Effect of Myricetin, Pyrogallol, and Phloroglucinol on Yeast Resistance to Oxidative Stress

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1. Introduction

Oxidative stress is a hallmark of human disorders such as cancer and age-associated diseases [1]. It results from an unbalance between the levels of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and cellular antioxidant defenses. The toxicity of high levels of ROS and RNS is associated with the accumulation of damaged molecules, including proteins, lipids, and nucleic acids [1]. Under normal physiological conditions, ROS are kept at low levels by antioxidant defenses such as superoxide dismutases (SOD) that catalyze the dismutation of superoxide radicals into hydrogen peroxide, catalases, or peroxidases that reduce \( \text{H}_2\text{O}_2 \) into water, as well as nonenzymatic defenses, including glutathione that plays critical roles in redox homeostasis and cellular detoxification [2]. In addition, antioxidants obtained in the diet, such as vitamins C and E and phenolic compounds, play essential role in cellular protection [3].

Phenolic compounds are natural antioxidants present in the human diet through the consumption of fruits, vegetables, and drinks such as juice, tea, coffee, and wine [4, 5]. Structurally, these compounds are characterized by having one or more hydroxyl groups attached to at least one aromatic ring [4]. The number and position of hydroxyl groups are important features that affect the antioxidant activity of phenolic compounds [6]. These compounds possess antiproliferative, proapoptotic, and anti-inflammatory properties and they have been associated with the prevention of cancer and cardiovascular, neurodegenerative, and metabolic disorders [7, 8]. The protective effects of these compounds have been
attributed not only to their intrinsic antioxidant activity but also to the modulation of cell signaling pathways, including mitogen-activated protein kinase cascades, which regulate oxidative stress responses [9–11].

The budding yeast *Saccharomyces cerevisiae* has been used as a eukaryotic model organism to characterize the molecular mechanisms underlying oxidative stress resistance and to evaluate the antioxidant potential of dietary extracts and phenolic compounds [12]. We have previously reported that quercetin, the most common flavonol in the diet, increases yeast oxidative stress resistance [13] and exerts its protective effects against oxidative stress by inducing the biosynthesis of trehalose, a stress protectant disaccharide, and the activation of the cell wall integrity pathway [14]. Other studies have shown that resveratrol and catechin increase oxidative effects against oxidative stress by inducing the biosynthesis of polyphenolic compounds [12]. We have previously reported that resveratrol and catechin protect yeast through activation of catalase [15], whereas delphinidin 3-glucoside increases stress resistance in yeast by mechanisms associated with the activation of the cell wall integrity pathway [14].

2. Materials and Methods

2.1. Reagents. All reagents and chemicals used were of analytical grade. Sodium or potassium phosphates, riboflavin, and H₂O₂ were purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO), myricetin, pyrogallol, phloroglucinol, and nitroblue tetrazolium were purchased from Sigma (Sintra, Portugal). Phenolic compounds were dissolved in DMSO at a 200 mM stock concentration and stored at −80°C. Solutions were prepared in ultrapure water (Milli-Q).

2.2. Yeast Strains and Growth Conditions. *Saccharomyces cerevisiae* cells (Euroscarf, Germany) used in this study were BY4741 (MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0; parental strain), sod1Δ (BY4741 sod1Δ::KanMX4), and sod2Δ (BY4741 sod2Δ::KanMX4). Yeast cells were grown in YPD medium [1% (w/v) yeast extract, 2% (w/v) bactopeptone, and 2% (w/v) glucose] or in synthetic complete (SC) drop-out medium containing 2% (w/v) glucose, 0.67% (w/v) yeast nitrogen base without amino acids supplemented with the appropriate amino acids (80 mg His L⁻¹, 400 mg Leu L⁻¹, and 80 mg trp L⁻¹), and nucleotides (80 mg Ura L⁻¹). Cultures were maintained in an orbital shaker, at 26°C and 120 rpm, with a ratio of flask volume/medium volume of 5:1.

