

# Effects of different cooking conditions on the anthocyanin content of a black rice (*Oryza sativa* L. 'Violet Nori')

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# Effects of different cooking conditions on the ~~antioxidant~~ anthocyanin content of a ~~violet~~ black rice (*Oryza ~~Sativa~~ sativa L.* 'Violet Nori')

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**Abstract**

The study is focused on the effect of different cooking conditions on the antioxidant content, particularly anthocyanins, of *Oryza sSativa* L. 'Violet Nori, a new violet black rice cultivar. 10 different cooking tests were performed. The selected cooking conditions allowed to evaluate the effect of boiling, roasting, oven cooking, *risotto* cooking, and *oriental* cooking. The total anthocyanins amounts were evaluated by both a spectrophotometric pH differential method and High-Performance Liquid Chromatography (HPLC), together with the Total Phenolic Content (TPC) and the Radical Scavenging Activity (RSA). The obtained results showed that boiling with a low water amount (100 g rice/650 mL water) and *oriental* cooking, which allows a reduction of boiling times thanks to the lid of the pota sealing cover, allows effectively saving at least part of their anthocyanins content. Similar results were obtained by boiling if rice was introduced in cold or boiling water, but on the contrary a ratio of 100 g rice-/1000 mL water greatly enhanced the loss of antioxidant compounds. *Risotto* and oven cooking allowed obtaining results that were roughly intermediate between the 'best' and the 'worst' results. A high correlation existed between the amounts of total anthocyanins and both TPCs and RSAs. The 'best' results show that although cooking necessarily decreases the rice content of valuable antioxidants, a careful choice of the operative conditions allows effectively preserving amounts of total anthocyanins higher than 100 mg/100 g rice portion (about 120-140 mg/100 g rice) which are close to or even higher than in other well-known sources of dietetic anthocyanins.

**Keywords:** 'Violet Nori' rice (*Oryza sSativa L.*), anthocyanins, cooking tests, antioxidants.

**Introduction**

The interest in the healthy properties of ~~colour~~pigmented rice is continuously expanding all over the world and in Italy its crop areas have increased more than seven times in the last years [1]. Several bioactive compounds have been identified in the coloured caryopses, most belonging to the class of phenolic compounds [2-5]. Anthocyanins, the major water-soluble pigments which accumulate in the grains during maturation [4, 6], are principally known for their antioxidant activity [7-10]. Moreover in 2016 an exhaustive review by Olivas Aguirre et al. [11] dealing with cyanidin-3-O-glucoside, which is by far the major anthocyanin in rice, underlines its intense anti-proliferative effect against different kinds of cancer cell, even though the amounts used both for *in vitro* tests and in laboratory animals were often higher than those obtainable from food sources. As far as the anti-inflammatory effect of anthocyanins is concerned, in 2015 Vendrame and Klimis-Zacas [12] examined the scientific literature on this matter: they concluded that, on the basis of both *in vivo* and *in vitro* evidences, these properties are to be mainly attributed to the anti-oxidant properties of anthocyanins, though other mechanisms could be partially involved and still need to be clarified. More generally, Olivas Aguirre reported that several biological activities of dietary anthocyanins, such as their cancer preventive activity [13], their anti-inflammatory one [12,14] and their possible role in the prevention of cardiovascular diseases (CVD) [15] are probably related to their primary antioxidant activity [11].

Recently, Zhu [16] confirmed that anthocyanins have both *in vitro* and *in vivo* antioxidant and retinal protection activities, together with the ability to inhibit cholesterol adsorption and to regulate lipid profile. Other interesting biological activities of dietary anthocyanins are their glycaemic regulation activity, at present confirmed only by *in vitro* tests [16], and their neuroprotective effect, probably due to the modulation of gut microbiota [16, 17, 18].

In addition, other classes of flavonoids having similar protective activity, such as flavonols and flavan-3-ols, have been identified in ~~colour~~pigmented rice, and the positive interaction of anthocyanins with them is now clearly established [16]. However, it is interesting to underline that after oral introduction anthocyanins have a peculiar metabolic fate, since they can be partially absorbed in their native form in the stomach [19, 20], after release from the food matrix thanks to the acidic conditions [16, 21]. However, many studies report that in the human body anthocyanins reach the highest concentration in intestine [18], thus the gastric adsorption is not relevant to the effect of anthocyanins on the axis gut-brain. Although some authors reported that only the 9-10 % of the intact anthocyanins can be absorbed in the stomach [11], a correct evaluation of the bio-activity of dietary anthocyanins should consider the concentrations of both the parent molecules and of their active metabolites [18]. Moreover, they are also absorbed in their intact form in the intestinal wall, where they can reach significant concentrations before undergoing a transformation to phenolic acids, thanks to the activity of colonic bacterial metabolism [18]. These acids, particularly the major metabolite protocatechuic

acid, are highly concentrated in the blood stream and probably responsible of another part of the biological activity of anthocyanins [18, 19].

