Yield vs selectivity in grape pomace polyphenol microwave extraction

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Abstract — A microwave pretreatment to the conventional solid-liquid extraction is proposed for the extraction of active compounds from grape pomace. This pretreatment allows to overcome industrial implementation limitations. With the addition of this step, it is possible not only to improve polyphenol yield, but also selectivity. The microwave enhancement is due to the effectiveness of microwaves towards polyphenol rather than in other substances. The microwave boost is even more notable in anthocyanin extraction, where an 85% richer extract than in the conventional extraction can be obtained. In addition, extract's bio-antioxidant activity is also enhanced.

In sum, the microwave pretreatment exposed in this work allows an easy industrial implementation and improves product quality and quantity.

Keywords — microwave pretreatment, polyphenols, yield, selectivity, antioxidant activity

I. INTRODUCTION

Polyphenols from natural resources are actually a high-valuable product, due to their ability to subdue free radical induce diseases [1], as well as to the large number of applications that they have [2].

Grape pomace is considered an outstanding source of polyphenols, since it still holds a great concentration of active compounds. Especially of anthocyanins, active pigments with large antioxidant activity [3]. The use of grape pomace as raw material allows to revalorize a valueless by-product and contributes to solve the ecological problem of its disposal.

Nonetheless, industrial extraction presents some limitations yet to overcome, such as bulky equipment and long extraction times. Novel intensification techniques have been proved to overcome these drawbacks. In particular, microwaves have been found to greatly enhance the extraction process of active compounds from grape pomace [4]. However, microwave intensification also presents significant drawbacks, such as the energy consumption and the penetration depth of the microwaves. Energy consumption is an inherent feature to this technology, although its efficiency can be improved by a thorough oven design taken into account the dielectric properties of the material. In the case of the penetration depth, a pretreatment of the material in a tubular duct prior to conventional extraction is proposed as an alternative to microwave assisted extraction in order to achieve a homogeneous irradiation of the material. During the microwave pretreatment an intense pulse of energy is given so temperature sharply increases, causing the disruption of the cell wall [5], although no thermal degradation of the active compounds take place due to the short exposure to high temperatures [6]. Once the solid structure has been distored, conventional solid-liquid extraction continues to draw the remaining active compounds.

Hitherto, all the studies about microwave extraction have been focus on yield improvement. However, from a commercial point of view, final extract quality is a crucial factor. The higher the quality of the product is, the more valuable the extract becomes. So, it is also important to take into account the extraction of undesired substances that reduce the polyphenol richness of the extract. In addition, these substances can hinder polyphenols antioxidant bioactivity, since they can reduce their bioaccessibility [7].

In summary, the aim of this work is to develop an intensification step able to overcome industrial limitations, and assess the final product quality, in terms of polyphenol richness and antioxidant activity.

II. MATERIALS AND METHODS

A. MATERIALS

Vintage 2014 red grape pomace from *Tempranillos* grape was kindly given by Bodega Matarromera (Spain). In order to preserve its activity, grape pomace was stored at -18ºC and thawed overnight at 4ºC before used.

A 50% (v/v) mixture of ethanol and acid water was employed as solvent. Sulfuric acid up to a water pH=1 was added to enhance polyphenol stability during storage.

B. EXTRACTION PROCEDURE

The effect of the microwave pretreatment was assessed by comparing it with an actual industrial process [8], based on a conventional solid-liquid extraction.

Conventional extraction was carried out with a solid-liquid ratio of 0.50 g/mL, and at a temperature of 60ºC during three hours with vigorous stirring.

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The microwave pretreatment was implemented before the conventional extraction, and after a 5 minute solid-liquid homogenization. A CEM Discover microwave was used in the pretreatment. Previous results suggested the convenience of using a different solid-liquid ratio than the one employed in conventional extraction. For the pretreatment, a ratio of 0.75 g/mL was preferred. Samples were irradiated with 300 W during 60 and 120 seconds to achieve sample temperatures of 80ºC and 100ºC. For the latter experiment, a pressure vessel (QianCap, QLabtech) was required to maintain the solvent in a liquid state. For now on, these experiments have been labeled as MW80 and MWP100.

When the irradiation was over, additional solvent was poured to achieve the conventional extraction ratio (from 0.75 g/mL in the pretreatment to 0.50 g/mL in the subsequent solid-liquid extraction). The grape pomace-solvent mixture was cooled down to 60ºC in an ice bath, and it continued with the conventional extraction at this temperature.

