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Recombinant hydroperoxide lyase for the production of aroma compounds: Effect of substrate on the yeast *Yarrowia lipolytica*

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Abstract

The aim of this study was to investigate the action mechanism of linoleic acid hydroperoxides (HPOD), which are the major substrates of hydroperoxide lyase for the production of flavour compounds, on the yeast *Yarrowia lipolytica* by evaluating their effect on the oxidative state of the cells. The total antioxidant capacity (TAC) and the activity of the main antioxidant enzymes, such as glutathione reductase, glutathione peroxidase and superoxide dismutase, of cells treated with HPOD were studied. The potential role of intracellular glutathione, including reduced glutathione (GSH) and oxidized glutathione (GSSG), in conferring HPOD resistance was also been examined. The experimental findings showed that HPOD induced a concentration-dependent decrease in TAC which could be an indication of the oxidative stress. The decrease in glutathione reductase activity and the increase in glutathione peroxidase and superoxide dismutase activities, following the treatment with HPOD may suggest a possible detoxification role for these enzymes. An important role of the intracellular glutathione in the cellular responses to HPOD was demonstrated, since the treatment of the cells with an increase in HPOD-concentration resulted by shifting the redox balance GSH/GSSG of the cells to a more oxidized state. Moreover, the increasing membrane permeability of the HPOD-treated cells, support the hypothesis that HPOD can interact with the membrane and penetrate into the cells for the induction of any oxidative stress.

Keywords: *Yarrowia lipolytica*; Antioxidant enzyme; Oxidative stress; Linoleic acid hydroperoxides; Glutathione

1. Introduction

The bioconversion of polyunsaturated fatty acids containing a 1(Z), 4(Z)-pentadiene moiety, into regio-specific hydroperoxides by lipoxygenase and the scission of C–C bond of these hydroperoxides between the carbon of the hydroperoxide group and the neighbouring double bond by hydroperoxide lyase (HPL) to produce volatiles aldehydes and ω-oxo-acids are the main steps of the biotechnological process for the production of natural flavours, recognized as "green notes" [1,2]. These flavours are composed of C6 or C9-aldehydes and the corresponding alcohols, formed by the action of HPL on the 13- and 9-hydroperoxides of linoleic (HPOD) or linolenic acid (HPOT) [2].

Hydroperoxide lyase has been well characterised and the gene encoding HPL from fruits has been cloned and sequenced such as bell pepper HPL in *Escherichia coli* [3,4], 13-HPL from alfalfa in *E. coli* [5] and HPL of tomato fruits in *E. coli* [6]. There is currently a strong interest in the development of new host for the secretion of heterologous proteins. Yeasts are attractive hosts for production of foreign proteins because they combine the advantages of procaryotic and higher eukaryotic systems. The yeast *Yarrowia lipolytica* has been identified as one of the more intensively studied species for fundamental research and also for biotechnological applications [7]; it uses efficiently the long- and short-chain fatty acids and the corresponding *n*-alkanes for its growth [8]. In our previous work, the HPL gene from green bell pepper fruit has been cloned and expressed in *Y. lipolytica*,

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with 350 mg/L of C6-aldehydes (hexanal and *trans*-2-hexenal) produced when HPOD were added directly in the medium of growing cells [9].

However, the literature reported that micro-organisms were sensitive to the reactive oxygen species, such as HPOD, which can form delocalized lipid radicals or react with other hydroperoxides to form the peroxy radicals, recognized as one of the more reactive species [10]. Masini et al. [11] have demonstrated that exogenous octadecanoic hydroperoxide-induced, in a concentration-dependent system, the membrane potential derangement. In addition, an unsaturated aldehydes 2,4-decadienal, derived from hydroperoxides, was found to induce apoptosis [12]. Moreover, one major target of the oxidative species attack is the unsaturated lipids, which could lead to lipid peroxidation causing many pathogenesis, in particular cancer [13] and inflammation [14] as well as the membrane fluidity changes and membrane damage of the mitochondrial cell [15]. Evans et al. [16] have shown that linoleic acid hydroperoxides (HPOD) are important source of biomembrane damage and are implicated in the onset of atherosclerosis, hepatic diseases and food rancidity; these authors have also demonstrated that HPOD, at very low concentrations, are toxic to *Saccharomyces cerevisiae*.

Previous research work done by our group [17] showed that HPOD undoubtedly presented a toxic effect on the yeast cells including a membrane fluidizing action. Indeed, the attack of a cell by an oxidative molecule resulted first by the contact of this molecule with the cell wall before the cell membrane could respond to this attack by activating the cell wall associated enzyme [12]. Moreover, each cell possess rather complicated antioxidant defence mechanisms, which include both low molecular weight scavengers, such as the reduced glutathione (GSH), and specialized enzymes such as superoxide dismutase (SOD), catalase, and peroxidases [18]. The objective of the present work was to investigate the effect of linoleic acid hydroperoxides on the yeast cell metabolism, by evaluating the activity of antioxidant enzymes, including glutathione reductase, glutathione peroxidase and superoxide dismutase, and by determining the amount of intracellular glutathione.

