm. One of the levers of action consists in reducing environmental impacts and requirement in key resources such as water usage or arable land surfaces for purposes other than food. The cosmetic industry is often a precursor in new approaches to satisfy consumer needs and expectations. Many attempts are made to improve environmental impacts of cosmetics. Among the actual trends, specific efforts are made in sustainability of vegetal sourcing and cosmetic formulas as well as the environmental impacts of production and packaging.

In this context, waterless formulas become more and more attractive to consumers who see water as a critical resource but paradoxically also as a useless ingredient, without activity on the skin. Formulators are thus looking for sustainable alternatives. Solid cosmetics become popular because they do not contain water. As vegetables contains high amounts of water, among the solutions, are emerging fruit water extracts. Beside avoiding the use of water in formulas, these extracts, as soon as they are organic certified, also facilitate to obtain organic certifications [1]. However, in the current context, the production of fruits to produce these fruit waters needs high amounts of water itself. The overall production cycle thus just leads to a counterproductive use of water and arable land surfaces.

On the other hand, food waste is a global issue, causing hunger in poor countries while 1/3 of total food grown globally is wasted, representing 1.3 billion tons per year [2]. In addition, 8 to 10 % of global greenhouse gas emissions out of a total of 24 % emitted by agri-food activities on a global scale would be caused by uneaten food [3]. There is thus an emergency in valorizing this food waste to decrease the global carbon imprint.

To answer those two topics, Biolie has developed organic certified vegetable waters [4], natural aqueous active extracts from organic zucchini, pear and cucumber coproducts to substitute water in cosmetic formulations.

Concomitantly, a consistent strategy must be implemented to consider the whole value chain of cosmetics. Therefore, attention must also be paid to the extraction process and the extract usage. With these objectives in mind, Biolie has applied its patented enzymatic assisted aqueous extraction (EAE) technology to obtain active vegetable waters. Indeed, implementing enzymatic processes in place of conventional processes generally results in a reduced contribution of global warming and contribution to acidification, eutrophication and a reduction of pollutant emission by up to 90 % and energy use for more than 60 % [5], [6].

Furthermore, pear (*Pyrus communis*) is known to have high antioxidant and anti-tyrosinase effects [7], [8]. Similarly, beside their nutritional composition, cucumber and zucchini extracts were able to inhibit hyaluronidase and elastase, two key enzymes of skin aging [9]–[11].

Food and cosmetic trends are very often related. Consumers see cosmetics as “food for skin” directly applied on the substrate. Cosmetics are thus expected to bring moisture and nutrients to the stratum corneum while eaten food brings water and nutrients in the whole body. During the last two years, the cosmetic industry has again adopted a “food industry” trend, looking for products able to protect the skin microbiota, which plays a very important role in the protection of the skin against pollution and environmental aggression [12]–[14].

Therefore, the vegetable waters obtained with the EAE approach from food by-products were investigated for their potential in skincare and particularly their activity on collagenase, tyrosinase, DPPH, IL-8 inhibitions and their ability to keep the balance within bacterial skin microbiota.

2. Materials and Methods

Vegetable water extractions

Biolie has applied its patented enzymatic extraction eco-process to organic fruits and vegetable coproducts. Vegan, food-grade, non-GMO enzymes digest the grinded plants, open the cells and release the water and molecules of interest under controlled temperature.

A first extraction study has been performed to compare extraction with or without enzymes. In this study, fruits or vegetables are grinded and poured into a reactor with or without enzymes. The mixture is stirred for 4 hours at 40-50 °C. After extraction, the content of the reactor is centrifuged, and the aqueous fraction is filtered up to 0.2 µm and kept at room temperature in the dark for further analyses.

Aqueous extract characterization.