Despite the great interest in the anthocyanins metabolism and bio-activity, few studies have been published about the effect of cooking on the content of anthocyanins and phenolic compounds in ~~colour~~pigmented rice [22, 24] and in 2009 Hiemori et al. reported a dramatic decrease in the anthocyanins content after cooking [23]. Thus, the aim of this study was to investigate if the residual amounts of these compounds after cooking could yet support the inclusion of ~~colour~~pigmented rice among the most interesting sources of dietary anthocyanins and antioxidant compounds. The study is particularly focused on *Oryza ~~Sativa~~ sativa* L. 'Violet Nori', a new variety of ~~violet~~ black rice growing in Piedmont and registered at the Community Plant variety Office, whose anthocyanins content is generally higher or at least comparable with that in other best known black cultivar, such as 'Venere', 'Nerone', and 'Artemide'[1].

## Materials and Methods

### Chemicals

Cyanidine-3-O-glucoside chloride, DPPH• (1,1-diphenyl-2-picrylhydrazyl), Folin-Ciocalteu reagents, gallic acid, Trolox (6-hydroxy-2,5,7,8-tertramethylchromane-2 carboxylic acid), ethyl alcohol p.a., acetonitrile HPLC grade and formic acid were supplied by SIGMA. ~~Deionised~~ Deionized water (18MΩ) was produced by a Milli-Q system (Millipore, USA).

### Equipment.

A Retsch Grindomix 200 M mill (Haan, Germany) and an IKA Ultra-Turrax T25 (Staufen im Breisgau, Germany) were employed for the homogenization of the samples in the extraction solvents. The direct Ultrasound Assisted Extraction was realized by Hielscher UP200St (Teltow, Germany). An UV-Vis Agilent 8453 (Waldbronn, Germany) allowed the determinations of the Total Phenolic Content (TPC) [25], Radical Scavenging Activity (RSA) and Total Anthocyanins by the pH differential method [26] in the extracted solutions. An Agilent 1100 Liquid chromatograph equipped with a Diode Array Detector (DAD) allowed the HPLC determination of anthocyanins [1].

~~5~~ **Samples:** 'Violet Nori' whole rice was supplied by Azienda Agricola Eleonora Bertolone (Collobiano, VC, Italy), which collected it in 2017.

### Cooking treatments

The cooking tests were carried out employing the same rice batch. Ten different cooking tests were performed with professional cooking equipment at the Hotel School, Genova. For each test 100 g of whole 'Violet Nori' rice were cooked either in pan or oven by employing different conditions, which are summarized in Table 1.

*Boiling* (tests 1-4). 100 g dried rice were added to 650 or 1000 mL of cold (20 °C) or boiling (100 °C) water; cooking required 40 min after return to boiling.

'*Oriental' cooking* (test 5). 100 g dried rice were added to 300 mL of boiling water (100 °C), boiled without covering for 10 min and then, with a tight ~~sealed cover~~lid on the pot, for 14 min, up to the incorporation of the cooking water.

*'Risotto' cooking* (tests 6 and 7). Although this peculiar Italian cooking style normally involves a slow cooking, under a careful mixing, covering the rice grains with hot broth, a significant variation was adopted in order to reduce the cooking times of the whole rice and to better preserve the water-soluble compounds. Thus, a preliminary 10 min boiling of 100 g dried rice in boiling water was realized. The boiling water was then drained and collected, and a classical '*risotto'* cooking was realized by employing the discarded and intensely coloured boiling water instead of broth, with or without a preliminary rice toasting. The amounts of the initial water for the test with and without the preliminary roasting were 750 mL and 1000 mL, respectively.

*Oven cooking* (tests 8-10). Rice was prepared in a kitchen oven employing an uncovered baking tray. The effects of toasting (test 9) and of a preliminary 10 min boiling (test 10) were also tested.

In order to evaluate the initial anthocyanin content, a control test (test 11) was prepared by drying, grinding and extracting the crude whole violet rice in the same conditions of the cooked samples.