2.3. Oxidative Stress Resistance Assays. Yeast cells were grown to the exponential phase (OD₅₆₀ = 0.5–0.6) in YPD medium, pretreated with polyphenols (myricetin, pyrogallol, or phloroglucinol at 300 μM) or equal volume of DMSO (vehicle) for 15 min, and subsequently exposed to 1.5 mM H₂O₂ for 1 hour. Cell viability was determined by dilution plate counts on YPD medium containing 1.5% agar. Colonies were counted after growth at 26°C for 3 days. Viability was expressed as the percentage of colony-forming units (CFU).

2.4. Intracellular Oxidation. The oxidant-sensitive probe 2′,7′-dichlorodihydrofluorescein (H₂DCF-DA) (Molecular Probes) was used to measure intracellular oxidation. Yeast cells grown to the exponential phase in YPD medium and pretreated with polyphenols for 15 min were subsequently exposed to 1.5 mM H₂O₂ for 1 hour in the absence or presence of 25 μM H₂DCF-DA. Cells were spun down (4,000 rpm, 4 min), washed twice, and suspended in filtered phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 1.8 mM KH₂PO₄, pH 7.4). Fluorescence was measured in FL-1 channel (excitation and emission wavelength at 488 nm and 525 nm, respectively) in a Becton-Dickinson FACSort flow cytometer. Autofluorescence was analyzed in cells untreated with H₂DCF-DA. Data was acquired from a total of 10,000 events/samples. BD CellQuest Pro Software was used for data acquisition and FlowJo Software for data analysis.

2.5. Protein Carbonylation. Protein extracts were prepared in 50 mM potassium phosphate buffer (pH 7.0) containing protease inhibitors (Complete, Mini, EDTA-free Protease Cocktail Inhibitor Tablets; Roche Applied Science), by vigorous shaking of the cell suspension, in the presence of glass beads, for 5 min. Short pulses of 1 min were used, with 1 min intervals on ice. Protein content was estimated by the Lowry method, with bovine serum albumin as a standard. Protein carbonylation assays were performed by slot-blot analysis, as previously described [13], using rabbit IgG anti-dinitrophenyl (DNP) (Sigma) at 1:1,000 dilution as the primary antibody and goat anti-rabbit IgG peroxidase (Sigma) at 1:5,000 as the secondary antibody. Immunodetection was performed by chemiluminescence, with a kit from GE Healthcare (RPN 2109). Quantification of bands was performed by densitometry.

2.6. Glutathione Levels and Enzymatic Activities. All the procedures were carried out at 4°C. Yeast cells were harvested by centrifugation. Glutathione levels were measured by the method of Tietze [22], as described in a previous work [13]. For enzyme activities, yeast extracts were prepared as described for the analysis of protein carbonylation.
The activity of catalase and SOD was analyzed in situ, after separation of proteins (50 μg) by native polyacrylamide gel electrophoresis (PAGE), as described previously [23, 24]. Quantification of bands was performed by densitometry.

2.7 Chronological Lifespan. Overnight cultures in SC medium were diluted to OD₆₀₀ = 0.5 and grown to the stationary phase for 3 days (in the case of BY4741 and sod1Δ cells) or for 1 day (in the case of sod2Δ cells). Then, the compounds (300 μM myricetin or pyrogallol) or DMSO (vehicle; volume identical to compounds) were added to the cultures (day 0). These cells were kept in culture media at 26°C and viability was analyzed at indicated times by standard dilution plate counts on YPD medium containing 1.5% agar. Colonies were counted after growth at 26°C for 3 days and viability was expressed as the percentage in CFU relative to day 0.

