UPTAKE OF *TRANS*-RESVERATROL

BY YEAST CELLS

Franklin Aiah Lawrence Lamin

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DECLARATION

The work presented in this thesis, is to the best of my knowledge and belief original except as indicated in the text. I hereby declare that I have not submitted this material either in full or part, for a degree in this or any other institution.

Candidate's signature: 

Franklin Aiah Lawrence Lamin

BMSc.

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DEDICATION

I would like to dedicate this work to my beloved wife Marian Lamin, my fabulous kids David, Mayullah, Patrick and Monica Lamin for putting a smile on my face through every step of the way; and to my elder sisters Judith and Victoria and my younger brother Komba Lamin for their various support and encouragement.

LIST OF ABBREVIATIONS

Abbreviation	Full term
ACN	Acetonitrile
AR	Analytical reagent grade
CAM	Complementary and alternative medicine
CompleMed	Centre for Complementary Medicine Research
CH_3Cl	Chloroform
r^2	Correlation coefficient
DHS	Department of Human Services
DPPH	1,1-diphenyl-2-picryhydrazyl
DMF	N'N dimethyl formamide
DMSO	Dimethyl sulfoxide
EtOH	Ethanol
E_1	1^{st} extraction
E_2	2^{nd} extraction
GC	Gas chromatography
g	Grams
GSM	Glucose synthetic media
h	Hour
HAL	Herbal Analysis Laboratory
HAPL	Herbal Analysis and Pharmacological Laboratory
HPLC – PDA	High performance liquid chromatography photodiode array detection
HPTLC	High performance thin layer chromatography
kg	Kilogram
LC-MS	Liquid chromatography mass spectrometry

LOD	Limit of detection
LOQ	Limit of quantitation
L	Litre
MS	Mass spectrometer
MeOH	Methanol
mM	Millimolar
min	Minute
µM	Micro molar
µL	Micro litre
µg	Microgram
mg	Milligram
ng	Nanogram
nm	nanometre
n	Number of replicates
C18	Octadecasilane
ppm	Part per million
%RSD	Percentage relative standard deviation (or coefficient of variation)
QC	Quality control
ROS	Reactive oxygen species
SIM	Selected ion monitoring
SPE	Solid phase extraction
SD	Standard deviation
Supt	Supernatant
MS/MS	Tandem mass spectrometer
TLC	Thin – layer chromatography

TGA	Therapeutic Goods Administration
TR	*Trans*-resveratrol
UV	Ultraviolet
H_2O	Water
λ	Wavelength of radiation (nm)

ABSTRACT

This study investigates the uptake of the stilbene *trans*-resveratrol into *Saccharomyces cerevisiae* yeast cells. Stilbenes are used in ageing studies and are believed to increase longevity, have strong antioxidant activity and show protective effect against atherosclerosis, coronary heart disease, postmenopausal problems, cancer and a broad spectrum of degenerative diseases. *S. cerevisiae* yeast has been used for thousands of years and is widely used today in genetics and cell biology research as it genome is known. In this study, *S.cerevisiae* yeast cell is used, for the first time, as a model eukaryotic system to determine the uptake of *trans*-resveratrol in all eukaryotes.

Synthetic yeast growth media containing 0.5% and 2% glucose were used to determine the effect of calorie variation on *trans*-resveratrol uptake rate. The yeast cells were exposed to 2.5, 5.0, 10, 50 and 100 µM (or 0.57, 1.14, 2.28, 11.41 and 22.82 µg/mL) *trans*-resveratrol in media and incubated for 1, 24, 96, 240 and 480 h. After incubation, the samples were centrifuged and the supernatant cleaned up by C18 solid-phase extraction before analysis of trans-resveratrol by high performance liquid chromatography with photodiode array detection (HPLC-PDA) at 307 nm. The yeast cells were lysed by ultrasonication in a 2:1 (*v*/*v*) chloroform-methanol mixture, then dried and weighed before analysis as for the supernatant.

The LOQ of *trans*-resveratrol in the matrices used in this study was found to be 0.57 µg/mL and the mean recovery was 88.1 ± 5.6 %. *Trans*-resveratrol uptake increased with increasing concentration in the media. Uptake in the calorie restricted medium (0.5% glucose) was found to steadily increase over the study time period while in the non-calorie restricted medium (2% glucose), uptake peaked at 10 days. A DPPH scavenging activity assay of the compound showed that it had high antioxidant activity.

Keywords: *trans-resveratrol analysis and uptake*, *HPLC analysis*, *yeast cell*

CHAPTER ONE

INTRODUCTION

1.0. Introduction

This chapter gives an overview of the project by first looking at the history of complementary medicine and its current practice followed by an introduction to the research topic with its aim and objectives.