2. Experimental

2.1. Strain and culture conditions

Yeast strain JMY 861 of *Y. lipolytica* used in this study expressing a 6-His-tagged green bell pepper hydroperoxide lyase was previously described [19]. The cells were grown on YTGA (5 g L^{-1} Yeast extract, 10 g L^{-1} Tryptone, 10 g L^{-1} Glucose, 15 g L^{-1} Agar) medium at 27 °C for 48 h. The biomass was harvested and re-suspended in physiologic water (9 g L^{-1} NaCl), the suspension was inoculated in 50 mL YTG (5 g L^{-1} Yeast extract, 10 g L^{-1} Tryptone, 10 g L^{-1} Glucose) liquid medium. After 24 h of pre-culture, the biomass was inoculated with an initial O.D. (600 nm) of 0.25 in 50 mL YTG medium (2.5 10^6 cells mL^{-1}). The cells were grown in 100 mL baffled erlenmeyer flasks, agitated on a rotary shaker at 140 rpm and 27 °C for 19 h. The biomass was then harvested for processing.

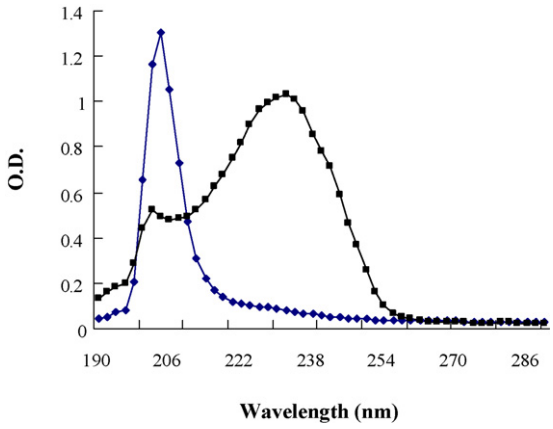

Fig. 1. Spectrum of linoleic acid (♦) and linoleic acid hydroperoxide (■).

2.2. Linoleic acid hydroperoxide preparation

The HPOD were prepared by oxidation of linoleic acid (Sigma Chemical Co.) as previously described [20]. Five hundred milligrams of linoleic acid were mixed with 5 mg of commercially purified soybean lipoxygenase type I-B (Sigma Chemical Co., 131 000 U mg^{-1} solid) in borate buffer (0.1 M, pH 9.6). The reaction mixture was incubated at 25 °C, for 1 h, with 300 rpm shaking and 30 mL O_2 min^{-1}. The enzymatic reaction was stopped by the addition of drop of H_2SO_4 until pH 4.0. HPOD were extracted with diethyl ether (Prolabo, France) for three times. The organic phase was dried with $MgSO_4$ (Sigma Chemical Co.) and evaporated under vacuum. The HPOD were solubilized in absolute ethanol and stored at −20 °C. The conversion of fatty acids into HPOD was followed up by measuring the absorbance at 234 nm (Fig. 1). The HPOD concentration was calculated using a molar extinction coefficient of 25 000 L mol^{-1} cm^{-1}.

2.3. Measure of membrane permeability

The membrane permeability was measured by a modification of the method of Aguedo et al. [21]. The yeast cells, grown for 19 h on YTG, were recovered by centrifugation (7000 × *g*, 5 min, 4 °C) and washed with distilled water until the absorbance (260 nm) of the supernatant was inferior to 0.1. The cells were then resuspended in 20 mL of MilliQ® water, containing HPOD at different concentrations (0–10 mM), and incubated at 27 °C, with a shaking of 140 rpm. At defined intervals (0–150 min), 1.4-mL samples were withdrawn and centrifuged (12 000 × *g*, 5 min, 4 °C). The supernatant was transferred to a 1-mL cuvette and the absorbance at 260 nm was measured.

