Re-use of grape pomace flour as new ingredient for pasta fortification

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Introduction

The winemaking industry produces large volumes of waste and by-products, among these, pomace represents 20% of the processed grape weight. Grape pomace is an important source of bioactive molecules like polyphenols, dietary fiber, unsaturated fatty acids, etc. Only small amount of this by-product is recycled thus potentially resulting in an environmental problem. Over the last years, the evaluation of grape pomace flour as functional alternative ingredient and its recovery into value-added food products have attracted great interest (1,2). The by-product can thus re-enter the food cycle, avoiding environmental complications (3) and contributing to a more sustainable winemaking activity. In this study fortified pasta was prepared through the replacement of durum wheat semolina with 5 % of pomace flour from one white grape cultivar (Fiano) and two red grape cultivars (Aglianico and Lambrusco). Fortified uncooked and cooked pasta samples were characterized by bioactive molecule content and profile. High Performance Liquid Chromatography analysis of both soluble and bound phenols, tocochromanols and carotenoids were performed.

Materials and Methods

Sample preparation

Three batches of wine pomace (grape harvest year 2019), *Vitis vinifera* L. varieties Aglianico and Lambrusco, (achieved after fermentation for red wine making), and Fiano (without fermentation, as it is used in white wine making) were obtained from a commercial winemaking facilities located in Salento (Apulia Region, Southern Italy). Pomace samples were dried in an oven at 50°C, until constant weight. The dried grape pomace were milled to flour and passed through a 1 mm sieve (Fig. 1). Pasta was produced by Del Duca pasta factory (Parabita, Italy). It was "fettuccine" made by durum wheat semolina added or not (control) with 5% (w/w) of grape pomace flour (Fig 1 and Fig 2).

Pasta extraction and HPLC analysis

Extraction of soluble and insoluble-bound phenolic compounds from raw and cooked pasta was carried out as described by Durante et al. (4). Briefly, aliquots of each sample were extracted twice with 80% v/v ethanol. The combined supernatants (soluble phenolic fraction) were collected, evaporated and hydrolyzed with 2M NaOH for 4 h. The insoluble-bound phenolic acids were extracted from pellets by hydrolysis with 2M NaOH for 4 h. Samples were acidified to pH 2.0 with 12M HCl and extracted twice with ethyl acetate. The upper phase was collected, evaporated, dissolved in 80% ethanol, and assayed by HPLC-DAD as reported by Gerardi et al.(5).

Isoprenoids (tocochomanols and carotenoids) were extracted from raw and cooked pasta as described by Durante et al. (4). Briefly, triplicate aliquots of each sample were suspended in 60% w/v KOH, 95% v/v ethanol, 1% w/v NaCl and 0.05% w/v BHT in aceton and incubated at 60 °C for 30 min. After hydrolysis, to each was added 1% w/v NaCl and extracted with 9:1 v/v n-hexane/ethyl acetate. The upper phases were evaporated re-dissolved in ethyl acetate and assayed by HPLC-DAD as reported by Durante et al. (6).

A B C

Fig. 1: A=Whole grape pomace (skins, seeds, stalks); B=Grape pomace flour; C= Fortified pasta with 5% of grape pomace flour.

A B C D

Fig. 2: A=Control Fettuccine made by durum wheat semolina; B=Fettuccine fortified with 5% of Fiano grape pomace flour; C= Fettuccine fortified with 5% of Aglianico grape pomace flour; D=Fettuccine fortified with 5% of Lambrusco grape pomace flour.