1.1 . Background on traditional medicine

Before the advent of modern medicine, mankind has been meeting its health needs through the use of botanical products which is the backbone of ancient medicine. This practice still forms a significant part of health care in many developing countries where Western medicine is expensive or inaccessible. The terms 'alternative' and 'complementary' are used interchangeably to refer to any form of medical practice that is not considered as mainstream or modern medicine. The history of complementary and alternative medicine (CAM) spans thousands of years.

1.2 . Types of plant metabolites and their uses

The main types of plant metabolites are primary and secondary metabolites. Primary metabolites take part in plant growth and development carrying out functions such as respiration, cell division, chemical storage, reproduction and photosynthesis (Bourgaud et al. 2001). Examples of primary metabolites include nucleotides, proteins, polysaccharides, amino acids and phytosterols.

Secondary metabolites are a group of structurally diverse compounds which are known to play a small but major role in the plants' adaptation to their environment (Bourgaud et al. 2001). They are present in every higher plant and are believed to help the plant interact

successfully with their ecosystem by acting as a defence mechanism against phytoalexins (pathogens), fungal, bacterial and viral infections and also protect the plant from UV damage. They are also known to act on insects and other animals such as cattle, by interfering with fertility (for example, forage grasses such as alfalfa and clover) (Zhong 2011). Secondary metabolites are generally classified under three major molecule groups according to their biosynthetic pathways – these are the phenolics, terpenes and steroids and alkaloids of which the most common is the phenolics because they are involved in lignin (integral part of the secondary cell walls of plants) synthesis (Zhong 2011). The other compound group that is the alkaloids are specific to the plant species.

Table 1.1 shows the major groups of products isolated from plant cells and tissues.

Phenylpropanoids	**Alkaloids**	**Terpenoids**	**Quinones**	**Steroids**
Anthocyanins	Acridines	Carotenes	Anthoquinones	Cardiac glycoside
Coumarines	Betalaines	Monoterpenes	Benzoquinones	Pregnenolone
Flavonoids	Quinolizidines	Sesquiterpenes	Naphthoquinones	
Isoflavonoids	Indole	Diterpenes		
Lignans	Purines	Triterpenes		
Stilbene	Pyridine			
Tanins	Tropane alkaloids			

Table 1.1. Groups of natural products isolated from plant and tissue culture. (Source: Zhong. 2011).

1.3. Research into the uptake of secondary metabolites in animal models and the use of yeast as a simpler system

The studies done on the biological effects of secondary metabolites so far have principally focused on complex biological systems. Examples of studies that have been carried out include the "quantification of *trans*-resveratrol in rat plasma and tissues" (Juan et al. 2009), "determination of baicalin in rat cerebrospinal fluid and blood…" (Huang et al. 2008), and "investigation of the absorption mechanisms of baicalin and baicalein in rats" (Taiming et

al. 2005) but so far none has been carried out on yeast cells as a model for a simpler system.

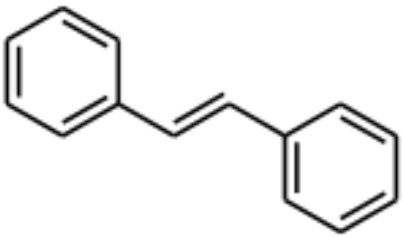
Yeasts are eukaryotic microorganisms belonging to the fungi kingdom. They are mostly unicellular and reproduce asexually by budding. *Saccharomyces cerevisiae* used in this study, is one of the important species of yeast. This yeast has been used for thousands of years in fermentation and baking and is widely used in modern genetics and cell biology research. As a simple eukaryote, it serves as a model for all eukaryotes including humans and hence can be used to gather information in relation to, for example, cell division, reproduction and metabolism (Gershon et al. 2000).

Analytical techniques such as high performance liquid chromatography with photodiode array detection (HPLC-PDA) or mass spectrometry detection (HPLC-MS) and high performance thin layer chromatography (HTLC) are the commonly used techniques to determine the concentration of analytes in biological matrices.

Trans-resveratrol is a well studied secondary metabolite in terms of its reported biological activity and is thus used in this yeast model study.

