Antimutagenic Activity of Raw Materials and By-Products from Production of Grape Wines

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Abstract: The inhibition of mutagenicity was assessed by Ames test by bacterial strains *Salmonella typhimurium* TA98 and TA100 using two mutagens and methanolic extracts of healthy fresh berries of blue grapevine varieties – St. Laurent, Portugal, André and white varieties – Chardonnay, Welschriesling, Pinot Gris and berries infected with *Botrytis cinerea* fungus. As model mutagens, two compounds whose presence in food is real, 2-amino-3-methyl-3H-imidazo-(4.5-f-)-quinoline (IQ), arising from certain heat treatments of meat and acting as indirect mutagen after metabolic activation, and *N*-nitroso-*N*-methylurea (MNU) acting as a direct mutagen, were applied. An increased risk of MNU is due to its possible endogenous formation. Fermentation sediment after vinification of the varieties Chardonnay, Welschriesling and André was tested by similar experimental system. All extracts showed strong positive inhibition of mutagenicity, berries infested with *Botrytis cinerea* also in diluted extracts. Positive inhibition was demonstrated also by fermentation sludge.

Keywords: wine; grape berries; polyphenolic compounds; antimutagenicity

A considerable attention has been paid in recent years to substances occurring naturally in foodstuffs of plant origin which can act positively in the prevention of tumorous diseases and coronary heart disease (Hertog 1998). Polyphenolic compounds have been applied mainly as antioxidants (Racek *et al*. 2001).

Grape wines, especially those from blue grape cultivars belong to the most attractive sources of polyphenolic compounds (Mikeš *et al*. 2008). In 1970's, resveratrol (3,4',5-trihydroxy-*trans*-stilbene) became a phenomenon, explaining the term "French paradox". The territories of south Moravia and of Polabí (Czech Republic) belong for ages to traditional, although to relatively small on a world scale production of grape wines. The content of polyphenolic substances, mainly stilbenes, in wines produced in these regions have been observed since 1990's (Totušek 2000; Melzoch *et al*. 2001; Kyseláková *et al*. 2003). The stilbene oligomers from grapes and wines can play also a role against oxidation and development of atherosclerosis. They have been shown to have a cancer chemopreventive activity and to protect lipoproteins from oxidative damage. Levels of stilbene oligomers and astilbin in French varietal wines and in grapes during noble rot (*Botryotinia fuckeliana* Whetzel anamorph *Botrytis cinerea*

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Pers.) development investigated Landrault *et al.* (2002).

The interest in explanation of mechanisms of beneficial to health action of regular moderate consumption of grape wine reached even the scientific institutes of the Czech Republic. One of the first studies dealing with resveratrol was published by Šmidrkal *et al.* (2001). Possible chemopreventive action of wine was mentioned in a study of Kopec (1999). At present, hundreds of studies concerning this topic from various viewpoints and on various levels of expert knowledge can by found in the world literature. Zendulka (2008) presented a review of these studies in his thesis. Resveratrol, as well as it is dimer (ε-viniferin), act as phytoalexins. They are synthesised in plant vine as products of response on the stress, either physical or biological one. Epidemiologic evidence indicates that red wine may contain phenolic compounds which protect against heart disease. Resveratrol and quercetin are wine phenolics which possess antioxidant and antimutagenic effects. The effect of red wine and its components on growth and proliferation of human oral squamous carcinoma cells studied Elattar and Virji (1999). Chan and Delucchi (2000) investigated the mechanism-based inactivation of cytochrome P450 3A4 by resveratrol as a red wine constituent. The studies on antimutagenic activity of polyphenolic fraction from grape seeds and grape extracts from varieties of *Vitis vinifera* were published by some authors (Agarwal *et al.* 2000; Stagos *et al.* 2005).

In the scope of a complex study of selected varieties of south Moravian wines and of grape cultivars Welschriesling, Pinot Gris, Chardonnay, Blauer Portugieser, André, and St. Laurent, this work presents the results of evaluation of antimutagenic activity of berries, both fresh and healthy, and of those infected with *Botrytis cinerea* fungus.