Dry matter. Samples are drought in oven at 110 °C for 24 hours. The percentage of dry matter is calculated with the formula:

$$DM = \frac{weight\ after\ 24\ h\ at\ 110\ °C}{initial\ weight} x100$$

Reducing sugars were measured according to Nelson Somogyi [15]. Namely, the aqueous samples and the standards are mixed with the copper reagent (1:1 V/V) in a 96 wells microplate. The samples are incubated for 10 minutes in a water bath at 95 °C. Arsenomolybdic reagent is added to the samples and the microplate is incubated for 10 minutes at room temperature for color development and read at 500 nm.

Proteins were evaluated in a 3-steps assay using the Kjeldahl method [16]. The samples (500 µL) are mineralized into ammonium under 4 mL hot concentrated sulfuric acid during 4 h. Ammonium is then converted to ammonia with 50 mL of concentrated NaOH and trapped into an aqueous solution of 4 % boric acid. The nitrogen content in the distillate is finally titrated using HCl (0.02 N). A conversion factor of 6.25 is applied to transform the nitrogen content into proteins.

Polyphenols are measured according to the Folin-Ciocalteu method with gallic acid as a standard [17]. Briefly, samples (20 µL) are mixed to 100 µL of Folin-Ciocalteu reactive (1/10) and 80 µL sodium carbonate solution (7 %) in a 96-wells microplate. The microplate is incubated during 30 minutes in the dark at room temperature and measured by spectrophotometry at 760 nm.

DPPH inhibition activity. A solution of DPPH is prepared at 2.5 % in methanol. A reference inhibitor, which is ascorbic acid, is prepared in stock solution at 1 mg/ml. Samples or ascorbic acid (100 µL) at accurate dilution are mixed with 200 µL of DPPH and homogenized in a microplate. After 30 minutes incubation at room temperature in darkness, absorbances are measured spectrophotometrically at 517 nm.

Collagenase inhibition activity. Samples (80 µL) are introduced in a 96 wells black microplate. 20 µL of the substrate (DQ gelatin at 125 mg/L in the buffer) and 100 µL of the enzyme (collagenase at 0.2 U/mL in the buffer) are added in each well. The plate is stirred at 900 rpm for 30 seconds and incubated for 1 h in the dark at room temperature. The fluorescence is read at 510 nm with an excitation at 485 nm. The percentage of inhibition is determined by comparison with the standard (phenanthroline at 10 mmol/L in the buffer).

Tyrosinase inhibition activity. Samples are prepared in a 0.08 M phosphate buffer (pH 6.8). In a microplate, 100 µL of inhibitor (sample or kojic acid) and 100 µL of DL-DOPA are added in wells. 30 µL of enzyme (at 66,67 U/ml) is added in wells. The microplate is then incubated 6 minutes at room temperature in the dark under stirring. The oxidation of DOPA is followed by reading the absorbance at 492 nm.

Glycation inhibition activity. Samples (100 µL) prepared in PBS buffer are introduced in a 96 wells black microplate and 100 µL of bovine serum albumin (15 g/L in PBS) are added. The microplate is sealed and incubated for one hour at 3 °C. The fluorescence is read at 460 nm after excitation at 360 nm (references for t0). In the wells, 50 µL of glycolaldehyde (30 g/L in PBS) are added. The plate is sealed and incubated for 24 h at 37 °C. The fluorescence is read at 460 nm after excitation at 360 nm. The percentage of inhibition is determined by comparison with the standard (aminoguanidine at 10 g/L in PBS).

IL-8 inhibition activity. A 96 wells microplate is coated with the capture antibody previously diluted in the coating buffer (PBS in distilled water) at the concentration of 1:250 V/V, sealed and incubated overnight at 4 °C. The wells are drained, washed 3 times with 300 µL of the wash buffer (PBS and Tween™-20 in distilled water) and drained again. Then 200 µL of ELISASPOT diluent are added in each well. The plate is incubated for 1h at room temperature (RT) and then drained, wash one time with 300 µL of wash buffer and drained again. The samples are diluted in the ELISASPOT diluent and 100 µL are introduced in the microplate. 20 µL of the human IL-8 standard are then added in each well. The plate is sealed and incubated for 2 h at RT. The wells are drained and washed as described before. 100 µL of the detection antibody are added in each well. The plate is then sealed and incubated for one hour at RT. The wells are drained and washed as described before. 100 µL of the diluted enzyme (Avidin-HRP) are introduced in the microplate, which is then incubated for 30 minutes at RT. The wells are drained and washed as described before. 100 µL of the substrate solution are added and the plate is incubated for 15 minutes at RT. 50 µL of the STOP solution are added in each well and the optic density is read at 450 nm and 570 nm. The anti-inflammatory activity is determined by comparison between the concentration of IL-8 in the blank condition and the sample condition.

