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ORIGINAL PAPER

Measurement of chemiluminescence induced by cytochrome c plus hydrogen peroxide to characterize the peroxidase activity of various wines and the *Botrytis cinerea* related quality of Aszú wines of Tokaj in Hungary

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Abstract
It was suggested that the measurement of chemiluminescence induced by cytochrome c plus hydrogen peroxide might characterize the peroxidase activity of various wines added and, furthermore, the *Botrytis cinerea* related quality of Aszú wines of Tokaj in Hungary. The chemiluminescence produced by cytochrome c plus hydrogen peroxide reaction was detected by a luminometer. The chemiluminescence stimulating effects of various wines ($n = 146$) including Aszú wines from Tokaj and other Hungarian white and red wines were compared and analysed by using an "Index of Stimulation". The content of gluconic acid in Aszú wines was determined colorimetrically. All types of wines tested could stimulate the intensity of chemiluminescence induced by the cytochrome c plus hydrogen peroxide expressed by an Index of Stimulation. This effect could mainly be regarded as a result of the peroxidase activity of wines. The highest values of the Index of Stimulation were found in the Aszú wines of Tokaj reflecting their 5–6 „baskets" types, which characterized their „*Botrytis* related qualities". Three categories of Aszú wines could be created on the basis of the Index of Stimulation. The stimulating effect was also proven by pure *Botrytis cinerea* particles owning peroxidase activity on the cytochrome c plus hydrogen peroxide reaction. The *Botrytis* influences and gluconic acid concentrations correlated positively. The augmentation of chemiluminescence in the cytochrome c plus hydrogen peroxide reaction could characterize the peroxidase activity of various wines and, furthermore, the „*Botrytis cinerea* related quality" of various *Botrytis* wines. However, this quality did not reflect completely the values of flavour and odour in these wines.

Keywords Cytochrome c · H_2O_2 · Chemiluminescence · *Botrytis cinerea* · Aszú wines of Tokaj · Peroxidases

Introduction

The various compounds which are plant metabolites found in a variety of plant-derived foods, including fruits, vegetables, oils, as well as wines are intensively studied for their potential health benefits [1]. Especially, the high dietary intake of flavonoids was studied and found to be associated with a reduced incidence of cardiovascular events [2]. Recently, a review declared that „the moderate wine consumption not only does not increase the risk of chronic degenerative diseases but is also associated with health benefits particularly when included in a Mediterranean diet model" [3]. Therefore, detailed biochemical studies on the effects of various wines which are historically stable elements of human nutrients can be of importance. In this field, we observed now a new phenomenon in vitro. Studying the interactions

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between two vital molecules, it was found that the chemiluminescence induced by cytochrome c (Cytc) and hydrogen peroxide (H_2O_2) could be increased by various wines. Cytc (an iron-containing haemprotein) is essential in mitochondrial electron transport and intrinsic type II apoptosis. Mammalian Cytc also scavenges reactive oxygen species (ROS) under healthy conditions, produces ROS, oxidizes cardiolipin during apoptosis and takes part in the regulation of various pathologic states [4]. Hydrogen peroxide (H_2O_2) modulates and signals the redox metabolism of cells by acting as a messenger together with hydrogen sulphide and nitric oxide radical, activating specific oxidations that determine the metabolic response, cell survival and apoptosis [5]. The interactions of these two molecules play crucial roles in early stage of cell apoptosis [6, 7]. Cytc as a peroxidase can catalyse the oxidation of organic substrates by H_2O_2 inducing apoptosis [8]. In this context, it was very important when Slawinski and co-workers discovered and detected the chemiluminescence (photon emission) in the reaction of Cytc with H_2O_2 resulting in a "ferryl peroxide complex" that could be measured by a luminometer precisely [9].

Peroxidases represent a type of constitutive enzyme in the grapevines interacting with H_2O_2 during the metabolism of plants [10]. The secretory peroxidase (class III) contains iron (Fe^{+++}) similar to Cytc [10]. Furthermore, fruit wines contain glutathione peroxidase [11]. Thus, the idea came to investigate whether the intensity of chemiluminescence induced by the Cytc plus H_2O_2 reaction might be modified by various wines showing peroxidase activity like Cytc [8]. If yes, how can be used this phenomenon to characterize certain quality of wines, especially that of Aszú wines from Tokaj. The Aszú wines of Tokaj were the most famous Hungarian wines in the last three hundred years. They belong to the great group of Botrytized wines [12]. The peroxidase enzymes of *Botrytis cinerea* were found to break down hydrogen peroxide [13]. Additionally, the components of *Botritis cinerea* could induce the production of H_2O_2, too [14, 15]. The chemiluminescent method was already used for the analysis of alcoholic beverages [16].