2.8 Statistical Analysis. Analysis was performed in GraphPad Prism. Data are expressed as the mean values ± standard error of the mean (SEM) of at least three independent experiments. The 0.05 probability level was selected as the point of statistical significance. Values of oxidative stress resistance assays were analyzed by one-way ANOVA and compared by Dunnett’s multiple comparisons test. Intracellular ROS and protein carbonyls were analyzed by two-way ANOVA and compared by Sidak’s multiple comparisons test. Statistical analysis of total and oxidized glutathione levels and the ratio GSSG/GSH₅ was performed by two-way ANOVA, Sidak’s multiple comparisons test (⁎ p < 0.05) for comparison of values between treatments in each condition (control or H₂O₂), and multiple t-tests using the Holm-Sidak method for corrections (⁎ p < 0.05) for comparison of values between control and H₂O₂ for all treatments. Lifespans were compared by Student’s t-test.
Figure 2: Effect of myricetin, pyrogallol, and phloroglucinol on intracellular oxidation and oxidative damage. Yeast cells were grown in YPD medium to the exponential phase and pretreated with compounds (300 μM) or equal volume of DMSO (control) for 15 min and subsequently treated with 1.5 mM H₂O₂ for 1 h. (a) Representative histograms of intracellular ROS analyzed by flow cytometry using H₂DCF-DA as a probe. (b) Quantification of intracellular ROS expressed by mean fluorescence intensity in 10,000 cells (arbitrary units) from at least 3 independent assays. (c) Quantitative analysis of protein carbonyl content was performed by densitometry using data taken from the same membrane. Proteins were derivatized with DNPH and slot-blotted into a PVDF membrane. Immunodetection was performed using an anti-DNP antibody. Values are mean ± SEM of at least 3 independent assays. Values were compared by two-way ANOVA, Sidak’s multiple comparisons test (* p < 0.05).

3. Results

3.1. Myricetin and Pyrogallol Increase Hydrogen Peroxide Resistance in Saccharomyces cerevisiae. To assess the effect of myricetin, pyrogallol, and phloroglucinol on oxidative stress resistance, exponential phase yeast cells were pretreated with these compounds individually (300 μM) or DMSO (control) for 15 min and subsequently exposed to 1.5 mM H₂O₂ for 1 h. The presence of polyphenols per se (in the absence of H₂O₂) did not affect cell viability, intracellular oxidation, or protein oxidation. Myricetin and pyrogallol, in contrast with phloroglucinol, increased cell viability from 33% (in control cells) to 64% and 51%, respectively (Figure 1(b)). To investigate if H₂O₂ resistance induced by these polyphenols was correlated with a decrease in oxidative stress markers, intracellular ROS levels were measured by flow cytometry using cells labeled with an oxidant-sensitive probe, H₂DCF-DA (Figures 2(a)-2(b)), and protein oxidation was assessed through the analysis of protein carbonyl content (Figure 2(c)). In control cells, exposure to H₂O₂ caused a 10-fold increase in intracellular ROS and a 3-fold increase in protein carbonylation. Myricetin and pyrogallol, but not phloroglucinol, significantly decreased H₂O₂-induced intracellular oxidation and protein carbonylation.

3.2. Myricetin and Pyrogallol Do Not Affect the Activity of Superoxide Dismutase or Catalase. To investigate if the protective effect of myricetin or pyrogallol was associated with an induction of antioxidant defenses, the activity of superoxide dismutase and catalase was determined. Consistent with published data [25], SOD activity decreased 31% in control cells (DMSO-treated) exposed to H₂O₂ (Figure 3(a)). Pretreatment with the phenolic compounds did not affect...
basal SOD activity or prevent its decrease upon exposure to H$_2$O$_2$. Catalase is not expressed in exponential phase cells [26] and, therefore, its activity was not detected in control cells. Moreover, it was not induced in cells treated with the tested compounds (data not shown). These results indicate that the increase of oxidative stress resistance in cells pretreated with myricetin or pyrogallol did not result from the induction of SOD and catalase.

3.3. Myricetin Suppresses H$_2$O$_2$-Induced Glutathione Oxidation. The tripeptide glutathione (GSH) is the most abundant low-molecular weight thiol that serves to maintain a reduced intracellular environment [27]. To assess the effect of myricetin, pyrogallol, and phloroglucinol on redox homeostasis, glutathione levels were determined in cells exposed to H$_2$O$_2$ (Figures 3(b)–3(d)). In control cells, after exposure to H$_2$O$_2$, total glutathione levels (GSH$_T$) decreased 37% whereas GSSG levels increased 70%, increasing the ratio between oxidized glutathione and total glutathione levels. Values are mean ± SEM of at least 3 independent assays. GSH$_T$ and GSSG levels were compared by two-way ANOVA, Sidak's multiple comparisons test (* p < 0.05) and the ratio was compared by Student's t-test (* p < 0.05).