Microbiota activity. Vegetable waters have been tested on face skin microbiota. Skin microbiota of 44 healthy participants with normal skin has been analyzed to identify the most representative species of bacteria on the face skin. On this basis, a microbiota model composed of 3 genera (*Cutibacterium, Staphylococcus.* and *Corynebacterium*) and 15 species was developed, allowing to predict the impact of the extract on the bacterial populations present on the skin. In this study, each bacteria type has been cultivated individually with different concentrations of vegetable waters (20 % and 40 %) and compared to control (water). After 24 h, the effect of vegetable waters on the growth of the bacteria population is compared to control.

3. Results

3.1. Process yields

Aqueous maceration of ground cucumber yielded to 73 ± 4 % (w/w) of water extraction compared to the input mass of vegetable in the process and 27 ± 4 % (w/w) were retrieved as wet solid residues (Figure 1). Supplementing the mixture with selected enzymes allowed to increase the weight of extracted water by 20 % while reducing the solid residues to 12 ± 2 % (w/w), saving 56 % of wasted mass. Similar gains were obtained on zucchini and pear coproducts.

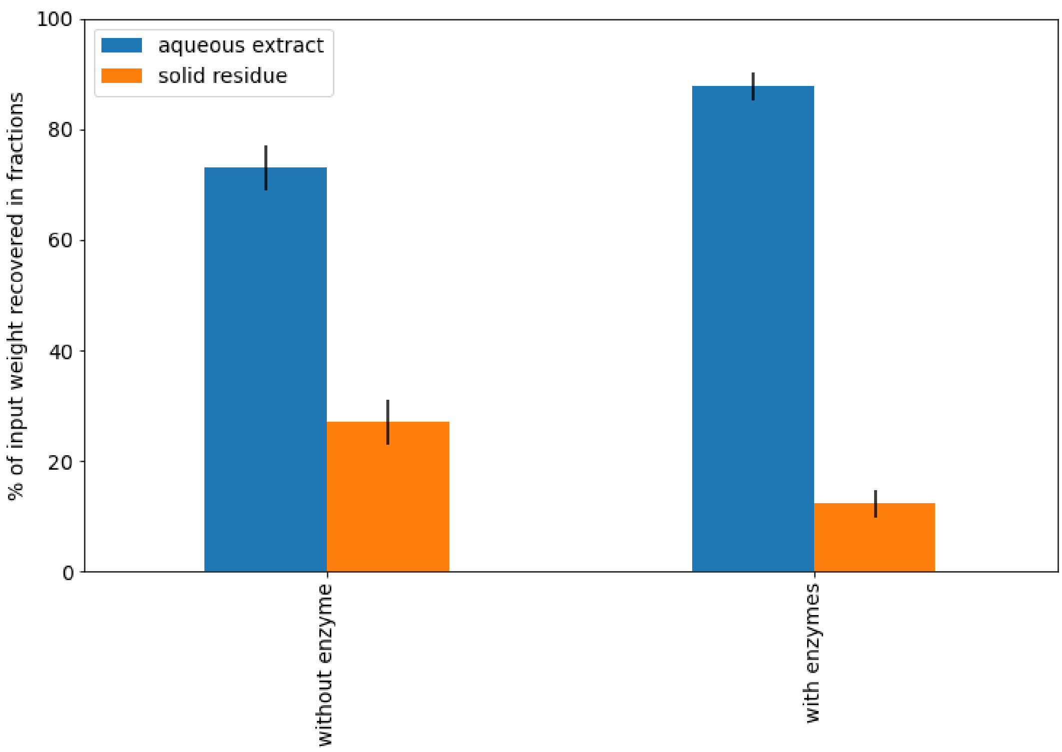

Figure 1. Water and solid residues extraction yields of vegetable co-products with or without enzymatic hydrolysis

