Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 349 (2008) 83–93
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Stabilization and encapsulation of photosensitive resveratrol within yeast cell

Guorong Shi [a,b,*], Liqun Rao [a,**], Huazhong Yu [c], Hua Xiang [d], Hua Yang [a], Runa Ji [b]

[a] College of Biological and Technology, Hunan Agricultural University, Changsha 410128, PR China
[b] College of Science, Hunan Agricultural University, Changsha 410128, PR China
[c] Key Laboratory of Hunan Forest Product and Biochemical Industry Engineering, Jishou University, Zhangjiajie 427000, Hunan, PR China
[d] Department of Biochemistry, Hunan Medical School, Changsha 410004, PR China

Received 26 December 2006; received in revised form 20 June 2007; accepted 22 July 2007
Available online 10 August 2007

Abstract

The photosensitive resveratrol was successfully encapsulated in yeast cells for the first time, as characterized by FT-IR spectra, fluorescence and confocal micrographs of the yeast cells, resveratrol and microcapsules. The release characteristic of the obtained yeast-encapsulated resveratrol in simulated gastric fluid was evaluated, and its storage stability as a powder was investigated at 25 °C/75% relative humidity (RH), 25 °C/90% RH and 60 °C under the laboratory fluorescent lighting conditions (ca. 300 lx) or in the dark. Also, the scavenging capacity of yeast-encapsulated resveratrol on DPPH radical was compared with that of non-encapsulated resveratrol. It could be demonstrated clearly that no chemical changes occurred during the encapsulation. Besides, the DPPH radical-scavenging activity increased after the encapsulation. In addition, the yeast-encapsulated resveratrol exhibited good stability, and its bioavailability was enhanced as a result of increased solubility of resveratrol and sustained releasing.

Keywords: Microencapsulation; Resveratrol; Yeast cells; Stability; Bioavailability

1. Introduction

Phenolic compounds have attracted considerable interest for their beneficial effects for human health (Haslam, 1998). Resveratrol, occurring in the *trans*- and *cis*-isomeric forms (Fig. 1), a naturally occurring nonflavonoid polyphenolic compound, presents both in edible materials, such as grapes, peanuts and peanut products, berries, red wine, and numerous nonedible plants including *Polygonum cuspidatum* Sieb. et Zucc. (Nonomura et al., 1963; Gao et al., 2002), and has drawn extensive attention from researchers for its various beneficial biological roles. Recently, it has been confirmed that coco and chocolate are new sources of resveratrol (Counet et al., 2006). It was reported that the roots of the weed *P. cuspidatum* Sieb. et Zucc. constituted one of the richest sources of resveratrol and have long been used in the traditional Chinese medicine as a drug against inflammation, allergy and hyperlipidemia (Vastano et al., 2000). It is clearly known that plants produce resveratrol in response to exogenous stress factors, such as injury, fungal infections or UV irradiation. Therefore, it is a phytoalexin that exhibits various biological (Fremont, 2000; King et al., 2006) and pharmacological activities (Gusman et al., 2001), including antioxidative activities (Li et al., 2006; Orallo, 2006), cardiovascular protective effects (Bradamante et al., 2004; Delmas et al., 2005), anti-inflammatory properties (de la Lastra and Villegas, 2005), platelet anti-aggregate (Olas and Wachowicz, 2005), anticancer activities (Jang et al., 1997; Bhat and Pezzuto, 2002; Savouret and Quesne, 2002; Aggarwal et al., 2004; Ulrich et al., 2005; Choi et al., 2006; Hsieh et al., 2005) and estrogenic functions (Gehm et al., 1997; Bhat et al., 2001).

The so-called "French Paradox" (Richard, 1987; Renaud and de Lorgeril, 1993) (i.e., low incidence of cardiovascular events in spite of diet relatively high in saturated fat in people of

* Corresponding author at: College of Biological and Technology, Hunan Agricultural University, Changsha 410128, PR China. Tel.: +86 731 4617022; fax: +86 731 8865515.
** Corresponding author. Tel.: +86 731 4673600; fax: +86 731 8865515.
E-mail addresses: shiguorong@56.com (G.R. Shi), raoliqun@163.com (L.Q. Rao).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.07.044

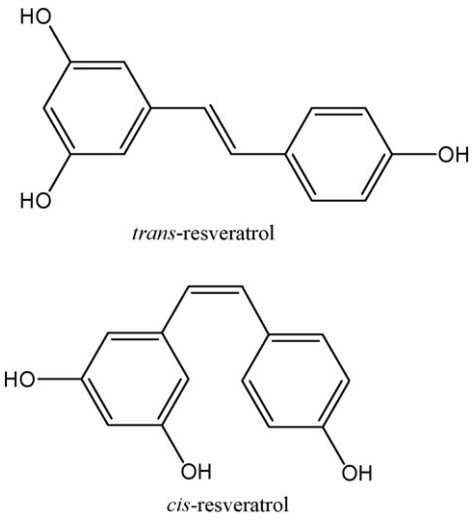

Fig. 1. Chemical structures of *trans*-/*cis*-resveratrol.

Southern France) and the publication of the pioneer work that resveratrol is effective in blocking in vivo the three stages of carcinogenesis: initiation, promotion and progression (Jang et al., 1997) have greatly increased the interest of the scientific community in resveratrol over the last year. It is reported that the possible mechanisms for its various pharmacological activities involve modulating lipid metabolism, platelet aggregation, and the inflammatory response (Norata et al., 2007; Shenouda et al., 2004; Jarolim et al., 2004; Pacifici, 2004). Many articles (Wu et al., 2006; Pozo-Guisado et al., 2005; Gosslau et al., 2005; Martin et al., 2004; Luzi et al., 2004) demonstrated that the apoptosis-inducing effect was one of the most striking biological activities of resveratrol soundly investigated during the late years. Szabolcs et al. (2006) concluded that the beneficial effects of resveratrol on acute pancreatitis seemed to be mediated by its antioxidant effect or by an NF-κB-independent anti-inflammatory mechanism. In summary, the biological properties of resveratrol are attributed to its ability to inhibit the oxidation of human low-density lipoprotein, while the suppression of cyclooxygenase-2 and inducible nitric oxide synthase (iNOS) activities also contributes to its anti-inflammatory and antioxidant effects (King et al., 2006).

However, the utilization of beneficial effects of resveratrol is limited because it is an easily oxidizable and extremely photosensitive compound (Piñeiro et al., 2006). It is susceptible to oxidative degradation (Signorelli and Ghidoni, 2005), and the half-life of *trans*-resveratrol is only 30–45 min (Bertelli et al., 1996). Abert Vian et al. (2005) demonstrated that 80–90% of the *trans*-resveratrol in solution was converted to *cis*-resveratrol if exposed to light for 1 h. Trela and Waterhouse (1996) also reported that *trans*-resveratrol was susceptible to UV-induced isomerization, and converted to *cis*-form by irradiation at 366 and 254 nm in yields of 90.6% and 10% after a 1 h exposure time, respectively. Moreover, after having investigated the relationship between resveratrol bioavailability and its effect on tumor growth, Asensi et al. (2002) concluded that the potential anticancer effects of resveratrol strongly related to its low bioavailability, while Wieder et al. (2001) observed that the presence of 4′-OH together with stereoisomery in the *trans*-conformation was absolutely required for the inhibition of cell proliferation. In addition, Matsuoka et al. (2002) also revealed that the 4′-hydroxy group was responsible for the in vitro cytogenetic activity of resveratrol. Thus, it is truly desirable and challenging to stabilize resveratrol in order to preserve its biological and pharmacological activities.

In addition, the poor solubility of the highly lipophilic resveratrol in water (López-Nicolás et al., 2006) and the rapid metabolism and elimination (Walle et al., 2004) emphasized this challenge. Wenzel and Somoza (2005) provided an overview of the metabolism and bioavailability of *trans*-resveratrol, and concluded that the oral bioavailability of resveratrol was almost zero due to the rapid and extensive metabolism.

Taking into account its short biological half-life, labile properties and rapid metabolism and elimination, it seems desirable to stabilize resveratrol while preserving its biological activities and enhance its bioavailability as it is the case of microencapsulation.