Figure 3: Effect of myricetin, pyrogallol, and phloroglucinol on antioxidant defenses. Yeast cells were grown in YPD medium to the exponential phase and pretreated with compounds (300 μM) or equal volume of DMSO (control) for 15 min and subsequently treated with 1.5 mM H$_2$O$_2$ for 1 h. (a) SOD activity was assessed in situ after native PAGE. Band intensities were measured by densitometry using data taken from the same gel; (b) GSH$_T$ levels, (c) GSSG levels, and (d) ratio between oxidized glutathione and total glutathione levels. Values are mean ± SEM of at least 3 independent assays. GSH$_T$ and GSSG levels were compared by two-way ANOVA, Sidak’s multiple comparisons test (* p < 0.05) and the ratio was compared by Student’s t-test (* p < 0.05).
3.4. Myricetin Increases the Chronological Lifespan of sod2Δ Mutant Cells. Aging has been associated with an increase in intracellular oxidation and accumulation of oxidative damage [28]. Mitochondria are a major source of ROS and its dysfunction has been implicated in aging [29, 30]. Mitochondria contain several antioxidant enzymes, including the superoxide dismutases Sod1p (CuZnSOD) that is present in the mitochondrial intermembrane space (and cytosol) and Sod2p (MnSOD) located in the mitochondrial matrix. Cells lacking Sod1p or Sod2p exhibit a decreased chronological lifespan associated with the accumulation of oxidative damage [31] (Figures 4(a)-4(b)). The protective effect of myricetin and pyrogallol against oxidative stress caused by H₂O₂ led us to assess its effect on the chronological lifespan (CLS) of parental cells and of sod1Δ and sod2Δ mutant cells. Parental cells showed a time-dependent loss of cell viability, which was not affected by pretreatment with myricetin, pyrogallol, or phloroglucinol (Figure 4(a)). These phenolic compounds also did not affect the lifespan of sod1Δ cells (data not shown). However, myricetin significantly increased the CLS of sod2Δ cells (Figure 4(b)), suggesting that this compound exerts a protective effect that is particularly relevant in cells that have a decreased capacity to scavenge superoxide radicals within the mitochondrial matrix. Consistently, myricetin decreased protein carbonylation in aged sod2Δ cells, although it had a modest effect in parental cells (Figures 4(c)-4(e)). In contrast, pyrogallol and phloroglucinol did not extend the CLS of sod2Δ cells (Figure 4(b)).

Mitochondria play an important function during oxidative stress. Indeed, ð0 petite strains, which lack mitochondrial DNA, and cells deficient in electron transport chain function are sensitive to H₂O₂ [32, 33]. A recent study showed that H₂O₂ increases the mitochondrial production of superoxide radicals, which have a protective effect at low concentrations [34]. However, high concentrations of superoxide radicals are detrimental. In agreement, sod2Δ cells were sensitive to H₂O₂ (Figure 5). We also assessed the effect of polyphenols in sod2Δ cells exposed to H₂O₂. The results show that pyrogallol pretreatment slightly increased H₂O₂ resistance of sod2Δ cells, although to levels below those observed in parental cells. In contrast, myricetin and phloroglucinol did not affect H₂O₂ resistance in these mutants (Figure 5).