Materials and methods

Wines and Aszú grapes

The results of 154 wines are presented here. The majority of exclusively white wines are derived from the whole Tokaj area comprising 55000 hectares. Mostly Botrytized Aszú wines of 5 and 6 baskets (puttony), furthermore, a small number of Aszú types of 3 and 4 baskets, besides some late harvested Szamorodni wines completed with white and red wines from other regions of Hungary were analysed. The base wines for Aszú were furmint, linden leaved and muscatel. „Essencia" was the sweet fluid pressed from noble-rotted berries representing no or only very few amounts of ethanol. The Tokaj area of Hungary has been the only place in the world where the „noble-rotted berries" affected by *Botrytis cinerea* (named Aszú grapes) could have been developed every year since more centuries as a consequence of the special geographic milieu created by these rivers and mountains. In the manufactory of Aszú wines, the following elements are given the specialities: (a.) the Botrytized Aszú grapes are collected manually in baskets (containing 23–25 kg of grapes); (b.) the combined mellowing of a base wine together with the Aszú grapes (deriving mainly from 5 or 6 baskets now) is taking place in a barrel of 136 L volume at least for 3 years. This procedure and technique ensure the application of especially high amounts of Botrytized Aszú grapes during wine making [17]. (Besides Tokaj, we tested Botrytized wines also from other countries, but their results were not documented here). Every wine was tested in the following form: 0.1 ml of wine in 3.0 ml of final reaction volume.

In order to get „pure" preparations of *Botrytis cinerea*, the fungal particles were collected manually and mechanically from the Aszú grapes. *Cladosporium cellare* was taken from the walls of cellars. *Saccharomyces cerevisiae* was purchased commercially. From all three yeasts, solutions were prepared with an equal concentration of 1 mg/1 ml H_2O which were sonicated and later centrifuged. Their supernatants were used for further studies.

Measurement of chemiluminescence

A Berthold Automat Plus BL 953 (Berthold, Germany) „tube" luminometer with a spectral width of 380–630 nm was applied for the measurement of chemiluminescence (photon emission). The numeric value of chemiluminescence was expressed in "Relative Light Unit" (RLU) using the whole spectrum of equipment at room temperature for 60 min. Duplicates of samples were used in the measurements.

Calculation of index of stimulation

The chemiluminescence stimulating effect of wine on the Cytc plus H_2O_2 reaction was characterized numerically by the calculation of an „Index of Stimulation (IS)", compared to a „control" solution. IS was calculated as follows: "wine" chemiluminescence (RLU) divided by "control" chemiluminescence (RLU). Two types of control solutions (Control A, Control B) were composed.

Control A

The "wine buffer" was the "control" for every wine that was a new form of "phosphate buffered saline (PBS)" representing an increased ionic power than the „traditional" PBS, as follows:

Wine Buffer (WB): 0.07 M phosphate buffer + 0.9% Na Cl, pH: 7.4; applied volume: 0.100 ml.

Calculation of Index of Stimulation for every wine: RLU of Wine/RLU of WB.

Control B

For the Aszú wines of Tokaj as "control" solution, the „shame furmint" was composed containing the most characteristic stable molecules of „classical furmint base wine", as follows:

Shame Furmint (SF): ethanol 14%, tartaric acid 2.5 g/l, malic acid: 1 g/l, glycerol 10 mg/l, glucose 3 g/l, $FeCl_2 \times 6H_2O$ 12 mg/l, in wine buffer.

Calculation of Index of Stimulation for Aszú wines of Tokaj: RLU Aszú/RLU SF.

The induction of chemiluminescence by the Cytc plus H_2O_2 reaction occurred in 3 ml of final volume as follows:

3×10^{-5} M cytochrome c (from equine heart, Sigma-Aldrich); 3×10^{-2} M H_2O_2 (Sigma-Aldrich) in WB.