**Extraction of anthocyanins and other antioxidant compounds**

After cooking at the Hotel S~~s~~chool Marco Polo (Genova), the samples were immediately transferred to the University laboratories and oven dried at 40 °C for 48 hours. A Grindomix 200 M mill (Retsch) (35 sec at 5000 rpm) finely ground the grains: 1 g powder was then suspended in 40 mL EtOH/$H_2O$ mixture (60:40 v/v) and homogenized by an Ultra-Turrax T25 homogenizer (IKA) in an ice bath (0.5 min at 8000 rpm, followed by 1.5 min at 24000 rpm). The final extraction was obtained by the direct ultrasound assisted extraction method optimized and described in a previous study [1]. The extracted solutions were separated by centrifugation (3000 rpm for 10 min), followed by a Whatmann n.1 paper disk filtration of the supernatant for the separation of the coarse solid material and a further filtration by an RC membrane syringe filter (0.45 µm) for the removal of the fine solid material. Two replicated extractions were performed for each sample.

**Analysis of the extracted solutions**

The total anthocyanin~~s~~ content was determined both by the previously described ~~HPCL~~HPLC-DAD method [1] (Figure 1) and by the spectrophotometric pH differential method [27]. The results were expressed ~~as~~ as µg cyanidin-3-

glucoside/mL in the extracted solutions and turned into mg/100 g crude rice on a dried matter basis (d.m.), using cyanidin-3-glucoside in the range between 1 and 100 µg/mL as external standard.

Other two tests were performed by UV-VIS spectrometry: the DPPH test [26] for the determination of the RSA, expressing the results as µmol TEAC/g sample ~~on a dried matter basis (~~d.m.~~),~~ and the Folin-Ciocalte~~a~~u test [25] which determines the value of the TPC, expressing the results as mg GAE/g sample d.m..

As far as DPPH test is concerned, briefly a DPPH radical solution approximately $10^{-4}$ M in methanol was daily prepared. The initial DPPH concentration in this solution was measured by the absorbance at 515 nm of the control sample $C_0$ (without extracts), which was obtained by diluting 0.250 mL of methanol with the DPPH solution into a 10 mL volumetric flask. Then, in order to measure the antiradical activity of each extract, 0.250 mL of the extracted solution were transferred into a 10 mL volumetric flask before adding the DPPH solution to the mark. The flask was gently shaken and kept in the dark for 30 min before reading the residual absorbance. Before each sample a blank absorbance (0.250 mL of the extracted solution diluted to 10 mL by pure methanol, without DPPH) was measured and then subtracted. For each sample, the amount of the reacted DPPH was obtained by subtracting the residual absorbance at 515 nm from the $C_0$ absorbance, and was transformed in antioxidant activity using 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) as reference standard (Trolox Equivalent Antioxidant Capacity, TEAC). Two replicated determinations were performed for each sample.

For the Folin-Ciocalteu test, after a 1:10 dilution of the extracted solution, 0.2 mL of the diluted solution, 1 mL of Folin–Ciocalteu reagent (diluted 1:10 with deionized water) and 0.8 mL of aqueous sodium carbonate 7.5% w/v were mixed together in a test tube and vortexed. After standing in the dark at room temperature (25 ± 2 °C) for 30 min, the absorbance was read at 760 nm and the total phenolic concentration was calculated using gallic acid for the calibration curve (Gallic Acid Equivalent, GAE). Two replicated determinations were performed for each sample.

**Statistical analysis**

Statistical analysis were performed by the MedCalc® statistic software, freely downloadable for a trial at https://www.medcalc.org/. ANOVA was employed to evaluate the statistical significance of the differences among samples.

**Results ~~and discussion~~**

As previously reported for other black rice [28], all the HPLC plots showed that cyanidine-3-glucoside was by far the major anthocyanin (about 90 % of total anthocyanins) in 'Violet Nori' caryopses, both before and after cooking, whereas peonidin-3-glucoside stood for about the 8-10 % of total anthocyanins. Only minor amounts of 2 other anthocyanins were detected (Figure1). The ratios among anthocyanins were almost preserved in the cooking tests, though in some samples (i.e. tests 3 and 4) only cyanidin-3-glucoside and peonidin-3-glucoside were detectable after cooking. Considering its simplicity, its low cost and low environmental impact, the pH differential method for the total anthocyanin~~s~~ determination [27~~7~~] was thus tentatively applied together with the previously employed HPLC-DAD method with the aim of comparing the results obtained by the two methods, both employing cyanidine-3-glucoside as standard reference. In order to evaluate if the two methods give comparable results, the obtained values of the total anthocyanins amount in the extracts obtained after the ten cooking tests were compared by Passing-Bablok regression (Figure 2), since the classical univariate regression is not fit for the comparison of two variables which are both affected by an experimental error. Although a high correlation existed between the two series of results ($R^2$= 0,~~.~~981), with ~~and there was~~ no deviation from linearity, the two methods ~~(Figure)~~ were not statistically ~~(p<0,001)~~ identical (p<0,001). In fact, the +3.473 intercept (≠ 0) and the 1.134 slope (≠ 1) highlight the probable presence of non-separated coloured compounds, which enhanced the values obtained by the directs spectrophotometric analysis.