4. Discussion

The increased production of ROS and RNS together with the decrease of antioxidant defenses has been implicated in the pathogenesis of numerous diseases and aging [28]. Thus, a diet containing natural compounds with antioxidant properties, such as phenolic compounds, may be beneficial to human health. The antioxidant activity of these compounds is determined by structural features, including the number and position of hydroxyl groups, polarity, solubility, and reducing potential [35, 36]. In this study, we used the yeast Saccharomyces cerevisiae to assess in vivo the antioxidant capacity of the flavonol myricetin and two simple phenols, pyrogallol and phloroglucinol. Myricetin was the most effective in increasing H₂O₂ resistance in yeast, whereas phloroglucinol had no protective effect. Consistently, H₂O₂-induced intracellular oxidation and protein carbonylation decreased in cells pretreated with myricetin and pyrogallol but not with phloroglucinol. Pyrogallol and phloroglucinol contain three hydroxyl groups in the ortho- and meta-position, respectively, of a benzene ring. The vicinal positions of hydroxyl groups in pyrogallol result in a lower bond dissociation energy of O–H, facilitating the donation of hydrogen to free radicals [37]. In accordance with that, our results show that pyrogallol, in contrast with phloroglucinol, increased the viability of yeast cells exposed to H₂O₂. Myricetin, which contains a pyrogallol structure in the B ring, provided an even higher resistance. Our results are in accordance with data demonstrating the importance of the pyrogallol structure for the bioactivity of phenolic compounds [38]. Our data is also consistent with several reports showing a protective effect of myricetin against oxidative stress in mammalian cells. For instance, myricetin decreases H₂O₂-induced DNA damage in Caco-2 and HepG2 cells [39] and decreases tert-butyl hydroperoxide-induced protein oxidation and lipid peroxidation in erythrocytes from T2DM patients [40].

Being redox-active compounds, phenolic compounds can also act as prooxidants and, therefore, induce stress responses leading to an increase in the levels of cellular antioxidant defenses [41, 42]. Our results indicate that this mechanism does not contribute to the protective effects of myricetin and pyrogallol in yeast, since these compounds did not increase intracellular oxidation or affect catalase and SOD activities under the conditions used in this study. We have previously observed that hydrogen peroxide resistance in yeast incubated with quercetin is also not associated with prooxidant effects or modulation of antioxidant defenses [13]. In contrast, other reports showed that catalase activity increases in yeast treated with resveratrol and catechin, enhancing cellular resistance to oxidative stress [15].

Glutathione is an important cellular small molecule responsible for the maintenance of redox homeostasis [27]. The reduced form (GSH) mediates H₂O₂ decomposition catalyzed by glutathione peroxidase [27] giving rise to oxidized glutathione (GSSG) which is then reduced to GSH by glutathione reductase [43]. Glutathione has also important functions in detoxification of toxic compounds [44] and in the protection of proteins from oxidation through glutathionylation [45]. Thus, glutathione oxidation is a biomarker of oxidative stress. In control (DMSO-treated) cells, exposure to H₂O₂ led to an increase in GSSG levels that, concomitantly with glutathione depletion, resulted in a higher GSSG/GSH ratio. In cells pretreated with myricetin, H₂O₂-induced glutathione oxidation and the increase in the ratio GSSG/GSH were suppressed, which is consistent with the reduction of intracellular oxidation. Pretreatment with pyrogallol, which had a lower protective effect comparing with myricetin, did not prevent glutathione oxidation. These results suggest a correlation between the protective effect of myricetin and maintenance of glutathione redox status. Treatment with both myricetin and pyrogallol per se led to a decrease in total GSH levels, which may result from the formation of GSH-compound adducts mediated by glutathione-S-transferases. Indeed, these adducts have been reported for quercetin [41,
Figure 4: Effect of myricetin, pyrogallol, and phloroglucinol on (a) BY4741 and (b) sod2Δ cells CLS. Cells were grown in SC-glucose medium to the stationary phase and treated with myricetin, pyrogallol, or phloroglucinol (300 μM). Viability was measured by standard dilution plate counts which were considered 100% on day 0 (first treatment day). (c, d) On the indicated days, the levels of protein carbonyls were analyzed during aging of BY4741 (c) and sod2Δ (d) cells pretreated with myricetin. Quantitative analysis of protein carbonyl content was performed by densitometry using data taken from the same membrane. Proteins were derivatized with DNPH and slot-blotted into a PVDF membrane. Immunodetection was performed using an anti-DNP antibody. A representative blot is shown in (e). Values are mean ± SEM of at least 3 independent assays. Viability values were compared by Student’s t-test (* p < 0.05) and protein carbonyl values were compared by two-way ANOVA, Sidak’s multiple comparisons test (* p < 0.05).
the protective effect of L-ascorbic acid against paraquat, a generator of superoxide radicals, is more pronounced in sod2Δ than in parental cells [56]. In addition, myricetin decreased the generation of H2O2 in isolated mouse skeletal muscle mitochondria [57] and decreased the depolarization of the inner mitochondrial membrane potential in C6 glial cells exposed to oxygen-glucose deprivation [58] and it was the most efficient among other phenolic compounds in the protection of mouse brain mitochondria against toxicity induced by methyl mercury [59]. Notably, myricetin was unable to protect sod2Δ cells against high doses of H2O2 whereas pyrogallol slightly increased the oxidative stress resistance of these mutants. It is likely that the excessive oxidative stress in sod2Δ cells treated with high doses of H2O2 overwhelms the protective effects of these compounds.

