CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN 978-88-95608- 56-3; **ISSN** 2283-9216

Biotechnological Strategies to Valorise Grape Pomace for Food Applications

Massimiliano Binaschi, Guillermo Duserm Garrido, Chiara Cirelli, Giorgia Spigno*

DiSTAS - Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.
giorgia.spigno@unicatt.it

In this work, a preliminary experimental plan was set up to investigate the effect of an enzymatic pre-treatment (ET) of grape skins (GS) on the release of total phenols and antioxidant compounds in a next step of aqueous ethanol extraction and on the content of total dietary fibre (TDF) and water retention capacity (WRC) of the GS.
Dried and milled GS were obtained from fermented pomace of different red grape cultivars: a mixture of San Giovese – Merlot (SGM) and Croatina (CRO). SGM-GS were micronized (250 µm), while CRO-GS were milled at particle size < 2 mm. A commercial food grade enzyme preparation (Viscozyme® L, by Novozyme Corp.), was used to carry out the ET at a 3 % enzyme load (w/w based on dry weight of GS), 24 °C, 4 h under stirring, at two different moisture levels (17 and 72 %). After the ET, a conventional solvent extraction with 60 % ethanol at a solid/solvent ratio (SSR) of 1/7 or 1/24 (for the 17 % and 72 % moisture level of the ET, respectively), at 40 °C and under stirring for 90' was conducted. The extracts were characterised for total free phenols (TP); total anthocyanins (TA) and antioxidant capacity (ABTS and FRAP assay) and compared with direct solvent extraction control treatments. For the micronized SGM-GS, the ET allowed for a slight but significant increase in TP and TA release only for the 1/24 SSR. For the coarser CRO-GS, the ET significantly increased the TP and TA release only for the 1/7 SSR, apparently compensating the limitation to mass transfer given by lower surface area, which could be exploited to reduce the energy cost of extensive GS milling. Almost the same trend was observed for the antioxidant capacity. Analysis of TDF revealed a significantly reduction of this parameter after ET confirming the enzyme action on the cell wall components.

1. Introduction

Grape is one of the largest fruit crops in the world, with reported 74.50 Mton in 2014 and with about 50 % of the world grapes processed into wine (FAOSTAT, 2014). Grape processing into wine generates huge amount of solid residues with grape pomace (GP) 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). GP consists in a mixture of skins and seeds and residual stalks. Grape skins (GS) represent indicatively the 50 % of the GP and are particularly interesting for their content in protein (5 -12 %), ash (2 - 8 %), soluble sugars (from 1 - 70 % depending on the applied process) and, above all, for the content in fibre and phenolic compounds. In fact, a total dietary fibre content up to almost 60 % of dry matter has been reported, dominated up to 98.5 % by insoluble dietary fibre. The cell wall is made up of neutral polysaccharides (cellulose, xyloglucan, arabinan, galactan, xylan and mannan), acidic pectin substances, insoluble proanthocyanidins, lignin and structural proteins (Pinelo et al., 2006). GS are well known and exploited for their content in phenolic compounds. These are present both as cell-wall phenols and non-cell-wall phenols. Regarding the cell-wall phenols, these are mainly bound to cellulose and hemicellulose which, on their turn, are tightly linked to lignin (Pinelo et al., 2006). Extraction processes which cannot act on the cell-wall structure will be essentially able to extract only the free phenols (Pedroza et al., 2015). On the other hand, the direct food application of GS as a source of antioxidant fibre (Lavelli et al., 2016) presents some critical aspects due to limited bioavailability of phenols bound to the cell wall, rheological and sensory problems defects and unbalanced ratio of insoluble/soluble fibre which, in the diet, should be 3/1. Enzymes are already used in the maceration step of the wine-making process to act on

the cell wall enhancing colour and phenolics release. Enzyme assisted extraction has also been studied for greener processes (Costoya et al., 2010; Ferri et al., 2016). However, this is the first time that enzyme treatments are investigated not only to enhance phenols extraction but also to assess their influence on the cell wall fraction with the aim of producing innovative antioxidant fibre ingredients for the food industry.

