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Enzymatic Pre-Treatment of Fruit Pomace for Fibre Hydrolysis and Antioxidants Release

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Processing of grapes and other fruits in the wine and fruit juice industry generates huge amounts of solid residues (pomace) which, if not properly disposed of, can represent an environmental concern due to their high organic load. To avoid landfill and incineration, different alternative uses are available and commonly applied. Among these, the most common are for feeding, composting and biogas production which are, anyway, not proper valorisation strategies. On the other hand, fruit pomaces are by-products still rich in bioactive components, such as dietary fibre and phenolic/antioxidant compounds. Considering the positive health potentials of such components, together with their potential technological role (as texturing and antioxidant ingredients), fruit pomaces may then be simply dried and milled to get functional food ingredients. It is known however, that incorporation of high levels of raw fibres into food products often causes unpleasant textures and colours. Furthermore, phenolic compounds only partially occur in fruit pomace as free compounds, while they are bound to cell wall (fibre) fractions. Enzymatic hydrolysis processes could be applied as a pre-drying treatment of fruit pomace to improve the functional properties of the final powders in terms of fibre composition and antioxidants release.
In this study different fruit skins separated from different pomaces (grape, apple and blackcurrant) were submitted to an enzymatic treatment before drying. Two different commercial pectinase preparations were used: one already used in the apple juice processing for the treatment of apple and blackcurrant skins, the other currently used in the winemaking process for the treatment of grape skins. Untreated and treated dried skins were analysed for structural carbohydrates, soluble and insoluble dietary fibre, free glucose and xylose, water holding capacity, water solubility and total phenolics and antioxidants release. The results were highly variable depending on the fruit type, probably due to a different cell wall composition which requires targeted enzyme selection. In general, the enzyme treatment led to an increase in water solubility, water and oil holding capacity and free monosaccharides. Release of antioxidant compounds was observed only for apple peels.

1. Introduction

Fruit and vegetable pomaces are generated in huge amounts in industries, hostels, juice centres and households. If dumped along with other wastes without segregation, they are unfit for further use, but they can be easily collected from the industry. Due to its organic nature, fruit pomace cannot be directly released in the environment, unless for a direct agronomic use in very limited and controlled amounts. Fruit pomace must then be disposed of in some way. Direct transport to landfill is the least desirable strategy in the waste management hierarchy (Directive 1999/31/EC). Energy recovery (such as for biogas production) is just above disposal while prevention and minimisation of waste generation are at the top of the waste uses pyramid. Recycling and reuse are in the middle and, when applied, they allow to convert the waste into a by-product. Fruit pomace consists of a mixture of skins, seeds and residual pulp and is still rich in many valuable compounds, first of all non-digestible carbohydrates (dietary fibre) (Canela-Xandri et al., 2018), often associated to phenolic compounds bound to the cell wall components cellulose and hemicellulose which, in turn, are tightly linked to lignin when present (Pinelo et al., 2016).

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The high fibre and phenolic content make fruit pomace a very interesting potential functional ingredient for the food industry. Fibre is well known for its important beneficial health effects such as appetite reduction, lowering variance in blood sugar levels, reduced risk of heart disease and onset risk or symptoms of metabolic syndrome and diabetes, reduced risk of colorectal cancers, facilitated regularity and alleviated constipation (Dhingra et al., 2011). However, direct incorporation of high amounts of raw fibre for the formulation of food products presents some critical aspects due to limited bioavailability of phenols bound to the cell wall, rheological and sensory defects and unbalanced ratio of insoluble/soluble fibre which, in the diet, should be 3/1 (Lavelli et al., 2016). If present in large amounts, fibre may compromise food texture and role. Fibre is often exploited in low sugar, low fat and gluten free products due to its technological and structuring role: swelling capacity, water holding capacity and oil holding capacity. These healthy and technological properties make fruit fibre interesting functional ingredients for the food industry but in their raw form they do not always match the desired technological properties for food applications. Enzymatic treatments might bring modifications of dietary fibre and improve functional properties (Canela-Xandri et al., 2018).
Under specific research projects and based on their industrial diffusion and healthy profiles, different fruit pomaces were used in the experimentation: apple, blackcurrant and apple pomaces.
Apple pomace is generated from cider and juice industry and is one of the most investigated fruit pomaces in the literature for food and other uses (Niglio et al, 2019).
Blackcurrant (*Ribes nigrum*) is primarily used in juice manufacturing, generating several thousand tonnes per annum of pomace (Alba et al., 2018). Finally, grape is one of the largest fruit crops in the world, with about 50 % of the world grapes processed into wine. Winemaking generates huge amounts of solid residues with grape pomace (GPS) being the main fraction of the solid wastes (up to 60% of their weight and the 20-25% of the received grape) (Spigno et al., 2017). The aim of this research was then to investigate the application of an enzymatic treatment to fruit skins from the above listed different pomaces, before drying and milling, to evaluate the enzyme effect on both technological (water holding capacity and solubility, oil holding capacity) and nutritional properties (composition and release of phenolic and antioxidant compounds. Enzymes (mainly pectinases and cellulases) are already commonly used in the fruit juice industry in the first juice extraction steps to enhance juice and colour release, therefore specific industrial enzymes from the cider and wine industry were used for apple / blackcurrant and grape treatment, respectively. Enzymatic action can increase the amount of soluble dietary fibre but also of hydroxycinnamic acids, free phenols concentration, water soluble antioxidant activity and phenol compounds availability (Liyana-Pathirana et al., 2006).