Samples were taken during the process to assess the improvement. Extraction followed a first order kinetic equation like the one shown in equation (1); where C represents the concentration in mg/g, C_0 the concentration of non-bound polyphenols in mg/g, C_f is a pre-exponential factor (mg/g) whose sum with C_0 computes the extraction yield, k is the rate extraction constant in min^{-1}, and t is the time in minutes. C_0, C_f and k are adjustable parameters whose value has been calculated by minimizing the average relative deviation (ARD, eq. (2)) between the experimental and calculated concentrations.

$$C = C_0 + C_f \cdot \left[1 - \exp(-kt)\right] \quad (1)$$

$$ARD = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{C_{\exp} - C_{cal}}{C_{\exp}}\right| \quad (2)$$

In order to study process yield and selectivity, concentrations have been expressed in two different bases: as $mg/g_{Dry\ Pomace}$ and as $mg/g_{Dry\ Extract}$. Each concentration represents, respectively, the amount of polyphenols extracted from the raw material (yield), and the polyphenol richness of the final extract (selectivity).

C. ANALYTHIC METHODS

Total polyphenol and anthocyanin content were measured by the spectrophotometric methods Folin-Ciocalteu and pH differential method [9,10]. Results were expressed as gallic acid equivalents (GA) for total polyphenol content and as cyaniding-glucoside equivalents (CG) for anthocyanins.

Chemical and cellular antioxidant activities were determined by ORAC and CAA methods. These techniques have been described in detail elsewhere [11,12]. Chemical antioxidant activity was computed in trolox equivalents ($\mu mol_{Trolox}/g_{Dry\ Extract}$) and cellular antioxidant activity in quercetin equivalents ($\mu mol_{Quercetin}/g_{Dry\ Extract}$).

III. RESULTS

MWP100 pretreatment led to the highest total polyphenol content. Figure 1 shows its yield kinetics whereas Figure 2 represents the selectivity of the extracts along the extraction. Both kinetic analyses are compared with the results obtained from the conventional extraction.

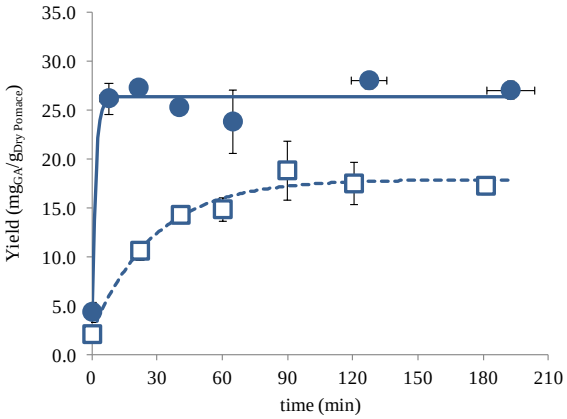

Fig. 1. Total polyphenol yield obtained for the MWP100 pretreatment (●) and the conventional (□) extraction.

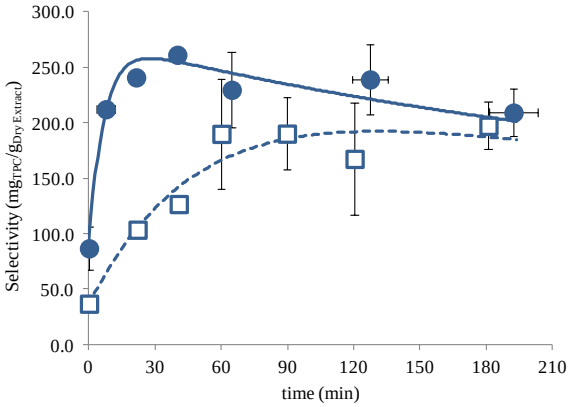

Fig. 2. Total polyphenol selectivity obtained for the MWP100 pretreatment (●) and the conventional (□) extraction.

On the other hand, MW80 pretreatment provided the highest anthocyanin yield and selectivity. Table 1 gathers the kinetic parameters obtained from the adjustments.

	C ($mg_{CG}/g_{Dry\ Pomace}$)		C (mg_{CG}/L)	
	MW80	Conventional	MW80	Conventional
C_0	0.21	0.19	70.39	68.67
C_f	1.42	0.96	224.45	117.65
k	0.18	0.03	0.093	0.029
ARD	0.02	0.07	0.078	0.065

Table 1. Anthocyanin yield and selectivity kinetic parameters for the MW80 pretreatment and the conventional extraction.

In both cases, for total polyphenol and anthocyanin selectivity, the kinetics of non-desired compounds are an important factor, since they greatly affects extract richness. The kinetic extraction parameters of non-polyphenol compounds are shown in Table 2.

	C (g/L)		
	MW100	MW80	Conventional
C_0	11.52	11.85	13.28
C_f	50.25	10.55	537.68
k	$4.76 \cdot 10^{-4}$	$2.72 \cdot 10^{-2}$	$5.25 \cdot 10^{-5}$
ARD	0.087	0.088	0.086

Table 2. Non-polyphenol extraction kinetic parameters.