Measurement of gluconic acid

The concentration of gluconic acid was determined by spectrophotometer (Thermo Scientific Genesys 150) using the enzyme kit of D-gluconic acid/D-glucono-delta lactone (R-Biopharm Hungary).

Mass spectrometry

Wine proteins were acetone precipitated; the pellet was redissolved in 8 M urea, reduced with 100 mM dithiothreitol and alkylated with 200 mM iodoacetamide. Before digestion with 0.5 µg MS grade trypsin (Sciex USA), the pH was set to 8 with NaOH. Sample clean-up with PierceC18 Tips (ThermoScientific USA) was performed and the presence of Cytc was examined using targeted proteomics. For the PRM assay performed on Orbitrap Fusion—Easy-nLC 1200 system (ThermoScientific USA) 17 selected Cytc peptides originating from grape, yeast and Botrytis were used, and data were analysed with Skyline [18].

Statistical analysis

The normal distributions of data concerning the RLU and IS were tested by the Shapiro–Wilk test. Depending on these results, test groups were formed and further evaluated by Kruskal–Wallis and Dunn's post hoc tests. For describing the analysis and calculation of correlations between IS and concentrations of gluconic acid, the GraphPad Prism 5.0 program was used.

Results

Stimulation of chemiluminescence by various types of Hungarian wines added to cytochrome c plus hydrogen peroxide

Table 1 demonstrates the results of 14 wines on the stimulation of chemiluminescence (IS values) in three groups of experiments: (1) Cytc plus H_2O_2 plus wines, (2) H_2O_2 plus wines and (3) Cytc plus wines. The following conclusions could be drawn: (a) In group 1, all wines (both white and red wines) increased the values of RLU and IS. The greatest elevations were found in the Aszú wines of Tokaj and Essencia (owning the greatest Botrytis contents). (b) The same tendency was observed also in group 2, however, with much lower numeric values. These data suggested that all wines tested here might show some "(plant) peroxidase" activity similar to Cytc reacting with hydrogen peroxide, as well. (c) It was surprising that in the Aszú wines and Essencia of Tokaj, traces of H_2O_2 could be detected producing detectable chemiluminescence interacting with Cytc.

Characterization of *Botrytis cinerea* related qualities of Aszú wines from Tokaj compared to the base wines measuring their chemiluminescence stimulating effects on the cytochrome c plus hydrogen peroxide reaction

The comparison of various wines from Tokaj (Aszú $n = 89$, Szamorodni $n = 19$, base wines (furmint, linden-leaved, muscatel $n = 30$, total number $n = 148$)) and solutions of „shape furmint" was occurred by the determination of their effects to stimulate the chemiluminescence induced in the Cytc plus H_2O_2 reaction. The stimulation was expressed by a value of IS. Using statistical analysis, three categories of Aszú wines could be created as follows:

„Aszú of Tokaj with weak Botrytis quality: IS < 2.79.

„Aszú of Tokaj with outstanding good Botrtyis quality": IS = 2.80–3.54.

„Aszú of Tokaj with extraordinary good Botrytis quality": IS > 3.55.

The „Essencia of Tokaj" could also be evaluated by this method but it was not regarded to be an „Aszú" product. The diagram of these results is documented in Fig. 1. It should be stressed, however, that the numeric values of the three categories were valid only for a very standardized protocol during the measurements regarding both the reagents and

Table 1 Stimulation of chemiluminescence by various types of Hungarian wines added to cytochrome c plus hydrogen peroxide

