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# Quercetin Increases Oxidative Stress Resistance and Longevity in *Saccharomyces cerevisiae*

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Quercetin, the major flavonol found in several fruits and vegetables, is a natural antioxidant with potential anticancer and antiaging activities. In this paper, the effect of quercetin in *Sacharomyces cerevisiae* cells submitted to oxidative stress was studied. Hydrogen peroxide resistance increased in cells pretreated with quercetin. Cellular protection was correlated with a decrease in oxidative stress markers, namely, levels of reactive oxygen species, glutathione oxidation, protein carbonylation, and lipid peroxidation. The acquisition of $H_2O_2$ resistance was not associated with the induction of antioxidant defenses or with iron chelation. Oxidative stress is a limiting factor for longevity. In agreement, quercetin also increased 60% chronological life span. These results support the utilization of yeast as a useful model to screen in vivo for natural antioxidants with putative health beneficial effects.

**KEYWORDS: Yeast; quercetin; oxidative stress; chronological life span; lipid peroxidation; protein carbonylation**

## INTRODUCTION

Reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide ($H_2O_2$), are produced as normal byproducts of cellular metabolism. In the presence of reduced metal ions ($Fe^{2+}$ or $Cu^{+}$), $H_2O_2$ originates hydroxyl radicals via the Fenton reaction. These ROS are able to oxidize nucleic acids, proteins, lipids and carbohydrates, affecting membrane integrity and cellular functions important for viability. Under physiological conditions, oxidative damage is prevented by antioxidant defenses, such as superoxide dismutase (SOD), catalase, peroxidases, and glutathione. When the levels of ROS exceed the antioxidant capacity, cells undergo an oxidative stress. This unbalanced situation has been associated with aging and numerous diseases, including cancer, cardiovascular disease, and neurodegenerative disorders (*1*).

In recent years there has been a growing interest in antioxidant molecules that could prevent oxidative damages by ROS. Flavonoids are a group of natural antioxidants found in fruits and vegetables as well as in foods and beverages of plant origin, such as olive oil, tea, and red wine. The health beneficial effects of these compounds have been associated with antitumor, antimutagenic, and antiaging activities (*2*). Flavonoids are probably located at the aqueous/membrane interface, where they trap aqueous free radicals to form more stable phenoxy radicals and therefore prevent the initiation of lipid peroxidation (*3*). The structure of these compounds is a key determinant of their antioxidant activity, which has also been associated with chelation of metal ions (*4*), inhibition of oxidative enzymes (*5*), and activation of antioxidant defenses (*6*−*9*). Recent data indicates that flavonoids may also function independently of their antioxidant properties, through cell cycle regulation, interaction with type II oestrogen binding sites, activation of the SIRT1 deacetylase that negatively regulates the p53 tumor suppressor, and inhibition of a number of protein kinase and lipid kinase signaling cascades (*10*−*13*).

Flavonols constitute a major group within the flavonoids present in several foodstuffs, such as apples, cherries, citrus fruits, black and green tea, onions, broccoli, and other green vegetables. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is the most common flavonol in the diet (*14*). The structural features of quercetin that have been associated with its antioxidant properties include a B-ring catechol group, a 2,3-unsaturated bond conjugated with a 4-oxo group in the C-ring, and functional hydroxyl groups (*15*). Despite the extensive studies to characterize the in vitro properties of quercetin, the molecular mecha-

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nisms of how it functions in vivo remains unclear. Yeast has been used as a eukaryotic model organism to study the molecular mechanisms associated with oxidative stress resistance and cell aging. In this work we studied the protective effect of quercetin in *Saccharomyces cerevisiae* cells and its correlation with oxidative stress markers. In addition, we investigated whether the biological effects of quercetin were associated with the induction of endogenous antioxidant defenses or mediated by iron deprivation.

## MATERIALS AND METHODS

**Reagents.** All reagents and chemicals were of analytical grade. Sodium or potassium phosphates, riboflavin, and $H_2O_2$ were purchased from Merck (Darmstadt, Germany); dimethyl sulfoxide (DMSO), quercetin, *o*-nitrophenyl β-D-galactopyranoside (ONPG), and nitro blue tetrazolium (NBT) were purchased from Sigma−Aldrich (Madrid, Spain). Solutions were prepared in ultrapure water (Milli-Q).