Overall, at least 82 % (w/w) of vegetable water were obtained using enzymatic aqueous assisted extraction (Table 1). In parallel, weights of wet solid residues were drastically reduced, reaching at maximum 15 % of the initial weight.

Table 1. Extraction outputs of enzymatic processing for vegetable waters production (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

	ZE	CE	PE
Outputs			
Vegetable water extract (w/w)	87 % ± 2 %	96 % ± 1 %	82 % ± 5 %
Solid residues (w/w)	12 % ± 3 %	3 % ± 1 %	15 % ± 3 %

3.2. Extract characterization

The biochemical profiles of each vegetable water were established, and the main criteria are summarized in the Table 2.

Table 2. Biochemical characterization of the vegetable waters (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

	ZE	CE	PE
Dry matter (% w/w)	3.8 ± 0.5	2.2 ± 0.1	12 ± 0.7
pH	5.7 ± 0.3	4.1 ± 0.2	4.4 ± 0.2
Proteins	3.7 ± 0.2	4.3 ± 0.7	1.9 ± 0.2
Polyphenols (mg/L GAE)	310 ± 45	84 ± 22	335 ± 50
Reducing sugars (g/L)	25 ± 3	10 ± 1	96 ± 6

Zucchini, cucumber, and pear aqueous extracts (ZE, CE and PE respectively) produced with enzymatic extraction are composed for 94, 97 and 88 % of water respectively. They have moderate acidic pH and low amounts of polyphenols and proteins. On the contrary, the water extracts have up to 10 % (w/w) of reducing sugars due to high sugar content in the raw fruits [18], [19] combined with enzymatic hydrolysis which specifically targets the hydrolysis of polysaccharides.

3.3. Skin care activity

3.3.1. Antioxidant

DPPH test showed different antioxidant profiles between the three extracts (Figure 2). ZE and CE show light free radicals scavenging effects, reaching a maximum inhibition of DPPH of 18 and 22 % respectively at doses of 100 %. PE on the other hand shows a high antioxidant potential. At a dose of 25 %, PE inhibits 86 % of free radicals. Topic application of vegetable waters may thus contribute to limit environmental stresses.