Microencapsulation, the envelopment of small solid particles, liquid droplets or gases in a coating, is of growing application in pharmaceuticals, cosmetic and food industries (Schrooyen et al., 2001; Shahidi and Han, 1993; Nelson, 2002; Gouin, 2004) due to its extension of shelf-life, protection against oxidation and control release of active component. Hence, those impediments in resveratrol application can be partially overcome by microencapsulation technology to protect it from light and oxidative stress and to dissolve it in an aqueous environment. The eukaryotic structure of yeast cell wall made it a potential excellent encapsulating wall material and its natural properties made it many benefits over other microencapsulation technology (Nelson, 2002). In fact, yeast-cell-based microencapsulation process has been successfully applied in the encapsulation of essential oil and flavor (Pannell, 1990; Nelson et al., 1991; Bishop et al., 1998). Moreover, baker's yeast (*S. cerevisiae*) has emerged as a convenient host for the development of a new kind of drug delivery system (Blanquet et al., 2001, 2005). In our previous work (Shi et al., 2007) we have encapsulated water-soluble antioxidant chlorogenic acid into the modified yeast cells successfully.

From the point of research on resveratrol, most of the studies thus far have focused on the demonstration of its biological activities, evidence for its potent biological effects from clinical studies and search for synergistic effect of resveratrol with other diet/beverage components, little has been considered for its stabilizing and the increasing of its bioavailability (Nam et al., 2005; López-Nicolás et al., 2006). According to Zlotnik et al. (1984), the structure of the yeast cell wall is permeable to both small polar and apolar molecules in aqueous solutions, but the size (or molecular weight) and polarity properties of the molecules are the two limiting factors. Scherrer et al. (1974) reported that a molecule, with a molecular radius smaller than 0.81 nm or molecular weight lower than 620 Da, could penetrate through the yeast cell wall freely. Moreover, the results of Normand et al. (2005) have shown that the dry yeast system is well locked for the encapsulated flavor molecules. Accordingly, the encapsulation of resveratrol with a molecular weight

of 228 g/mol and a log *P* value of 3.08 into yeast cells is expected to be feasible.

The objective of this study was to encapsulate resveratrol in yeast cell, with an aim to achieve the sustained release and improved solubility and bioavailability, to evaluate its characterizations and to test its stability as a powder under different conditions. Furthermore, in vitro releasing property of resveratrol was investigated to show applicable potential of yeast cell as an effective control drug delivery carrier.

2. Materials and methods

2.1. Materials

Resveratrol was extracted and purified from *P. cuspidatum* Sieb. et Zucc. Resveratrol standard was purchased from Sigma. All the chemicals used for HPLC analysis were HPLC grade and were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). All the products for yeast culture media were from Dingguo Biochemical Ltd. (Beijing, China) and other chemicals were analytical grade and supplied by Shanghai Chemicals Reagent Factory (Shanghai, China). Double-distilled water was used throughout the experiments.

2.2. Encapsulation of resveratrol

Yeast used in the experiments was *S. cerevisiae* (ATCC code 9804). Cells were grown in liquid culture medium to the early stationary phase under optimal conditions obtained by response surface method. Cells were harvested by centrifugation and washed five times in water. Then the yeast cells were pre-treated with a plasmolyser in appropriate experimental conditions to remove most of the cytoplasmic material that occupies the cells (Shi et al., 2007). Cells were centrifuged again, washed five times in water and then lyophilized.

The preparation of microcapsules was based on the technique adapted from Bishop et al. (1998). One hundred milligrams resveratrol, 300 mg of cells and 5 ml of absolute ethanol and 5 ml of water were mixed and stirred in a 100 ml beaker to form homogeneous solution. Then the beaker was put into a close container under 25 MPa at 40 °C for 4 h. The cells were then centrifuged for 10 min at 12,000 rpm (Himac CR 21G, Japan). After decanting the supernatant solution, the cells were washed three times with absolute ethanol and freeze dried for 48 h.

2.3. Determination of the encapsulated resveratrol and encapsulation yield (EY)

Twenty milligrams yeast encapsulated resveratrol were extracted exhaustively with 50 ml of 50% ethanol at ambient temperature protected from the light, the total resveratrol was determined by HPLC (Shimadzu LC-10ATvp, consisting of a binary pump and diode array detector). A C_{18} column (Waters Symmetry 150 mm × 3.9 mm, 5 μm), coupled with a Waters guard column (Waters Symmetry 5 mm × 3.9 mm, 5 μm), kept at 40 °C, was used under isocratic conditions with a mobile phase consisting of water:methanol:glacial acetic acid (440:550:10, v/v/v). The flow rate was 1.0 ml/min and the volume of injection was 20 μl. Quantification was performed at 306 nm by external standard calibration. Also, the spectrum of yeast encapsulated resveratrol was recorded between 210 and 450 nm. The EY was calculated as the ratio of the mass of resveratrol encapsulated to the mass of the final product, and the result was reported as milligram resveratrol per 100 mg of microcapsules ($n = 5$).

2.4. Microscopic observation

2.4.1. Fluorescence microscopy

Yeast encapsulated resveratrol was dispersed in double-distilled water to form a suspension. The suspension was then mounted on a glass slide and enclosed with a cover slip. The particles were observed for fluorescence resulting from entrapment of yeast encapsulated resveratrol. Brightfield and fluorescence micrographs were acquired with the use of fluorescence microscope (Motic BA 400, Xiamen, China), fitted with a high-pressure mercury burner, DC 100 W, as light source (Osram, Germany), the blue filter with an exciter filters 470/37 nm and barrier filters 515 LP nm was used, the objective used was 20× and digital images were processed by Adobe Photoshop software. For comparison, the yeast cells used for the encapsulation were also observed.

2.4.2. Laser confocal microscopy

Wet mounts of yeast encapsulated resveratrol and yeast cells were observed under a laser scanning confocal microscope (FV 500 + IX 70) equipped with a blue exciter filter. The microscope was set up so that both confocal and brightfield images could be observed simultaneously via a monitor and the image recorded digitally. The overlapped micrographs of confocal and brightfield images were recorded also.

2.5. Fourier-transform infrared (FT-IR) spectroscopy

The IR spectra (KBr pellet) of resveratrol, yeast cells, encapsulating powder and physical mixture of resveratrol and yeast cells were collected on a Fourier-transform infrared spectroscope (WQF-310, Beijing, China). For reliable sample comparison, all the resveratrol-involved samples for the pellets were the mixtures of 2 mg resveratrol and 200 mg KBr.

2.6. In vitro releasing property of resveratrol

In vitro resveratrol release was determined according to the method adapted from Luan et al. (2006) with a slight modification. In brief, 10 mg of microcapsules were suspended in 5 ml of simulated gastric fluid without pepsin (pH 1.2). The suspension was incubated in Eppendorf tube at 37 °C in an incubation shaker (150 rpm). The sample was centrifuged at predetermined time points and the supernatant was collected and replaced by fresh medium. The collected supernatant was then filtered directly by membrane filter of poly(tetrafluoroethylene) type with pore size of 0.45 μm and analyzed by HPLC method described in Section

2.3. The total mass of released resveratrol in each moment of the experiment was calculated and expressed as a percentage relative to the total resveratrol in the sample ($n = 3$).

2.7. Effect of encapsulation on the solubility of resveratrol

The solubility of non-encapsulated resveratrol (crystal and amorphous form) and encapsulated powder in water were measured at 25 °C. Excess amount of microcapsules or non-encapsulated resveratrol was placed in a 100 ml crown volumetric flask filled with 100 ml water. The samples were placed in a shaking incubator and shaken for 8 h at 25 °C. The saturated solutions were filtered by membrane filter of poly(tetrafluoroethylene) type with pore size of 0.45 μm and then determined by the HPLC method given in Section 2.3. The results were reported as milligram per 100 ml of water ($n = 5$).

2.8. Light stability

For light stability, 50% ethanol solution extracted from yeast encapsulated resveratrol or non-encapsulated resveratrol was exposed to laboratory fluorescent lighting conditions (ca. 1000 lx) at room temperature for 1 h. Aliquots of the solutions were withdrawn, filtered and then determined for the resveratrol by the HPLC method given in Section 2.3 ($n = 3$) at predetermined time points. The effect of encapsulation on the light stability of resveratrol was evaluated by the retention percentage, the ratio of the content of resveratrol retained to the original one in the sample ($n = 3$).