As far as the remaining anthocyanins content in the cooked samples is concerned, in the 10 cooking tests (tests 1-10) it always appears statistically ~~certainly~~ lower (p<0,001) than in the crude rice (test 11, 466 ± 30 mg/100g) (Figure ~~2~~3), with a reduction between ~~72~~ 70 and 92 %. Nevertheless, in some samples, for example those obtained in tests 1, 2 and 5, the residual amounts of anthocyanins were 120-140 mg/100g rice. The best results (138 ± 6 mg/100g) were obtained in the cooking test 5, which involved the employment of water in a 1:3 (p/v) rice /water ratio, its total adsorption into rice grains within the cooking times and the reduction of the cooking time thanks to the increase of the pressure in the pot due to the ~~sealed cover~~tight lid. The results obtained in the tests 1 and 2 were close to 120 mg/100 g rice, i.e slightly but not significantly ($p$<0,05) lower than in the test 5 ~~but still satisfying, close to 120 mg/100 g rice~~: in these tests the rice/water ratio was enhanced at 1:6.5, since the longer cooking time makes it essential to avoid rice drying. Since in these tests too the added water was completely adsorbed, the anthocyanins decrease with respect to test 5 was probably related to the longer boiling time. No statistically significant difference (p<0.05) was observed between sample 1 and 2, i.e. if rice was added to cold water (T = 20 °C) (test 1) or boiling water (test 2). As far as the sample~~s~~ appearance is concerned, the appearance and texture after the 5 test ~~was~~ were by far more attractive: in fact after *oriental* cooking rice grains were well-separated, with a bright violet color and a shiny appearance, whereas the two boiled samples appeared rather sticky.

In the simple and traditional *Pilaf* cooking (test 8) anthocyanins were lost in a larger extent than in test 1 and 2 ($p< 0.1$) although their amount was still close to 100 mg/100 g, whereas ~~*Risotto* (tests 6 and 7) and~~ *Pilaf + roasting and Pilaf + boiling* (tests ~~8,~~ 9 and 10, respectively) and *Risotto* cooking (tests 6 and 7) ~~cooking~~ caused much significant reductions ($p<0,05$) of the final anthocyanin amount. ~~allowed obtaining similar results, roughly intermediate between the "best" and the "worst" results.~~ As far as *risotto* is concerned, the preliminary ten minutes boiling and the substitution of broth with the discarded boiling water was not enough to preserve anthocyanins (test 6), and toasting (~~sample~~ test 7) led to a sticky consistence. *Pilaf* oven cooking, both with and without preliminary rice roasting or boiling, did not lead to the hoped results: the final aqueous consistency of rice grains and the amounts of preserved anthocyanins were not satisfactory, although the ratios rice/water were close to those of test 1 and 2.

As can be expected, as far as anthocyanins amounts are concerned the worst cooking conditions were by far those tested in tests 3 and 4, which preserved significantly lower amount ($p<0.02$) than in *Risotto* and *Pilaf* samples. It is possible to presume that ~~since~~ the high rice/water ratio (1:10) had caused a high loss of these compounds in the discarded residual water, and this loss was similar if rice ~~was~~ had been introduced in cold water or at its first boil ~~or in cold water~~.

With respect to TPCs and RSAs, the detected values appeared highly correlated with the total content of anthocyanins. In order to evaluate this correlation, the crude rice sample was not considered in sight of its possible leverage. When the 10 cooking tests are considered, the correlation between the anthocyanins amounts expressed as mg/100 g crude rice detected by the HPLC method and the RSAs appeared very high, with ~~r~~ R = 0,~~.~~9998; this value confirms the powerful antiradical activity of the extracted anthocyanins after heating and the possibility to partially preserve this valuable activity even after cooking. The correlation between the anthocyanins content by HPLC and the TPCs appeared slightly lower but still high (R~~r~~ = 0,~~.~~9319): the residuals plot shows the higher scatter for test 7, when rice had undergone the risotto cooking with a 3 min preliminary toasting that probably had determined a thermal degradation of anthocyanins. Similar results were obtained for the correlation between the total anthocyanins detected by the pH differential method and both TPCs (R = 0,9574) and RSAs (0,9902), all expressed on a dry matter basis (Figure ~~3~~4). Nevertheless, the correlation with RSAs appeared slightly lower than with HPLC-DAD, thus confirming the spectrophotometric interference of molecules which do not belong to the class of anthocyanins and are indeed separated by HPLC.