2. Materials and Methods

A preliminary experimental plan was set up to try to develop a low-cost and environmentally friendly enzymatic hydrolysis treatment (**ET**) of three different fruit pomaces. Fermented grape pomace skins (**GPS**) of *Croatina* red grape variety from winemaking process was kindly provided by Cantina F.lli Bonelli (Rivergaro, PC, Italy). Apple pomace (**AP**) and blackcurrant pomace (**BCP**) from cider and fruit juice industry were kindly provided by UK companies.
For all the collected pomaces, the skins were manually separated from seeds and other impurities and then submitted to the enzymatic hydrolysis treatment. The aim of this enzymatic treatment was to improve the content of soluble phenolic and antioxidant compounds together with the fibre composition and technological properties of the skins. In order to make the process more environmentally friendly and industrially implementable, it was decided to test the enzymatic treatment directly on fresh fruit pomace as a pre-treatment before final drying and milling. Two commercial enzymatic preparations were used. For AP and BCP a pectinase-based enzyme preparation provided by the cider company was used (for non-confidentiality reasons it is not possible to provide here further details of the product). For GPS, based on previous works (Gruppi et al., 2017; Binaschi et al., 2018) and literature on grape skins (Costoya et al., 2010), a commercial enzyme preparation specifically formulated for oenological applications was used: LAFASE® XL PRESS (kindly provided by Laffort), suggested for the grape pressing step with pectinase activity and low level of cinnamyl-esterase.
For the treatment of AP and BCP, 100 g of whole fresh skins were mixed with 5 % and 10 % (only 10 % on BCP) of enzyme preparation (w/w based on wet weight of skins) and put in an oven at 30 °C for 2 h with manual mixing every 15 minutes. After the treatment, the apple skins were dried at 120 °C for 2 h, while the blackcurrant skins were dried at 60 °C for 9 h. The drying time/temperature combinations were selected through preliminary trials (no data shown) in order to find the best conditions to limit degradation of phenolic compounds. The anthocyanins of BCP requires lower temperatures. For the control samples, the skins were directly dried under the same conditions.
For the treatment of GPS, 100 g of whole fresh skins were treated with 10 % of LAFASE® XL PRESS (w/w based on wet weight) diluted ten times in water, as indicated in the technical data sheet. The skins were

maintained at 40 °C in oven, for 4 h under stirring. Then the skins were dried at 60 °C until the moisture of samples was less than 10 % (around 24 h). After drying, the skins were ground to a particle size < 1 mm. AP and BCP skin powder were further sieved to obtain two fractions with particle size lower or higher than 750 µm. The flours were then analysed for the soluble and insoluble dietary fibre content, oil retention capacity (ORC), water holding capacity (WHC), water solubility (WS), structural carbohydrates (SC), and free reducing sugars (glucose, xylose, arabinose), and for the content of extractable phenolic and antioxidant compounds.

2.1 Analytical methods

2.1.1 Dietary fibre

The analysis of soluble and insoluble dietary fibre was assessed through an enzymatic assay (Megazyme, K-TFDR-200 A), according to AOAC Method 991.43.