2.9. Effect of the relative humidity and illumination on the stability of microcapsules

Microcapsules were stored in desiccators of constant temperature and humidity to evaluate the stability of yeast encapsulated resveratrol under light conditions. Approximately 30 mg microcapsules was weighed into open dishes, and placed in desiccators containing a saturated salt solution of 75% relative humidity (RH) and 90% RH, using sodium chloride for 75% RH and potassium nitrate for 90% RH, respectively. The desiccators were sealed and held in an air bath for 10 days under fluorescent light (ca. 300 lx) at 25 ± 2 °C. The indicated samples were removed and analyzed for their content of *trans*-resveratrol by HPLC method given in Section 2.3 ($n = 3$). The results were expressed as retention percentage, shown in Section 2.8. For comparison, non-encapsulated resveratrol was also stored and determined at the same time.

2.10. Thermal stability

Microcapsules (30 mg) and non-encapsulated resveratrol (10 mg) were stored in closed containers at 60 °C for 10 days in dark condition. The retention percentage shown in Section 2.8 was used to evaluate the thermal stability of resveratrol.

2.11. Assessment of DPPH radical-scavenging activity

The 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical-scavenging activity was assessed using the method of Okada and Okada (1998) with a slight modification. One milliliter of 0.80 mM DPPH radical in ethanol was placed in Eppendorf tube, 4 ml of ethanol extraction extracted from the microcapsules or resveratrol standard solution at different concentration was added. The solution was mixed instantly, 15 min later, the absorbance was measured at 517 nm with a spectrophotometer (Agilent 8453, USA). The DPPH radical-scavenging activity was estimated based on the method of Maisuthisakul et al. (2007) with a slight modification and calculated as

$$\text{DPPH radical-scavenging activity (\%)} = \frac{A_0 - (A_1 - A_s)}{A_0} \times 100$$

where A_0 is the absorbance of the control solution (containing only DPPH), A_1 the absorbance of the DPPH containing resveratrol and A_s is the decrease of the absorbance of DPPH caused by blank sample (extraction of the same weight of empty yeast cells). The actual resveratrol concentrations of the microcapsules were confirmed by HPLC method described in Section 2.3. The data obtained at each point was the average of three measurements.

2.12. Statistical analysis

The statistical processing of the data obtained from all studies was implemented by means of dispersion analysis with the SigmaPlot 8.0 Demo Stat (version 8.0, SPSS Inc.) software. Data are expressed as means ± standard deviations (SD). Mean values were compared with Duncan's multiple range test.

3. Results and discussion

3.1. Analysis of resveratrol

Fig. 2 shows the typical HPLC chromatograms (1) and UV–vis spectra (2) of the resveratrol standard and the yeast encapsulated resveratrol. It can be seen from Fig. 2(1) that the peak of resveratrol standard and the yeast encapsulated resveratrol had the same retention time at 2.52 min under the same chromatographic conditions, and both of them were purity peaks identified by the DAD detector. Besides, it can also be observed from Fig. 2(2) that the UV–vis spectra of the resveratrol standard and the yeast encapsulated resveratrol had the same outline: both of them had the same absorption maximum at 306 nm, the second maximum absorption peak at 218 nm and the same absorption minimum at 254 nm. This indicates that the two spectra are homology and are in accordance with the results of Abert Vian et al. (2005) and Trela and Waterhouse (1996). Thus, the same HPLC retention time and UV–vis spectrum between the standard and microcapsules indicate that no chemical change has taken place during the encapsulation process. Besides, there was a new peak at 2.92 min if the sample was exposed to light

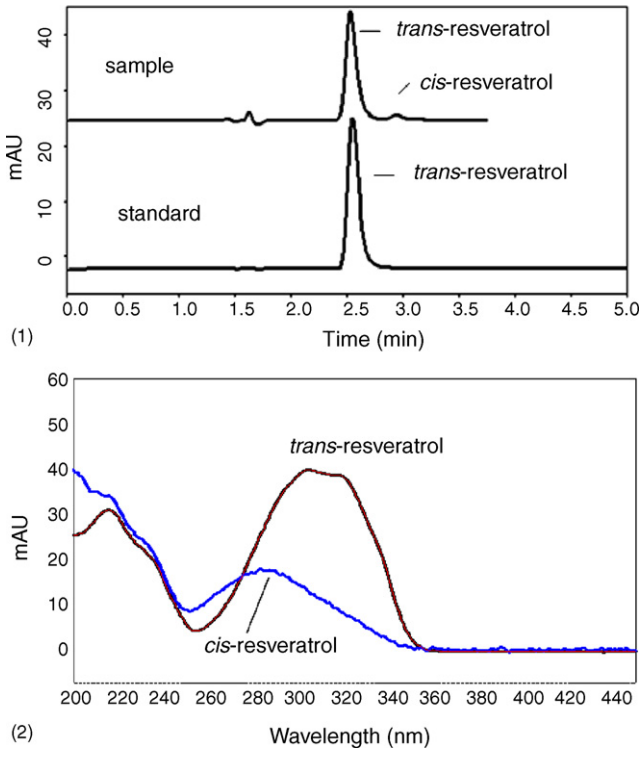

Fig. 2. Chromatograms (1) and spectra (2) of the resveratrol standard and the yeast-encapsulated resveratrol.

illumination, and the area of this new peak increased with the exposure time while the area of *trans*-resveratrol at 2.52 min decreased. In addition, its UV spectrum is consistent with that of *cis*-resveratrol (Abert Vian et al., 2005). Taking together, this new peak can be deduced as *cis*-resveratrol that is converted from *trans*-resveratrol due to the light illumination.

According to Zlotnik et al. (1984), the major mannoproteins situated both on the surface and in deeper layers of the yeast wall. It was reported that the outer layer of mannoproteins was heavily glycosylated (Cappellaro et al., 1994) and resveratrol was more associated with lipoproteins than with lipoprotein-free proteins (Belguendouz et al., 1998). Hagerman et al. (1998) illustrated that hydrogen bonding and hydrophobic interactions were the main driving forces of phenolic compound–protein interactions, and hydrophobic interactions were the main forces responsible for the interactions with nonpolar phenolic compounds.

Moreover, according to Dardelle et al. (2007), the theoretical encapsulation efficiency of resveratrol (E_F) is calculated to be 18.75%, and the encapsulation yield (EY) should be 6.25%. However, the experimental encapsulation yield was (4.52 ± 0.05)% and the yeast cells have lost viability after encapsulation. The difference may be made for two possible reasons. The solvent we used was not water but 50% of ethanol and the encapsulation efficiency for one single molecular species depended not only on the log P, volume of yeast and water, but the size, shape, affinity with the wall material affected the yield also (Dardelle et al., 2007). This indicates that resveratrol was inside the yeast cells.

Besides, the encapsulation yield positively correlated with the resveratrol concentration in the external medium and temperature (data not shown), suggesting that the loading process of resveratrol occurred by means of passive diffusion, similar to the encapsulation of orange peel oil in the cell (Bishop et al., 1998).

Taking together, although the external mannoprotein layer can play a role in the stabilization of resveratrol, it is conceivable that the resveratrol loading in the yeast cells was initially driven by the concentration discrepancy between the external medium and internal yeast cells, enhanced by the hydrophobic interactions. However, the hydrogen bonds between the OH group of resveratrol and the NH_2, OH, COOH in the polar headgroups of lipids at the lipid–water interface should be helpful to attract and stabilize the penetrated resveratrol. This is in accordance with the result of Lania-Pietrzak et al. (2004), that resveratrol was potentially able to perturb the model phospholipids bi-layers by decreasing enthalpy and temperature of phase transition, influencing membrane fluidity and order. They deduced that resveratrol can penetrate the hydrophobic core of bi-layer, but its exact positioning in lipid membranes need to be determined by further investigations. Thus, the dry system of yeast is well locked for encapsulated resveratrol, the interactions between resveratrol and yeast cells prevented the release of resveratrol.