**Discussion**

The comparison between ~~Though~~ the fast UV-VIS and the HPLC methods for the determination of total anthocyanins confirmed that the pH differential method may slightly overestimate the anthocyanin content [29] since the two methods are not statistically identical, but the~~ir~~ high correlation and the high similarity of the obtained results allow to

look at the former for a preliminary fast and cheap tool for the evaluation of the total anthocyanins in ~~colour~~pigmented rice.

As far as the cooking tests are concerned, pressure boiling was not tested since previous study had shown that it causes the higher losses of anthocyanins [23, 24]. The cooking tests confirmed that cooking always significantly decreases rice anthocyanins [23, 24], thus negatively affecting the RSAs. Nevertheless, t~~T~~he results obtained in the cooking tests of 'Violet Nori', show that a careful choice of the cooking conditions allows preserving its healthy peculiar features ~~although cooking necessarily decreases its content of valuable anthocyanins and total phenolic compounds, thus negatively affecting the RSAs~~. The detected loss of total anthocyanin was in the 70-92 % range, i.e. not far from the previously reported 65,4-74,2 % for cyiandin-3-glucoside [23] and from the average degradation rate of 73,5 % and 72,5 % for cyanidin-3-glucoside and peonidin-3 glucoside, respectively [24]. Since neutral water was employed for the cooking tests, it can be assumed the flavylium cation had been broken in the B ring to form protocatechuic acid [30], which in this study was only tentatively identified but not quantified at 280 nm. The detected loss was also in agreement with the data reported by Zaupa et al. [31] for boiled rice, whereas the better performances that the same authors obtained for *risotto* were not confirmed. It is possible to assume that the lab-conditions of the risotto cooking [31], i.e. boiling in a test tube up to water sdsorption, had a different effect from the conditions of the 'real' risotto cooking: the direct gas heating of the pan probably involves a higher temperature on the inner pot surface, thus promoting anthocyanin thermal degradation. On the other hand, the conditions applied in lab [31] are much similar to the conditions employed in test 5, giving the best results. It is also interesting to note that the *oriental* cooking conditions employed in test 5 are quite similar to the conditions of a rice porridge preparation (without milk) and that porridge preparation has been previously identified as the better cooking method both for waxy and non-waxy black rice [24].

As far as the individual anthocyanin is concerned, in accordance with some previous studies [23, 24] no differences were observed in the tested cooking conditions in the percentage of losses of peonidin-3-glucoside and cyianidin-3-glucoside, which were on the contrary highlighted in the study of Zaupa et al. [31] reporting a higher stability of peonidin-3-glucoside.

The reduction of total anthocyanins was always paired with a reduction of TPC and RSA, thus to a reduction of the ability for free radical scavenging. Nevertheless, since the amount of anthocyanins in the crude 'Violet Nori' rice was generally higher than in other reported black rice [23, 24, 31, 32], a~~A~~fter 'Violet Nori' cooking, the total amount of anthocyanins ~~can be~~was preserved at values higher than 100 mg/100 g portion when the cooking water is completely adsorbed. The comparison between tests 1, 2, 3, 4 and 5 shows the importance of the ratio g rice/mL water in cooking

operations, since increasing the amounts of the employed water caused a significant loss of antioxidant compounds in the discarded water.

MoreoverOtherwise, the values obtained in the a 100 g portion in the 'best' cooking conditions are comparable to or even higher than the values reported in the fresh tissues of those fruits and vegetables which are generally considered the best sources of dietetic anthocyanins [2338], such as cranberries (140±30 mg/100g), black plums (140±26 mg/100g), red raspberries (92±20 mg/100 g), red grapes (27±11 mg/100g) and eggplants (87 mg/100g).These results are particularly interesting since fruits and vegetables which are generally considered, together with red wines, the best sources of these valuable compounds, had severe limitations. In fact, fruit consumption is limited due to its content of simple carbohydrates, the availability of fresh vegetable and fruits is often limited to the harvest period and the wine consumption should be limited for its content of ethanol and its neurological and carcinogenic effects. On the contrary, the great interest for colored rice is based on their availability all the year round and to the primary position of rice in a balanced human diet, which allows to favorably eat it on an everyday basis. Thus, further studies are in progress aiming and extending the observation to other colored rice cultivars in order to expand their interest as sources of valuable anthocyanins.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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**Figure captions**

**Fig. 1.** Anthocyanin profile obtained by HPLC. Peak 1, cyanidin-3,5-diglucoside; Peak 2, cyanidin-3-glucoside; Peak 3, cyanidin-3-rutinoside; Peak 4, peonidin-3-glucoside.

~~**Figure**~~ **Fig. ~~1~~2**. The Passing Bablok regression between the total anthocyanins amounts obtained by the UV-VIS (Y axes) and HPLC-DAD (X axes) methods. Results are expressed as µg cyanidin-3-glucoside/mL extracted solution.