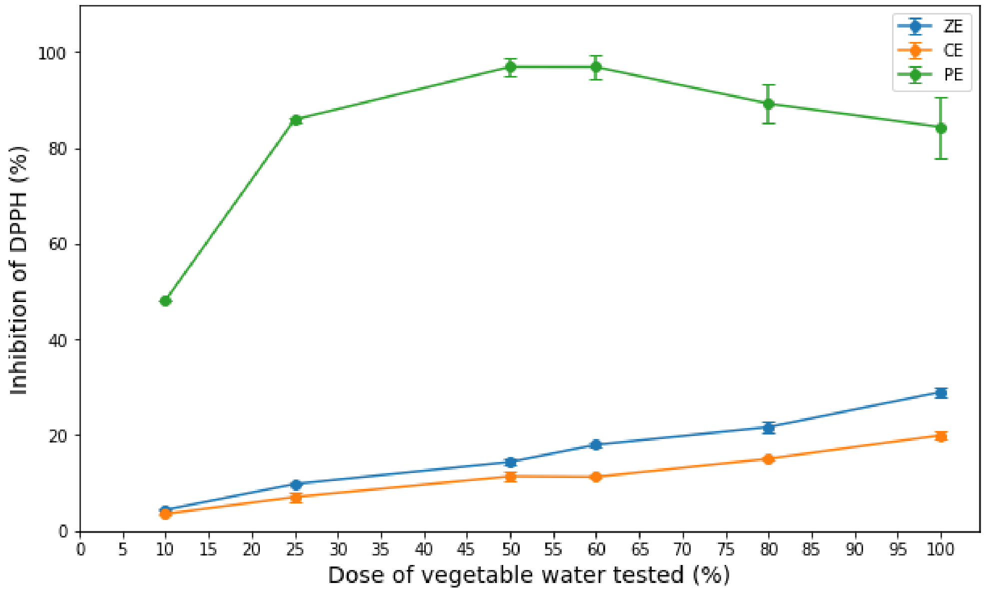

Figure 2. DPPH inhibition of vegetable waters at increasing doses (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

3.3.2. Collagenase

The aqueous vegetable waters were tested for the inhibition of the collagenase, a protein involved in the degradation of the collagen leading to the remodeling of the extracellular matrix of skin causing loss of firmness and wrinkle formation [20]. Figure 3 shows that all three waters have strong effects on collagenase inhibition. At doses over 20 %, the collagenase inhibition reaches 100 % for all vegetable waters. These results prove that the vegetable waters could help the skin keeping its softness and moderate wrinkle formation.

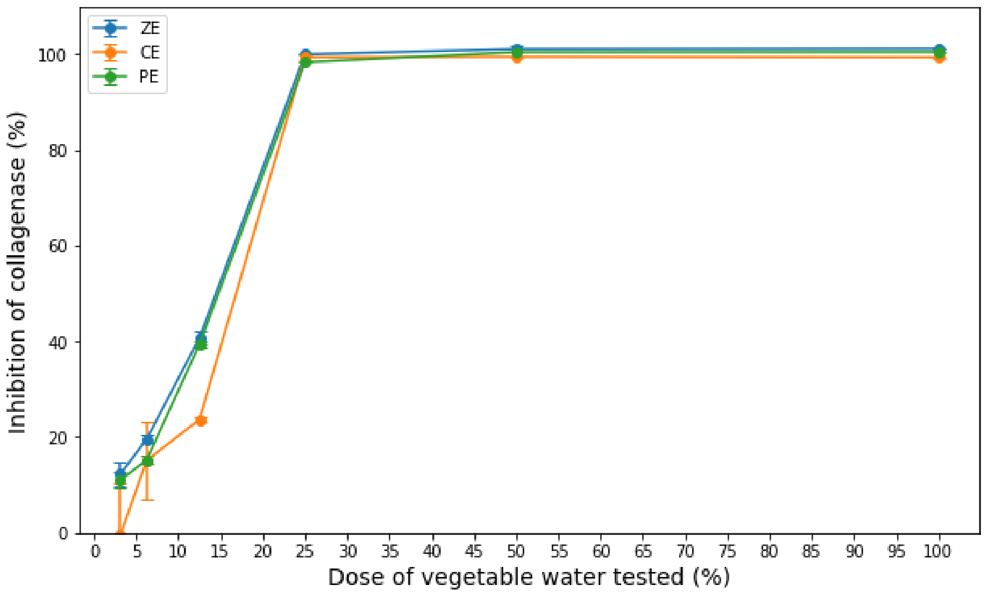

Figure 3. Collagenase inhibition of the vegetable waters at increasing doses (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

3.3.3. Glycation

Protein glycation is an endogenous reaction between sugars and dermis fibers, such as collagen and elastin. Glycation leads to skin degradation and hardening. Inhibition of this phenomenon is thus crucial to keep a soft and tonic skin. The vegetable waters were tested for the inhibition of protein glycation (Figure 4). With 20 % of vegetable extracts in the test, all three extracts inhibit protein glycation from 30 % for ZE and CE to 50 % for PE. At higher doses, the inhibition effect increases up to 70 % for CE and PE and 85 % of glycation inhibition was reached with ZE. According to these results, the vegetable waters could help maintaining skin tonicity.