2.1.2 Structural carbohydrates

Cellulose, hemicellulose and lignin contents were assessed as reported by according to the method proposed by Sluiter et al. (2011) based on a quantitative saccharification of polysaccharides through a strong acid hydrolysis followed by a dilute acid hydrolysis. Acid soluble lignin (ASL) is determined from absorbance reading at 320 nm of the hydrolysate (applying the absorbance coefficient of 30 L g^{-1} cm^{-1}), while acid insoluble lignin (AIL) is the solid residue of the saccharification after the determination of moisture and ash content. The xylose content in the hydrolysate is measured (Megazyme kit, K-Xylose) and multiplied by the correction factor of 0.90 to estimate hemicellulose content. Similarly, the glucose content is measured (Megazyme kit, K-FRUGL) and multiplied by the correction factor of 0.88 to estimate the cellulose (glucan) content.

2.1.3 Free reducing sugars

The content of free monosaccharides glucose, xylose and arabinose was evaluated through an aqueous extraction (solid/water ratio = 0.07) under stirring (SKI 4 ARGOLAB) for 2 h at room temperature. The extract was filtered through a paper filter (Whatman 595 ½) and analysed by enzymatic kits (Megazyme kit, K-FRUG, K-Xylose and K-ARGA).

2.1.4 Water holding capacity, oil adsorption capacity, water solubility

WHC and ORC were evaluated as reported by (Mateos-Aparicio et al., 2010). Briefly for WHC 500 mg of sample were hydrated with 30 ml distilled water for 18 h at room temperature. The sample was then centrifuged at 3000 g for 20 min and the residue fresh weight recorded. WRC was calculated as the amount of water retained by the pellet (g water/g sample dw). For the ORC the same protocol was followed, but with extra virgin olive oil (acidity 0.7°) for distilled water. ORC was expressed as g oil/g sample dw.

The method described by Tuyen et al. (2010) was used to evaluate WS. The sample (2.5 g) was vigorously mixed with 30 ml of distilled water in a 50 ml centrifuge tube, incubated at 37 °C for 30 min and then centrifuged at 11,410 g for 20 min. The supernatant was oven dried at 103 ± 2 °C and the WS was calculated as the percentage of dried supernatant with respect to the initial sample weight.

2.1.5 Total phenolic content and antioxidant capacity

The content in free extractable phenolic compounds and antioxidant compounds was assessed through extraction of the dried skins powders with ethanol 60 % (1/8 w/v) at 40 °C for 1 h 30 min under stirring (SKI 4 ARGOLAB) and analysis of the extracts separated by centrifugation for the following parameters:

- Total phenols (TP), based on the Folin-Ciocalteu's assay (García et al., 2011), expressing the results as mg of gallic acid equivalents (GAE, based on a calibration curve with standard of gallic acid) on dry weight of the samples (mg_{GAE}/g_{dw}).
- Antioxidant activity was evaluated according to FRAP assay (Vellingiri et al., 2014) and the results were expressed as $\mu mol_{Fe(II)}/g_{dw}$ (based on a calibration curve with standard solution of Fe(II)).

2.2 Statistics

All the trials and the analytical measurements were carried out in triplicates. The values are reported as means ± SD. The significance of the influence of the enzymatic treatment on the measured parameters, was assessed by one-way ANOVA (IBM SPSS Statistics v.25) and Tukey's post-hoc test for means discrimination at a confidence level of 95 % ($p < 0.05$).

3. Results and Discussion

The results of the chemical composition, functional properties and extractable antioxidants are reported in Tables 1, 2 and 3.

Table 1 Characterisation of AP powder of different granulometry (< 750 µm and > 750 µm), obtained from untreated (no enzyme) or enzymatically treated (5 % and 10 % enzyme) fresh skins. Different letters for the same parameter indicate means significantly different (ANOVA and post-hoc of Tukey, p < 0.05).