5. Conclusion

In summary, our data show that myricetin and, to a lesser extent, pyrogallol, increased yeast resistance to H2O2. This protective effect was correlated to a reduction in intracellular oxidation and protein carbonylation and maintenance of GSSG/GSH ratio. However, changes in catalase or superoxide dismutase activities were not associated with the protective effects. Furthermore, myricetin attenuated the shortened CLS of yeast cells lacking the mitochondrial superoxide dismutase (sod2Δ mutants).

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| CLS          | Chronological lifespan |
| DMSO         | Dimethyl sulfoxide |
| GSH          | Reduced glutathione |
| GSH_T        | Total glutathione |
| GSSG         | Oxidized glutathione |
| H2DCF-DA     | 2′,7′-Dichlorodihydrofluorescein diacetate |
| PAGE         | Polyacrylamide gel electrophoresis |
| RNS          | Reactive nitrogen species |
| ROS          | Reactive oxygen species |
| SC           | Synthetic complete |
| SOD          | Superoxide dismutase |

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] B. Halliwell and J. Gutteridge, “Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death,” in Free Radicals in Biology and Medicine, pp. 187–267, Oxford University Press, New York, NY, USA, 4th edition, 2007.
to study the effect of cocoa polyphenols in the resistance to oxidative stress,” *Journal of Agricultural and Food Chemistry*, vol. 59, no. 5, pp. 2077–2085, 2011.

[18] C. Müller, R. Lang, and T. Hofmann, “Quantitative precursor studies on di- and trihydroxybenzene formation during coffee roasting using ‘in bean’ model experiments and stable isotope dilution analysis,” *Journal of Agricultural and Food Chemistry*, vol. 54, no. 26, pp. 10086–10091, 2006.

[19] T. Yasuda, A. Inaba, M. Ohmori, T. Endo, S. Kubo, and K. Ohsawa, “Urinary metabolites of gallic acid in rats and their radical-scavenging effects on 1,1-diphenyl-2-picrylhydrazyl radical,” *Journal of Natural Products*, vol. 63, no. 10, pp. 1444–1446, 2000.

[20] L. Montero, M. Herrero, E. Ibáñez, and A. Cifuentes, “Separation and characterization of phlorotannins from brown algae *Cystoseira abies-marina* by comprehensive two-dimensional liquid chromatography,” *Electrophoresis*, vol. 35, no. 11, pp. 1644–1651, 2014.

[21] M. G. L. Hertog, E. J. M. Feskens, P. C. H. Hollman, M. B. Katan, and D. Kromhout, “Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study,” *The Lancet*, vol. 342, no. 8878, pp. 1007–1011, 1993.

[22] F. Tietze, “Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues,” *Analytical Biochemistry*, vol. 27, no. 3, pp. 502–522, 1969.

[23] L. Flohe and F. Otting, “Superoxide dismutase assays,” *Methods in Enzymology*, vol. 105, pp. 93–104, 1984.

[24] S. M. Conyers and D. A. Kidwell, “Chromogenic substrates for MAPK and caspase pathways in homeostatic response and induction of apoptosis,” *Archives of Pharmacal Research*, vol. 23, no. 1, pp. 1–16, 2000.

[25] A. B. Demir and A. Koc, “Assessment of chronological lifespan-effectors of cellular senescence: beyond the free radical theory,” *Journal of Biological Chemistry*, vol. 271, no. 21, pp. 12275–12280, 1996.

[26] D. V. Ziegler, C. D. Wiley, and M. C. Velarde, “Mitochondrial oxidative stress in aging and healthspan,” *Longevity & Healthspan*, vol. 3, article 6, 2014.