1.4. Trans-resveratrol – its chemistry, sources and medicinal properties

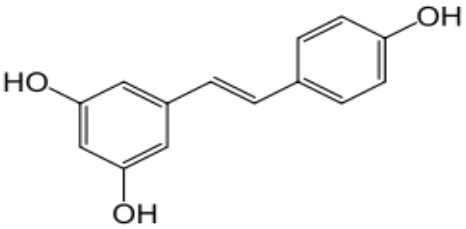
Trans-resveratrol ([(*E*)-5-[2-4(hydroxyphenyl) ethenyl]-1, 3-benzenediol]) belongs to a group of polyphenolic compounds called stilbenes (general structure shown in Fig. 1.1). Stilbenes are relatively small molecular weight (210-270 g/mol) compounds produced by a wide range of plants in response to environmental threat such as microbial and viral attack, and excessive UV exposure.



Trans-1, 2-diphenylethylene

Fig.1.1. General structure of trans-stilbene.

The derivatives of stilbenes are called stilbenoids, of which *trans*-resveratrol (Fig. 1.2) is an example.



Trans-resveratrol

Fig.1.2. Structure of *trans*-resveratrol.

Trans-resveratrol is found in grapes, peanuts and other plants such as *Polygonum cuspidatum.* In grapes, it is found mainly in the skin and hence is a key ingredient in red wine and is present in between 0.2 to 5.8 mg/L. In general, resveratrol is said to be present in 72 species of plants (Havet et al. 2011) and the content in these sources varies widely and is influenced by factors such as plant variety, location, season, climate, UV exposure, and microbial infection (Signorelli et al. 2005).

Resveratrol in red wine has long been speculated to help explain the 'French Paradox' – the low incidence of heart disease among the French who consumed relatively high amounts of fat and red wine. The potent antioxidant, anti-cancer, anti-inflammatory, antiviral and anti-ageing properties (Wang et al. 2010) of *trans*-resveratrol have been

widely reported. It has also been reported to exhibit protective effect against coronary heart disease, postmenopausal problems, atherosclerosis, platelet aggregation (Babu et al. 2005) and a range of degenerative diseases such as Parkinson's (Wang et al. 2010).

Like all stilbenes, resveratrol exists as a stereoisomer in *E* (trans) and *Z* (cis) forms but predominantly it is present in the *E* form. Studies report that the *Z* and *E* forms of resveratrol exhibit different pharmacological activities with the *E* form being more potent (Roupe et al. 2006). Thus for this project, the *E* form is used. It is extracted from *Polygonum cuspidatum* (Fig**.** 1.3).

Figure 1.3 *Polygonum cuspidatum.*

(With permission from: http://www.mdidea.com/products/herbextract/resveratrol/research.html)

Polygonum cuspidatum, also known as Japanese Knotweed or Mexican bamboo, belongs to the polygonaceae family that is widely distributed in Asia and North America. *Polygonum cuspidatum*, known as *Hu Zhang* (meaning "tiger's cane") in Chinese medicine is used for chronic bronchitis, jaundice, cancer and high blood pressure. It is also used as an analgesic, anti diuretic, antipyretic and an expectorant. Extracts of *Polygonum cuspidatum* are reported to have high antioxidant and anti-inflammatory properties and due to its high concentration of resveratrol, it is also used as an

ingredient in nutraceutical product formulations. The extract is used commercially as an additive to standardise resveratrol concentration in extracts such as grapes pomace (skins and seeds) that have low or variable natural concentration of resveratrol (Bralley et al. 2008). Standardised extracts of *Polygonum cuspidatum* typically contain 50% resveratrol (Babu et al. 2005). The extract of *P. cuspidatum* is also claimed to contribute to longevity (Wang et al. 2010).

Trans-resveratrol has been the major focus in medicinal plant metabolomics mainly because of its reported strong antioxidant and anti-ageing properties. It has been estimated that 5% of the oxygen living things inhale is reduced to oxygen derived free radicals (Yu 1994). These oxygen free radicals have been found to be involved in the pathogenesis of numerous human diseases such as cancer, coronary heart disease and ageing (Moskovitz et al. 2002). As part of the normal metabolic processes, reactive oxygen species such as superoxide anion ($^{\cdot}O_2$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2) are continually being formed in the body (Valko et al. 2007). As we are also constantly exposed to oxidants from exogenous factors and agents, there needs to be a constant balance between oxidants and antioxidants for the body to function properly (Ames 1979). *Trans*-resveratrol is also a free radical scavenger that can neutralise free radicals in the body (Balkir et al. 2011). *Trans*-resveratrol cannot be synthesised by the body and hence needs to be taken in the form of supplements or by consuming the fruits that contain them.

To better derive the benefits of *trans*-resveratrol, we first need to determine the uptake with regards to factors such as dosage, time and concomitant calorie intake which may affect that process. This project uses *Saccharomyces cerevisiae* yeast cells to study the influence of these variables.