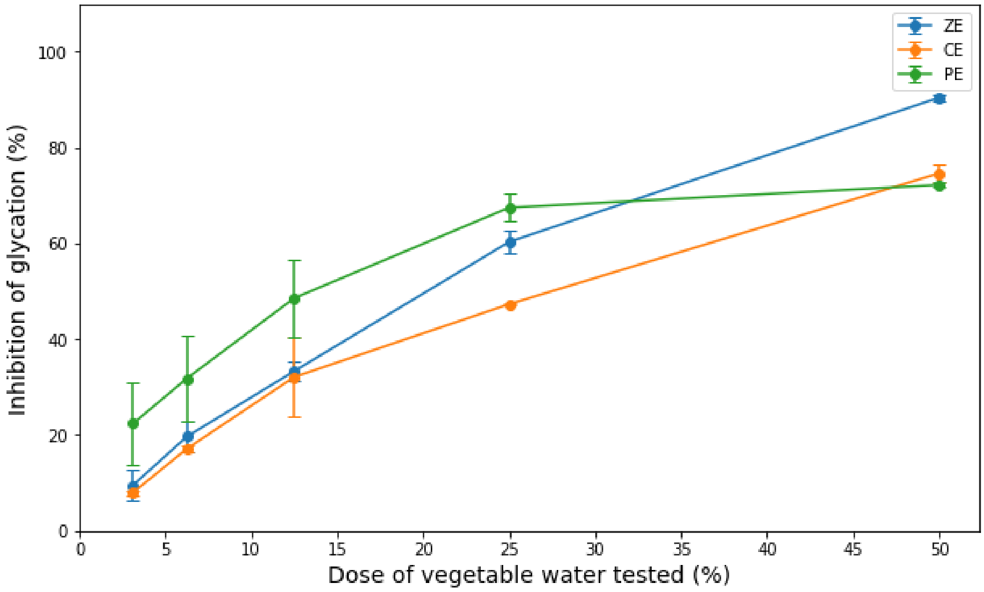

Figure 4. Glycation inhibition of the vegetable waters at increasing doses (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

3.3.4. Tyrosinase

Tyrosinase is an enzyme playing a key role in the melanogenesis, responsible for skin pigmentation [20]. If these pigments are important for protection against solar radiation, the hyperpigmentation also leads to age spots [21]. The inhibition potential of tyrosinase of the vegetable waters was evaluated (Figure 5). All extracts have shown similar inhibition behavior. At a dose of 20 %, the ZE inhibits 50 % of the tyrosinase activity compared to water. CE and PE at the same dose were able to inhibit more than 60 % of the enzyme activity. At a dose of 50 %, all three extracts were able to inhibit more than 80 % of the tyrosinase activity. Topic application of vegetable waters may thus contribute to reduce age spot appearance.