Parameter	Apple					
	No enzyme		5 % enzyme		10 % enzyme	
	< 750 µm	> 750 µm	< 750 µm	> 750 µm	< 750 µm	> 750 µm
Soluble Dietary Fibre (% g_{dw})	6.95 ± 1.52 ^{a}	7.78 ± 2.95 ^{a}	7.32 ± 0.20^{a}	6.27 ± 0.16^{a}	4.33 ± 0.22^{a}	5.03 ± 0.45^{a}
Insoluble Dietary Fibre (% g_{dw})	53.54 ± 0.68^{d}	59.05 ± 1.64^{e}	44.52 ± 0.84^{b}	47.64 ± 0.73^{c}	39.89 ± 0.31^{a}	43.85 ± 0.70^{b}
Oil Retention Capacity (g/g_{dw})	2.91 ± 0.02^{b}	2.89 ± 0.00^{b}	2.88 ± 0.13^{b}	2.78 ± 0.00^{ab}	2.85 ± 0.01^{ab}	2.71 ± 0.00^{a}
Water holding Capacity (g/g_{dw})	4.53 ± 0.36^{d}	4.30 ± 0.05^{d}	3.28 ± 0.01^{bc}	3.60 ± 0.05^{c}	2.75 ± 0.25^{a}	3.09 ± 0.26^{ab}
Water Solubility (%)	9.27 ± 1.47^{a}	9.16 ± 1.17^{a}	18.74 ± 1.94^{c}	13.40 ± 0.98^{b}	22.86 ± 0.19^{d}	20.55 ± 0.49^{cd}
TPC (mg_{GAE}/g_{dw})	2.53 ± 0.02^{a}	3.11 ± 0.11^{ab}	3.60 ± 0.21^{bc}	4.19 ± 0.21^{bc}	4.30 ± 0.37^{d}	4.87 ± 0.37^{d}
FRAP ($\mu mol_{FeII}/ g_{dw}$)	36.04 ± 0.39^{ab}	33.07 ± 0.87^{a}	46.03 ± 1.16^{cd}	42.37 ± 3.18^{bc}	60.09 ± 4.11^{e}	50.59 ± 1.65^{d}
Free Glucose (% g_{dw})	2.59 ± 0.00^{cd}	2.68 ± 0.08^{d}	2.40 ± 0.00^{b}	2.25 ± 0.03^{a}	2.50 ± 0.05^{bc}	2.50 ± 0.02^{bc}
Free Xylose (% g_{dw})	0.04 ± 0.001^{a}	0.04 ± 0.009^{a}	0.07 ± 0.003^{b}	0.08 ± 0.00^{bc}	0.10 ± 0.004^{cd}	0.11 ± 0.01^{cd}
Free Arabinose (% g_{dw})	0.10 ± 0.00^{a}	0.12 ± 0.02^{a}	0.55 ± 0.03^{b}	0.56 ± 0.00^{b}	0.79 ± 0.01^{c}	0.77 ± 0.00^{c}

Table 2 Characterisation of BCP powder of different granulometry (< 750 µm and > 750 µm), and of GPS obtained from untreated (no enzyme) or enzymatically treated (10 % enzyme) fresh skins. Different letters within the same column indicate means significantly different (ANOVA and post-hoc of Tukey, p < 0.05).