1.4.1. Biosynthesis of trans-resveratrol

The biosynthesis of *trans*-resveratrol is via the phenylpropanoid pathway (Fig. 1.4) which is common to all higher plants and is responsible for the synthesis of a wide range of phenolic compounds such as flavonoids, lignins and proanthocyanindins.

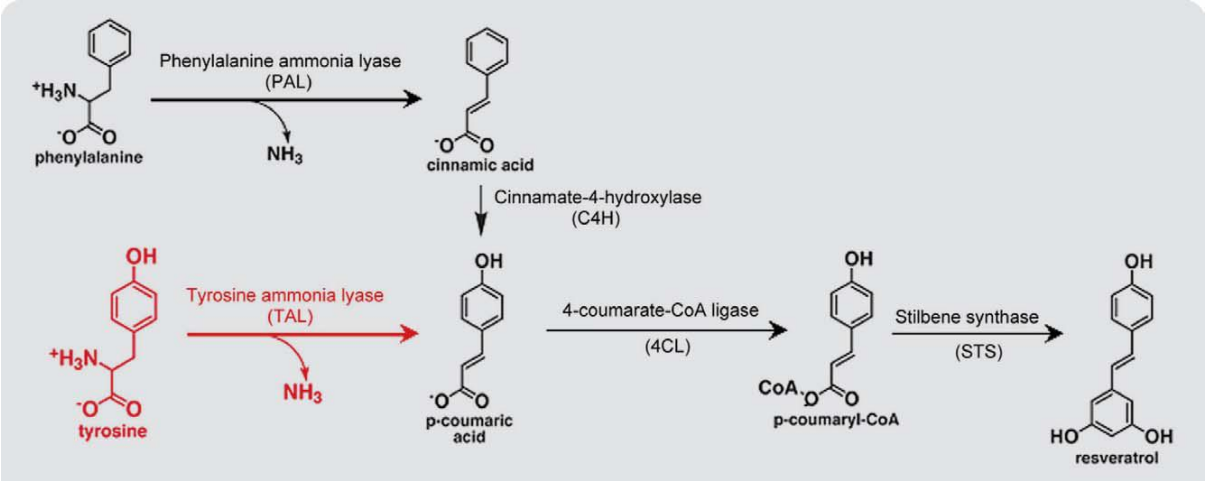


Figure 1.4. Biosynthetic pathway of resveratrol (Wang et al. 2010).

The first step in resveratrol biosynthesis is the deamination of phenylalanine by phenylalanine ammonia lyase to produce cinnamic acid which is a substrate for cinnamate-4-hydroxylase to produce *p*-coumeric acid which is in turn attached to the pantetheine group of coenzyme A, producing *p*-coumaroyl-CoA, an important intermediate of all higher plants. *P*-coumaroyl-CoA can also be converted into monolignols for lignin biosynthesis or flavonoids for anthocyanin and proanthocyanidin biosynthesis. For resveratrol accumulating plants such as grapes, and *Polygonum cuspidatum*, the last step of resveratrol synthesis is via a decarboxylation condensation reaction facilitated by three molecules of malonyl CoA and a molecule of 4-coumaroyl CoA, catalysed by stilbene synthase (Wang et al. 2010). As a defence mechanism, the biosynthesis of resveratrol is only activated under stressful situations, or during excessive UV exposure or viral or fungal attack.

Further reactions conjugates native resveratrol to glucosyl or sulfate residues at the 3-position of the biphenolic ring (Tosun et al. 2010).

Resveratrol can be degraded by oxidation but the glycosylated form is more stable and water soluble and can be readily absorbed by the human intestine (Wang et al. 2010).

1.4.2. Pharmacokinetic studies of trans-resveratrol

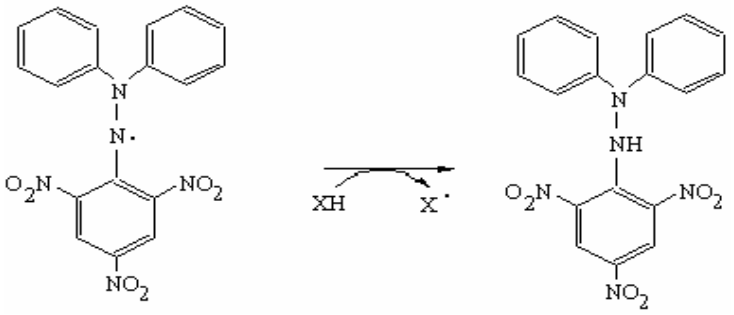
Pharmacokinetics studies of resveratrol have revealed that glucuronidation is the predominant metabolic pathway of resveratrol. A sulfated metabolite of resveratrol has also been reported to be formed but is less apparent compared to the glucuroninated metabolite (Wenzel et al. 2005). In rats, resveratrol is known to be glucuroninated in the small intestine whereas in humans, resveratrol glucuronidation occurs in the gastrointestinal tract and the liver (Kuhnle et al. 2000). The glucuronidation process is facilitated by a group of enzymes called uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase, or UGT) which catalyses the transfer of the glucuronic acid moiety from the cosubstrate UDP-glucuronic acid (UDPGA) to the substrate. This generally results in the formation of a *trans*-resveratrol metabolite with greater polarity and hence solubility in water (Iwuchkwu et al. 2007). Resveratrol glucuronidation in the human liver is both stereospecific and regioselective which usually result in the production of two major metabolites, namely resveratrol 3-*O*-glucuronide (R3G) and resveratrol-4'-*O*-glucuronide (R4'G) (Aumont et al. 2001).