3.2. Fluorescence and confocal microscopy analysis

3.2.1. Fluorescence microscopy analysis

The brightfield and fluorescence micrographs of yeast cells and microcapsules were photographed in Fig. 3. As can be seen, the brightfield micrograph of yeast cells (Fig. 3(1)) showed a granular cytoplasm visible within each cell, surrounded by a thick cell wall. Besides, the brightfield micrograph of microcapsules (Fig. 3(3)) illustrated almost the same granular cytoplasms within each intact mother cell and bud. In addition, nothing can be seen in the fluorescence micrograph of yeast cells (Fig. 3(2)), indicating that the yeast cells themselves could not emit fluorescence under the experimental conditions, as confirmed by the results of Bishop et al. (1998). However, the fluorescence image (Fig. 3(4)) of microcapsules has shown the bright profiles of yeast cells. Considering the fluorescence property of resveratrol (Gürbüz et al., 2007; Piñeiro et al., 2006) and our experimental result showing the maximum emission wavelength of resveratrol at 499 nm under the excitation wavelength 470 nm, as well as the potential interactions between resveratrol and the proteins or polysaccharides (Kroll et al., 2003; Jannin et al., 2004), it can be deduced reasonably that the fluorescence of the microcapsules was emitted by the encapsulated resveratrol where the fluorescence of resveratrol corresponded well within the position of the yeast cells.

3.2.2. Confocal microscopy analysis

The confocal micrograph of yeast encapsulated resveratrol and yeast cells are given in Fig. 4. The results of confocal micrograph of yeast encapsulated resveratrol (Fig. 4(1)) and yeast cells (Fig. 4(2)) indicated that resveratrol existing in the yeast cell wall was not so significant, since there was no fluorescence emitted from the cell wall of the microcapsules. It is confirmed

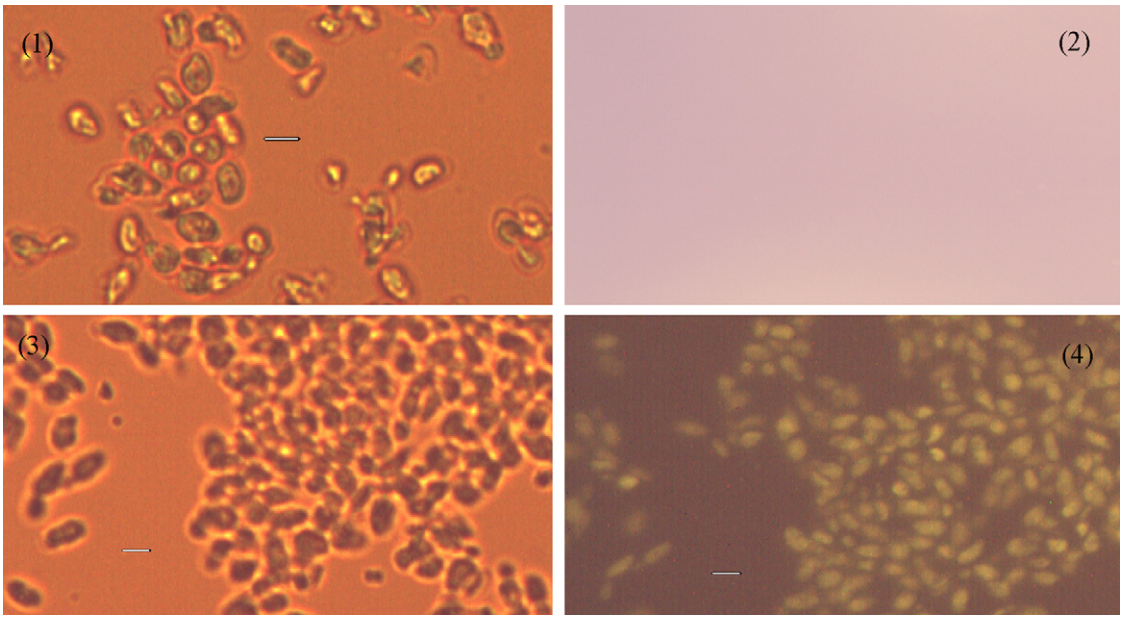

Fig. 3. Brightfield micrographs and fluorescence micrographs of yeast cells (1 and 2) and encapsulated resveratrol cells (3 and 4). The scale bar is 5 μm.

that the fluorescence of the microcapsules was emitted by the encapsulated resveratrol that had stayed inside the yeast cells.

3.3. FT-IR analysis

Fig. 5 illustrated the IR spectra of resveratrol, yeast cells, encapsulating powder and physical mixture of resveratrol and yeast cells. As can be seen, the spectrum for yeast cells presented in Fig. 5 is consistent with the results of Ci et al. (2002). By analogy with the results of our previous study (Shi et al., 2007) and the results of Larionova et al. (1999) and Ci et al. (2002), the broad absorption band at ~3342 cm^{-1} may be attributed to the absorption bands of OH vibrations in polysaccharides of yeast cells and the resveratrol molecules. The absorption band

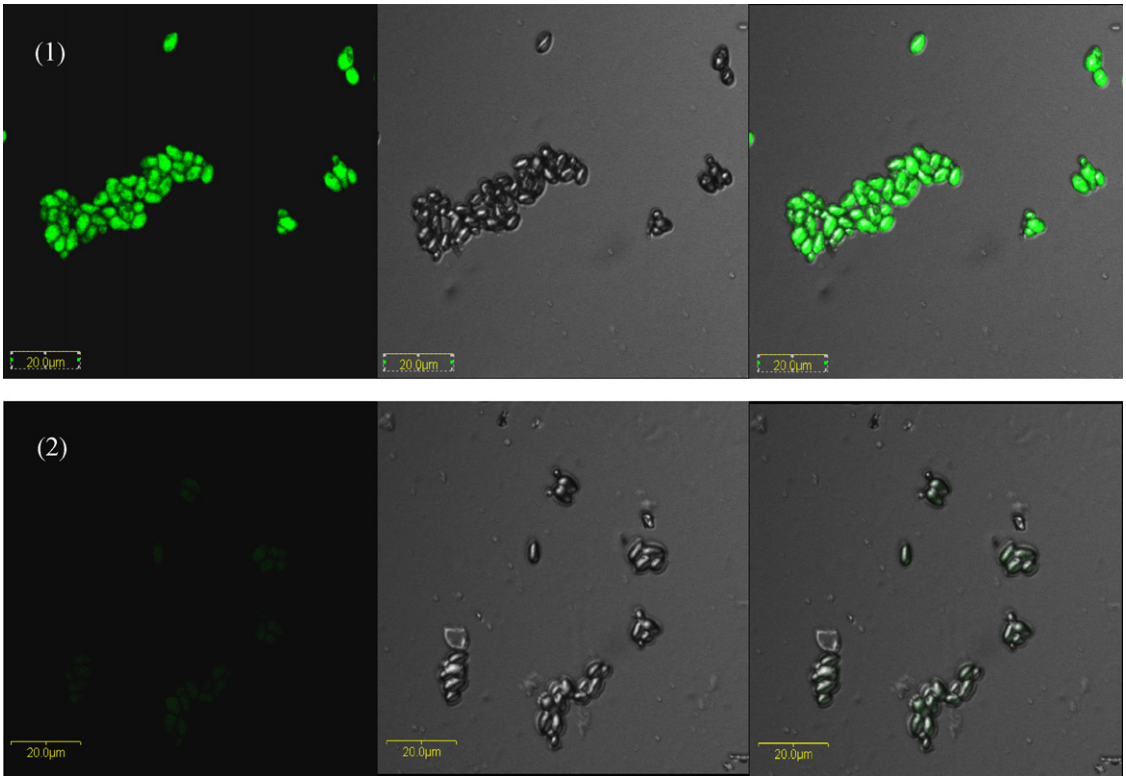

Fig. 4. Confocal (left), brightfield (middle) and overlapped (right) micrographs of yeast-encapsulated resveratrol (1) and yeast cells (2). The objective used was 40× and the scale bar is 20 μm.