2.4. Estimation of total antioxidant capacity

The total antioxidant capacity (TAC) in cellular homogenates was obtained by disruption of the cells with glass beads (diameter 425–600 μm, Sigma Chemical Co.) in the presence of protease inhibitors. TAC was estimated by a modified method of $ABTS^{\circ+}$ decolorization [22]. In summary, ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium

salt] was solubilized in deionized water to a 7 mM concentration. The ABTS radical cation ($ABTS^{\circ+}$) was produced by reaction of the ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was allowed to stand in the dark at room temperature for 12–16 h before its use. Although the oxidation of ABTS was started immediately, its maximum absorbance and stability were obtained after 6 h. The radical was stable in this form for more than 2 days when stored in the dark and at room temperature. The $ABTS^{\circ+}$ solution was diluted with phosphate buffer saline (PBS) of 10 mM and pH 7.4 to an absorbance of 1 (±0.05) at 414 nm and equilibrated at 30 °C [23]. For each assay, after addition of 1.0 mL of diluted $ABTS^{\circ+}$ solution ($O.D._{414\,nm} = 1 \pm 0.05$) to 100 μL of the cell extract or Trolox standards (final concentration 0–15 μM) in PBS, where the absorbance was measured at 30 °C, 10 s exactly after the initial mixing and up to 5 min. Solvent blank was run in tandem of each assay. Each assay was carried out at least three times and in triplicate, with different concentrations of the standard and samples. The relative percent of inhibition, measured at 414 nm, was calculated from its plots as a function of concentrations of antioxidants and of Trolox for the standard reference data.

2.5. Measure of antioxidant enzyme

2.5.1. Preparation of yeast extracts

The treated cells with HPOD were harvested by centrifugation (7000 × *g*, 5 min) and washed with potassium phosphate (K-phosphate) buffer (50 mM, pH 7.5). The yeast pellets were re-suspended in the same buffer, but containing 1 mM phenylmethanesulfonyl fluoride (PMSF) and 0.5 mM ethylenediaminetetraacetic (EDTA). The cells were disrupted (2 × 20 s) with 1 volume of glass beads (425–600 μm, Sigma Chemical Co.), followed by 1 min of cooling in ice. The cell debris was removed by centrifugation (15 000 × *g*, 10 min, 4 °C). The cell extract was kept in ice for immediate use.

2.5.2. Enzyme activities assays

The superoxide dismutase (SOD) activity was essayed spectrophotometrically at 550 nm and was determined as the inhibition rate of reduction of cytochrome *c* by the superoxide radical, in a medium containing K-phosphate (50 mM, pH 7.8), containing 0.1 mM EDTA, 0.01 mM cytochrome *c*, 0.05 mM xanthine, 0.005 U of xanthine oxidase and 100 μL supernatant in a final volume of 3.0 mL. A blank trial was run without cell extract and xanthine oxidase. One unit of SOD was defined as 50% of the inhibition rate of reduction of cytochrome *c* in a coupled system, using xanthine and xanthine oxidase at pH 7.8 and 25 °C.

The glutathione reductase (GR) activity was measured spectrophotometrically at 340 nm, using a molar extinction coefficient of $6.22 \times 10^3\ M\,cm^{-1}$. The reaction was followed by the decrease in NADPH in the reaction medium of K-phosphate buffer (75 mM, pH 7.6), containing 2.6 mM EDTA, 1.0 mM oxidized glutathione, 0.09 mM NADPH, and 100 μL supernatant in a final volume of 3.0 mL. Two blanks trials were run in tandem with this assay, without glutathione or cell extract. One unit of GR was defined as the amount of supernatant protein that reduced 1.0 μmole of oxidized glutathione per minute at pH 7.6 and 25 °C.

The glutathione peroxidase (GPX) activity was measured spectrophotometrically at 340 nm, using a molar extinction coefficient of $6.22 \times 10^3\ M\,cm^{-1}$. The reaction was followed by the decrease in NADPH in the reaction medium of K-phosphate buffer (48 mM, pH 7.0), containing 0.38 mM EDTA, 0.95 mM sodium azide, 3.2 U of glutathione reductase, 1.0 mM reduced glutathione, 0.12 mM NADPH, 0.0007% (w/w) hydrogen peroxide and 50 μL supernatant in a final volume of 3.05 mL. A blank was run without cell extract. One unit of GPX was defined as the amount of supernatant protein that catalyzed the oxidation by H_2O_2 of 1.0 μmole of reduced glutathione into oxidized glutathione per minute at pH 7.0 and 25 °C.

2.6. Glutathione measurements

2.6.1. Glutathione extraction

The yeast cells, after HPOD treatment at different concentrations, were centrifuged (7000 × *g*, 10 min). The recovered cells were washed twice with distilled water, suspended in 1 mL H_2O and thermally treated at 100 °C for 6 min before it was cooled in ice [24]. The suspension was centrifuged (10 000 × *g*, 10 min) and the supernatant was used for glutathione determination.

2.6.2. Analytical procedures

Glutathione identification and quantification was obtained by high-performance liquid chromatography (HPLC) at 30 °C, equipped with an UV detector (210 nm), using a (250-4) mm Purospher RP-18 endcapped column (Merck). The elution solution was performed with dihydrogen phosphate NaH_2PO_4 (25 mM, pH 3.5) at a flow rate of 0.3 mL min^{-1}.