In vivo pharmacokinetics studies of orally administered resveratrol in humans and rats show that the plasma concentration of resveratrol is usually low due to poor bioavailability as it is completely conjugated to its glucuronide and sulfate metabolites. The resulting sulfate metabolite, resveratrol-3-sulfate, is reported to affect the metabolism of resveratrol

by interfering with its bioavailability (Santi et al. 2000) suggesting that a higher dose of resveratrol is required for a significant pharmacological effect to be realised.

1.5. DPPH scavenging activity studies of trans-resveratrol

DPPH (1, 1-diphenyl-2-piccrylhdraxyl) is used in this project to determine the claimed antioxidant effect of *trans*-resveratrol. DPPH is a fast and inexpensive colorimetric method widely used to test for free radical scavenging activity and to ascertain the antioxidant capacity of foods (Balkir et al. 2011). It is a free radical which is stabilised by a delocalised electron and is readily reduced when reacted with an antioxidant. DPPH is purple when dissolved in methanol and has a λ_{max} = 517 nm. In the presence of an antioxidant, DPPH changes from purple to a strong yellow colour (Fig. 1.5).



Purple Yellow

Figure 1.5. Reaction of DPPH with antioxidant.

1.6. Yeast cell reproduction

Yeast reproduce asexually by asymmetric division otherwise known as budding or mitosis and sexually or meiosis. Yeast cells are about the same size as human red blood cell, measuring between 3–40 µm in diameter depending on species.

Asexual (mitosis) propagation takes place when nutrient is abundant and mitosis occurs between every 2-3 h. However under stressful conditions such as carbon and nitrogen starvation, sexual reproduction (meiosis) is favoured leading to spore formation (haploid spores) which can germinate once conditions improve, leading back to the formation of the diploid (Ausubel et al. 1997).

1.7. Aim and objectives

The aim of this study is to determine the uptake of *trans*-resveratrol by *Saccharomyces cerevisiae* yeast cells under different growth conditions.

The objectives are to:

- determine *trans*-resveratrol uptake by *Saccharomyces cerevisiae* grown in media containing 2.5, 5.0, 10.0, 50 and 100 µmol/L of trans-resveratrol.
- determine the uptake of *trans*-resveratrol by *Saccharomyces cerevisiae* grown in synthetic media containing 0.5% and 2.0% glucose.
- determine *trans*-resveratrol uptake over 1 h, 1 day, 4 days, 10 days and 20 days for each of the above mentioned media

1.8. Significance of study

Most studies on plant secondary metabolite uptake use animal models. This study uses yeast cells as a simple model system, representative of all eukaryotic systems.

1.9. Method validation parameters

1.9.1. Method validation

This is the process by which an analytical method is assessed for its suitability for a particular application and its performance characterised. In complementary medicine research, method validation is a regulatory requirement (Harris 2007). In Australia, this regulatory body is the Therapeutic Goods Administration (TGA). The main aim of the TGA is to ensure the quality, safety, efficacy, and timely availability of therapeutic goods imported into or exported out of Australia (TGA 2011).

To validate the analytical technique for this study, recovery studies are carried out at three spiking levels with seven replicates per spike level. From the precision (or standard deviation (SD)) obtained, the limit of detection (3x SD) and limit of quantitation (10x SD) are determined.

1.9.2. Analytical Techniques

Since most plants metabolites are polar the obvious method of choice for their analysis is by liquid chromatography and because *trans*-resveratrol has a chromophore, UV detection is suitable. With a PDA detector, a reasonable degree of peak identity confirmation is possible as the analyte has been reported to show a broad absorption peak between 304 nm and 321 nm.

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CHAPTER TWO

LITERATURE REVIEW

2.0. Background to chapter

This chapter presents a summary of the published literature on uptake studies of *trans*-resveratrol by cell lines and animals focussing on the analytical methodologies employed for its analysis.