~~**Figure**~~ **Fig. ~~2~~3.** Total anthocyanins content (mg/100g rice d.m.) in the ten cooked samples (1-10) and in the crude rice (11).

~~**Figure**~~**Fig. ~~3~~4**. Correlation between the anthocyanins content detected by HPLC-DAD and the RSAs (a, regression line; b, residuals) and TPCs (c, regression line; d, residuals) of the ten cooked rice samples. Values are expressed as µg/g dried matter.

**Table 1**

**The tested cooking conditions**

| | Appliance | Cooking Method | Init. Temp °C | Initial Water mL | Cooking time min |
|---|---|---|---|---|---|
| **1** | Stove | Boiling | 20 | 650 | 40 |
| **2** | Stove | Boiling | 100 | 650 | 40 |
| **3** | Stove | Boiling | 20 | 1000 | 40 |
| **4** | Stove | Boiling | 100 | 1000 | 40 |
| **5** | Stove | *Oriental* Cooking | 100 | 300 | 14 uncovered + 14 with a sealed cover |
| **6** | Stove | *Risotto* Cooking | 100 | 1000 | 10 (boiling) + 25 |
| **7** | Stove | Toasting + *Risotto* Cooking | 100 | 750 | 10 (boiling) +3 (toasting) + 25 |
| **8** | Oven | Pilaf | 20 | 500 | 30 |
| **9** | Oven | Pilaf | 20 | 500 | 3 (toasting)+ 30 |
| **10** | Oven | Pilaf | 20 | 650 | 10 (boiling) + 10 |