3. Results and discussion

All organisms that use oxygen as a terminal electron acceptor in oxidative phosphorylation have to be protected from reactive oxygen species (ROS), which damages proteins, DNA, and membrane fatty acids. In order to cope with the oxidative stress, cells have a range of non-enzymatic and enzymatic defence systems, including glutathione, thioredoxin, glutaredoxin, superoxide dismutase and peroxidases [25]. A series of protecting systems can be classified into two subsets; one includes catalase, peroxidase, and superoxide dismutase that can reduce the endogenous levels of ROS, whereas the other one includes enzymes for the reparation of ROS damage (oxidized lipid, DNA, or proteins). In this study, the effect of linoleic acid hydroperoxides (HPOD) on the yeast cells during the aroma production was investigated. Our group has recently demonstrated [17] that HPOD inhibited the cell growth inducing hence toxic effect on the yeast *Y. lipolytica* and showed a fluidizing action on the yeast membrane; however, the mechanism of HPOD action in the cells was not clear. It is possible that after entry into the yeast, HPOD caused an oxidative stress which may be due to their peroxide group that can induce the cell mortality. Therefore, the enzymes involved in the detoxification of H_2O_2, such

as catalase [26], glutathione peroxidase [27], could play a role in the cellular response to HPOD. Moreover, glutathione is an abundant intracellular thiol found to be important in the response of cells to other types of oxidative stress [28]. Hence, the intracellular glutathione in the yeast cells treated with HPOD was also investigated. But firstly, in order to clarify the penetration way of HPOD into the cells, the membrane permeability of cells in the medium containing HPOD at different concentrations was studied.

3.1. Effect of HPOD on the membrane permeability

The literature [29] indicated that the stability and permeability of the cellular membranes play a fundamental role for their adaptation to different kinds of stresses, which might be closely related to the lipid and fatty acid composition. In the present work, when the yeast cells were maintained in water for different periods of time, a slight increase in absorbance at 260 nm was obtained in the supernatant (Fig. 2); this absorbance may be due to a leakage from an intracellular free pool of amino acids and 260-nm-absorbing compounds that were shown to diffuse from cells by a passive mechanism [30]. The presence in the medium of agents that enhance the membrane permeability would stimulate the leakage of these compounds [31]. After the addition of 2.5 mM HPOD to the medium, there was no change in the absorbance at 260 nm of the supernatant of the cells; however, the HPOD at concentrations higher than 5.0 mM resulted by markedly increase in the absorbance, demonstrating hence their effect on the cell membrane integrity. These results are in agreement with our previous study of the cell membrane fluidity [17], where the toxic effect of HPOD on the yeast *Y. lipolytica* was shown to be related to a strong interaction of these compounds with the cell membrane phospholipids and their components. The literature [32] indicated that the main function of the cell membrane is the permeability barrier, which regulate the flow solutes between the cell and its external environment. The barrier properties of the cytoplasmic membrane is of special importance for the energy transduction of the cell [32]. An increase in the permeability of the membrane for proton or other ions may lead to a dissipation of the proton motive force, resulting in a less effective energy transduction. In this study, the presence of HPOD in the medium resulted by an increase in the cell membrane permeability for intracellular free pool of amino acids and other 260-nm-absorbing compounds. Hence, HPOD could penetrate the cells and be converted by hydroperoxide lyase into aroma compounds. However, HPOD with their peroxide group were able to induce a stress in the cells, resulting in the cell mortality. Ninety percent of the yeast cells died after 120 min of exposition in 100 mM HPOD [17].

3.2. Total antioxidant capacity

Total antioxidant capacity (TAC) is the sum of activities of all non-enzymatic antioxidants present in the analyzed material. According to the literature, the determination of TAC may be a useful tool of assessment of oxidative stress, where the low TAC could be indication of the oxidative stress or increased susceptibility to oxidative damage [33,34]. The experimental findings (Fig. 3) demonstrate that the incubation with HPOD, at different concentrations, resulted by a decrease in TAC of cell extracts. A 90-min treatment of the cells with 25 and 50 mM HPOD resulted by 10% and 19% decrease in TAC, respectively, whereas the treatment for 150 min resulted by 31% and 42% decrease, respectively. Hence, the decrease in TAC was not only HPOD-concentration dependant, but also contact time-dependant. These results were consistent with the considerable drop in glutathione, which is the main low-molecular cellular thiol (Fig. 5A). Generally, the TAC of cellular homogenate is determined largely by the intracellular thiol content [33] and it decrease with conditions associated with the oxidative stress [35]. The overall experimental results may highlight the effect of HPOD on the cells; however, the TAC assays may not be sufficient to provide a better idea about the antioxidant defence of the cells. For that reason, it was interesting to measure the main antioxidant enzymes as well as the intracellular glutathione in the HPOD-treated yeast cells.