[27] D. V. Longo, E. B. Gralla, and J. S. Valentine, “Superoxide dismutase activity is essential for stationary phase survival in *Saccharomyces cerevisiae*: mitochondrial production of toxic oxygen species in vivo,” *Journal of Biological Chemistry*, vol. 271, no. 21, pp. 12275–12280, 1996.

[28] D. F. Dai, Y. A. Chiao, D. J. Marcinek, H. H. Szeto, and P. S. Rabinovitch, “Mitochondrial oxidative stress in aging and healthspan,” *Longevity & Healthspan*, vol. 3, article 6, 2014.

[29] A. Meister and M. E. Anderson, “Glutathione,” *Annual Review of Biochemistry*, vol. 52, pp. 711–760, 1983.

[30] D. F. Dai, Y. A. Chiao, D. J. Marcinek, H. H. Szeto, and P. S. Rabinovitch, “Mitochondrial oxidative stress in aging and healthspan,” *Longevity & Healthspan*, vol. 3, article 6, 2014.

[31] A. B. Demir and A. Koc, “Assessment of chronological lifespan-dependent molecular damages in yeast lacking mitochondrial antioxidant genes,” *Biochemical and Biophysical Research Communications*, vol. 400, no. 1, pp. 106–110, 2010.

[32] C. M. Grant, F. H. MacIver, and I. W. Dawes, “Mitochondrial function is required for resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*,” *FEBS Letters*, vol. 410, no. 2–3, pp. 219–222, 1997.
G. W. Thorpe, C. S. Fong, N. Alic, V. J. Higgins, and I. W. Dawes, "Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 17, pp. 6564–6569, 2004.

G. W. Thorpe, M. Reodica, M. J. Davies et al., "Superoxide radicals have a protective role during H2O2 stress," *Molecular Biology of the Cell*, vol. 24, no. 18, pp. 2876–2884, 2013.

W. Bors, C. Michel, and K. Stettmaier, "Structure-activity relationships governing antioxidant capacities of plant polyphenols," *Methods in Enzymology*, vol. 335, pp. 166–180, 2001.

J. C. J. M. D. S. Menezes, S. P. Kamat, J. A. S. Cavaleiro, A. Gaspar, J. Garrido, and F. Borges, "Synthesis and antioxidant activity of long chain alkyl hydroxycinnamates," *European Journal of Medicinal Chemistry*, vol. 46, no. 2, pp. 773–777, 2011.

V. Thavasi, L. P. Leong, and R. P. A. Bettens, "Investigation of the influence of hydroxy groups on the radical scavenging ability of polyphenols," *The Journal of Physical Chemistry A*, vol. 110, no. 14, pp. 4918–4923, 2006.

S. Mitsuhashi, A. Saito, N. Nakajima, H. Shima, and M. Ubukata, "Pyrogallol structure in polyphenols is involved in apoptosis-induction on HEK293T and K562 cells," *Molecules*, vol. 13, no. 12, pp. 2998–3006, 2008.

S. A. Aherne and N. M. O’Brien, "Protection by the flavonoids myricetin, quercetin, and rutin against hydrogen peroxide-induced DNA damage in Caco-2 and Hep G2 cells," *Nutrition and Cancer*, vol. 34, no. 2, pp. 160–166, 1999.

K. B. Pandey, N. Mishra, and S. I. Rizvi, "Myricetin may provide protection against oxidative stress in type 2 diabetic erythrocytes," *Zeitschrift für Naturforschung Teil C: Biochemie, Biophysik, Biologie, Virologie*, vol. 64, no. 9–10, pp. 626–630, 2009.

M. Kessler, G. Ubeaud, and L. Jung, "Anti- and pro-oxidant activity of rutin and quercetin derivatives," *Journal of Pharmacy and Pharmacology*, vol. 55, no. 1, pp. 131–142, 2003.

V. Calabrese, C. Cornelius, A. T. Dinkova-Kostova et al., "Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity," *Biochimica et Biophysica Acta—Molecular Basis of Disease*, vol. 1822, no. 5, pp. 753–783, 2012.