**Yeast Strains, Plasmids, and Growth Conditions.** The following *Saccharomyces cerevisiae* strains were used in this study: By4741 (Matα *his3*$\Delta_1$ *leu2*$\Delta_0$ *met15*$\Delta_0$ *ura3*$\Delta_0$), By4741 *CTH2*-LacZ ([By4741] pCM64-*CTH2*-FeRE-*CYC1*-LacZ), and By4741 *CTH2*-LacZ M3 ([By4741] pCM64-*CTH2*-FeRE-*CYC1*-LacZ M3). By4741 cells (obtained from Euroscarf) were transformed by electroporation with *CTH2*-LacZ or *CTH2*-LacZ M3 plasmids that contain consensus or mutant Aft1 binding sequences from *CTH2* promoter fused to the *CYC1* minimal promoter−LacZ reporter (*16*). By4741 cells were grown in YPD medium [1% (w/v) yeast extract, 2% (w/v) bactopeptone, and 2% (w/v) glucose] to early exponential phase ($OD_{600}$ = 0.6), in an orbital shaker, at 26 °C, and 120 rpm, with a ratio of flask volume/medium volume of 5:1. Yeast cells expressing *CTH2*-LacZ or *CTH2*-LacZ M3 were grown in minimal medium-GLU [0.67% (w/v) yeast nitrogen base without amino acids, plus 2% (w/v) glucose] supplemented with appropriate amino acids (40 mg of histidine $L^{-1}$, 80 mg of leucine $L^{-1}$, and 40 mg of methionine $L^{-1}$).

**Oxidative Stress Resistance and Chronological Life Span Assay.** Cells were grown to the exponential phase in YPD medium and pretreated with 0.10 mg of quercetin $mL^{-1}$ (from a stock solution at 20 mg $mL^{-1}$ prepared in dimethyl sulfoxide (DMSO); 50 μL/10 mL of culture) or DMSO (vehicle; volume identical to quercetin) for 15 min. Oxidative stress resistance was determined in cells treated with 1.5 mM $H_2O_2$. Chronological life span was assayed as previously described (*17*). Cells were centrifuged at 4000 rpm for 5 min, resuspended in ultrapure water, and recentrifuged (repeated twice). Washed cells were resuspended in water and incubated at 26 °C for the indicated times. Cell viability was determined by standard 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.

**β-Galactosidase Assay.** Yeast expressing consensus or mutant Aft1-LacZ reporter were grown in minimal medium to exponential phase and treated with 0.1 mg of quercetin $mL^{-1}$ or 30 μM bathophenantholinedisulfonic acid (BPS) for 4 h. Cells were harvested and β-galactosidase activity was measured in permeabilized cells as previously described (*18*).

**Oxidative Stress Markers.** Protein oxidation was determined by immunodetection of protein carbonyls, as previously described (*19*). Protein content of cellular extracts was estimated by the method of Lowry (*20*), with bovine serum albumin as a standard. Protein carbonylation assays were performed by slot blot analysis with rabbit IgG anti-dinitrophenyl (DNP) (Dako, Glostrup, Denmark) at a 1:5000 dilution as the primary antibody and goat anti-rabbit IgG−peroxidase (Sigma, St. Louis, MO) at a 1:5000 dilution as the secondary antibody. Immunodetection was performed by chemiluminescence, with a kit from Amersham (RPN 2109). Quantification of carbonyls was performed by densitometry.

For lipid peroxidation analysis, yeast extracts were prepared in 20 mM sodium phosphate buffer (pH 7.2), by vigorous shaking of the cell suspension, in the presence of glass beads, for 3 min. Short pulses of 1 min were used, with 1 min intervals on ice. Trichloroacetic acid (10% w/v) was added and two more pulses of 1 min were performed. Protein content was estimated as described above. Lipid peroxidation was assayed in 600 μL of 1% (w/v) thiobarbituric acid, 0.05 M NaOH, 0.025% (w/v) butylated hydroxytoluene, 100 μL of 0.1 M EDTA, and 50 μg of total cytoplasmic proteins. Malondialdehyde (MDA) concentration was determined spectrophotometrically at 532 nm, and expressed as nanomoles of MDA (milligram of protein)$^{-1}$ (*21*).

The oxidant-sensitive probe dihydrorhodamine 123 (DHR) was used to measure intracellular oxidation. Cells were pretreated with DMSO or quercetin for 15 min and then incubated with dihydrorhodamine 123 (0.025 μg $\mu L^{-1}$) for 1 h in the dark, spun down, and resuspended in PBS. Treatment with 1.5 mM $H_2O_2$ was made simultaneous with DHR incubation. A negative control was prepared with cells untreated with DHR. Fluorescence was measured on the FL-1 channel of a Becton-Dickinson FACSort flow cytometer (excitation and emission 490 and 530 nm, respectively).

**Glutathione Determination.** Yeast extracts were prepared by combining equal volumes of 2 M perchloric acid and a cell suspension in 100 mM potassium phosphate buffer (pH 7.0) with 2 mM EDTA. The mixture was vigorously shaken, in the presence of glass beads, for 5 min. Short pulses of 1 min were used, with 1 min intervals on ice. Cell debris was removed by centrifugation at 5000 rpm for 5 min. The supernatant was neutralized to pH 7.0 with 2 M KOH and 0.3 M 4-morpholinepropanesulfonic acid. Protein content was estimated as described above. For glutathione disulfide determination, the samples were treated with 2% (v/v) 2-vinylpyridine and incubated for 1 h at 4 °C with agitation. Glutathione was assayed by the method of Tietze (*22*). The rate of color development was monitored at 405 nm. The concentration was determined by reference to a GSSG standard added to the assay cuvette (internal standard) and expressed as nanomoles of glutathione (microgram of protein)$^{-1}$.