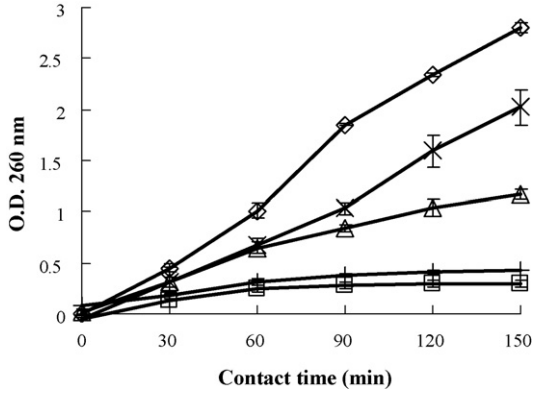

Fig. 2. Effect of HPOD on 260-nm-absorbing compounds release by reference yeast cells (+) and in presence of HPOD 2.5 mM (□), 5.0 mM (△), 7.5 mM (×) or 10.0 mM (◊).

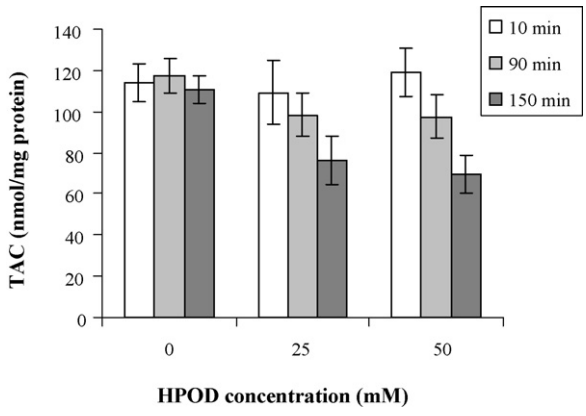

Fig. 3. Total antioxidant capacity (TAC) of cell extract after 10-, 90-, 150-min incubation of the cells with HPOD at various concentrations. TAC was measured by monitoring the $ABTS^{\circ+}$ solution decolorization in supernatants of cell homogenates and presented as Trolox equivalents (nmol/mg protein).

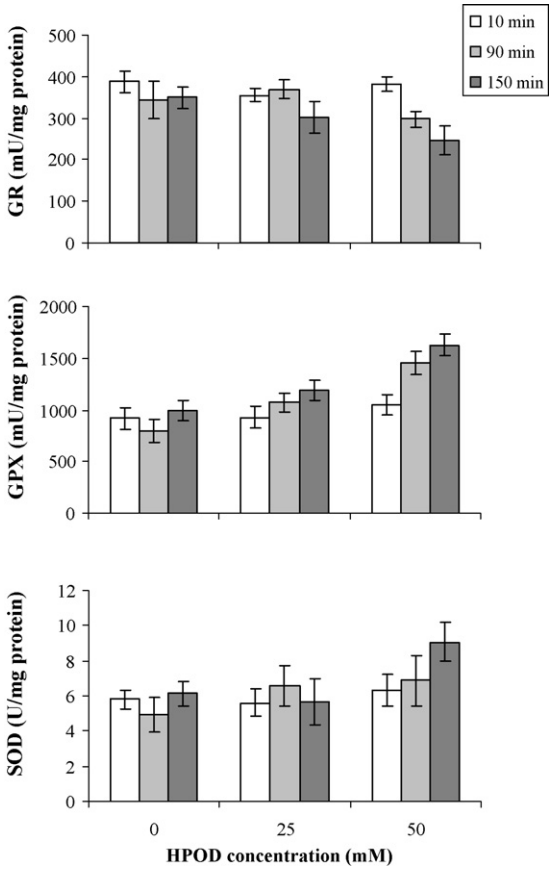

Fig. 4. Effect of HPOD on the antioxidant enzymes of yeast cells. Detailed conditions for the experiments are described in the text. (GR: glutathione reductase; GPX: glutathione peroxidase; SOD: superoxide dismutase).