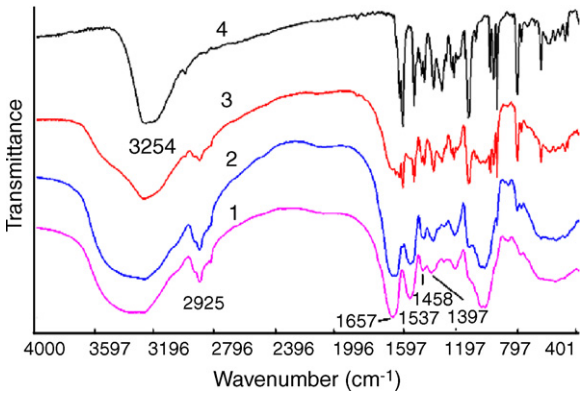

Fig. 5. FT-IR spectra of yeast cells (1), encapsulating powders (2), physical mixture of resveratrol and yeast cells (3) and resveratrol (4).

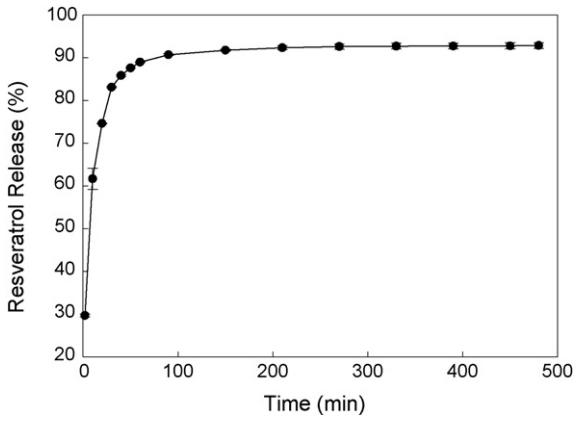

Fig. 6. Resveratrol release of microcapsules in simulated gastric fluid. Data are given as means ± S.D. ($n = 3$).

at ~2854, ~2925 and ~2958 cm^{-1} of microcapsules can be assigned to the overlapping of the symmetrical CH_2 stretching vibrations, asymmetrical CH_2 stretching vibrations, and asymmetrical CH_3 stretching vibrations of nucleic acids, proteins and lipids. While the absorption band at ~1537 and ~1657 cm^{-1} of microcapsules can be attributed to the protein amide II and I bands of yeast cells overlapped by the conjugated C=C stretching vibrations and aromatic ring skeletal vibrations of resveratrol. In addition, the CH_3 group of yeast cells gave rise to the symmetric bending mode at ~1397 cm^{-1} and the asymmetric bending mode at ~1458 cm^{-1} of the microcapsules, the absorbance at ~1458 cm^{-1} may be overlapped by the aromatic ring skeletal vibrations of resveratrol also.

In summary, the microcapsules spectrum was almost the overlaid spectrum of resveratrol and yeast cells in the region 4400–1300 cm^{-1}. However, the differences between the microcapsules spectrum and the spectrum of yeast cells in the fingerprint region (1300–650 cm^{-1}) are rather complicated, and a detailed interpretation is difficult to make at present.

It is worth noting that the large discrepancy existed in the FT-IR spectrum of encapsulating powder and the physical mixture of resveratrol and yeast cells. The characteristic IR absorption peaks of resveratrol are almost as clear as that of the pure resveratrol in the physical mixture, which disappear in the encapsulating powder. As the content of resveratrol in the pellet of encapsulating powder and physical mixture is identical with that of pure resveratrol, therefore the disappearance of the absorbance of resveratrol in microcapsules at 1600, 1587, 1500 and 987 cm^{-1} indicated that resveratrol was encapsulated into the yeast cells.

3.4. In vitro resveratrol release

Fig. 6 illustrated the release profile in terms of percentage release of resveratrol from yeast cells. The resulting release profile showed that about 90% of resveratrol was released within 90 min in simulated gastric fluid. The present result is consistent with the basical structure of *S. cerevisiae* (Lipke and Ovalle, 1998; Feuillat, 2003; Klis et al., 2002). It was reported that the outer protein layer and the plasma membrane were responsible for permeability barrier to the permeating molecule (Zlotnik et al., 1984; Bishop et al., 1998). As they can be destroyed by acid, associated with the trigger of the liquid water (Dardelle et al., 2007), it makes the release of resveratrol possible.

It is worthy to note that there were no differences in the chromatograms and the spectra between the collected supernatants of the simulated gastric fluid and the 50% ethanol extraction of the encapsulating powder during the detection of the released resveratrol by the HPLC method with the DAD detector. This indicates that no new compound appeared during the release of resveratrol from the yeast cells.

Hence, this release property is in favor of the enhancement of its poor bioavailability due to the rapid metabolism and elimination of resveratrol. According to Asensi et al. (2002), the highest concentration of resveratrol in plasma, either after i.v. or oral administration, reached within the first 5 min in rabbits, rats, and mice, the highest levels in brain, lung, liver and kidney were found within the first 10 min after administration and the resveratrol content was always below 1 nmol/g of fresh tissue due to the removing of the circulating resveratrol by the liver. Although resveratrol does not accumulate extravascularly, the 14.4 min half-life of resveratrol in plasma, after i.v. administration of 20 mg t-resveratrol/kg b.wt., in rabbits, can be supplemented by the sustained release, to some extent, on maintaining its biological activities.

3.5. Solubility

The comparison of the solubility of encapsuled resveratrol and the non-encapsulated resveratrol (crystal and amorphous form) in water at 25 °C was listed in Table 1. As can be seen, the solubility of resveratrol is significantly different ($p < 0.001$) among the three forms of resveratrol. The extraction solution of microcapsules contained more than three times of the resveratrol

Table 1
Water solubility of resveratrol in different forms ($n = 5$)

Resveratrol form	Solubility[a] (mg/100 ml)
Crystal	1.96 ± 0.01
Amorphous	3.06 ± 0.02
Microcapsules	6.67 ± 0.03

[a] Solubility values for the three forms are significantly different ($p < 0.001$).

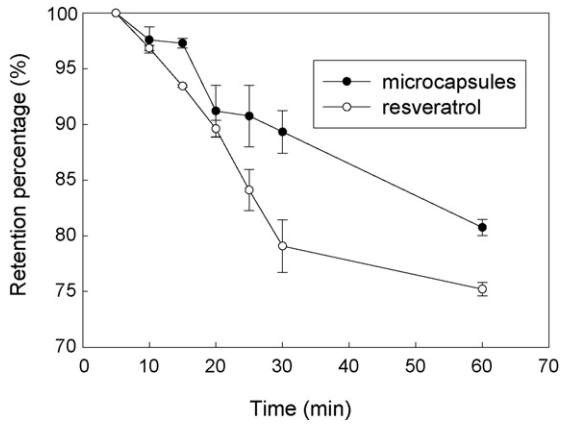

Fig. 7. Kinetic degradation curve of encapsulated and non-encapsulated resveratrol exposed to laboratory fluorescent lighting conditions. Each value represents mean ± S.D. ($n = 3$).

than that of the crystal resveratrol, and more than two times than the amorphous resveratrol in the same volume of water. This is in accordance with the data reported by Asensi et al. (2002), who reported that the resveratrol solubility in drinking water was 0.023 mg/ml. Thus, the bioavailability of resveratrol can be enhanced with the increased water solubility by yeast cell encapsulation processing.

However, the exact reason is unclear at present. While López-Nicolás et al. (2006) revealed that the formation of β-cyclodextrin (β-CD) inclusion complex increased the solubility and bioavailability of resveratrol. It is possible that the increased water solubility of yeast encapsulated resveratrol is associated to the interactions among resveratrol, the yeast protein and polysaccharide.