2. Materials and Methods

2.1 Grape skin powders preparation

The GS powder (GSP) samples were obtained from fermented red GP of a mix of Sangiovese and Merlot (SGM) varieties, vintage 2015, gently supplied by Casa Emma s.r.l. (Barberino Val d'Elsa, FI, Italy) and of Croatina (CRO) variety, vintage 2016, kindly provided by Cantina F.lli Bonelli (Rivergaro, PC, Italy).
The GP were dried at 60 °C for about 24 h (until a moisture content < 7-10 %). The skins were, then, separated through screening from stalks and seeds and milled until a final powder size $\leqslant$ 2 mm for CRO, and $\leqslant$ 250 µm for SGM. The obtained GSP were characterized for: dry matter (by oven drying at 105 °C ± 2 °C for 24 h); water activity (a_w) (Rotronic Hygropalm instrument);content of nitrogenous substances (Kjeldahl method); free sugars (D - Glucose and D - Fructose) content on the aqueous extract obtained from 7 g GSP and 100 ml H_2O (Megazyme enzymatic kit , K–FRUGL); total fat content (by Soxhlet extraction with hexane); ash content (oven drying at 550 °C ± 10 °C for 5 h); total dietary fiber (TDF) (Megazyme enzymatic kit, K-TFDR).

2.2 Enzymatic pre-treatment

After some preliminary tests to evaluate the water absorption by SGM and CRO GSP under different stirring speeds (150 rpm and 280 rpm in the orbital shaker HT INFORS AG CH-4103), incubation temperature (24 °C and 40 °C) and time (2, 4, 6 h), the following operating conditions for the enzymatic pre-treatment step were chosen: 24 °C, 280 rpm, 4 h.
The commercial enzymatic preparation used for the pre-treatment was based on previous work carried out by the research group (Gruppi et al., 2017) and literature reported use on grape skins (Costoya et al., 2010). Viscozyme® L is an enzyme complex that includes various carbohydrate activities: arabinase, cellulase, β-glucanase, emicellulase and xylanase. Cellulase and xylanase activity at pH 6 was assessed by specific Megazyme kits giving a cellulase activity of 25.28 ± 0.47 BGU/g (beta glucanase units) and a xylanase activity of 15.53 ± 1.03 units/g.
A high enzyme load, 3 % w/w of dried GSP, was applied to emphasize the enzyme action. The trials were carried, in triplicate, on 25 g of GSP. The required amount of enzyme was solubilized in distilled water and then added and mixed to the GSP. Two different amounts of water were added to obtain a mixture with a low water content (17 %) or a high water content (72 %). The samples were then incubated at the selected working conditions.At the end of the ET, for each sample an aliquot was dried and analyzed for the TDF content and water retention capacity (WRC) (Mateos-Aparicio et al., 2010). The rest of the sample was subjected to solvent extraction.

2.3 Solvent extraction

According to previous works (Pedroza et al., 2015; Moncalvo et al., 2016), solvent extraction was carried out with 60 % (v/v) aqueous ethanol, at 40 °C, 250 rpm (in the same orbital shaker used for the ET) for 90', with different solid/solvent ratios (SSR) based on dry weight of GSP: 1/7 and 1/24 g/mL.
Direct solvent extraction on GSP was carried out as control treatment. In the case of solvent extraction after the ET, samples enzymatically treated at 17 % moisture were subjected to 1/7 SSR extraction, while those treated at 72 % moisture to 1/24 SSR extraction.
After extraction, the liquid extract was separated by centrifugation (10314.4*g*, 2' at 24 °C) measuring the volume, stored at 4.0 ± 0.2 °C in closed containers sealed with parafilm and wrapped in aluminium foil and analysed in maximum 3 days. The extracts were characterised 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 gallic acid) on dry weight of GSP (mg_{GAE}/g_{dw}).
- ✓ Total anthocyanins (TA), estimated by dilution with acid–ethanol (ethanol:water:HCl, 7:3:1) and reading of the absorbance at 538 nm (Spigno et al., 2015). Results were expressed as mg total anthocyanins (TA) on dry weight of GSP (mg_{TA}/g_{dw}).
- ✓ Antioxidant activity was evaluated according to two different tests. In the radical ABTS test (García et al., 2011) the results were expressed as Trolox equivalent antioxidant capacity by means of calibration curve with Trolox® as $\mu mol_{Trolox}/g_{dw}$ (TEAC' based on GSP dry weight) and as mol_{Trolox}/mol_{GAE} (TEAC based on

total phenols content). In the FRAP test (Vellingiri et al., 2014) the results were expressed as $\mu mol_{Fe(II)}/g_{dw}$ and as $mol_{Fe(II)}/mol_{GAE}$.