Figure x

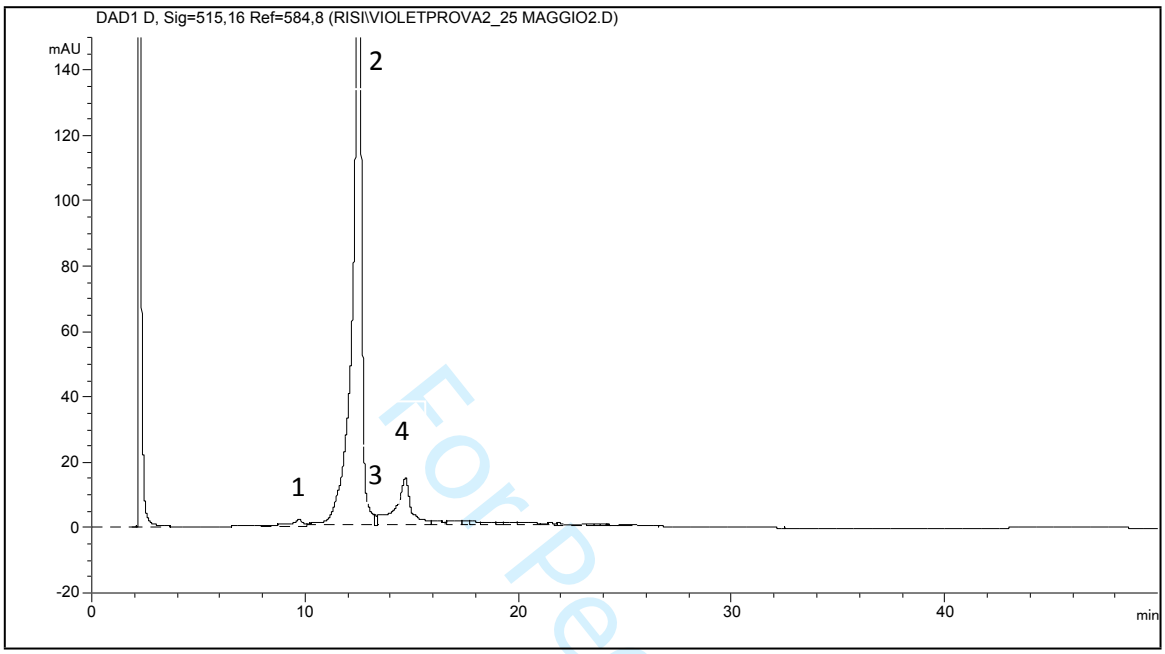


**Figure 2**

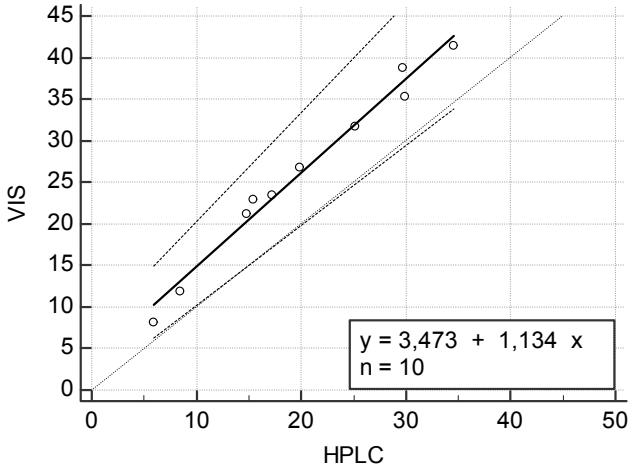


Figure 3

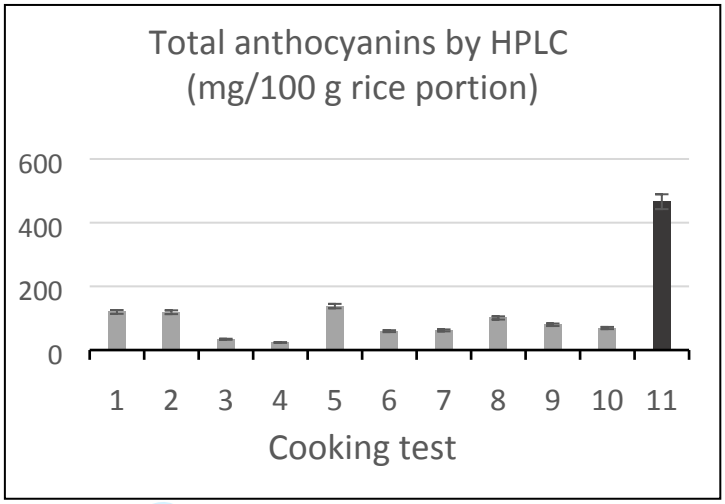
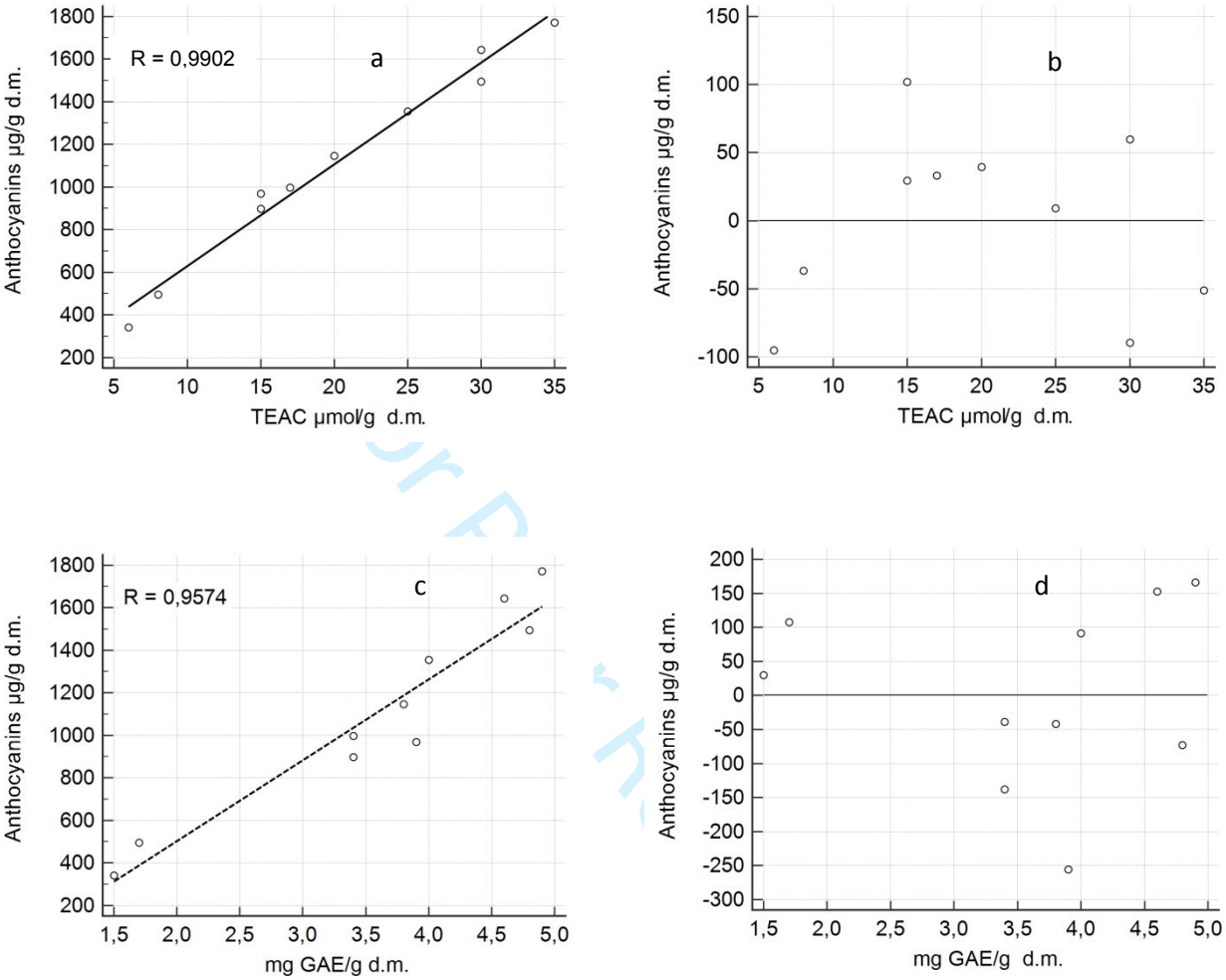