Results and Discussion

The HPLC analysis of uncooked pasta fortified with both white and red grape pomace showed a significantly increased soluble phenolic molecules content while the content of bound phenols increases slightly in pasta added with red grape pomace only (Tab.1). As showed in Tab. 2, during cooking process, the bound phenols were lost, while soluble phenols showed a net increase both in control and fortified pasta samples. Moreover cooked fortified pasta showed a higher soluble phenolic content than the control (Tab.2). Both tocochromanols and carotenoids showed a net increase in uncooked pasta added with either white grape pomace or red grape pomace (Tab. 1). In the cooked samples, tocochromanol and carotenoid content slightly decreased; despite the losses, cooked pasta still presented a significative higher content of these molecules with respect to the control (Tab 2). Phenols, tocochromanols and carotenoids possess different biological activities such as antibacterial, antitumor, antioxidant, anti-inflammatory effects (7, 8).

These results show that pasta fortified with Fiano, Aglianico and Lambrusco grape pomace flour could represent a food product enriched with functional molecules and a potential technological alternative for the food industry by-products re-use.

Composition of polyphenols, tocochromanols and carotenoids in uncooked (Tab1) and cooked (Tab2) pasta. CTRL: durum wheat fettuccine; Fiano, Aglianico, Lambrusco: durum wheat fettuccine added with 5% of Fiano, Aglianico and Lambrusco grape pomace flower.
Values represent the mean ± standard deviation of three independent replicate.

Tab.1

	CTRL		Fiano		Aglianico		Lambrusco	
	µg/g FW							
Phenols	**Soluble**	**Bound**	**Soluble**	**Bound**	**Soluble**	**Bound**	**Soluble**	**Bound**
Gallic acid	nd	nd	3.33±0.06	nd	1.40±0.01	nd	1.91±0.01	nd
Coutaric acid	nd	0.05±0.001	nd	nd	nd	nd	nd	nd
Catechin	nd	nd	3.89±0.05	nd	2.20±0.03	nd	nd	nd
Epicatechin	nd	5.18±0.02	1.53±0.04	4.36±0.04	2.09±0.03	0.09±0.001	4.67±0.04	3.33±0.03
Vanillic acid	nd	7.72±0.03	nd	nd	nd	10.00±0.03	nd	11.41±0.19
Sinapic acid	nd	10.72±0.39	2.75±0.02	9.25±0.52	nd	9.03±0.03	nd	7.85±0.05
Syringic acid	nd	nd	nd	nd	nd	15.71±0.32	nd	18.65±0.75
p Coumaric acid	1.31±0.01	4.76±0.06	nd	6.63±0.05	1.61±0.01	30.92±0.41	1.44±0.01	34.47±0.40
Total anthocyanins	nd	nd	0.26±0.001	0.52±0.03	1.40±0.001	0.22±0.001	2.08±0.02	0.64±0.02
Quercetin	nd	nd	1.72±0.02	nd	nd	nd	nd	19.14±0.17
Rutin	nd	13.48±0.98	nd	5.57±0.02	nd	5.24±0.002	nd	4.03±0.05
Quercetin-3-Glc	nd	0.46±0.001	0.48±0.01	0.86±0.01	0.10±0.001	0.40±0.005	nd	nd
Oenin	nd	nd	nd	nd	nd	nd	nd	nd
Kanpherol	nd	nd	0.71±0.02	0.60±0.001	0.36±0.001	0.23±0.003	nd	nd
Kanpherol-3-Glc	nd	nd	0.37±0.02	0.24±0.09	nd	0.09±0.001	0.35±0.01	0.15±0.01
Kanpherol-3-Rut	nd	nd	nd	0.14±0.01	nd	nd	nd	nd
Ferulic acid	0.14±0.02	224.02±2.71	1.03±0.01	210.80±1.14	0.09±0.02	214.38±2.19	0.11±0.02	186.41±0.71
Caftaric acid	nd	nd	nd	nd	nd	0.16±0.001	nd	nd
Total	***1.45±0.03***	***266.39±4.19***	***16.07±0.25***	***238.67±1.91***	***9.25±0.10***	***286.47±2.99***	***10.57±0.11***	***286.08±2.38***
Tocochromanols								
ß tocotrienols	1.81±0.03		5.29±0.05		3.22±0.02		3.65±0.02	
α tocopherols	nd		nd		0.59±0.01		0.89±0.03	
Total	***1.81±0.03***		***5.29±0.05***		***3.81±0,03***		***4.54±0.05***	
Carotenoids								
Lutein	0.72±0.01		1.79±0.01		1.06±0.02		1.16±0.05	
Zeaxanthin	0.01±0.0005		0.03±0.0005		0.016±0.001		0.02±0.002	
ß carotene	Nd		0.04±0.001		0.01±0.001		0.05±0.001	
Total	***0.73±0.03***		***1.86±0.01***		***1.09±0.03***		***1.22±0.05***	