2.4 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 extraction process on the measured parameters, was assessed by one-way ANOVA (IBM SPSS Statistics v.23) and Tukey's post-hoc test for means discrimination at a confidence level of 99 % ($p < 0.01$). In case of inhomogeneous variances, was applied Browne-Forsythe's ANOVA analysis with Games-Howell's post-hoc test.

3. Results and Discussion

3.1 Grape skin powder characterization

The characterization of the two GSP used in this study is reported in Table 1. The composition is in line with literature data (Moncalvo et al., 2016) and confirms the high potential of GSP as a source of dietary fibre. The residual level of free sugars is very low as expectable for fermented grape pomace. The SGM and CRO samples did not substantially differ for the main chemical composition.

*Table 1: Composition of grape skin powders. The values are expressed as mean ± d.s. The * symbol indicates a significant difference between the two powders for the same parameter (ANOVA, p <0.01).*

Parameter	Sangiovese Merlot	Croatina
Moisture (g/100 g_{dw})	5.09 ± 0.05 *	7.56 ± 0.1 *
a_w	0.36 ± 0.01	0.34 ± 0.02
Nitrogenous substances (g/100 g_{dw})	11.93 ± 0.19	11.74 ± 0.19
Total fat (g/100 g_{dw})	6.47 ± 1.38	5.42 ± 0.23
D-Glucose (g/100 g_{dw})	0.25 ± 0.00 *	1.06 ± 0.14 *
D-Fructose (g/100 g_{dw})	0.30 ± 0.01 *	1.62 ± 0.12 *
Ash (g/100 g_{dw})	7.38 ± 0.07 *	6.47 ± 0.10 *
Total Dietary Fibre (g/100 g_{dw})	65.57 ± 1.16	63.45 ± 0.83

3.2 Influence of enzyme pre-treatment on solvent extraction

Figure 1 reports the results of TP and TA recovered from different GSP samples obtained through direct solvent extraction with 60 % aqueous ethanol.

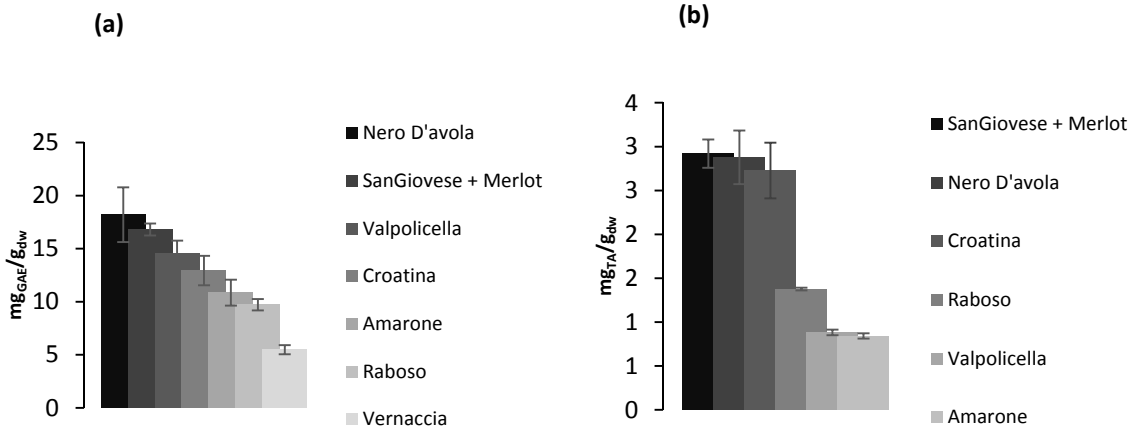

Figure 1 - Yields of total phenols (a), expressed as mg_{GAE}/g_{dw} (GAE = gallic acid equivalents) and total anthocyanins (TA) (b) expressed as mg_{TA}/g_{dw}, by direct solvent extraction, solid/solvent ratio 1/7, 40 °C, 90 min for SanGiovese+Merlot and Croatina, 1/8, 60 °C, 120 min for other samples. Error bars represent ± s.d. of the mean values.