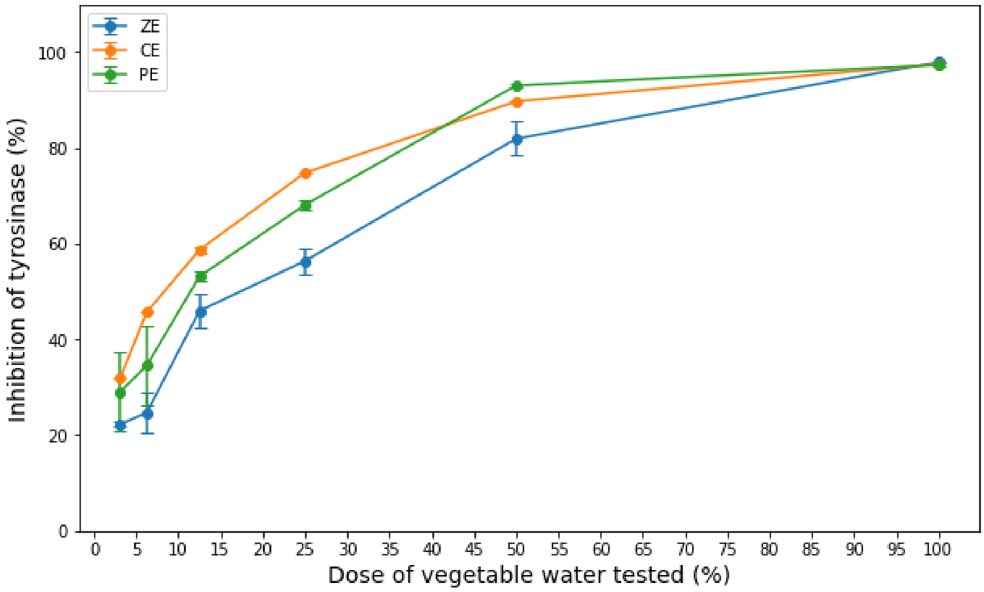

Figure 5. Tyrosinase inhibition of the vegetable extracts at increasing doses (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

3.3.5. Interleukin-8

During inflammatory stimuli, skin cells produce Interleukin-8. This is a proinflammatory cytokine, secreted by skin after detection of pathogen agents to recruit immune cells like polynuclear neutrophils [22]. Inhibition of interleukin-8 recognition by anti-interleukin-8 antibody is a way to reduce inflammation of the skin. All extracts were tested at a dose of 20 %. In all cases, vegetables extracts were able to inhibit at least 96 % of the recognition of IL-8 by their anti-human IL-8 antibody. According to these results, the vegetable waters could help the skin to preserve its softness, elasticity and clearness by reducing inflammations and their consequences on skin.

Table 3. (ZE = Zucchini extract, CE = Cucumber extract, PE = Pear extract)

	ZE	CE	PE
IL-8 recognition inhibition (% of total)	100 ± 0.6	98.0 ± 2.3	95.7 ± 1.6

3.3.6. Microbiota

Influence of vegetable waters on microbiota has been investigated (Table 4). In this study, the growth of individual species isolated from healthy human skin in presence of 20 % of 40 % of vegetable extracts has been measured and compared to water. Regarding the genera, *Cutibacterium spp.* are not or negatively impacted by vegetable waters, whereas the growth of *Corynebacterium spp.* are not or positively impacted. For *Staphylococci*, it is rather fuzzy and species dependent. The growth of *Staphylococcus aureus* and *S. capitis* are significantly reduced by all extracts whereas, *S. hominis* and *S. lugdunensis* are increased. Overall, zucchini extracts showed a neutral effect on 67 % of the bacterial strains at a dose of 20 % and 55 % at a dose of 40 %. Similarly, at the same dose, the growth of 55 % of the strains were not impacted by cucumber extract at a dose of 20 % and 47 % at a dose of 40 %. However, for pear extract, only 40 % had similar growth to control at both doses.

Table 4. Influence of vegetable waters on skin microbiota at doses 20 % and 40 % in tests (→ no significant growth change, ↘ growth inferior to control at $p \leq 0.01$, ↗ growth superior to control at $p \leq 0.01$)

Bacteria strains	Zucchini extract		Cucumber extract		Pear extract	
	20 %	40 %	20 %	40 %	20 %	40 %
Cutibacterium						
C. acnes	→	→	→	→	↘	↘
C. namnetense	→	→	→	→	↘	↘
C. granulosum	→	→	→	→	→	→
Staphylococcus						
S. epidermis	→	→	↘	↘	→	↘
S. c.	→	→	↘	↘	→	→
S. capitis	↘	↘	↘	→	→	→
S. hominis	↗	↗	↗	↗	↗	↗
S. lugdunensis	↗	↗	↗	↗	↗	↗
S. p.	→	↘	→	→	↘	↘
S. aureus	↘	↘	→	↘	↘	↘
S. sciuri	→	↗	→	↗	→	→
Corynebacterium						
Co. tuberculostearicum	→	→	→	→	→	↘
Co. amycolatum	↗	↗	↗	↗	↗	→
Co. kroppenstedtii	→	→	↗	↗	↗	→
Co. pseudotuberculosis	→	→	→	→	↗	↘