3.6. The influence of light irradiations on the resveratrol stability

Photosensitive compound can be protected from photodegradation by microencapsulation technique. This has been confirmed by the results of Barbosa et al. (2005) who reported that the carotenoid bixin, 6-methyl hydrogen 9′-*cis*-6,6′-diapocarotene-6,6′-dioate, could be protected successfully from both oxidative and photochemical degradation by encapsulated in edible polysaccharide preparations. The similar result has obtained in our present study. The protective effect of the yeast-cell-based microencapsulation on the light stress of resveratrol was investigated based on the 50% ethanol extraction of yeast encapsulated resveratrol and resveratrol standard. Fig. 7 shows the kinetic curve for the degradation of encapsulated resveratrol exposed to laboratory fluorescent lighting conditions as compared to the photodegradation of non-encapsulated resveratrol. As can be seen, resveratrol is a highly photosensitive compound, this is in accordance with the data reported by Abert Vian et al. (2005) and Trela and Waterhouse (1996), who observed that more than 80% of the *trans*-resveratrol in solution was converted to *cis*-resveratrol if exposed to light for 1 h, besides, the different conversion ratio resulted most likely from the specific spectrum of light reaching the sample (Trela and Waterhouse, 1996). However, it is also remarkable that the extract of microcapsules exhibited greater stability than non-encapsulated resveratrol, which indicates that the strong photodegradation effect of light on resveratrol can be inhibited, to some extent, by the yeast-cell-based encapsulation processing. Taking together, the previous reports (Regev-Shoshani et al., 2003; Krasnow and Murphy, 2004) that glycosylation can inhibit enzymatic oxidation of resveratrol, thereby preserving its biological activity and increasing its stability and bioavailability, and the formation of inclusion complex (López-Nicolás et al., 2006) increased the bioavailability and solubility of resveratrol, the yeast cell wall component mannoprotein and fibrous β1,3-glucan (Lipke and Ovalle, 1998) is likely to be attributed to this inhibition of degradation influence of light on resveratrol.

3.7. Storage stability

One of the purposes of microencapsulation is to improve the stability of active compounds. Taking into account the easily oxidizable and extremely photosensitive properties of resveratrol and the hygroscopic characteristic of yeast cells, we investigated the storage stabilities of encapsulated and non-encapsulated resveratrol at 25 °C/75% RH, 25 °C/90% RH and 60 °C under light/dark conditions. The influence of moisture on resveratrol stability, under light conditions, is listed in Table 2. As can be seen, the retention percentage values showed that the stability of resveratrol was good during the thermal storage, in addition, there was no significant difference ($p > 0.001$) between the average of thermal retention percentage of microencapsules and non-encapsulated resveratrol. However, this is not the case at 25 °C/75% RH, 25 °C/90% RH conditions. The retention percentage values are different significantly ($p < 0.001$) between the encapsulated and the non-encapsulated resveratrol. Moisture and illumination have negative effect on the stability of both the encapsulated and non-encapsulated resveratrol. In addition, moisture has a significant effect ($p < 0.05$) on the retention percentage values of encapsulated resveratrol while no significant effect on that of the non-encapsulated resveratrol ($p > 0.05$). Nevertheless, yeast cells have protected resveratrol, to some extent, from oxidative degradation and photodegradation as the retention percentage values of encapsulated resveratrol are

Table 2
Retention percentage (mean ± S.D., $n = 3$) of microcapsules and non-encapsulated resveratrol under 25 °C/75% RH, 25 °C/90% RH and 60 °C for 10 days

Retention percentage (%)	25 °C/75% RH	25 °C/90% RH	60 °C
Microcapsules	97.58 ± 1.81 b	93.13 ± 1.81 c	99.39 ± 0.37 d
Non-encapsulated resveratrol	79.88 ± 2.05 a	77.77 ± 1.50 a	99.63 ± 0.66 d

Values in the column marked with a different letters are significantly different ($p < 0.05$).

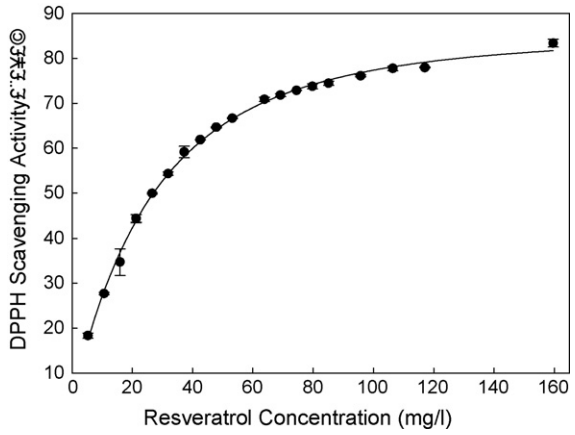

Fig. 8. Scavenging effects of resveratrol on DPPH radicals. Each value represents mean ± S.D. ($n = 3$).

significant ($p < 0.001$) greater than those of non-encapsulated. According to Lancon et al. (2004), both the passive diffusion and carrier-mediated process were involved in the uptake of the highly lipophilic resveratrol by cells. Taking together, similar to the CD complexation (Ueda et al., 1998), this fact may be a result of insulation of resveratrol against various degradation factors due to the particular structure of yeast cells, the network composed mostly of mannoprotein and fibrous β1,3-glucan and the bi-layer membrane of yeast cells inhibited the degradation influence of light and moisture on resveratrol.

These results are supported by the results of Bishop et al. (1998) and Nelson (2002), who had suggested that the bi-layer membrane of yeast cell may act as a liposome during the microencapsulation of essential oils and allowed the stabilization of oil droplets within the cell, thus can provide a highly stable product. Therefore, our results obtained here are in accordance with the above observations, and it can be deduced that yeast cells have effectively prevented the damage of resveratrol from oxygen and light, etc., during storage.

3.8. DPPH radical-scavenging activity

Fig. 8 illustrated the DPPH radical-scavenging activities of resveratrol standard solutions at different concentrations ($n = 3$). As can be seen, this result can be fitted fairly well with the modified hyperbola III nonlinear regression function with a regression coefficients of $R^2 = 0.9978$. The determined DPPH radical-scavenging activity values were (34.98 ± 1.12)% at concentration 10.18 μg/ml, (50.68 ± 1.36)% at concentration 19.28 μg/ml and (63.35 ± 0.31)% at 31.21 μg/ml, respectively, which were significantly higher than those of the predicted values 27.37% at concentration 10.18 μg/ml, 41.79% at 19.28 μg/ml and 53.84% at 31.21 μg/ml, respectively, obtained from nonlinear regression function of the standard resveratrol solutions, using Sigma Stat (version 8.0). This is consistent with the results of Nam et al. (2005), who reported that the bioactivity of resveratrol was not affected by immobilization in the porous substrate particles as over 93% of antioxidant effect was sustained after stabilized in the porous particles. The present results have indicated that the DPPH radical-scavenging activity of resveratrol was enhanced significantly ($p < 0.001$) by yeast cell microencapsulation.

Thus, the DPPH radical-scavenging activity was not affected by the wall material yeast cells. In contrast, the DPPH radical-scavenging activity of microcapsules was stronger than that of non-encapsulated resveratrol. As many of the biological properties of resveratrol are related to its antioxidant ability (Szabolcs et al., 2006), therefore, the biological activities of resveratrol can be enhanced by the yeast-cell-based microencapsulation.

4. Conclusions

In summary, we have demonstrated the successful preparation of yeast-encapsulated photosensitive resveratrol, as confirmed by the fluorescence and confocal micrographs and FT-IR spectra. Moreover, the same retention time and UV spectrum between the standard and microcapsule suggested that no chemical change had taken place during the encapsulation process. The yeast-encapsulated resveratrol showed two to three times higher the water solubility, the slower photodecomposition and stronger free radical scavenging activity than that of non-encapsulated resveratrol. In addition, the microcapsule was more stable under wet and illumination stresses, and the sustaining release profile suggested that the poor bioavailability due to the rapid metabolism and elimination of resveratrol can be supplemented, to some extent, by the yeast cell encapsulation technology and thus maintained its biological activities. This study would be helpful to promote the application of resveratrol and may have some general interests for the stabilizing of other photosensitive substances and for potentially enhancing the bioavailability of other lipophilic drugs.

Acknowledgements

This project was supported by Hunan Provincial Natural Science Foundation of China (06JJ4006). The authors would like to thank Prof. Qingji Xie, Zemin Luo and Yong Cao for helpful suggestions.

References

Abert Vian, M., Tomao, V., Gallet, S., Coulomb, P.O., Lacombe, J.M., 2005. Simple and rapid method for *cis*- and *trans*-resveratrol and piceid isomers determination in wine by high-performance liquid chromatography using Chromolith columns. J. Chromatogr. A 1085, 224–229.

Aggarwal, B.B., Bhardwaj, A., Aggarwal, R.S., Seeram, N.P., Shishodia, S., Takada, Y., 2004. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res. 24, 2783–2840.