The results obtained in this study were in line with those obtained by the authors with GSP from different red grape cultivars, even though under different extraction conditions (1/8, SSR, 60 °C, 2 h).
The yields of TP obtained by direct solvent extraction (1/7 SSR), were significantly different in relation to the type of GSP. SGM gave higher yield of both TP and TA, than CRO (Table 2), even though the latter is a well-known cultivar for the high content in anthocyanins (on average higher than SGM) . The higher yield was probably due to the finer granulometry of the SGM compared to CRO.
The use of a higher SSR (1/24 vs 1/7) obviously allowed for a higher recovery of phenolic compounds for both the GSP (Table 2). This was particularly evident for the coarser powder (CRO), for which the higher SSR allowed a yield increase of 134 % and 140 % for TP and TA, respectively. For SGM the increase was only 30 % probably due, again, to the finer particles size which, obviously, decreases the resistance to mass transfer.

Table 2: Total phenols anthocyanins recovered from different grape skin powders. DE: direct solvent extraction at 1/7 or 1/24 solid/solvent ratio. ETE: solvent extraction preceded by enzymatic pre-treatment at 17 or 72 % moisture content. The results are expressed as mean ± s.d. GAE: gallic acid equivalents. Different letters within the same column indicate significant differences (ANOVA and post-hoc of Tukey, p <0.01).

Variety	Treatment	Total phenols (mg_{GAE}/g_{dw})	Total anthocyanins (mg_{TA}/g_{dw})
Sangiovese Merlot	DE - 1:7	16.92 ± 0.32 d-e	2.92 ± 0.16 c
	ETE 17 % - 1:7	15.27 ± 0.55 e-f	2.90 ± 0.08 c
	DE - 1:24	22.10 ± 1.90 c	3.84 ± 0.23 b
	ETE 72 % - 1:24	27.91 ± 0.01 b	4.27 ± 0.09 b
Croatina	DE - 1:7	12.94 ± 1.36 f	2.73 ± 0.37 c
	ETE 17 % - 1:7	20.19 ± 1.26 c-d	4.58 ± 0.30 b
	DE - 1:24	30.29 ± 0.46 a-b	6.54 ± 0.36 a
	ETE 72 % - 1:24	31.60 ± 0.77 a	6.59 ± 0.21 a

Application of an enzymatic pre-treatment was investigated for the development of a greener extraction process with a reduced use of solvent. From a sustainability point of view, the ideal would always be to work with limited water addition and at room temperature, even though, the latter, does not favour the enzymatic activity. For this reason the ideal low moisture level of 17 % was investigated and compared with the 72 % moisture level.
For SGM powder, the ET led to a slight but significant increase in yields in TP (+ 26%) and TA (+ 11.2%) only for the solvent extraction carried out using 1/24 SSR. For the 1/7 SSR, the pre-treatment appeared usefulness.
In the case of CRO flour, the trend was the opposite, since the ET led to a significant increase in TP yield (+ 56%) and TA (+ 67.7%) only in the case of low-SSR solvent extraction. These differences may be due, as commented for direct extraction, to the different granulometry. However, in this case, also a different composition of the fibrous fraction of the two skins and the types of respective fibre-phenol bonds may have influenced the enzyme action.
The antioxidant capacity of the different extracts is reported in Table 3.
Based on the FRAP assay, the antioxidant capacity of SGM extracts, expressed as $mmol_{Fe(II)}/g_{dw}$, reflected the yields of TP and TA, increasing only for the SSR 1/24. However, the ET gave a 100% increase, compared to the 30 % increase calculated for TP and TA. This suggests a poor correlation of the FRAP assay with the Folin's analysis and is confirmed by the higher specific antioxidant capacity (mol_{Trolox}/mol_{GAE}) of the enzyme extract. This could be explained by an effective action of the enzyme in releasing some phenol compounds otherwise bound to the fibre fraction and should be confirmed by a chromatographic evaluation of the phenolic profile.
Also in the case of CRO, the $mmol_{Fe(II)}/g_{dw}$ data confirmed what observed for TP and TA yields, that is a positive effect of the pre-treatment only at the 1/7 SSR. However, for CRO, the mol_{Trolox}/mol_{GAE} was not influenced by the extraction conditions suggesting an influence on the amount but not on the composition of the recovered compounds.
The results obtained with the ABTS assay did not completely agree with the FRAP assay, which can be partly due to the different antioxidant mechanisms detected by the two methods (Gruppi et al., 2017). In fact, the TEAC data (specific antioxidant capacity) were not significantly affected by the extraction process.
The TEAC' data indicated that the ET did not increase the recovery of antioxidant compounds from SGM powder, independently of the SSR ratio, whereas for CRO powder they confirmed the positive enzyme effect.