**Enzymatic Activity Measurement.** Yeast extracts were prepared in 50 mM potassium phosphate buffer (pH 7.0) and protease inhibitors, 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 as described above. Catalase activity was determined spectrophotometrically at 240 nm, following $H_2O_2$ decomposition (*23*). Superoxide dismutase activity was analyzed in the presence of riboflavin and NBT, after native polyacrylamide gel electrophoresis (60 μg of protein), as described (*24*). Bands were quantified by densitometry. Glucose-6-phosphate dehydrogenase activity was assayed spectrophotometrically at 340 nm by incubating 25 μg of protein with 0.5 mmol/L $NADP^+$ 0.7 mmol/L glucose 6-phosphate, and 100 mM phosphate buffer (pH 7.4) (glucose-6-phosphate dehydrogenase kit, 345-B, Sigma).

**Statistical Analysis.** Data are expressed as mean values ± SD of at least three independent experiments. Values were compared by Student's *t*-test. The 0.05 probability level was chosen as the point of statistical significance throughout.

## RESULTS

**Quercetin Increases Oxidative Stress Resistance in *S. cerevisiae*.** In the present work, the protective effect of quercetin in *S. cerevisiae* By4741 cells exposed to oxidative stress conditions was analyzed. Yeast cells grown to the logarithmic phase and preincubated with DMSO (vehicle) or 0.10 mg of quercetin $mL^{-1}$ for 15 min were exposed to 1.5 mM $H_2O_2$ for 1 h. The analysis of stress resistance shows that quercetin increased cellular viability from 13% (DMSO) to 40% (quercetin) (**Figure 1a**). To investigate if the higher resistance of yeast cells pretreated with quercetin was correlated with decreased levels of reactive oxygen species, intracellular oxidation was monitored by use of the molecular probe dihydrorhodamine (DHR) 123. The results obtained by flow cytometry show that $H_2O_2$-induced DHR fluorescence decreased 5−6-fold in cells preincubated with quercetin (**Figure 1b,c**).

The nonenzymatic defense glutathione contains a redox-active sulfhydryl group that reacts with oxidants. To investigate the

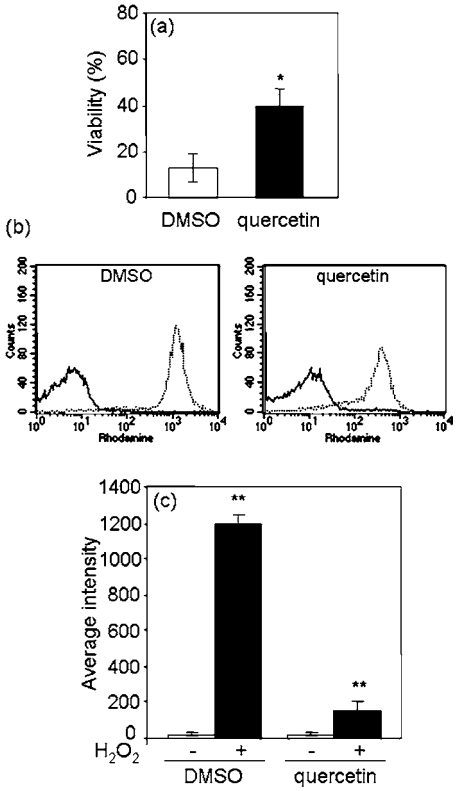


**Figure 1.** Effect of quercetin in $H_2O_2$-induced cell death and intracellular oxidation. Exponential-phase *S. cerevisiae* By4741 cells were pretreated with 0.10 mg of quercetin $mL^{-1}$ or with DMSO (vehicle) for 15 min and exposed to 1.5 mM $H_2O_2$ for 1 h. (**a**) Cellular viability was determined by standard dilution plate counts and expressed as the percentage of the colony-forming units of nonstressed cells. (**b**) Intracellular oxidation: cells were labeled with 0.025 $\mu$g of dihydrorhodamine 123 $\mu L^{-1}$ simultaneously with $H_2O_2$ treatment and analyzed by flow cytometry: (—) autofluorescence; (···) $H_2O_2$. (**c**) Fluorescence intensity of rhodamine-labeled cells. Data are mean ± SD of at least three independent experiments. $^{**}p < 0.01$; $^{*}p < 0.05$.