3.3. Effect of HPOD on antioxidant enzymes of yeast cells

In order to clarify the role of HPOD, the potential effect of HPOD on the main antioxidant enzymes, such as superoxide dismutase, glutathione reductase and glutathione peroxidase, which are known to be involved in the maintenance of cellular oxidative status was investigated. The activity of these enzymes in the cell extract after 10-, 90- and 150-min incubation of the cells with HPOD at various concentrations was determined. Fig. 4 shows no change in glutathione reductase, glutathione peroxidase and superoxide dismutase activities in the control trial (0 mM HPOD), after 150 min of incubation. Although the cells treated with 25 mM HPOD showed no significant change in superoxide dismutase activity, a slight decrease in glutathione reductase activity with a concomitant increase one in glutathione peroxidase of the cells incubated for up to 150 min. The changes in the antioxidant enzymes activities were more significant in the cells treated with 50 mM HPOD. The increase in superoxide dismutase and glutathione peroxidase activities as well as the decrease in glutathione reductase activity was time-dependant. In comparison to previous work done by our group [17], the cell viability dropped to 30% and 52% in the presence of 25 and 50 mM HPOD, respectively; these results on the activity of the main cellular antioxidant enzymes confirm the toxic effect of HPOD, inducing hence an oxidative stress in the cells. Grant et al. [36] have demonstrated that glutathione reductase is required for protection against oxidative stress, where the yeast mutant deleted for GLR1, encoding glutathione reductase, lacks the GR activity that resulted by an increased accumulation of oxidized glutathione leading to the cell death. Much is known about the physiological role of SOD in different aerobic species, where in the case of some bacteria and yeasts, the enzyme provides for micro-organisms survival under various type of stress (such as heat and osmotic shock), with the oxidative stress being responsible for cell protection against reactive oxygen species (ROS) [37–40]. Moreover, glutathione peroxidase (GPX) is one of the most important anti-oxidant enzymes in yeast [41]. Tran et al. [42] have reported that the yeast *Hansenula mrakii* IFO 0895 was able to grow in a medium containing 4 mM HPOD; such resistance resulted from GPX, which was induced when the yeast was incubated in the presence of lipid hydroperoxides. It seems that the increase in GPX and SOD activities mirrors the increase in HPOD cell mortality, suggesting hence that oxidative stress could be a major event in HPOD stress of cells.

3.4. Measurement of intracellular glutathione

In oxidative stress conditions, reduced glutathione (GSH) levels may be altered as a result of four main processes, including its oxidation to oxidized one (GSSG) which can be recycled back to GSH by the action of glutathione reductase, the formation of mixed disulfides with proteins (GSSP), the active pumping from the cell following conjugation reactions, catalysed by glutathione transferases and the degradation of GSH, initiated by the action of γ-glutamyltranspeptidase. In this study, the redox state of GSH was analysed at 0, 25 and 50 mM HPOD (Fig. 5). Fig. 5A shows that use of 25 and 50 mM HPOD resulted by an approximate 50% and 90% in decrease of GSH, respectively, after 150 min of treatment; however, there was no significantly change in GSH between 90- and 150-min treatment. Although there was an increase in the GSSG levels of the treated cells, no significant difference in the use of different HPOD concentrations and different treatment periods of times (Fig. 5B). Taking into account the reduced glutathione (GSH) and the oxidized one (GSSG), the total glutathione concentration (GSH + 2 × GSSG) did not changed by the treatment with HPOD (Fig. 5C). However, the change of total glutathione levels could be explained by the fact that HPOD are able to cause membrane damage resulting hence in increased membrane permeability and the cells could rapidly lose low-molecular-weight molecules, such as glutathione.

One important parameter of GSH metabolism is the redox ratio (GSH/GSSG), which provides a measure of the proportion of GSH present in the oxidized state relative to that of the reduced one. The GSH redox ratio was substantially reduced by all investigated HPOD concentrations (Fig. 5D), indicating hence that the HPOD shifted the redox balance of the cell to a more oxidized state and interestingly, the decrease in the redox ratio correlated with the loss of cell viability [17].

The literature [28] showed that GSH is an essential metabolite required for the resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*. The use of GSH in both enzymatic and