The literature was obtained from Science Direct and SciFinder Scholar starting from 2002 to the present. Earlier years were examined but nothing pertinent to this study was obtained.

Details of the method validation parameters reported for each reference are presented here.

2.1. General overview

Gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography with mass spectrometry (HPLC-MS and HPLC-MS/MS) or photodiode array detection (HPLC-PDA), high-performance thin layer chromatography (HPTLC), fluorescence microspray, capillary electrophoresis with electrochemical detection, are the analytical techniques typically employed.

2.2. Fluorescence microspray

Fluorescence microspray was used to map out the uptake and transport of *trans*-resveratrol in human hepatoblastoma (HepG2 tumor cells) and human hepatocytes (Lancon et al. 2004). The uptake of resveratrol was studied over a time course of 0-240 min at 60 min intervals. The fluorescence property of resveratrol was used to track the intracellular transport of resveratrol in the cell and ^{3}H-labelled resveratrol to quantify the uptake.

The concentration range of the analyte used was 0.1 – 60 µM (or 0.02-13.68 µg/mL) and the cells were incubated at 4 and 37^{o}C to determine the influence of *trans*-resveratrol concentration and temperature on resveratrol uptake. The uptake was reported to be linear at the concentration range 0.1 – 60 µM and the optimum uptake temperature was 37 ^{o}C. The investigators concluded that the uptake under those conditions could have been the result of passive and mediated processes. From the time course investigation, the maximum uptake of resveratrol in both types of cell lines occurred at 10 min with values of 0.5 µmol per 10^6 cells for human hepatocytes and 1.1 µmol per 10^6 for HepG2 cells. Each study was carried out in triplicate only.

2.3. HPLC

The authors (He et al. 2008) used HPLC-PDA ($\lambda_{detection}$ = 303 nm) to analyse *trans*-resveratrol in cell suspension, intestinal Krebs' buffer and rat plasma. The *trans*-resveratrol concentrations studied were 1, 10 and 100 µM (0.23, 2.28 and 23.20 µg/mL) for cell suspension; 0.10, 0.5 and 5.0 µg/mL for intestinal Krebs' buffer; and 0.10, 5.0, and 25 µg/mL for rat plasma. Extraction of analyte was achieved with acetonitrile. The LOQ in cell suspension was reported to be 0.02 µg/mL, 0.05 µg/mL for Krebs buffer and was 0.05 µg/mL for rat plasma with recoveries ranging from 87-102%. A Hypersil C18 column was used with a mobile phase of 0.025 % triethylamine in a 45:55% mixture of methanol and water. The uptake of *trans*-resveratrol was found to be concentration dependent.

Trans-resveratrol was quantified in human plasma and urine using reverse-phase HPLC-UV ($\lambda_{detection}$ = 325 nm) (Boocock et al. 2007). A Waters Atlantis C18 column (4.6 mm x 150 mm, pore size 3 µm) was used and the mobile phase consisted of aqueous ammonium

acetate (5 mM) containing 2% propan-2-ol and methanol containing 2% propan-2-ol. *Trans*-resveratrol (1 g) was administered as an oral dose with water. Blood and urine samples were collected from volunteers' pre and post dosage of *trans*-resveratrol and stored on ice. Plasma was separated from blood by centrifuging. The plasma and urine samples were treated with methanol and acidified with conc. HCl prior to HPLC analysis. The LOD of resveratrol in plasma and urine were both reported to be 0.002 µg/mL. The recoveries were found to be reproducible though low at 58.0 ± 3.0% respectively for each matrix. The number of subjects used in this study was four and there was no mention of dosage time in any of the analysis conducted.

He et al. (2006) developed a HPLC-UV ($\lambda_{detection}$ = 303 nm) method for the determination of *trans*-resveratrol in rat plasma. The stationary phase was a Hypersil ODS_2 C18 column (4.6 mm x 250 mm, particle size 5 µm) with a mixture of 0.5% acetic acid in methanol and water (52:48 *v/v*) as mobile phase. A correlation coefficient (r^2) of 0.9997 was achieved for the standard curve. A dosage of 20 mg/kg per rat and administered orally. Blood samples were collected pre and post dosage at eight time points (0, 2, 5, 10, 20, 45, 60 and 90 min) and centrifuged immediately to obtain the plasma. *Trans*-resveratrol was extracted with acetonitrile and analysed. Three spike concentrations (0.02, 0.40, and 40.0 µg/mL) of the analyte were determined and the reported recoveries were 98.7 ± 2.7, 91.3 ± 3.5 and 84.4 ± 2.8 % respectively. The LOQ was reported to be 0.02 µg/mL. There was no mention of the number of replicate rats used in the study.