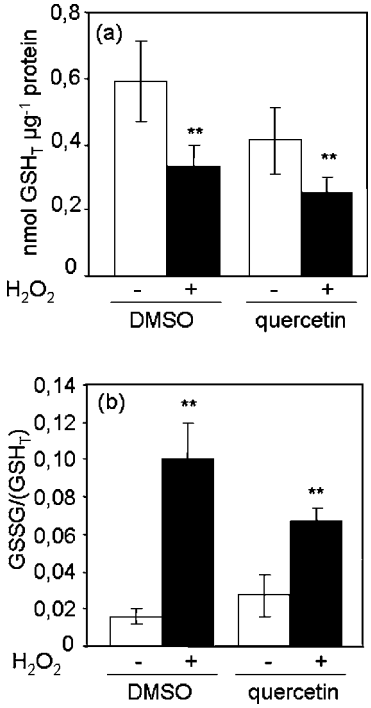


**Figure 2.** Effect of quercetin on glutathione levels. Exponential-phase *S. cerevisiae* By4741 cells were pretreated with 0.10 mg of quercetin $mL^{-1}$ or with DMSO (vehicle) for 15 min and treated with 1.5 mM $H_2O_2$ for 1 h. Total glutathione ($GSH_T$) levels (**a**) and GSSG/($GSH_T$) ratio (**b**) were determined as described under Materials and Methods. Data are mean ± SD of three independent experiments. $^{**}p < 0.01$.

effect of quercetin on redox homeostasis, glutathione levels were determined in cells exposed to $H_2O_2$. In DMSO-treated cells (control), glutathione levels decreased 40% and the ratio GSSG/(GSH + GSSG) increased 5.5-fold after exposure to $H_2O_2$ (**Figure 2**). In cells pretreated with quercetin, $H_2O_2$-induced glutathione depletion was similar but glutathione oxidation (2.5-fold) was significantly lower (**Figure 2**). These results are in agreement with the reduction of intracellular oxidation and suggest a correlation between the protective effect of quercetin and maintenance of a redox homeostasis.

Cellular death induced by oxidative stress is associated with DNA damage, accumulation of oxidized proteins, and lipid peroxidation (*1*). To investigate whether quercetin reduces oxidative damages, protein carbonylation and lipid peroxidation levels were analyzed. As previously shown (*19*, *21*), exposure of DMSO-treated cells (control) to $H_2O_2$ increased both protein carbonyl content and lipid peroxidation (**Figure 3**). In contrast, oxidative damages induced by $H_2O_2$ were not observed in cells pretreated with quercetin. Notably, quercetin decreased the constitutive levels of oxidized proteins and lipids (**Figure 3**). These results indicate that quercetin protects cells against oxidation induced not only by $H_2O_2$ but also by reactive oxygen species generated as byproducts of normal respiratory metabolism.

**Quercetin Does Not Induce Glucose-6-phosphate Dehydrogenase, SOD, or Catalase and Does Not Function through Iron Chelation.** Glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose phosphate pathway, produces NADPH that is required to reduce glutathione. As glutathione oxidation decreased in cells pretreated with quercetin, we raised the hypothesis that this flavonol might induce glucose-6-phosphate dehydrogenase. However, the results show that quercetin did not increase glucose-6-phosphate dehydrogenase activity (**Figure 4a**). Aiming at testing whether the induction of other enzymatic defenses contributes to the protective effect of quercetin, the activity of superoxide dismutase (SOD) and catalase was quantified. In DMSO-treated cells (control), SOD specific activity was 1.0 ± 0.1 unit (mg of protein)$^{-1}$. Quercetin is a scavenger of superoxide radicals and, therefore, samples from cells treated with quercetin show a 2.5-fold higher SOD activity when analyzed by a spectrophotometric assay (data not shown). Thus, to estimate endogenous SOD without quercetin interference, enzyme activity was determined in situ, after gel electrophoresis under nondenaturing conditions. The results obtained show that quercetin did not significantly affect SOD activity (**Figure 4a**). Catalase activity was not detected in DMSO-treated cells (control). This is consistent with the fact that catalase genes are expressed only in the postdiauxic growth phase or in cells exposed to stress conditions (*25*). Exposure to quercetin did not affect catalase activity (data not shown). These results suggest that quercetin improves oxidative stress resistance by a mechanism independent of these endogenous antioxidant defenses.

Hydrogen peroxide toxicity is associated with the production of the highly reactive hydroxyl radicals catalyzed by transition metals, such as iron and copper (Fenton reaction). The antioxidant capacity of flavonoids, including quercetin, has also been attributed to their capacity to bind these metals (*26*). Thus, we raised the hypothesis that the protective effect afforded by quercetin could result from iron chelation. It was previously