Wines	Year	Cytc + H_2O_2		Wine + H_2O_2		Wine + Cytc	
		RLU	IS	RLU	IS	RLU	IS
Wine buffer (control)		328	1.00	149	1.00	143	1.00
Dry Furmint (Tokaj)	2020	542	1.65	182	1.22	134	0.94
Sweet Furmint (Tokaj)	2015	580	1.77	302	2.03	nm	nm
Linden—leaved (Tokaj)	2020	493	1.50	191	1.28	nm	nm
Linden—leaved (Tokaj)	2013	597	1.82	329	2.21	nm	nm
Sweet Muscatel (Tokaj)	2020	553	1.69	184	1.23	nm	nm
Sweet Szamorodni (Tokaj)	***2016***	***697***	***2.13***	***261***	***1.75***	nm	nm
Tokaj Aszu (6 baskets)	***2006***	***875***	***2.67***	***345***	***2.32***	***149***	***1.04***
Tokaj Aszu (6 baskets)	***1994***	***914***	***2.79***	***432***	***2.90***	***173***	***1.21***
Tokaj Aszu (5 baskets)	***1936***	***1092***	***3.33***	***416***	***2.79***	***166***	***1.16***
Tokaj Aszu (6 baskets)	***1912***	***1153***	***3.52***	***451***	***3.03***	***173***	***1.21***
Tokaj Aszu Essencia	***1942***	***1210***	***3.69***	***498***	***3.34***	***167***	***1.17***
Furmint (Balaton)	2019	595	1.81	215	1.44	141	0.99
White Dry (Balaton)	2020	489	1.49	179	1.20	nm	nm
Red wine (Eger)	***2015***	***847***	***2.58***	***220***	***1.48***	***141***	***0.99***

Remarkable elevations are shown in bolditalics

Cytc cytochrome c, *RLU* Relative Light Unit, *IS* Index of Stimulation, *nm* not measured

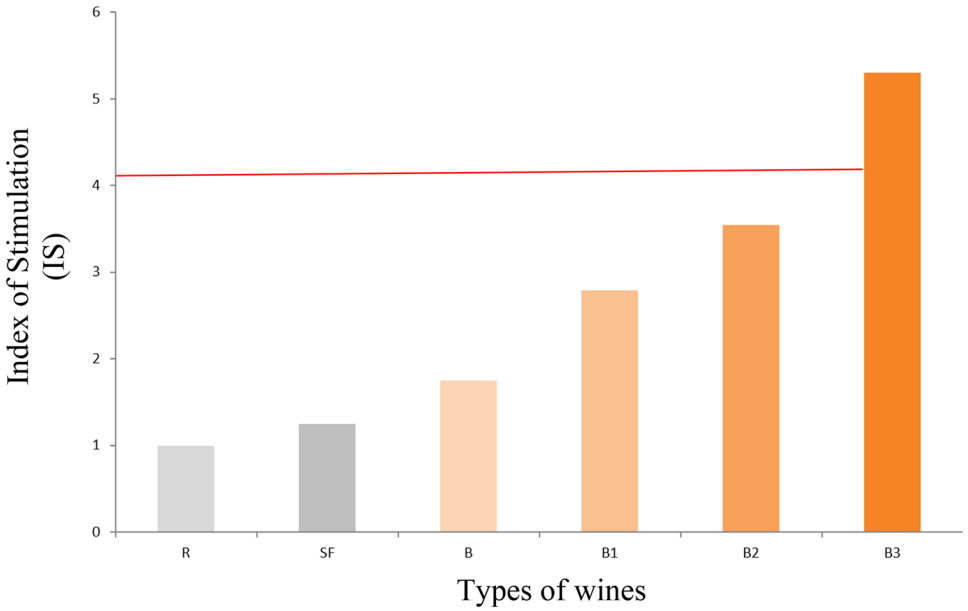

Fig.1 Characterization of *Botrytis cinerea* related qualities of Aszú wines by chemiluminescence measurements expressed by Indices of Stimulation compared to "shame furmints" ($n = 148$). (*R*) cytochrome c + H_2O_2, *SF* Shame Furmint, *B* Base wines, *B1* Aszu of Tokaj with weak *Botrytis* quality, *B2* Aszu of Tokaj with outstanding good *Botrytis* quality, *B3* Aszu of Tokaj with extraordinary good *Botrytis* quality, *Red line* Historical top *Botrytis* quality

the types of luminometers. It could also be seen from the diagrams that the IS values of "historical top Aszú wines" (older than 50 years) exceeded the IS value of 4.05.