Asensi, M., Medina, I., Ortega, A., Carretero, J., Bano, M.C., Obrador, E., Estrela, J.M., 2002. Inhibition of cancer growth by resveratrol is related to its low bioavailability. Free Radic. Biol. Med. 33, 387–398.

Barbosa, M.I.M.J., Borsarelli, C.D., Mercadante, A.Z., 2005. Light stability of spray-dried bixin encapsulated with different edible polysaccharide preparations. Food Res. Int. 38, 989–994.

Belguendouz, L., Fremont, L., Gozzelino, M.T., 1998. Interaction of transresveratrol with plasma lipoproteins. Biochem. Pharmacol. 55, 811–816.

Bertelli, A.A.E., Giovannini, L., Stradi, R., Urien, S., Tillement, J.P., Bertelli, A., 1996. Kinetics of *trans*- and *cis*-resveratrol (3,4′,5-trihydroxystilbene) after red wine oral administration in rats. Int. J. Clin. Pharmacol. Res. 16, 77–81.

Bhat, K.P.L., Lantvit, D., Christov, K., Mehta, R.G., Moon, R.C., Pezzuto, J.M., 2001. Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. Cancer Res. 61, 7456–7463.

Bhat, K.P.L., Pezzuto, J.M., 2002. Cancer chemopreventive activity of resveratrol. Ann. NY Acad. Sci. 957, 210–229.

Bishop, J.R.P., Nelson, G., Lamb, J., 1998. Microencapsulation in yeast cells. J. Microencapsul. 15, 761–773.

Blanquet, S., Garrait, G., Beyssac, E., Perrier, C., Denis, S., Hébrard, G., Alric, M., 2005. Effects of cryoprotectants on the viability and activity of freeze dried recombinant yeasts as novel oral drug delivery systems assessed by an artificial digestive system. Eur. J. Pharm. Biopharm. 61, 32–39.

Blanquet, S., Marol-Bonnin, S., Beyssac, E., Pompon, D., Renaud, M., Alric, M., 2001. The biodrug concept: an innovative approach to therapy. Trends Biotechnol. 19, 393–400.

Bradamante, S., Barenghi, L., Villa, A., 2004. Cardiovascular protective effects of resveratrol. Cardiovasc. Drug Rev. 22, 169–188.

Cappellaro, C., Baldermann, C., Rachel, R., Tanner, W., 1994. Mating type-specific cell–cell recognition of *Saccharomyces cerevisiae*: cell wall attachment and active sites of a- and alpha-agglutinin. EMBO J. 13, 4737–4744.

Choi, H.K., Yang, J.W., Kang, K.W., 2006. Bifunctional effect of resveratrol on the expression of ErbB2 in human breast cancer cell. Cancer Lett. 242, 198–206.

Ci, Y.X., Zang, K.S., Gao, T.Y., 2002. FTIR study of microbes. Chem. J. Chin. Univ. 6, 1047–1049.

Counet, C., Callemien, D., Collin, S., 2006. Chocolate and cocoa: new sources of *trans*-resveratrol and *trans*-piceid. Food Chem. 98, 649–657.

Dardelle, G., Normand, V., Steenhoudt, M., Bouquerand, P.E., Chevalier, M., Baumgartner, P., 2007. Flavour-encapsulation and flavour-release performances of a commercial yeast-based delivery system. Food Hyd. 21, 953–960.

de la Lastra, C., Villegas, A.I., 2005. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. Mol. Nutr. Food Res. 49, 405–430.

Delmas, D., Jannin, B., Latruffe, N., 2005. Resveratrol: preventing properties against vascular alterations and ageing. Mol. Nutr. Food Res. 49, 377–395.

Feuillat, M., 2003. Yeast macromolecules: origin, composition, and enological interest. Am. J. Enol. Viticul. 54, 211–213.

Fremont, L., 2000. Biological effects of resveratrol. Life Sci. 66, 663–673.

Gao, L.Y., Chu, Q.C., Ye, J.N., 2002. Determination of *trans*-resveratrol in wine, herbs and health food by capillary electrophoresis with electrochemical detection. Food Chem. 78, 255–260.

Gehm, B.D., McAndrews, J.M., Chien, P.Y., Jameson, J.L., 1997. Resveratrol a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. Proc. Natl. Acad. Sci. U.S.A. 94, 14138–14143.

Gosslau, A., Chen, M., Ho, C.T., Chen, K.Y., 2005. A methoxy derivative of resveratrol analogue selectively induced activation of the mitochondrial apoptotic pathway in transformed fibroblasts. Br. J. Cancer 92, 513–521.

Gouin, S., 2004. Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci. Technol. 15, 330–347.

Gürbüz, O., Göçmen, D., Daðdelen, F., Gürsoy, M., Aydin, S., Sahin, I., Büyükuysal, L., Usta, M., 2007. Determination of flavan-3-ols and *trans*-resveratrol in grapes and wine using HPLC with fluorescence detection. Food Chem. 100, 518–525.

Gusman, J., Malonne, H., Atassi, G.A., 2001. A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. Carcinogenesis 22, 1111–1117.

Hagerman, A.E., Rice, M.E., Ritchard, N.T., 1998. Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin16 (4 → 8) catechin (procyanidin). J. Agric. Food Chem. 46, 2590–2595.

Haslam, E., 1998. Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action. Cambridge University Press, Cambridge.

Hsieh, T.C., Wang, Z., Hamby, C.V., Wu, J.M., 2005. Inhibition of melanoma cell proliferation by resveratrol is correlated with upregulation of quinone reductase 2 and p53. Biochem. Biophys. Res. Commun. 334, 223–230.

Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W., Fong, H.H., Farnsworth, N.R., 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275, 218–220.

Jannin, B., Menzel, M., Berlot, J.P., Delmas, D., Lancon, A., Latruffe, N., 2004. Transport of resveratrol, a cancer chemopreventive agent, to cellular targets: plasmatic protein binding and cell uptake. Biochem. Pharmacol. 68, 1113–1118.

Jarolim, S., Millen, J., Heeren, G., Laun, P., Goldfarb, D.S., Breitenbach, M., 2004. A novel assay for replicative lifespan in *Saccharomyces cerevisiae*. FEMS Yeast Res. 5, 169–177.

King, R.E., Bomser, J.A., Min, D.B., 2006. Bioactivity of resveratrol. CRFSFS 5, 65–70.

Klis, F.M., Mol, P., Hellingwerf, K., Brul, S., 2002. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 26, 239–256.

Krasnow, M.N., Murphy, T.M., 2004. Polyphenol glucosylating activity in cell suspensions of grape (*Vitis vinifera*). J. Agric. Food Chem. 52, 3467–3472.

Kroll, J., Rawel, H., Rohn, S., 2003. A review: reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. Food Sci. Technol. Res. 9, 205–218.

Lancon, A., Delmas, D., Osman, H., Thénot, J.P., Jannin, B., Latruffe, N., 2004. Human hepatic cell uptake of resveratrol: involvement of both passive diffusion and carrier-mediated process. Biochem. Biophys. Res. Commun. 316, 1132–1137.

Lania-Pietrzak, B., Hendrich, A.B., Michalak, K., 2004. Perturbation of model lipid membrane structure by resveratrol. Curr. Topics Biophys. 28, 17–60.

Larionova, N.V., Kazanskaya, N.F., Larionova, N.I., Ponchel, G., Duchene, D., 1999. Preparation and characterization of microencapsulated proteinase inhibitor aprotinin. Biochemistry (Moscow) 64, 857–862.

Li, Y.B., Cao, Z.X., Zhu, H., 2006. Upregulation of endogenous antioxidants and phase 2 enzymes by the red wine polyphenol, resveratrol in cultured aortic smooth muscle cells leads to cytoprotection against oxidative and electrophilic stress. Pharmacol. Res. 53, 6–15.

Lipke, P.N., Ovalle, R., 1998. Cell wall architecture in yeast: new structure and new challenges. J. Bacteriol. 180, 3735–3740.

López-Nicolás, J.M., Núñez-Delicado, E., Pérez-López, A.J., Barrachina, A.C., Cuadra-Crespo, P., 2006. Determination of stoichiometric coefficients and apparent formation constants for β-cyclodextrin complexes of *trans*-resveratrol using reversed-phase liquid chromatography. J. Chromatogr. A 1135, 158–165.