P. Mullineaux, G. Creissen, P. Broadbent, H. Reynolds, B. Kular, and A. Wellburn, "Elucidation of the role of glutathione reductase using transgenic plants," *Biochemical Society Transactions*, vol. 22, no. 4, pp. 931–936, 1994.

M. V. St-Pierre, S. Ruetz, L. F. Epstein, P. Gros, and I. M. Arias, "ATP-dependent transport of organic anions in secretory vesicles of Saccharomyces cerevisiae," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 20, pp. 9476–9479, 1994.

M. M. Gallogly and J. J. Mieyal, "Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress," *Current Opinion in Pharmacology*, vol. 7, no. 4, pp. 381–391, 2007.

J. P. E. Spencer, G. G. C. Kuhnele, R. J. Williams, and C. Rice-Evans, "Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites," *Biochemical Journal*, vol. 372, no. 1, pp. 173–181, 2003.

L. G. Higgins and J. D. Hayes, "Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents," *Drug Metabolism Reviews*, vol. 43, no. 2, pp. 92–137, 2011.

K. J. Bitterman, O. Medvedik, and D. A. Sinclair, "Longevity regulation in Saccharomyces cerevisiae: linking metabolism, genome stability, and heterochromatin," *Microbiology and Molecular Biology Reviews*, vol. 67, no. 3, pp. 376–399, 2003.

P. Fabrizio, L. Battistella, R.vardavas et al., "Superoxide is a mediator of an altruistic aging program in Saccharomyces cerevisiae," *The Journal of Cell Biology*, vol. 166, no. 7, pp. 1055–1067, 2004.

Y.-S. Ho, D. C.-H. Poon, T.-F. Chan, and R. C.-C. Chang, "From small to big molecules: how do we prevent and delay the progression of age-related neurodegeneration?" *Current Pharmaceutical Design*, vol. 18, no. 1, pp. 15–26, 2012.

R. N. Alcalay, Y. Gu, H. Mejia-Santana, L. Cote, K. S. Marder, and N. Scarmeas, "The association between Mediterranean diet adherence and Parkinson’s disease," *Movement Disorders*, vol. 27, no. 6, pp. 771–774, 2012.

N. Vassallo and C. Scerri, “Mediterranean diet and dementia of the Alzheimer type,” *Current Aging Science*, vol. 6, no. 2, pp. 150–162, 2013.

K. T. Howitz, K. J. Bitterman, H. Y. Cohen et al., "Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan," *Nature*, vol. 425, no. 6954, pp. 191–196, 2003.

L. Xiang, K. Sun, J. Lu et al., "Anti-aging effects of phloridzin, an apple polyphenol, on yeast via the SOD and Sir2 genes," *Bioscience, Biotechnology and Biochemistry*, vol. 75, no. 5, pp. 854–858, 2011.

V. Palermo, F. Mattivi, R. Silvestri, G. la Regina, C. Falcone, and C. Mazzoni, "Apple can act as anti-aging on yeast cells," *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 491739, 8 pages, 2012.

J. Safi, L. Sonego, Q. D. Varela, and M. Salvador, "Antioxidant activity of L-ascorbic acid in wild-type and superoxide dismutase deficient strains of Saccharomyces cerevisiae," *Redox Report*, vol. 11, no. 4, pp. 179–184, 2006.

G. Grünz, K. Haas, S. Soukop et al., "Structural features and bioavailability of four flavonoids and their implications for lifespan-extending and antioxidant actions in C. elegans," *Mechanisms of Ageing and Development*, vol. 133, no. 1, pp. 1–10, 2012.

K. S. Panickar and R. A. Anderson, "Mechanisms underlying the protective effects of myricetin and quercetin following oxygen-glucose deprivation-induced cell swelling and the reduction in glutamate uptake in glial cells," *Neuroscience*, vol. 183, pp. 1–14, 2011.

J. L. Franco, T. Posser, F. Missau et al., "Structure-activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction," *Environmental Toxicology and Pharmacology*, vol. 30, no. 3, pp. 272–278, 2010.

Oxidative Medicine and Cellular Longevity