Effects of sonicated extracts of *Botrytis cinerea*, *Cladosporium cellare* and *Saccharomyces cerevisiae* on the chemiluminescence induced by cytochrome c plus hydrogen peroxide

During the long procedure of making an Aszú wine *Botrytis cinerea, Cladosporium cellare* and *Saccharomyces cerevisiae* used to be the most important microbes which could involve the mellowing of a wine at different extents. Therefore, it was of worth testing their direct capabilities to induce chemiluminescence in the Cytc plus H_2O_2 system. Water solutions of 1 mg/1 ml concentration were prepared from all of them. After centrifugation of sonicated samples, their supernatants were used for further investigations. Table 2 shows that only the extract of *Botrytis* was able to stimulate directly the chemiluminescence, whereas the two other samples caused inhibitions. These results demonstrated the possibility of direct participation of *Botrytis*

Table 2 Effects of the sonicated extracts of *Botrytis cinerea*, *Cladosporium cellare* and *Saccharomyces cerevisiae* on the chemiluminescence induced by cytochrome c + H_2O_2

Reactions	RLU	IS
Cytc + H_2O_2 + wine buffer	324	1.0
Cytc + H_2O_2 + *Botrytis cinerea* extract	*461*	*1.42**
Cytc + H_2O_2 + *Cladosporium cellare* extract	229	0.71
Cytc + H_2O_2 + *Saccharomyces cerevisiae* extract	124	0.38

cytc cytochrome c, * *increase*, *IS* Index of Stimulation

cinerea derived substances in the stimulation of chemiluminescence induced by Cytc plus H_2O_2 reaction. These molecules could become fundamental parts of the Aszú wines in Tokaj. Thus, their amounts in the Aszú wines could reflect quantitatively the "Botrytis effects" establishing the chance to use them as 'qualitative markers of Botrytis character" in the Aszú wines. They could be peroxidases [13].

Chemiluminescence induced by sonicated extracts of *Botrytis cinerea* particles prepared from noble-rotted berries representing various classes derived from various wine yards of Tokaj

During the harvest of 2021, various samples of Aszú berries were collected manually showing or not showing *Botrytis cinerea* particles on their surfaces. Depending on their yeast contents, the berries were classified visually by the hill mayors as class I (rich), class II (poor) grapes, or berries without *Botrytis cinerea* (B.c.). The samples were derived from different villages of the Tokaj region (Mád and Erdőbénye) and from different parts of wine hills (top or foot). Water solutions of 1 mg/ml concentration were prepared from the Botrytis particles, then they were sonicated and centrifuged as it was mentioned above. The supernatants were tested in the Cytc plus H_2O_2 reaction. The results showed that the strongest chemiluminescence stimulating effects were caused by the yeasts from class I berries and by those derived from the top of the hill (Table 3). These data confirmed further the view that the molecules of Botrytis taken from the Aszú berries (with peroxidase activity) could be able to stimulate directly the chemiluminescence induced by Cytc plus H_2O_2. Besides, this observation could be the base for a new biochemical method to classify the Aszú berries during harvests in the future.

Effect of gluconic acid on the chemiluminescence induced by cytochrome c plus hydrogen peroxide

Glugonic acid (D-gluconic acid, gluconate) is a metabolite of *Botrytis cinerea* and a useful marker for *Botrytis* activity in the vineyard [13]. In our experimental system,

Table 3 Chemiluminescence caused by solutions of sonicated berries with and without *Botrytis cinerea* representing various qualities (classes) derived from various wine yards

Botrytis cinerea Noble-rotted berries ($n = 8$)	RLU	IS
Buffer (control)	319	1.00
Extract from berries of Mád without B.c. No.1	672	2.11
Extract from berries of Mád without B.c. No.2	563	1.76
Extract from berries of Mád with B.c. (class II.)	698	2.19
Extract from berries of Mád with B.c. (class I.)	***894***	***2.80***
Extract from berries of Mád with B.c. (top of the hill)	***824***	***2.58***
Extract from berries of Mád with B.c. (foot of the hill)	791	2.48
Extract from berries of Erdőbénye with B.c	758	2.38

Remarkable elevations are shown in bolditalics

B.c. Botrytis cinerea

gluconic acid dose dependently, significantly and linearly increased the chemiluminescence induced by Cytc plus H_2O_2 (Fig. 2). There was a significant correlation between the values of IS and the concentrations of gluconic acid ($r = 0.9969$; $p < 0.00001$).

Correlation between the values of indices of stimulation and concentrations of gluconic acid in Aszú wines of Tokaj

Testing a collection of Aszú wines from Tokaj ($n = 41$) including „historical," (more than 50 years old) Aszú wines and Essencias with extra high values of IS, a significant positive correlation was found between IS and concentrations of gluconic acid ($r = 0.419$; $p = 0.0073$). (Fig. 3.)