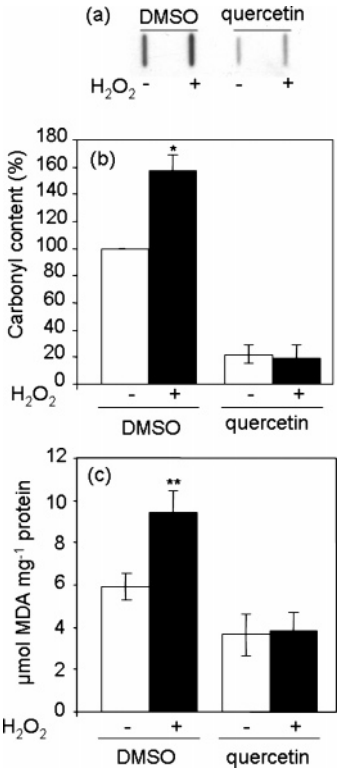


**Figure 3.** Effect of quercetin on oxidative damages. Exponential-phase *S. cerevisiae* By4741 cells were pretreated with 0.10 mg of quercetin $mL^{-1}$ or with DMSO (vehicle) for 15 min and treated with 1.5 mM $H_2O_2$ for 1 h. Controls were prepared from cells treated only with DMSO or quercetin for 75 min. (**a**) Protein oxidation: proteins were isolated, derivatized with DNPH, and slot-blotted into a PVDF membrane. Immunodetection was performed with anti-DNP antibodies, as described under Materials and Methods. (**b**) Quantitative analysis of carbonyls was performed by densitometry, with data taken from the same membrane. (**c**) Lipid peroxidation: MDA content was quantified by spectrophotometry at 523 nm, as described under Materials and Methods. Data are mean ± SD of at least four independent experiments. *$p < 0.05$; **$p < 0.01$.

shown that iron deprivation activates the Aft1p transcription factor that increases the expression of genes associated with iron uptake (*27*). To test whether quercetin activates Aft1p, $\beta$-galactosidase activity was measured in cells expressing the consensus (*CTH2*-LacZ) or mutant (*CTH2*-LacZ M3) Aft1 binding sequences from *CTH2* promoter fused to a LacZ reporter (*16*). The iron chelator bathophenantholinedisulfonic acid (BPS), which also increases $H_2O_2$ resistance, was used as control. Cellular viability in controls and BPS-pretreated cells was 26% ± 4% and 71% ± 7%, respectively. The results show that quercetin, in contrast to BPS, did not increase $\beta$-galactosidase activity in *S. cerevisiae* CTH2-LacZ (**Figure 4b**). As expected, both quercetin and BPS did not induce *CTH2*-LacZ M3 (data not shown). These results indicate that cellular protection by quercetin is not due to iron chelation.

**Quercetin Increases Chronological Life Span.** Aging has been associated with an increased production of reactive oxygen species associated with a decrease in antioxidant defenses. This oxidative damage theory of aging is supported by data showing that increased scavenging of reactive oxygen species by overexpression of antioxidant defenses delays aging (*17*, *28*). The protection against oxidative stress conferred by quercetin led us to analyze the effect of this compound on yeast chronological life span. The results show that quercetin increased the mean life span by 60% (**Figure 5**). After 7 days of aging,

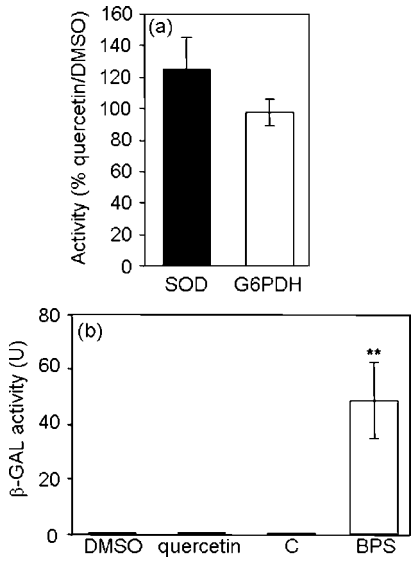


**Figure 4.** Quercetin does not induce antioxidant enzymes or function as iron chelator. (**a**) Exponential-phase *S. cerevisiae* By4741 cells were treated with 0.10 mg of quercetin $mL^{-1}$ or with DMSO (vehicle) for 75 min. Cu–Zn superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G6PDH) activities were determined as described under Materials and Methods. The G6PDH-specific activity in control (DMSO) cells was 0.046 unit (mg of protein)$^{-1}$. (**b**) *S. cerevisiae* By4741 *CTH2*-LacZ cells were grown on minimal medium to exponential phase. $\beta$-Galactosidase activity was measured in cells untreated (C) or treated with 0.10 mg of quercetin $mL^{-1}$, with DMSO (vehicle), or with 20 $\mu$M BPS for 4 h. Values are means ± SD of three independent experiments. **$p < 0.01$.