MATERIALS AND METHODS

Chemicals and bacterial strains. 2-amino-3-methyl-3H-imidazo-(4,5-f-)-quinoline (IQ) and *N*-nitroso-*N*-methylurea (MNU) were used as model mutagens. IQ was obtained from MP Biomedicals, MNU and other chemicals were obtained from Sigma-Aldrich (Prague, Czech Republic). Liver microsomal fraction S9 (metabolic activator) was obtained from District Institute of Public Health, Ostrava, Czech Republic, bacterial strains of *Salmonella typhimurium* TA98 and TA100 from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic.

Preparation of berries and of fermentation sediment. Healthy berries of grape varieties Welschriesling, Pinot Gris, Chardonnay, Blauer Portugieser, André, and St. Laurent, and berries infested with *Botrytis cinerea* fungus originated from harvest 2006. After the harvest, both healthy and botrytic berries were stored in a freezing box at –28°C. Fermentation sediments after the vinification of varieties Chardonnay, Welschriesling, André were also sampled and stored under the same condition as the berries. Frozen healthy and botrytic berries were homogenised in 90% methanol, extracted for 30 min, and shaken up at 4°C and centrifuged at 1990 g for 10 minutes. This was followed by the second extraction for 30 min and centrifugation. The sediment was rinsed with 90% methanol and centrifuged. Supernatants were pooled, the total volume was recorded. Methanolic extracts were evapotared in a vacuum evaporator at 40°C, residues were dissolved in such volumes of dimethylsulphoxide (DMSO), that 200 mg of original native material corresponded to 100 µl. Then the solutions were diluted 2×, 5×, 10×, and 20× and 100 µl of both original and diluted extracts were applied by pipette on a plate.

Mutagenicity test system. The mutagenicity of the applied mutagens, or possibly its inhibition by the action of studied material was evaluated by the Ames test using the plate method (Maron & Ames 1983; Mortelmans & Zeiger 2000). In this test, reverse $His^- \rightarrow His^+$ mutations are visualised by plating *Salmonella typhimurium* bacteria in a histidine poor growth medium. In this medium only His^+ mutants are able to form visible colonies. Different bacterial strains are available to identify different type of mutations. We used the strains TA98 and TA100. These strains have been actually the most often used as they detect the great majority of mutagens. Strain TA98 gives an indication of frame-shift mutations, while a positive response from strain TA100 indicates base-pair substitution. For a substance to be considered genotoxic in the Ames test, the number of revertant colonies on the plates containing the test compounds must be more than twice the number of colonies produced on the solvent control plates (i.e., a ratio above 2.0). In addition, a dose-response relation should be evident for various concentrations of the mutagen tested.

The mutagenicity of 2-amino-3-methyl-3H-imidazo-(4,5-f-)-quinoline (IQ) as of an indirect

mutagen, e.g. acting after the activation by the enzymatic complex of cytochrom P450, has been evaluated from the dependence of number of revertants on the dose of mutagen, using the strain *Salmonella typhimurium* TA98 in the presence of metabolic activating system S9 (liver microsomal fraction of mice). The mutagenicity of *N*-nitroso-*N*-methylurea (MNU) as of a directly acting mutagen has been evaluated on the strain *Salmonella typhimurium* TA100. 10 ng and 100 μg of IQ and MNU, respectively per plate was applied in all performed tests, which corresponds to 344 (R_M = 8.2) and 475 revertants per plate (R_M = 3.8), respectively.

Also fermentation sediments after the vinification of varieties Chardonnay, Welschriesling, and André were tested by the same experimental system. The tested samples were stored in a freezing box at –28°C. Sediments were filtered by a micro-porous filter (Minisart , Sartorius, with size of pores 0.45 μm), filtered solution was dosed on a plate in the original concentration and in dilution 2×, 5×, 10×, and 20×, always in the volume of 100 μl.