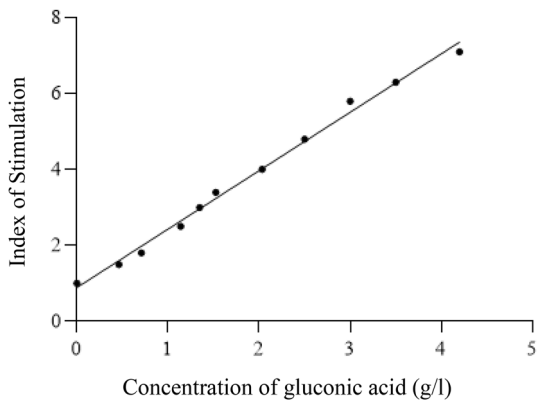

Fig. 2 Linear correlation between the values of Indices of Stimulation and concentrations of gluconic acid ($n = 11$). $y = 1.539$* $x = 0.8890$, $r = 0.9969$, $p = 0.00001$

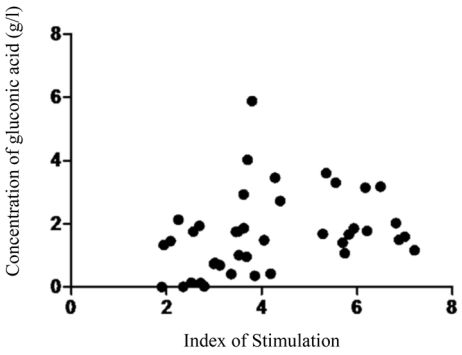

Fig. 3 Correlation between the values of Indices of Stimulation (IS) and the concentrations of gluconic acid in Aszu wines of Tokaj ($n=41$), $r=0.419$, $p=0.0073$

Discussion

Both Cytc and H_2O_2 are molecules of vital importance for every living being owning mitochondria. They are taking part in the energy metabolism and regulation of cellular survival [1–8]. However, it is only a rarely evaluated fact that the interaction of these molecules can continuously cause an oxidative, excited state in the cells producing a „ferryl peroxide complexes" which finally result in chemiluminescence (photon emission) [9]. Besides, Cytc interacting with H_2O_2 shows „peroxidase activity", too [8].

The novelties in this work could mainly be coupled to the "peroxidase" phenomenon as follows: (1) The standardized method of chemiluminescence measurement induced by Cytc plus H_2O_2 reaction was used and could be offered to evaluate the „ plant peroxidase activity" in various wines comprising different types of plant peroxidases [10, 11]. The chemiluminescence increasing effect of wine compared to the chemiluminescence produced in the reaction of Cytc plus H_2O_2 could be characterized numerically by the calculation of the Index of Stimulation (IS). This number was an "arbitrary" value but allowed to introduce a very sensitive, standardized, automated method which was applicable for comparative determinations in many wine samples simultaneously. (2) The further new merits of IS were realized concerning the Aszú wines of Tokaj characterizing their *Botrytis cinerea* related qualitative categories which correlated positively with the gluconic acid contents. (3) Gluconic acid alone could increase the chemiluminescence induced by Cytc plus H_2O_2 where its interaction with H_2O_2 could be involved [19]. Therefore, in the extra high chemiluminescence increasing effects of Aszú wines not only the plant (grape) peroxidases but some direct peroxidase molecules from Botrytis and gluconic acid produced during the Botrytis generated mellowing of wines could take part, additively. (4) On the basis of these observations, three categories representing the *Botrytis* related qualities were created for the Aszú wines of Tokaj compared to the values of a standardized „shame" furmint. (5) The direct linkage of chemiluminescence increasing effects of Aszú wines from Tokaj with *Botrytis cinerea* was proven by pure *Botrytis* particles (containing peroxidases) derived from noble-rotted Aszú berries collected from various places of wine region. (6) Besides, this work intends to draw attention to the „ferryl peroxide molecules" from those nutritional aspects that these important free radicals are continuously produced during all "Cytc plus H_2O_2" reactions at both intra and extracellular levels in all living organisms owning mitochondria. They can regulate not only the energy metabolism and cell survival, but their „side and end" products, the „biophotons" may also have an importance which is still hardly explored [20]. Thus, the plant peroxidases of wines may have individual, dose dependent and lifelong nutritional influences on these vital processes of all persons consuming wines.