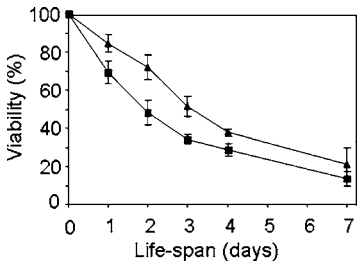


**Figure 5.** Effect of quercetin on yeast life span. Cells were preincubated with 0.10 mg of quercetin $mL^{-1}$ (▲) or with DMSO (■) for 15 min, washed with $H_2O$, and kept in $H_2O$. The viability was determined by standard dilution plate counts and expressed as the percentage of the colony-forming units at $t_0$. Data are mean ± SD of three independent experiments.

the viability of cells pretreated with quercetin or DMSO (control) was similar, possibly due to oxidation of quercetin in the medium.

## DISCUSSION

Oxidative stress is a hallmark of many acute and chronic diseases as well as of aging. Much interest has therefore emerged in the use of natural antioxidants, including flavonoids, as potential therapeutic agents in diseases associated with oxidative stress. The health beneficial effects of these phenolic compounds have been attributed to their free radical scavenging and metal chelating properties as well as to modulation of cell signaling pathways (*10*−*13*).

Quercetin is the most common flavonol in the diet and its properties has been extensively studied in vitro. In this work, we used yeast cells as an eukaryotic model organism to investigate the antioxidant effects of quercetin at the cellular level. The results obtained show that quercetin significantly

increased $H_2O_2$ resistance in *S. cerevisiae* cells. This protective effect was correlated with a reduction in the levels of ROS and a lower degree of glutathione oxidation. Glutathione (GSH) is a low molecular weight thiol that is important for the reduction of $H_2O_2$ and lipid hydroperoxides catalyzed by glutathione peroxidases and forms protein mixed disulfides (protein-S-thiolation), preventing the irreversible oxidation of protein-cysteine residues (*28*). Oxidized glutathione is reduced at the expense of NADPH that is produced in the pentose phosphate pathway. However, glucose-6-phosphate dehydrogenase activity was not affected by quercetin. These results suggest that quercetin prevents a redox imbalance by a mechanism independent of the upregulation of the pentose phosphate pathway.

Polyphenolic compounds are preferentially incorporated into membrane lipid bilayers and act as hydrogen donors, trapping free radicals, inhibiting the formation of lipid radicals, and recycling other antioxidants, such as α-tocopherol (*3*, *15*). In agreement, quercetin completely suppressed lipid peroxidation and protein carbonylation induced by $H_2O_2$. The decrease in lipid peroxidation by quercetin is in agreement with the fact that dietary polyphenols have been associated with a decrease of coronary heart diseases (*29*, *30*), and show high antioxidant activity in some lipid systems, in particular in low-density lipoproteins (*31*, *32*). Importantly, quercetin also decreased constitutive levels of oxidative damages, indicating that this phenolic compound protects cells against oxidation induced not only by $H_2O_2$ but also by reactive oxygen species generated as byproducts of normal cell metabolism (aerobic respiration and several metabolic reactions).

The effects of polyphenolic compounds may result from $H_2O_2$ production due to oxidation in cell culture medium (*33*). Sublethal doses of $H_2O_2$ induce a stress response that increases antioxidant defenses and other stress proteins, leading to cellular resistance to a subsequent lethal stress (*28*). This adaptive response could therefore mediate the acquisition of oxidative stress resistance afforded by quercetin. However, a number of lines of evidence shown in this study indicate that quercetin does not promote the production of significant $H_2O_2$ levels when added to YPD medium. Indeed, quercetin did not increase protein carbonyl content (constitutive levels even decreased) or upregulate antioxidant defenses.

The antioxidant protection of quercetin has also been associated with its capacity to chelate transition metals, such as iron and copper, that catalyze the conversion of $H_2O_2$ into the highly reactive hydroxyl radicals (Fenton reaction) (*4*, *26*, *34*−*36*). In the present study, iron chelation in vivo was monitored by measuring the activation of a LacZ reporter driven by Aft1p binding sites. The Aft1p transcription factor is activated by iron deprivation and increases the expression of genes associated with iron uptake and vacuolar iron utilization (*37*). Our results show that quercetin, in contrast with the iron chelator bathophenantholinedisulfonic acid (BPS), did not activate Aft1p. BPS also increased oxidative stress resistance, supporting the hypothesis that the Fenton reaction contributes to $H_2O_2$ toxicity in vivo. The overall results suggest that quercetin protects yeast cells from $H_2O_2$ stress by a mechanism independent of its metal-chelating properties and of the induction of glutathione, SOD, and catalase. It has been suggested that polyphenolic compounds may exert their effects through interactions with specific proteins of cell signaling pathways (*10*−*13*). The identification of signal transduction pathways modulated by quercetin will contribute to the characterization of how this natural compound exerts its protective effects.