Analysis	Blackcurrant				Grape	
	No enzyme		10 % enzyme		No enzyme	10 % enzyme
	<750 µm	>750 µm	<750 µm	>750 µm	1 mm	
Soluble Dietary Fibre (% g_{dw})	4.31 ± 0.72^{c}	1.30 ± 0.76^{a}	2.93 ± 0.25^{bc}	2.01 ± 0.25^{ab}	5.69 ± 0.56^{a}	1.18 ± 0.37^{b}
Insoluble Dietary Fibre (% g_{dw})	63.09 ± 0.63^{b}	66.14 ± 1.73^{c}	54.36 ± 0.38^{a}	70.00 ± 0.71^{b}	44.71 ± 0.70^{a}	35.34 ± 0.20^{b}
Oil Retention Capacity (g/g_{dw})	2.50 ± 0.01^{b}	2.43 ± 0.01^{a}	2.58 ± 0.01^{c}	2.48 ± 0.02^{b}	2.18 ± 0.03^{a}	1.95 ± 0.15^{a}
Water holding Capacity (g/g_{dw})	3.55 ± 0.14^{c}	2.63 ± 0.02^{b}	2.66 ± 0.02^{b}	2.31 ± 0.03^{a}	2.78 ± 0.02^{a}	2.28 ± 0.01^{b}
Water Solubility (%)	2.57 ± 0.02^{a}	3.85 ± 0.01^{b}	10.58 ± 0.40^{d}	7.39 ± 0.01^{c}	16.56 ± 0.02^{a}	26.35 ± 0.84^{b}
TPC (mg_{GAE}/g_{dw})	6.70 ± 0.19^{b}	4.35 ± 0.05^{a}	6.54 ± 0.51^{b}	4.50 ± 0.31^{a}	53.05 ± 0.06^{a}	47.04 ± 1.86^{b}
FRAP ($\mu mol_{FeII}/ g_{dw}$)	171.14 ± 2.84^{b}	102.84 ± 5.57^{a}	163.48 ± 13.30^{b}	87.41 ± 3.35^{a}	667.28 ± 6.04^{a}	545.40 ± 30.94^{b}
Free Glucose (% g_{dw})	0.12 ± 0.00^{a}	0.28 ± 0.05^{b}	0.32 ± 0.00^{b}	0.32 ± 0.00^{b}	2.53 ± 0.06^{a}	4.41 ± 0.11^{b}
Free Xylose (% g_{dw})	0.03 ± 0.003^{b}	0.02 ± 0.001^{a}	0.04 ± 0.003^{c}	0.02 ± 0.003^{a}	0.011 ± 0.002^{a}	0.33 ± 0,01^{b}
Free Arabinose (% g_{dw})	0.81 ± 0.01^{c}	0.85 ± 0.00^{c}	0.46 ± 0.01^{b}	0.26 ± 0.00^{a}	not detected	0.68 ± 0.01

Table 3 Analysis of structural carbohydrates of AP, BCP and GPS powders of different granulometry (< 750 µm and > 750 µm, < 1 mm for GPS), obtained from untreated (no enzyme) or enzymatically treated (5 % and 10 % enzyme) fresh skins. Different letters within the same parameter and fruit type indicate means significantly different (ANOVA and post-hoc of Tukey, p < 0.05).
CL: cellulose, HCL: hemicellulose; ASL: acid soluble lignin; AIL: acid insoluble lignin.

% dw	Apple				Blackcurrant				Grape	
	No enzyme		10 % enzyme		No enzyme		10 % enzyme		No enzyme	10 % enzyme
	<750 µm	>750 µm	<750 µm	>750 µm	<750 µm	>750 µm	<750 µm	>750 µm	1 mm	
CL	22.94±0.45^{c}	17.41±0.58^{b}	18.38±1.20^{b}	14.19±1.71^{a}	12.43±1.06^{c}	7.81±0.21^{a}	10.77±0.07^{b}	7.25±0.15^{a}	8.46±0.21^{a}	13.58±0.32^{b}
HCL	2.41±0.05^{b}	2.30±0.05^{b}	1.93±0.20^{a}	1.76±0.03^{a}	0.76±0.10^{b}	0.45±0.04^{a}	0.79±0.05^{b}	0.53±0.05^{a}	0.35±0.05^{a}	1.22±0.03^{b}
ASL	0.80±0.04^{a}	0.96±0.02^{b}	0.81±0.09^{ab}	0.96±0.05^{b}	0.34±0.02^{a}	0.47±0.01^{b}	0.30±0.05^{a}	0.57±0.04^{b}	0.30±0.02^{a}	0.87±0 .07^{b}
AIL	24.86±1.04^{a}	21.19 ±4.06^{a}	26.57±1.36^{a}	24.39±3.24^{a}	44.85±8.97^{a}	44.52±7.03^{a}	43.24±0.90^{a}	36.21±1.65^{a}	39.57±2.27^{a}	24.34±1.91^{b}

The results revealed that the enzymatic pre-treatment was particularly effective on the apple skins, leading to a reduction of dietary fibre (both soluble and insoluble), an increase in free xylose and arabinose content and an increase in the extractable total phenolic compounds and antioxidant compounds (Table 1). The effect was correlated to the enzyme dosage and confirmed the efficacy of the enzymatic preparation on this substrate (the enzyme is already used in the processing line in the initial step of apples pressing for juice extraction). In spite of the constant level of free glucose, the analysis of structural carbohydrates revealed a reduction in cellulose content after enzymatic treatment and a slight reduction in hemicellulose (Table 2). As expected, the lignin, or rather the lignin-like fraction, was not affected by the enzyme.