We assumed that the chemiluminesce increasing effects of various wines in our Cytc plus H_2O_2 experimental system could be explained as a „summarized peroxidase" influence of wines and Botrytis peroxidases associated with Cytc being also a peroxidase. The results that the wines could induce a weaker chemiluminescence alone without Cytc reflected the direct "plant peroxidase" activities of wines in the presence of H_2O_2. However, it was an advantage to test these activities also in the "Cytc plus H_2O_2" milieu because much greater chemiluminescence reactions were produced here. It was also a crucial finding that we could not detect any Cytc molecule in the Aszú wines using the highly sensitivity mass spectrometry [18]. Reviewing the literature regarding wine proteome analysis, none of the studies could identify Cytc molecule in wine. Therefore, we regarded the group of various plant plus Botrytis peroxidases of wines to play the dominating role in the augmentation of chemiluminescence (photon) production in our "artificial system of H_2O_2 plus Cytc" where Cytc derived from equine heart. The exclusive detection of "traces of H_2O_2" in the Aszú wines of Tokaj, however, could be a proof for the earlier observation on the oxidation of ethanol by *Botrytis cinerea* forming aldehydes and hydrogen peroxide [13] and an oenological confirmation of the existence of an H_2O_2 generating system in *Botrytis cinerea* [14, 15].

Concerning the sensitivity of the method what we used to differentiate the *Botrytis* related qualities of various wines on the basis of IS, it should be stressed that it needed very strictly standardized protocols regarding the equipment, reagents and temperature. Already a small change could lead to some alterations in the numeric data. Inversely, if the conditions were stable, the method could help the evaluation of Botrytis effects very well in the Aszú wines of Tokaj. It was a great advantage that no luminol was required, which might disturb the red wine measurements (not documented observation). However, it was also an important recognition that

other values of wines, e.g., the flavour [21, 22] and odour [23] could not be estimated by this method as well as they were already worked out for other wines. Although, there were frequent positive coincidences between them.

The presented chemiluminescence method may have both theoretical and practical advantages by introducing the numerical, comparative evaluation of "peroxidase activities" of various wines, besides, giving a sensitive scientific chance to differentiate the Botrytis-related qualities of *Botrytis* wines helping to defend their special character and values. However, this method can be offered mainly for specialized laboratories that are going to elucidate, for example, the biological effects of wines from the new aspect of their peroxidase activities [24], or to find an explanation for the observation that in the Tokaj region the least cancer mortality rate was found among the historical wine territories of Hungary in the period of 2000–2010 when the inhabitants still were the consumers of their own wines, mostly [25].

These results draw attention to notice the hardly estimated extracellular importance of Cytc being a marker of mitochondria and cellular damages [26, 27]. Thus, the chance for real interactions between the plant peroxidases of wines and the naturally produced Cytc and H_2O_2 molecules in humans cannot be denied since the production of H_2O_2 is also continuous in the organism, and the interactions between Cytc and hydrogen peroxide are permanent, resulting in „ferryl-peroxide radicals" [8] and biophotons [20]. One nutritional merit of the present work can be the chemical demonstration of the remarkable stimulating effects of various wines on these interactions. Therefore, further works are needed to investigate the nutritional relevance of presented results in human systems.

Conclusions

A new enzymatic functional assay has been established to measure the „peroxidase activity" in various wines based on the measurement of chemiluminescence induced by Cytc plus hydrogen peroxide reaction and using the calculated, arbitrary values of Indices of Stimulation. In addition, the presented biochemical method is also able to characterise the *Botrytis cinerea* related quality of Botrytis wines. Furthermore, a special nutritional attention is paid to stress the importance of cytochrom c and hydrogen peroxide interactions from the aspect of "peroxidase activity" of wines, but not neglecting the importance of investigations on the unique characters of Aszú wines from Tokaj [28].

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Author contributions The concept and preparation of the manuscript derived mainly from SS. The statistical analysis was done by SB. All authors: SS, AN, JN, EN, ÉCS, and SB took part in the laboratory measurements and completed the manuscript.

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Declarations

Conflict of interest There is no conflict of interest among the authors.

Compliance with ethics requirements During the study, all ethical standards were applied.

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