An increased production of reactive oxygen species, mainly due to mitochondrial dysfunction, and the consequent accumulation of oxidative damages that lead to neuronal death are associated with aging and age-related diseases, such as Alzheimer and Parkinson diseases (*38*). In agreement, a diet replete in flavonoid-rich foods (Mediterranean diet) increases longevity, decreases the incidence of cardiovascular disease, and reduces the risk of aging-related pathologies (*39*). Yeast cells have been used as a model system to identify cell functions important for two different aspects of life span: replicative life span, measured as the number of buds generated by each mother cell, and chronological life span, measured as the capacity of nondividing (G0-arrested) cultures to maintain viability over time (*40*). Howitz et al. (*41*) showed that resveratrol, a phenolic compound found in red wine, extends replicative longevity of *S. cerevisiae*, through activation of sirtuins. Yeast cells overexpressing free-radical scavenging enzymes display an increased chronological life span (*17*, *40*). Results presented here show that quercetin extends chronological life span of yeast cells. This increase is more significative in the first 3 days, possibly due to posterior oxidation of the compound in the medium.

In conclusion, this study reveals that the flavonol quercetin increases oxidative stress resistance in yeast cells by scavenging free radicals, maintaining the redox homeostasis, and preventing protein carbonylation and lipid peroxidation. Quercetin also enhances the life span of nondividing cells, which is known to be limited by oxidative stress. These beneficial effects of quercetin are due to its intrinsic antiradical properties and not to iron chelation or the induction of endogenous antioxidant defenses. These results support the use of yeast as a model system to screen in vivo for natural antioxidants with putative health beneficial effects.

## ABBREVIATIONS

SOD, superoxide dismutase; ROS, reactive oxygen species; DMSO, dimethyl sulfoxide; DNPH, 2,4-dinitrophenylhydrazine; EDTA, ethylenediaminetetraacetic acid; MDA, malondialdehyde; DHR, dihydrorhodamine; PBS, phosphate-buffered saline.

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## LITERATURE CITED

(1) Halliwell, B., Gutteridge, J. M. C., Eds. *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: London, 1999.
(2) Zaveri, N. T. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sci.* **2006**, *78*, 2073−2080.
(3) Cotelle, N. Role of flavonoids in oxidative stress. *Curr. Top Med. Chem.* **2001**, *1*, 569−590.
(4) Van Acker, S. A. B. E.; Van den Berg, D. J. Structural aspects of antioxidant activity of flavonoids. *Free Radical Biol. Med.* **1996**, *20*, 331−342.
(5) Cos, P.; Ying, L.; Calomme, M.; Hu, J. P.; Cimanga, K.; Vanpoel, B.; Pieters, L.; Vlietinck, A. J.; Vanderberghe, D. Structure−activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.* **1998**, *61*, 71−76.
(6) Roig, R.; Cascon, E.; Arola, L.; Blade, C.; Salvado, M. J. Procyanidins protect Fao cells against hydrogen peroxide-induced oxidative stress. *Biochim. Biophys. Acta* **2002**, *1572*, 25−30.