The previous kinetic analysis leads to the selection of the optimal extraction time that allows to improve extraction yield and product richness. In the case of polyphenols, after the MWP100 pretreatment, extraction was stopped at 40 minutes; whereas for anthocyanin 20 minutes of extraction after the MW80 pretreatment provided a substantial rich extract in these compounds. Representative samples of each process were used to further analyze the antioxidant activity of each product. Figure 3 represents the chemical and cellular antioxidant activity obtained for each extract.

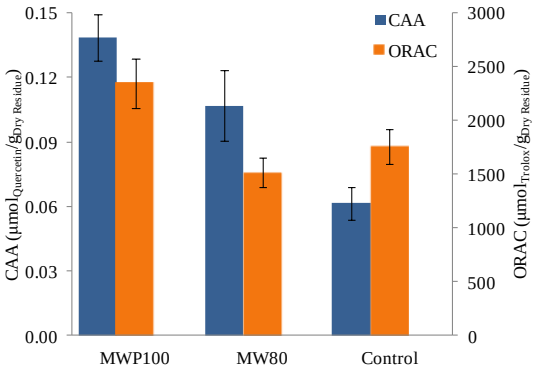

Fig. 3. Chemical and cellular antioxidant activity.

IV. DISCUSSION

Conventional solid-liquid extraction employs long extraction times in order to deplete grape pomace. However, it also entails the extraction of other undesired substances that reduce polyphenol richness. This is greatly notable in anthocyanin extraction, in which selectivity decreases a 45% in favor of extraction yield. Nonetheless, with the addition of a microwave pretreatment it is possible to improve both quantity and extract quality. Microwave advantages towards yield improvement are widely recognized, but nothing has been said about the extract quality. The pretreatments proposed here allow an offset between yield and selectivity, not possible to achieve by the conventional process.

Regarding total polyphenol content, a microwave and pressure pretreatment up to 100ºC (MWP100) allows to obtain the maximum amount of active compounds. Figure 1 represents the extraction yield of this pretreatment compared to the conventional process, whereas Figure 2 shows the extraction selectivity. As it can be seen in Figure 1, MWP100 enables the quick extraction of all the polyphenols available. No further yield improvement is found in the subsequent extraction. In comparison with conventional extraction, a 48% yield improvement takes place. In addition, by stopping the extraction at 40 minutes, also a 35% richer polyphenol extract is obtained. If the extraction continues up to 3 hours, as in the conventional process, no more polyphenols are leaked, but undesired substances like sugars and fibers that reduce the extract richness.

In the case of anthocyanins, a microwave pretreatment up to 80ºC (MW80) provides the best results. For this group the microwave boost is more pronounced than in polyphenols. An 85% more concentrated extract is obtained by MW80 pretreatment at 40 minutes. Table 1 shows the anthocyanin extraction kinetic parameters. This prominent improvement is due to the ease of anthocyanins to be drawn out. Anthocyanins are located in grape skin vacuoles and their extraction does not present such large mass transfer limitations as polyphenols placed in the grape seed do [13]. So, anthocyanins are quickly extracted before other undesired substances begin to leak, what results in the extraction of substantial rich extract.

These simultaneous yield and selectivity optimums are due to the prominent effect of microwaves on polyphenols rather than in other substances. Microwave polyphenol and anthocyanin extraction are mostly accomplished at 10-20 minutes, whereas undesired substances present their maximum extraction at 30-40 minutes. This is proven by the non-polyphenol extraction kinetics gathered in Table 2. The extraction yield (C_0+C_f) and the extraction constant of the conventional process are some order of magnitude larger than the microwave pretreatment ones. So, this selective effectiveness of microwaves on polyphenols allows not only to improve the process yield, but also, by selecting the proper extraction time, obtain a richer polyphenol extract.

Higher and lower energy pretreatments have been also tested. A higher microwave-pressure pretreatment does not enhance polyphenol extraction further than MWP100 does, but instead, undesired substances. Lower energy pretreatments present similar extraction yield and selectivity to conventional extraction, no microwave improvement is found.

Representative extracts obtained at the optimal conditions from each pretreatment were selected for further biofunctionality assays. Chemical and cellular antioxidant activity results are presented in Figure 3. Chemical antioxidant activity is computed by ORAC analysis and cellular by CAA tests. Chemical antioxidant activity was found to be proportional to the concentration of active compounds, whereas cellular bioactivity does not. MWP100 has the lowest proportion of non-polyphenol compounds, so it is unlikely that hinder interactions take place [14]. On the other hand, MW80 contains a large fraction of anthocyanins, compounds that greatly contribute to increase the antioxidant power, so its bioactivity is boosted by this fraction.

V. CONCLUSIONS

In sum, the pretreatments proposed here to optimize polyphenol and anthocyanin extraction have been proved to be a good alternative to conventional process and to overcome microwave industrial limitations.

The addition of the pretreatment allow to improve the process yield as well as the product quality. In particular, it has been found that the microwave pretreatment enhance the biofunctionality of the final extracts.

VI. ACKNOWLEDGEMENTS

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