The aim of microbiota friendly extracts is to keep balance of the microorganism diversity present on skin to maintain its protection role against foreign pathogens [23]. In this study, all extracts were able to maintain the growth profile of most strains. *Staphylococcus aureus* is one on the strain that is negatively impacted by all extracts but the most pathogenic species of its genus, responsible for deadly infections [24], [25]. Discussion remains thus open whether it is a desirable outcome or not. However, cosmetic formulations are often used with preservatives to avoid microorganism spoilation which, by nature, are used to prevent contaminations and thus contribute to microbiota disruption [26], [27]. Therefore, for instance, skin microbiota friendliness extracts are only accurate in formulas avoiding preservatives.

4. Discussion

A range of natural vegetable waters has been developed thanks to enzymatic assisted extraction, a patented eco-process able to produce natural active ingredients, rich in molecules of interest.

The enzymatic assisted extraction improved the extraction yields of bound water within fruits and vegetables compared to standard procedures. In addition to processing yields, this approach also decreased the mass of solid residues that must be handled afterwards, reducing the amount of extraction wastes.

The different extracts obtained with this approach have shown interesting and consistent potential towards skincare. Indeed, all extracts have shown *in tubo* inhibition properties of key enzymes involved in skin-aging such as collagenase, tyrosinase, glycation or in inflammatory process (IL-8). Furthermore, the extracts have shown no to moderate *in vitro* effect on the skin microbiota.

In addition, the full ingredient life cycle analysis, from the ground to the formulator, must also include the culture of the plants and the extraction waste management. Vegetable production mobilizes land surfaces otherwise dedicated to food production and significant amounts of water for their production [28], [29]. Therefore, careful attention to the real environmental impacts should be given to replacing water in cosmetics formulation and avoid counterproductive initiatives.

To minimize these impacts, the vegetables can be picked up among the wastes of the food industry. In this paper, the fruits are off-size or ugly and not departed from the food market. In addition, the maximum distance between sourcing and the extraction plant are 50 km, 60 km and 300 km for pears, cucumbers, and zucchinis respectively, limiting the transport of "water".

Standard enzymatic assisted aqueous extraction of vegetable water implies five main steps (grinding, hydrolysis, enzyme deactivation, separation, filtration). EAE is carried out under mild conditions. Temperature is only controlled during hydrolysis (around 50 °C) and deactivation (80 °C for 10min.) whereas pH is left to its natural level in the vegetables. Process duration and enzyme amounts are optimized to their minimum and no water is added.

Although the use of enzymes requires an additional step of deactivation at relatively high temperature during a very limited period, this approach allows to increase extracts yields of 20 % and thus decreases solid residues in addition to contribute to enrichment of aqueous extract in biomolecules. Overall, the process generates very small amounts of wastes, which are then valorized by anaerobic digestion and composting.

5. Conclusion

Vegetable waters offer an alternative to water in cosmetic formulation, representing 20 to 80 % of the final product. Organic certified, based on coproducts upcycling, vegetable waters are multifunctional ingredients bringing water, nutrients and efficacy, like tyrosinase inhibition or antioxidant activity. Thus, those natural ingredients help the formulator using less ingredients in the formula, following the emerging skinimalism trend, aiming for less ingredients and/or more natural and sustainable ones.

In conclusion, the enzymatic assisted extraction process starts with food industry coproducts (off-size or "ugly" vegetables), produces organic natural active waters for the cosmetic industry with limited energy consumption and a low amount of extraction wastes, which are valorized into energy thanks to anaerobic digestion or valorized with composting, giving back residual biomass to the ground to grow new plants.

Acknowledgements
We are grateful to Bpifrance for the financial support of this work

Conflict of interest statement
All authors of this paper are employed or related to the French company BIOLIE, which possesses a patent on enzyme-assisted extraction technology and sells on the cosmetic market the extracts presented in this paper.

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