**Figure 4**


R = 0,9902
a
Anthocyanins µg/g d.m.
TEAC µmol/g d.m.
b
Anthocyanins µg/g d.m.
TEAC µmol/g d.m.
R = 0,9574
c
Anthocyanins µg/g d.m.
mg GAE/g d.m.
d
Anthocyanins µg/g d.m.
mg GAE/g d.m.

Supplementary materials to

**Effects of different cooking conditions on the antioxidant content of a violet rice (*Oryza Sativa* L. 'Violet Nori')** **EFRT**

Silvia Catena, Federica Turrini, Raffaella Boggia, Matilde Borriello, Marco Gardella and Paola Zunin

**Corresponding author: Paola Zunin - Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148, Genova, Italy. zunin@difar.unige.it**

| Test | Total Antho by HPLC (μg/mL extracted solution)* | dev.st | Total Antho by pH differential method (μg/mL extracted solution)* | dev.st |
|---|---|---|---|---|
| 1 | 29,9 | 1,7 | 35,3 | 1,9 |
| 2 | 29,7 | 1,4 | 38,8 | 1,8 |
| 3 | 8,5 | 0,5 | 11,9 | 0,6 |
| 4 | 5,9 | 0,3 | 8,1 | 0,3 |
| 5 | 34,5 | 1,3 | 41,4 | 1,4 |
| 6 | 14,8 | 0,7 | 21,2 | 0,8 |
| 7 | 15,4 | 0,6 | 22,9 | 0,7 |
| 8 | 25,2 | 1,5 | 31,8 | 1,6 |
| 9 | 19,8 | 0,9 | 26,8 | 0,8 |
| 10 | 17,3 | 0,9 | 23,4 | 1,1 |

*expressed as cyanidin-3-glucoside

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**Viale Cembrano 4, 16148, Genova, Italy. zunin@difar.unige.it**

| Test | TEAC µmol/g (d.m.) | dev.st | TPC mg GAE/g (d.m.) | dev.st | Anthocyanins µg/g (d.m.) * | dev.st |
|---|---|---|---|---|---|---|
| 1 | 30 | 1,6 | 4,8 | 0,31 | 1495 | 7,0 |
| 2 | 30 | 1,35 | 4,6 | 0,24 | 1644 | 5,8 |
| 3 | 8 | 0,4 | 1,7 | 0,1 | 497 | 2,0 |
| 4 | 6 | 0,36 | 1,5 | 0,09 | 343 | 1,2 |
| 5 | 35 | 1,72 | 4,9 | 0,27 | 1772 | 5,1 |
| 6 | 15 | 0,78 | 3,4 | 1,15 | 898 | 2,8 |
| 7 | 15 | 1 | 3,9 | 0,18 | 970 | 2,5 |
| 8 | 25 | 1,2 | 4,0 | 0,22 | 1355 | 6,0 |
| 9 | 20 | 0,91 | 3,8 | 0,22 | 1146 | 3,8 |
| 10 | 17 | 0,9 | 3,4 | 0,2 | 996 | 3,7 |
| 11 | 67 | 3,57 | 8,8 | 0,43 | 4972 | 30,0 |

*expressed as cyanidin-3-glucoside

Supplementary materials to

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| Test | Total antho by HPLC mg/100 g * | Dev.st |
|---|---|---|
| 1 | 119,7 | 7,0 |
| 2 | 118,8 | 5,8 |
| 3 | 33,8 | 2,0 |
| 4 | 23,7 | 1,2 |
| 5 | 138,1 | 5,1 |
| 6 | 59,3 | 2,8 |
| 7 | 61,7 | 2,5 |
| 8 | 100,8 | 6,0 |
| 9 | 79,4 | 3,8 |
| 10 | 69,1 | 3,7 |
| 11 | 465,9 | 30,0 |

*expressed as cyanidine-3-glucoside