Juan et al. (2010) developed a HPLC method to quantify *trans*-resveratrol and its metabolites in rat plasma, brain, testes, lung, and kidney. An Agilent model 1100 HPLC

system was used with a Phenomenex C18 column (4.6 mm x 250 mm; particle size 4 µm) equipped with a C18 guard column cartridge. The mobile phase was 3% aqueous acetic acid and mixture of 3% aqueous acetic acid and acetonitrile (20:80 *v*/*v*). Identity confirmation of *trans*-resveratrol conjugates was achieved with an API 3000 triple quadrupole MS equipped with a Turbo Ionspray source. Sprague-Dawley rats (male adults) weighing from 275-300 g (n = 18) were used for the study. *Trans*-resveratrol at a dose of 15 mg/kg per rat weight was administered intravenously for 90 min. The plasma was extracted via a C18 SPE. Following elution with methanol, the eluate was acidified with ascorbic acid, reconstituted with a concentrator and HPLC analysed with $\lambda_{detection}$ = 306 nm. *Trans*-resveratrol in the tissue samples were extracted by homogenizing 1 g of the sample with acidified MeOH. The mixture was centrifuged and the organic supernatant reconstituted with a concentrator prior to HPLC analysis. The recoveries were reported as 98.5 ± 3.2, 100.1 ± 1.8, 96.5 ± 7.6, 99.0 ± 0.7 and 103 ± 2.7% for liver, kidney, lung, brain and testes respectively. The LOD ranged from 1.25 x 10^{-3} µg/mL in testes to 2.55 x 10^{-3} µg/mL in kidney. *Trans*-resveratrol was found in mainly the glucuronide form with high concentrations in the kidney and lower in the brain and only one time point was explored.

Rudolf et al. (2005) developed a HPLC method for analysing *trans*-resveratrol in peanut kernels. Analysis was done on a Waters HPLC system using a C18 column (4.6 mm x 250 mm, particle size 5 µm) with $\lambda_{detection}$ = 307 nm. The mobile phase comprised of aqueous 0.1% acetic acid and acetonitrile. Phenolphthalein was used as internal standard and *trans*-resveratrol was extracted from peanut by homogeneously mixing ground peanut, phenolphthalein and 80% ethanol (Sanders et al. 2000). The mixture was centrifuged (5 min at 1380 *g*) and the supernatant cleaned up with an aluminium oxide and silica gel packed column using 80% ethanol eluante. The eluate was evaporated to dryness and the

residue dissolved in 10% ethanol and analysed. The LOD was 0.010 µg/mL and the LOQ 0.04 µg/mL. The correlation coefficient (r^2) of the standard curve was 1.0 and recoveries of added *trans*-resveratrol at concentration levels of 0.5, 1.0, and 1.5 µg/mL were found to be 71.20 ± 3.42%, 96.08 ± 1.00% and 93.02 ± 2.35% respectively. The natural concentration of *trans*-resveratrol in peanut was reported as 0.50 ± 0.03 µg/mL (n = 5).

Hanzlikova et al. (2004) used HPLC with electrochemical and UV detectors to determine resveratrol in red and white wine, vine leaf, rachises and grape berry. Resveratrol in the vine leaf, rachises and grape was extracted with 80% ethanol prior to direct HPLC analysis. Both plant material and wine samples were analysed for their *cis*- and *trans*-resveratrol content. A C18 Nucleosil (250 mm x 4 mm, particle size 5 µm) column was used with $\lambda_{detection}$ = 306 nm and 295 nm for *trans*- and *cis*-resveratrol respectively. The gradient mobile phase was 25% ACN, 0.1% aqueous phosphoric acid and 5 mM sodium chloride in water. The LODs were found to be 0.03 µg/mL for *trans*-resveratrol and 0.015 µg/mL for *cis*-resveratrol. The recoveries for *trans*-resveratrol in red and white wines were 98.63 ± 1.45% and 97.35 ± 2.16% respectively and the recoveries for vine leaf, rachises and grape were 97.18 ± 1.89%, 98.75 ± 2.64% and 101.3 ± 3.04% respectively. Resveratrol in vine leaves ranged from 2.8-46 mg/kg and the concentration in rachises was 490 mg/kg. *Cis*-resveratrol was not detected in the berry but the *trans*-resveratrol content was found to be 5.8 mg/kg. The wine samples were analysed at three time points (0 h, 48 h and 17 days) but no time point data was reported for the plant materials.