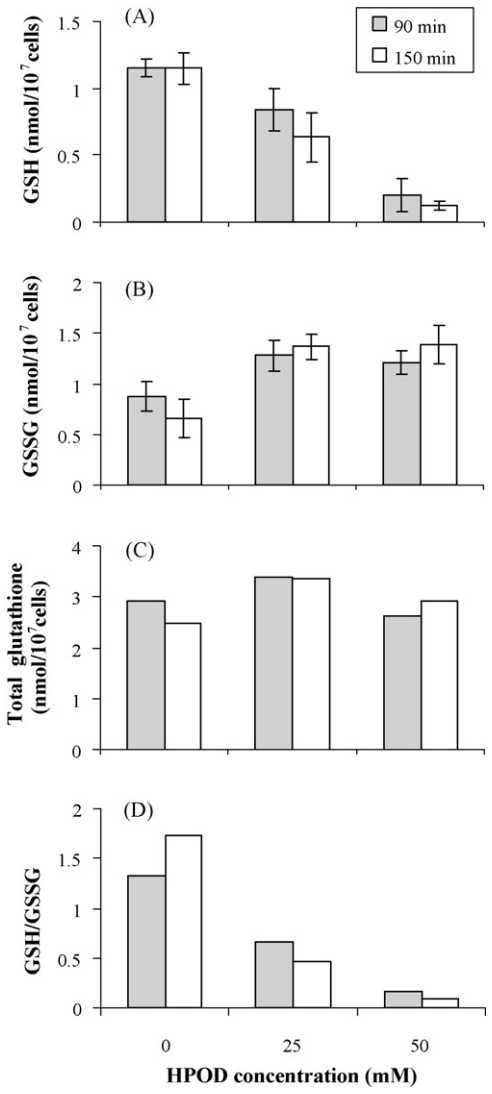

Fig. 5. Effect of HPOD on glutathione levels and redox state. (A) Levels of reduced glutathione (GSH). (B) Levels of oxidized glutathione (GSSG). (C) Levels of total glutathione (GSH + 2 × GSSG). (D) GSH redox ratio (GSH/GSSG).

non-enzymatic defence mechanisms resulted in its conversion to the oxidized form (GSSG), and it must be recycled to GSH to maintain the high intracellular ratio of GSH to GSSG [36]. Indeed, the requirement for GSH in protection against oxidative stress is similar to that in higher eukaryotes, but unlike to that in bacteria, where it is dispensable for the growth during both normal and oxidative stress conditions.

4. Conclusion

The results of this study have laid foundations for a more thorough understanding of how eukaryotic cells cope with lipid peroxide stress, and this has implications for both the medical and industrial fields, since lipid peroxidation plays a major role in both atherosclerosis and the food rancidity. Analysis has revealed several important features of the response to HPOD in yeast: membrane permeability increased with increasing HPOD concentration confirming the interaction between the hydrophobic carbon chains of HPOD with that of the acyl chain of phospholipids, the total antioxidant capacity of cells decreased after HPOD treatment expressing the oxidative stress caused by HPOD in the cells, glutathione and its related enzymes play key roles in the cellular defence against HPOD. Moreover, glutathione is the main thiol compound in yeasts, but its metabolism and functions in micro-organisms have been recently studied. Strong incidences of GSH-dependent pathways in the response against different stresses that can be generated by industrial exploitation of yeasts have emerged. A better understanding of yeast GSH appears to be a basic need for microbial technology.

The aim of future research is to study the relation between the redox potential (Eh), which is a physicochemical parameter that determines the oxidizing or reducing properties of the medium, and the resistance of the yeast *Y. lipolytica* to HPOD because Eh plays an important role in the cellular physiology of micro-organisms such as growth capacity, enzyme expression and thermal resistance. The previous study of our group has shown that oxidizing conditions were favourable for the growth of *Y. lipolytica*, whereas reducing conditions were more appropriate for the biosynthesis of hydroperoxide lyase [43]. Therefore, it will be very interesting to elucidate the role of Eh in the cellular response to HPOD by evaluating the viability, the intracellular glutathione and its related enzymes of cells after HPOD treatment in the different medium containing oxidizing or reducing Eh. The expected results could be an appropriate reference to improve the production of aromatic compounds called "green notes" by *Y. lipolytica*.

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References

[1] B.A. Vick, D.C. Zimmerman, Plant Physiol. 57 (1976) 780–788.
[2] A. Hatanaka, Phytochemistry 34 (1993) 1201–1218.
[3] K. Matsui, M. Shibutani, T. Hase, T. Kajiwara, FEBS Lett. 394 (1996) 21–24.
[4] E. Psylinakis, E.M. Davoras, N. Ioannidis, M. Trikeriotis, V. Petrouleas, D.F. Ghanotakis, Biochim. Biophys. Acta 1533 (2001) 119–127.
[5] M.A. Noordermeer, A.J. Van Dijken, S.C. Smeekens, G.A. Veldink, J.F. Vliegenthart, Eur. J. Biochem. 267 (2000) 2473–2482.
[6] K. Matsui, C. Miyahara, J. Wilkinson, B. Hiatt, V. Knauf, T. Kajiwara, Biosci. Biotechnol. Biochem. 64 (2000) 1189–1196.
[7] S. Muller, T. Sandal, P. Kamp-Hansen, H. Dalboge, Yeast 14 (1998) 1267–1283.
[8] G. Barth, C. Gaillardin, FEMS Microbiol. Rev. 19 (1997) 219–237.
[9] G. Bourel, J.M. Nicaud, B. Nthangeni, P. Santiago-Gomez, J.M. Belin, F. Husson, Enzyme Microb. Technol. 35 (2004) 293–299.
[10] G.J. Garssen, J.F. Vliegenthart, J. Boldingh, Biochem. J. 122 (1971) 327–332.
[11] A. Masini, D. Ceccarelli, D. Gallesi, F. Giovannini, T. Trenti, Biochem. Pharmacol. 47 (1994) 217–224.
[12] G. Spiteller, Prostag. Leukot. Essent. Fatty Acids 67 (2002) 151–162.
[13] N. Udilova, D. Jurek, B. Marian, L. Gille, R. Schulte-Hermann, H. Nohl, Food Chem. Toxicol. 41 (2003) 1481–1489.
[14] G. Spiteller, Med. Hypotheses 60 (2003) 69–83.
[15] J.J. Chen, B.P. Yu, Free Radical Biol. Med. 17 (1994) 411–418.