Table 3: Antioxidant capacity (FRAP and ABTS assays) of grape skin powders extracts. DE: direct solvent extraction at 1/7 or 1/24 solid/solvent ratio. ETE: solvent extraction preceded by enzymatic pre-treatment at 17 or 72 % moisture content. The results are expressed as mean ± d.s. CRO: Croatina; GAE: gallic acid equivalents; SGM: Sangiovese and Merlot; TEAC: Trolox equivalent antioxidant capacity. Different letters within the same column indicate significant differences (ANOVA and post-hoc of Tukey, p <0.01).

Variety	Treatment	FRAP assay		ABTS assay	
		$mmol_{Fe(II)}/g_{dw}$	$mol_{Fe(II)}/mol_{GAE}$	TEAC (mol_{Trolox}/mol_{GAE})	TEAC' ($\mu mol_{Trolox}/g_{dw}$)
SGM	DE - 1:7	346.06 ± 24.49 b	3.51 ± 0.25 a	1.07 ± 0.10	105.22 ± 9.56 c
	ETE 17 % - 1:7	295.50 ± 32.49 b-c-d	3.26 ± 0.33 a-b	1.11 ± 0.12	100.75 ± 10.80 c-d
	DE - 1:24	271.08 ± 53.30 c-d	2.06 ± 0.32 d	1.10 ± 0.13	151.76 ± 12.98 b
	ETE 72 % - 1:24	524.14 ± 35.20 a	3.19 ± 0.21 a-b-c	0.86 ± 0.18	141.69 ± 29.54 b
CRO	DE - 1:7	224.53 ± 27.36 d	2.96 ± 0.26 b-c-d	1.09 ± 0.14	75.81 ± 10.49 d
	ETE 17 % - 1:7	335.30 ± 44.86 b-c	2.82 ± 0.31 c-d	1.07 ± 0.12	126.98 ± 15.51 c
	DE - 1:24	482.80 ± 59.22 a	2.71 ± 0.33 c	1.03 ± 0.21	183.22 ± 36.61 b
	ETE 72 % - 1:24	516.06 ± 15.81 a	2.78 ± 0.08 c	1.08 ± 0.09	199.82 ± 17.21 a

The effect of the ET on the fibrous fraction was evaluated measuring the TDF content and the WRC of the treated samples after ET (Table 4).

Table 4: Characterisation of grape skin powder before. AP: as presented; ET: after enzymatic pre-treatment (ET) at 17 or 72 %different moisture %). The results are expressed as mean ± d.s. Different letters within the same column indicate significant differences (ANOVA and Tukey's post-hoc test, p <0.01).

Variety	Treatment	Total Dietary Fibre (g/100 g_{dw})	Water Retention Capacity (g H_2O/100 g_{dw})
Sangiovese - Merlot	AP	65.57 ± 1.16 a	1.84 ± 0.03
	ET 17 %	54.11 ± 0.04 cd	1.85 ± 0.02
	ET 72 %	52.95 ± 0.39 d	1.75 ± 0.12
Croatina	AP	63.45 ± 0.83 a	1.49 ± 0.06
	ET 17 %	59.98 ± 0.32 b	1.48 ± 0.05
	ET 72 %	55.82 ± 0.85 c	1.51 ± 0.07

TDF content revealed a significant reduction after the ET for both the SGM and CRO powders. The reduction was higher when the ET was carried out at a higher moisture content, confirming that this condition favours the enzyme activity. The reduction in TDF, however, did not lead to any difference in WRC.

4. Conclusions

The present study investigated the exploitation of an enzymatic treatment of fermented grape skins to catalyse the hydrolysis of the skin fibrous component. The objective may be different. The treatment could, in fact, promote the release of the phenolic compounds enhancing the yield and/or reducing the solvent use in a following conventional solvent extraction. Still, the treatment could increase the bioavailability of the active compounds and improve the fibre composition of grape skin powders as healthy ingredients for the food sector.

The effect of enzymatic treatment on the solvent extraction (with 60 % aqueous ethanol) of antioxidant compounds, revealed different results depending on the type of GSP used for the process.

In the case of extracts obtained from micronized SGM flour (250 µm), ET application before solvent extraction resulted in a limited (30 %), but significant, increase in yields of TP and TA only with a high SSR (1/24).