Tests were evaluated by counting of colonies of revertants on plates using equipment Colony Counter Q-count (Spiral Biotech, U.S.A.). Antimutagenicity was expressed as % of inhibition of mutagenicity according to the formula given by Ong *et al.* (1986) for Ames test:

$$\text{Inhibition [\%]} = 100 - [(R_T/R_M) \times 100]$$

where:

R_T – number of revertants per plate in the presence of mutagen and of the test sample

R_M – number of revertants per plate in the positive control (mutagen alone)

the result is evaluated on the scale

0–20	negative
20–40	slightly positive
40–60	positive
> 60	strongly positive

The tests were also performed without the application of mutagens to find out eventual mutagenic potential of tested wines alone. All results were negative.

RESULTS AND DISCUSSION

Results of tests of inhibition of mutagenicity of applied mutagens (IQ and MNU) through the action of extracts of fresh healthy grape berries of studied vine cultivars and of berries of the same cultivars infested with *Botrytis cinerea* fungus are presented in Table 1, results for fermentation sediments are presented in Table 2.

Extracts of the tested varieties of healthy berries showed the inhibition of mutagenicity on the scale as strongly positive in original concentration

Table 1. Inhibition of mutagenicity of IQ and of MNU by extracts of berries of grape vine

Vine variety	Berries	Inhibition of mutagenicity (coefficient of dilution) in %									
		IQ					MNU				
		1	2	5	10	20	1	2	5	10	20
Sain Laurent	healthy	96	92	70	47	6	61	59	51	43	24
	botrytic		97	96	97	86	55	22	28	27	14
Blauer Portugieser	healthy	97	96	73	32	1	74	76	74	58	51
	botrytic	98	97	95	84	31	67	68	57	59	50
Charsonnay	healthy	95	95	74	38	6	58	59	52	39	27
	botrytic	98	97	97	94	75	77	71	62	46	37
Welschriesling	healthy	96	94	82	31	21	55	47	41	41	25
	botrytic	96	97	96	90	57	53	58	57	43	37
André	healthy	97	96	95	77	19	60	52	42	33	24
	botrytic	97	98	97	96	83	71	64	52	57	30
Pinot gris	healthy	95	83	30	17	1	53	48	53	48	49
	botrytic	98	96	95	76	49	63	66	63	64	59

Table 2. Inhibition of mutagenicity of IQ and of MNU by wine fermentation sludges

Fermentation sludges – Vine variety	Inhibition of mutagenicity (coefficient of dilution) in %									
	IQ					MNU				
	1	2	5	10	20	1	2	5	10	20
Chardonnay	56	35	29	5	2	76	62	60	60	56
Welschriesling	87	75	58	38	30	70	66	59	62	61
André	83	72	48	40	4	71	70	58	54	60

eventually in dilution 2–5×, by dilution 10× and 20× the degree of inhibition decreased. In accordance with our assumption, botrytic berries showed a higher inhibition even in more diluted extracts. This phenomenon can be explained by higher contents of antioxidants – mainly of resveratrol, which is just like a phytoalexin formed in berries attacked by fungi (Melzoch *et al*. 2000; Landrault *et al*. 2002). This finding is in accordance with results of determination of polyphenolic substances including stilbenes in samples of the same materials (Tříska *et al*. 2004). This effect was more distinct towards IQ (strain *S. typhimurium* TA98, +S9), while at MNU, the inhibition effects towards strain TA100 were weaker.

It is evident from our results that relatively large amount of effective substances of grape vine, which are carriers of antimutagenic potential, penetrate even to the fermentation sediments.

CONCLUSIONS

In vine grape samples of varieties Welschriesling, Pinot Gris, Chardonnay, Blauer Portugieser, André, and St. Laurent the antimutagenic activity of methanolic extracts of berries infested with the *Botrytis cinerea* fungus was proved, using Ames test, in comparison to healthy berries. 2-amino-3-methyl-3H-imidazo-(4,5-f-)-quinoline was applied as an indirect mutagen, e.g. acting after the activation by the enzymatic complex of cytochrom P450, and *N*-nitroso-*N*-methylurea as a directly acting mutagen.

Fermentation sediments of varieties Chardonnay, Welschriesling, and André also showed a high degree of inhibition of the applied mutagens. It can be concluded that wine fermentation sediments represent at least the precious raw material for the potential isolation of these substances.

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