The action of the same enzyme on blackcurrant skins was different. In this matrix, insoluble dietary fibre content was reduced only in the finer fraction; glucose content slightly increased while arabinose slightly decreased (strangely). The amount of extractable phenolic compounds and antioxidants did not depend on the type of enzyme used but was higher for the finer particles. This might be due both to a higher extraction rate from smaller particles but also to a different composition of the two fractions, as commented for the dietary fibre. The analysis of structural carbohydrates showed, as for apple skins, a slight reduction in the cellulose content.
Regarding the evaluation of non-starch polysaccharides and lignin (or lignin-like) content, it must be underlined how this is generally quite complicated in plant tissues. In the work of Nawirska and Kwaśniewska (2005), the procedure for their determination, involved enzymic hydrolysis of the starch, precipitation in ethanol and acid hydrolysis obtaining fractions of pectin, hemicellulose, cellulose and lignin. The authors underline that the fractions determined in this way may not be clearly defined. In the case of blackcurrant, a slightly different composition than ours, was reported with 25.3 % hemicellulose, 12.0 % cellulose and 59.3 % lignin. The presence of the seeds in the pomace might partly explain the differences.
For the pre-treatment of grape skins, a specific oenological enzymatic preparation was used. As observed for the other fruit skins, the hydrolysis led to a reduction in soluble and insoluble dietary fibre, and an increase in free monosaccharides. However, in grape skins there was an apparent increase in cellulose and hemicellulose and a reduction in phenolics and antioxidants. This might be due to some side esterase activity in the enzyme or to a partial degradation of the phenolic compounds during the enzymatic treatment or maybe due to some residual polyphenol oxidases present in the pomace. The study of Costoya et al., (2010) tested three kinds of different enzyme (Cellubrix®, Neutrase® and Viscozyme®) on two different grape pomaces, Cabernet-Sauvignon (red) and Garnatxa (white) varieties, to enhance the yield of the extraction in terms of antioxidant compounds. The trials were carried out as in our case on fresh samples but ground which, of course, enhanced enzyme-substrate interaction. For both varieties, only the mixture of the three enzymes had a significant effect, increasing by 21 % for Garnatxa and 46 % for Cabernet the amount of extractable phenolics. Interestingly, the enzymatic treatment brought a higher water solubility in all the tested fruit skins, and especially on apple skins, even though the highest WS was shown by hydrolysed grape skins. Water solubility is an important property for food application. On the other hand, the increase in water solubility resulted in a lower water holding capacity which can be another important property depending on the expected technological role of the fibre in food products. Oil retention capacity was not affected by the enzymatic pre-treatment. The work of Reibner et al. (2018) reports for *Ribes nigrum* powder values of WHC and ORC in line with our results, 3.20 ± 0.20 g/g_{dw} and 2.00 ± 0.09 g/g_{dw}, respectively. Beyond this, the work by Canela-Xandri et al. (2018) revealed an increase in WHC of different fruit pomaces (apple, peach and citrus) after three different enzymatic treatments (pectin methyl-esterase, pectinase and cellulase).

4. Conclusions

The main aim of this study was to evaluate the effect of an enzymatic hydrolysis treatment of residual skins from fruit pomace, before drying and milling to get fruit fibre powders. Different industrial fruit pomaces were tested: apples, blackcurrants and grapes. Commercial enzymatic preparations mainly based on pectinases activity and already used in the respective fruit processing lines, were used to check the possibility of increasing the nutritional and functional profile of the fruit fibres in terms of extractable antioxidants and technological properties.
The results showed that the enzymatic treatment was effective in increasing the release of phenolic and antioxidant compounds only in the case of apple skins, while the release remained constant for blackcurrant skins and was reduced in grape skins. However, for all the matrixes, the effect of the enzyme was evident since there was a reduction in the fibre content or a change in its composition, an increase in free monosaccharides and an increase in water solubility. Other important technological properties, such as water holding capacity and oil retention capacity were not improved by the treatment.
As expected and anticipated by the selection of different enzymes for grape skins and apple/blackcurrant skins, it is clear that the enzymatic treatment must be tailored to skin cell wall composition.
Further research will help understanding the phenomena occurring with the treatment by analysing, for example, the phenolic profile of the extract, the generation of oligosaccharides and by electron microscope observations to evaluate structural effects of enzyme on the tissues.

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