Luan, X., Skupin, M., Siepmann, J., Bodmeier, R., 2006. Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide-containing poly(lactide-*co*-glycolide) microparticles. Int. J. Pharm. 324, 168–175.

Luzi, C., Brisdelli, F., Cinque, B., Cifone, G., Bozzi, A., 2004. Differential sensitivity to resveratrol -induced apoptosis of human chronic myeloid (K562) and acute lymphoblastic (HSB-2) leukemia cells. Biochem. Pharmacol. 68, 2019–2030.

Maisuthisakul, P., Pongsawatmanit, R., Gordon, M.H., 2007. Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (*Cratoxylum formosum* Dyer). Food Chem. 100, 1620–1629.

Martin, A.R., Villegas, I., La Casa, C., de la Lastra, C.A., 2004. Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. Biochem. Pharmacol. 67, 1399–1410.

Matsuoka, A., Takeshita, K., Furuta, A., Ozaki, M., Fukuhara, K., Miyata, N., 2002. The 4′-hydroxy group is responsible for the in vitro cytogenetic activity of resveratrol. Mutat. Res. 521, 29–35.

Nam, J.B., Ryu, J.H., Kim, J.W., Chang, I.S., Suh, K.D., 2005. Stabilization of resveratrol immobilized in monodisperse cyano-functionalized porous polymeric microspheres. Polymer 46, 8956–8963.

Nelson, G., 2002. Application of microencapsulation in textiles. Int. J. Pharm. 242, 55–62.

Nelson, G., Wales, D.S., Sagar, B.S., 1991. Treating Materials. European Patent, EP 0511 258, B1.

Nonomura, S., Kanagawa, H., Makimoto, A., 1963. Chemical constituents of polygonaceous plants. I. Studies on the components of Ko-J O-Kon (*Polygonum cuspidatum* Sieb. Et Zucc.). Yakugaku Zasshi 83, 988–990.

Norata, G.D., Marchesi, P., Passamonti, S., Pirillo, A., Violi, F., Catapano, A.L., 2007. Anti-inflammatory and anti-atherogenic effects of cathechin, caffeic acid and trans-resveratrol in apolipoprotein E deficient mice. Atherosclerosis 191, 265–271.

Normand, V., Dardelle, G., Bouquerand, P.E., Nicolas, L., Johnston, D., 2005. Flavor encapsulation in yeasts: limonene used as a model system for characterization of the release mechanism. J. Agric. Food Chem. 53, 7532–7543.

Okada, Y., Okada, M., 1998. Scavenging effect of water soluble proteins in broad beans on free radicals and active oxygen species. J. Agric. Food Chem. 46, 401–406.

Olas, B., Wachowicz, B., 2005. Resveratrol, a phenolic antioxidant with effects on blood platelet functions. Platelets 16, 251–260.

Orallo, F., 2006. Comparative studies of the antioxidant effects of *cis*- and *trans*-resveratrol. Curr. Med. Chem. 13, 87–98.

Pacifici, G.M., 2004. Inhibition of human liver and duodenum sulfotransferases by drugs and dietary chemicals: a review of the literature. Int. J. Clin. Pharmacol. Ther. 42, 488–495.

Pannell, N.A., 1990. Microencapsulation in microorganisms. European Patent, EP 0242 135, B1.

Piñeiro, Z., Palma, M., Barroso, C.G., 2006. Determination of *trans*-resveratrol in grapes by pressurised liquid extraction and fast high-performance liquid chromatography. J. Chromatogr. A 1110, 61–65.

Pozo-Guisado, E., Merino, J.M., Mulero-Navarro, S., Lorenzo-Benayas, M.J., Centeno, F., Alvarez-Barrientos, A., Femandez-Salguero, P.A., 2005. Resveratrol-induced apoptosis in MCF-7 human breast cancer cells involves a caspase-independent mechanism with downregulation of Bcl-2 and NF-kappaB. Int. J. Cancer 115, 74–84.

Regev-Shoshani, G., Shoseyov, O., Bilkis, I., Kerem, Z., 2003. Glycosylation of resveratrol protects it from enzymic oxidation. Biochem. J. 374, 157–163.

Renaud, S., de Lorgeril, M., 1993. The French paradox: dietary factors and cigarette smoking-related health risks. Ann. NY Acad. Sci. 686, 299–309.

Richard, J.L., 1987. Coronary risk factors. The French paradox. Arch. Mal. Coeur Vaiss. 80, 17–21.

Savouret, J.F., Quesne, M., 2002. Resveratrol and cancer: a review. Biomed. Pharmacother. 56, 84–87.

Scherrer, R., Louden, L., Gerhardt, P., 1974. Porosity of the yeast cell wall and membrane. J. Bacteriol. 118, 534–540.

Schrooyen, P.M.M., van der Meer, R., De Kruif, C.G., 2001. Microencapsulation: its application in nutrition. Proc. Nutr. Soc. 60, 475–479.

Shahidi, F., Han, X.Q., 1993. Encapsulation of food ingredients. Crit. Rev. Food Sci. Nutr. 33, 501–547.

Shenouda, N.S., Zhou, C., Browning, J.D., Ansell, P.J., Sakla, M.S., Lubahn, D.B., Macdonald, R.S., 2004. Phytoestrogens in common herbs regulate prostate cancer cell growth in vitro. Nutr. Cancer 49, 200–208.

Shi, G.R., Rao, L.Q., Yu, H.Z., Xiang, H., Pen, G.P., Long, S., Yang, C., 2007. Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. J. Food Eng. 80, 1060–1067.

Signorelli, P., Ghidoni, R., 2005. Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. J. Nutr. Biochem. 16, 449–466.

Szabolcs, A., Varga, I.S., Varga, C., Berkó, A., Kaszaki, J., Letoha, T., Tiszlavicz, L., Sári, R., Lonovics, J., Takács, T., 2006. Beneficial effect of resveratrol on cholecystokinin-induced experimental pancreatitis. Eur. J. Pharmacol. 532, 187–193.

Trela, B.C., Waterhouse, A.L., 1996. Resveratrol: isomeric molarabsorptivities and stability. J. Agric. Food Chem. 44, 1253–1257.

Ueda, H., Ou, D., Endo, T., Nagase, H., Tomono, K., Nagai, T., 1998. Evaluation of a sulfobutyl ether beta-cyclodextrin as a solubilizing/stabilizing agent for several drugs. Drug Dev. Ind. Pharm. 24, 863–867.

Ulrich, S., Wolter, F., Stein, J.M., 2005. Molecular mechanisms of the chemopreventive effects of resveratrol and its analogs in carcinogenesis. Mol. Nutr. Food Res. 49, 452–461.

Vastano, B.C., Chen, Y., Zhu, N., Ho, C.T., Zhou, Z., Rosen, R.T., 2000. Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. J. Agric. Food Chem. 48, 253–256.

Walle, T., Hsieh, F., Delegge, M.H., Oatis Jr., J.E., Walle, U.K., 2004. High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab. Dispos. 32, 1377–1382.

Wenzel, E., Somoza, V., 2005. Metabolism and bioavailability of *trans*-resveratrol. Mol. Nutr. Food Res. 49, 472–481.

Wieder, T., Prokop, A., Bagci, B., Essmann, F., Bernicke, D., Schulze-Osthoff, K., Dorken, B., Schmalz, H.G., Daniel, P.T., Henze, G., 2001. Piceatannol, a hydroxylated analog of the chemopreventive agent resveratrol, is a potent inducer of apoptosis in the lymphoma cell line BJAB and in primary, leukemic lymphoblasts. Leukemia 15, 1735–1742.

Wu, S.L., Yu, L., Pan, C.E., Jiao, X.Y., Lv, Y., Fu, J., Meng, K.W., 2006. Apoptosis of lymphocytes in allograft in a rat liver transplantation model induced by resveratrol. Pharm. Res. 54, 19–23.

Zlotnik, H., Fernandez, M.P., Bowers, B., Cabib, E., 1984. *Saccharomyces cerevisiae* mannoproteins from an external cell wall layer that determines wall porosity. J. Bacteriol. 159, 1018–1026.