Tab.2

	CTRL		Fiano		Aglianico		Lambrusco	
	µg/g FW							
Phenols	**Soluble**	**Bound**	**Soluble**	**Bound**	**Soluble**	**Bound**	**Soluble**	**Bound**
Gallic acid	nd	nd	1.88±0.01	nd	1.39±0.04	nd	1.01±0.02	nd
Coutaric acid	nd	nd	nd	nd	nd	nd	nd	nd
Catechin	nd	nd	4.66±0.09	nd	6.92±0.28	nd	1.81±0.08	nd
Epicatechin	nd	nd	13.26±0.99	nd	2.82±0.002	nd	nd	nd
Vanillic acid	nd	nd	2.37±0.01	nd	2.56±0.01	nd	2.77±0.04	nd
Sinapic acid	1.19±0.01	nd	1.76±0.003	nd	nd	nd	nd	nd
Syringic acid	nd	nd	nd	nd	1.05±0.16	nd	1.01±0.16	nd
p Coumaric acid	0.54±0.01	nd	0.98±0.03	nd	1.02±0.01	nd	0.88±0.01	nd
Total anthocyanins	nd	nd	nd	nd	1.94±0.01	nd	2.23±0.02	nd
Quercetin	nd	nd	2.13±0.08	nd	1.79±0.02	nd	7.73±0.18	nd
Rutin	0.03±0.001	nd	8.44±0.07	nd	1.94±0.03	nd		nd
Quercetin-3-Glc	nd	nd	0.44±0.01	nd	0.51±0.03	nd	1.50±0.007	nd
Oenin	nd	nd	nd	nd	0.40±0.001	nd	0.53±0.002	nd
Kanpherol	nd	nd	0.49±0.02	nd	0.21±0.003	nd	0.26±0.001	nd
Kanpherol-3-Glc	nd	nd	0.48±0.03	nd	nd	nd	nd	nd
Kanpherol-3-Rut	nd	nd	nd	nd	nd	nd	nd	nd
Ferulic acid	0.64±0.01	nd	1.93±0.08	nd	0.37±0.02	nd	0.91±0.01	nd
Caftaric acid	nd	nd	nd	nd	nd	nd	nd	nd
Total	***2.40±0.03***		***39.26±1.42***		***22.92±0.62***		***20.64±0.53***	
Tocochromanols								
ß tocotrienols	1.35±0.01		2.50±0.13		3.03±0.02		3.43±0.09	
α tocopherols	nd		nd		0.47±0.01		0.46±0.01	
Total	***1.35±0.01***		***2.50±0.13***		***3.50±0.03***		***4.19±0.10***	
Carotenoids								
Lutein	0.61±0.01		0.92±0.06		1.03±0.03		0.99±0.01	
Zeaxanthin	0.01±0.0004		0.03±0.002		0.01±0.0002		0.03±0.003	
ß carotene	nd		0.03±0.0004		0.01±0.004		0.04±0.003	
Total	***0.62±0.01***		***0.99±0.06***		***1.05±0.03***		***1.06±0.02***	

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Acknowledgements

This research was partially funded by CNR project NUTR-AGE (FOE2019, DSB.AD004.271), and ValBioVit-SNSvS NP-2.75 project- "2.2 Circular Economy" MITE-Italy.