(7) Coskun, O.; Kanter, M.; Korkmaz, A.; Oter, S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. *Pharmacol. Res.* **2005**, *51*, 117−123.
(8) Puiggros, F.; Llopiz, N.; Ardevol, A.; Blade. C.; Arola, L.; Salvado, M. J. Grape seed procyanidins prevent oxidative injury by modulating the expression of antioxidant enzyme systems. *J. Agric. Food Chem.* **2005**, *53*, 6080−6086.
(9) Alia, M.; Mateos, R.; Ramos, S.; Lecumberri, E.; Bravo, L.; Goya, L. Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). *Eur. J. Nutr.* **2006**, *45*, 19−28.
(10) Spencer, J.; Schroeter, H.; Kuhnle, G.; Srai, S.; Tyrrell, R. Hahn, U.; Rice-Evans, C. Epicatechin and its in vivo metabolite, 3′-*O*-methyl epicatechin, protect human fibroblasts from oxidative-stress-induced cell death involving caspase-3 activation. *Biochem. J.* **2001**, *354*, 493−500.
(11) Williams, R. J.; Spencer, J. P.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radical Biol. Med.* **2004**, *36*, 838−849.
(12) Vaziri, H.; Dessain, S. K.; Ng Eaton, E.; Imai, S. I.; Frye, R. A.; Pandita, T. K.; Guarente, L.; Weinberg, R. A. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* **2001**, *107*, 149−159.
(13) Lamson, D. W.; Brignall, M. S. Antioxidants and Cancer III: Quercetin. *Altern. Med. Rev.* **2000**, *5*, 196−208.
(14) Erlund, I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr. Res.* **2004**, *24*, 851−874.
(15) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure−antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.
(16) Puig, S.; Askeland, E.; Thiele, D. J. Coordinated remodeling of cellular metabolism during iron deficiency through targeted mRNA degradation. *Cell* **2005**, *120*, 99−110.
(17) Harris, N.; Costa, V.; MacLean, M.; Mollapour, M.; Moradas-Ferreira, P.; Piper, P. W. Mn-Sod overexpression extends the yeast chronological (G0) life span, but acts independently of Sir2p histone deacetylase to shorten the replicative life span of dividing cells. *Free Radical Biol. Med.* **2003**, *34*, 1599−1606.
(18) Ausubel, F. A., Brent, R., Kingston, D., Moore, D., Seidman, J. G., Smith, J. A., Struhl, K., Eds. *Current protocols in molecular biology*; John Wiley and Sons: New York, 1998.
(19) Costa, V.; Amorim, M. A.; Quintanilha, A.; Moradas-Ferreira, P. Hydrogen peroxide-induced carbonylation of key metabolic enzymes in *Saccharomyces cerevisiae*: the involvement of the oxidative stress response regulators Yap1 and Skn7. *Free Radical Biol. Med.* **2002**, *33*, 1507−1515.
(20) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, L. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(21) Steels, E. L.; Learmonth, R. P.; Watson, K. Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically. *Microbiology* **1994**, *140*, 569−576.
(22) Tietze, F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal. Biochem.* **1969**, *27*, 502−522.
(23) Aebi, H. Catalase *in vitro*. *Methods Enzymol.* **1984**, *105*, 121−126.
(24) Flohe, L.; Otting, F. Superoxide dismutase assays. *Methods Enzymol.* **1984**, *105*, 93−104.
(25) Belazzi, T.; Wagner, A.; Wieser. R.; Schanz, M.; Adam, G.; Hartig, A.; Ruis, H. Negative regulation of transcription of the *Saccharomyces cerevisiae* catalase T (CTT1) gene by cAMP is mediated by a positive control element. *EMBO J.* **1991**, *10*, 585−592.
(26) Mira, L.; Fernandez, M. T.; Santos, M.; Rocha, R.; Florencio, M. H.; Jennings, K. R. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. *Free Radical Res.* **2002**, *36*, 1199−1208.
(27) Rutherford, J. C.; Jaron, S.; Winge, D. R. Aft1p and Aft2p mediate iron-responsive gene expression in yeast through related promoter elements. *J. Biol. Chem.* **2003**, *278*, 27636−27643.
(28) Costa, V.; Moradas-Ferreira, P. Oxidative stress and signal transduction in *Saccharomyces cerevisiae*: insight into ageing, apoptosis and diseases. *Mol. Aspects Med.* **2001**, *22*, 217−246.
(29) Esterbauer, H.; Gebicki, J.; Puhl, H.; Jurgens, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.* **1992**, *3*, 341−390.
(30) Hergot, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Kantan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary disease. *Lancet* **1993**, *342*, 1007−1011.
(31) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.
(32) Porto, P. A. L.; Laranjinha, J. A. N.; Freitas, V. A. P. Antioxidant protection of low density lipoprotein by procyanidins: structure/activity relationships. *Biochem. Pharmacol.* **2003**, *66*, 947−954.
(33) Long, L. H.; Clement, M. V.; Halliwell, B. Artifacts in cell culture: rapid generation of hydrogen peroxide on addition of (−)-epigallocatechin, (−)-epigallocatechin gallate, (+)-catechin and quercetin to commonly used cell culture media. *Biochem. Biophys. Res. Commun.* **2000**, *273*, 50−53.
(34) Morel, I; Cillard, P.; Cillard, J. Flavonoid−metal interactions in biological systems. In *Flavonoids in Health and Disease*; Rice-Evans, C., Packer, L., Eds.; Marcel Dekker: New York, 1998; pp 163−177.
(35) Ferrali, M.; Signorini, C.; Caciotto, B.; Sugherini, L.; Ciccoli, L.; Giacchetti, D.; Comporti, M. Protection against oxidative damage or erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. *FEBS Lett.* **1997**, *416*, 123−129.
(36) Shackelford, R. E.; Manuszah, R. P.; Johnson, C. D.; Hellrung, D. J.; Link, C. J.; Wang, S. Iron chelators increase the resistence of *Ataxia telangeictasia* cells to oxidative stress. *DNA Repair* **2004**, *3*, 1263−1272.
(37) Rutherford, J. C.; Ojeda, L.; Balk, J.; Muhlenhoff, U.; Lill, R.; Winge, D. R. Activation of the iron regulon by the yeast Aft1/Aft2 transcription factors depends on mitochondrial but not cytosolic iron-sulfur protein biogenesis. *J. Biol. Chem.* **2005**, *280*, 10135−11040.
(38) Esposito, E.; Rotilio, D.; Matteo, D. M.; Giulio, C. D.; Cacchio, M.; Algeri, S. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiol. Aging* **2002**, *23*, 719−735.
(39) Scarmeas, N.; Stern, Y.; Tang, M. X.; Mayeux, R.; Luchsinger, J. A. Mediterranean diet and risk for Alzheimer's disease. *Ann. Neurol.* **2006**, *59*, 912−921.
(40) Piper, P. W. Long-lived yeast as a model for ageing research. *Yeast* **2006**, *23*, 215−226.
(41) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **2003**, *425*, 191−196.

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