In the case of extracts obtained from a coarse CRO powder (2 mm), an opposite trend was observed, since the solvent recovery was increased only in the case of extraction with a low SSR (1/7), compensating the limitation to mass transfer caused by the larger particle size. The increase was very high (135 %) and this is a positive result since the ET could be exploited to reduce the energy cost needed to mill the GSP down to 250 µm. The evaluation of the extracts' antioxidant capacity generally confirmed what observed for the recovery of

TP and TA. However, depending on the GSP and on the used assay, a different phenolic profile of the extract may be hypothesized as a consequence of the enzymatic action of specific phenols bound to the cell walls.
In the case of SGM, the FRAP assay showed a 100 % increase in the recovery of antioxidant compounds thanks to the ET when it was carried out with a high-water content (72 %).
In the case of CRO, significant increases in the recovery of antioxidant compounds were measured only for the ET carried out a low water content (17 %), even though the % increase (50-67 %) compared to direct solvent extraction was not as high as estimated based on TP and TA yield.
The analysis of TDF of the GSP before and after ET, confirmed the activity of the enzyme as a significant reduction of TDF content was assessed for both SGM and CRO powders. However, the decrease in TDF did not lead to a reduction in the water retention capacity of the powder which is very high and can give negative technological effects, for example, for a use in bakery products.
Further research will be required to verify the release of phenolic and anthocyanin compounds immediately after conditioning and to determine the phenolic profile of liquid extracts with HPLC analysis. Also, due to the low measured enzymatic activity of the selected commercial enzyme (Viscozyme®), different combinations of enzyme preparations need to be tested and optimised.

Acknowledgments

This research was supported by Fondazione Cariplo through the project “ReMarcForFood - Biotechnological strategies for the conversion of Winemaking by-products and their recycling into the food chain: development of new concepts of use” (2016-0740 grant).

Reference

Costoya N., Sineiro J., Pinelo M., Rubilar M., Nuñez M. J., 2010, Enzyme-aided extraction of polyphenols from grape pomace, EJEAFChe. 9 (4), 696-705.
FAOSTAT, Food and Agriculture Organization of the United Nations—Statistics Division, 2014. Available from: http://www.fao.org/faostat/en/#data
Ferri M., Bin S., Vallini V., Fava F., Michelini E., Roda A., Minnucci G., Bucchi G., Tassoni A., 2016,. Recovery of polyphenols from red grape pomace and assessment of their antioxidant and anti-cholesterol activities. N. Biotechnol. 33 (3), 338-344.
García A., Amendola D., González M., Spigno G., Labidi J., 2011, Lignin as natural radical scavenger. Study of the antioxidant capacity of apple tree pruning lignin obtained by different methods. Chemical Engineering Transactions 24, 925-930, DOI: 10.3303/CET1124155
Gruppi A., Garrido G.D., Dordoni R., De Faveri D.M., Spigno G., 2017, Enzymatic wheat conditioning, Chemical Engineering Transactions 57, 1777-1782, DOI: 10.3303/CET1757297
Lavelli V., Torri L., Zeppa G., Fiori L., Spigno G., 2016, Recovery of winemaking by-products for innovative food applications, It. J. Food Sci. 28(4), 542-564.
Mateos-Aparicio I.; Redondo-Cuenca A.; Villanueva-Suárez M.J., 2010, Isolation and characterisation of cell wall polysaccharides from legume by-products: Okara (soymilk residue), pea pod and broad bean pod. Food Chem. 122, 339-345.
Moncalvo A., Marinoni L., Dordoni R., Duserm Garrido G., Lavelli V., Spigno G., 2016, Waste grape skins: Evaluation of safety aspects for the production of functional powders and extracts for the food sector, Food Addit. Contam. Part A 33:7, 1116-1126.
Pinelo M., Arnous A., Meyer A.S., 2006, Upgrading of grape skins: significance of plant cell-wall structural components and extraction techniques for phenol release, Trends Food Sci. Technol. 17, 579–590.
Pedroza M.A., Amendola D., Maggi L., Zalacain A., De Faveri D.M., Spigno G., 2015, Microwave-assisted extraction of phenolic compounds from dried waste grape skins, Int. J. Food Eng. 11(3), 359-370.
Spigno G., Marinoni L., Duserm Garrido G., 2017, State of the Art in Grape Processing By-Products, In: Handbook of Grape Processing By-Products: Sustainable Solutions, Ed. Galanakis C.,Elsevier AP, 1,27.
Vellingiri V., Amendola D., Spigno G., 2014, Screening of four different agro-food by-products for the recovery of antioxidants and cellulose, Chemical Engineering Transactions, 37, 757-762, DOI:10.3303/CET1437127