[16] M.V. Evans, H.E. Turton, C.M. Grant, I.W. Dawes, J. Bacteriol. 180 (1998) 483–490.
[17] H. Tran Thanh, L. Beney, H. Simonin, T.X.S. Nguyen, P. Gervais, J.-M. Belin, F. Husson, Biochim. Biophys. Acta (BBA)-Biomembranes 1768 (2007) 2256–2262.
[18] V.I. Lushchak, Biochemistry (Mosc) 66 (2001) 476–489.
[19] M.P. Santiago-Gomez, C. Vergely, C. Policar, J.-M. Nicaud, J.-M. Belin, L. Rochette, F. Husson, Enzyme Microb. Technol. 41 (2007) 13–18.
[20] M. Gargouri, P. Drouet, M.D. Legoy, J. Biotechnol. 111 (2004) 59–65.
[21] M. Aguedo, Y. Wache, J.M. Belin, FEMS Microbiol. Lett. 200 (2001) 185–189.
[22] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radical Biol. Med. 26 (1999) 1231–1237.
[23] A. Robaszkiewicz, A. Balcerczyk, G. Bartosz, Cell Biol. Int. 31 (2007) 1245–1250.
[24] M. Rollini, M. Manzoni, Process Biochem. 41 (2006) 1501–1505.
[25] Y. Inoue, T. Matsuda, K. Sugiyama, S. Izawa, A. Kimura, J. Biol. Chem. 274 (1999) 27002–27009.
[26] W.H. Mager, P.M. Ferreira, Biochem. J. 290 (Pt 1) (1993) 1–13.
[27] C. Michiels, M. Raes, O. Toussaint, J. Remacle, Free Radical Biol. Med. 17 (1994) 235–248.
[28] C.M. Grant, F.H. MacIver, I.W. Dawes, Curr. Genet. 29 (1996) 511–515.
[29] M.C. Machado, C.S. Lopez, H. Heras, E.A. Rivas, Arch. Biochem. Biophys. 422 (2004) 61–70.
[30] T.C. Lee, M.J. Lewis, J. Food Sci. 33 (1968) 124–128.
[31] I. Sa-Correia, S.P. Salgueiro, C.A. Viegas, J.M. Novais, Yeast 5 (1989) S123–S127.
[32] F.J. Weber, J.A. de Bont, Biochim. Biophys. Acta 1286 (1996) 225–245.
[33] A. Balcerczyk, G. Bartosz, Free Radical Res. 37 (2003) 537–541.
[34] I.S. Young, J. Clin. Pathol. 54 (2001) 339.
[35] F.P. Woodford, T.P. Whitehead, Ann. Clin. Biochem. 35 (Pt 1) (1998) 48–56.
[36] C.M. Grant, L.P. Collinson, J.H. Roe, I.W. Dawes, Mol. Microbiol. 21 (1996) 171–179.
[37] V.D. Longo, E.B. Gralla, J.S. Valentine, J. Biol. Chem. 271 (1996) 12275–12280.
[38] J.I. Park, C.M. Grant, M.J. Davies, I.W. Dawes, J. Biol. Chem. 273 (1998) 22921–22928.
[39] J. Wawryn, A. Swiecilo, G. Bartosz, T. Bilinski, Biochim. Biophys. Acta 1570 (2002) 199–202.
[40] M.D. Pereira, R.S. Herdeiro, P.N. Fernandes, E.C. Eleutherio, A.D. Panek, Biochim. Biophys. Acta 1620 (2003) 245–251.
[41] C.W. Kho, P.Y. Lee, K.-H. Bae, S. Cho, Z.-W. Lee, B.C. Park, S. Kang, D.H. Lee, S.G. Park, Biochem. Biophys. Res. Commun. 348 (2006) 25–35.
[42] L.-T. Tran, Y. Inoue, A. Kimura, Biochim. Biophys. Acta (BBA)-Protein Struct. Mol. Enzymol. 1164 (1993) 166–172.
[43] F. Husson, V.P. Tu, M. Santiago-Gomez, R. Cachon, G. Feron, J.-M. Nicaud, S. Kermasha, J.-M. Belin, J. Mol. Catal. B: Enzym. 39 (2006) 179–183.