2.4. Laser desorption resonance enhanced multiphoton ionization coupled with time-of-flight mass spectrometry (LD–REMPI–TOFMS), and HPLC

Sanchez et al. (2005) compared LD–REMPI–TOFMS and HPLC for the analysis of *trans*-resveratrol in vine leaves. *Trans*-resveratrol was extracted by macerating the vine leaves in ethanol for 3 weeks and collecting the sample every 2 days. Following the maceration, the collected sample solution was filtered via cotton wool and the residue analysed by LD-REMPI-TOFMS to confirm the completion of the extraction process. The filtrate was then analysed by HPLC using a Tecknokroma Cromasyl C18 column (150 mm x 4.6 mm, particle size 5 µm) with a mobile phase consisting of MeOH-acetic acid-water (10:2:88) and MeOH-acetic acid-water (90:2:8). The $\lambda_{excitation}$ and $\lambda_{emission}$ used for the fluorescence detector to analyse *trans*-resveratrol were 330 nm and 374 nm respectively. The LOD and LOQ were 20 and 67 µg/mL respectively; compared to the LD-REMPI-TOFMS technique for which those values were 2.1 and 6.3 µg/mL respectively. The authors concluded that the HPLC method was the most efficient and economical method for the analysis of *trans*-resveratrol in plant extract.

Table 2.1 gives a summary of the analytical method performance characteristics summarised from the literature that has just been presented.

The review shows that thus far, the studies on *trans*-resveratrol uptake involve animal or human cell lines but none have been in yeast cells.

Most of the studies reported recovery data but two did not and only two reported the LOQ.

HPLC with a C18 column is almost exclusively used with the most common detector being the UV. The most commonly employed mobile phase components are acetonitrile and water. The other popular mobile phase components are methanol and water, trifluoroacetic acid and water, and formic acid and water. The $\lambda_{detection}$ is typically between 300-390 nm.

Matrix	Analytical method	Mobile phase	Recoveries (%)	LOD (µg/mL)	LOQ (µg/mL)	Reference
Hepatoblastoma and human hepatocytes	Fluorescence microspray	-	-	-	-	Lancon et al. 2004
Cell suspension, intestinal Krebs' buffer and rat plasma	HPLC-PDA (λ_{det} = 303 nm)	0.025 % TEA in a 45:55% mixture of MeOH and H_2O (isocratic elution)	87.0 & 102 respectively for each matrix	0.0023, 0.05 & 0.05 respectively for each matrix	-	He et al. 2008
Human plasma and urine	HPLC-UV (λ_{det} = 325 nm)	ammonium acetate in H_2O and MeOH with 2% propan-2-ol (gradient elution)	58.0 ± 3.0% for each matrix	0.002 for each matrix	-	Boocock et al. 2007
Rat plasma	HPLC-UV (λ_{det} = 303 nm)	0.5% acetic acid in MeOH and H_2O (52:48) (isocratic elution)	98.7 ± 2.7, 91.3 ± 3.5 & 84.4 ± 2.8 respectively for the three conc. Levels (0.02, 0. 40 and 40.0 µg/mL) examined	-	0.02	He et al. 2006

Rat plasma and brain, testis, lungs, and kidneys	HPLC-UV (λ_{det} = 306 nm)	3% acetic acid solution and a mixture of acetic acid and ACN (20:80) (gradient elution)	98.5 ± 3.2, 100.1 ± 1.8, 96.5 ± 7.6, 99.0 ± 0.7 & 103 ± 2.7 respectively for each matrix	0.0025 in testis & 2.55 in kidney	-	Juan et al. 2010
Peanut kernels	HPLC-PDA (λ_{det} = 307 nm)	0.1% acetic acid in H_2O and ACN (gradient elution)	71.2 ± 3.42, 96.1 ± 1.00 & 93.02 ± 2.35 respectively for the three replicates examined.	0.01	0.04	Rudolf et al. 2005
Red wine, white wine, vine leaf, rachises and grape	HPLC-UV (λ_{det} = 306 nm)	25% ACN, 0.1% H_3PO_4 and 5 mM NaCl in H_2O (isocratic elution)	98.6 ± 1.45, 97.1 ± 2.16, 97.2 ± 1.89, 98.8± 2.64 & 101.3 ± 3.04 respectively for each matrix	0.03 for each matrix	-	Hanzlikova et al. 2004
Human intestinal cell line	HPLC-fluorescence	H_2O:TFA and TFA:ACN (gradient elution)	-	-	-	Henry et al. 2005
Vine leaf	LD-REMPI-TOFMS and HPLC-fluorescence (λ_{ex} = 330 nm, λ_{em} = 374 nm)	MeOH- ?% acetic acid-H_2O (10:2:88) and (MeOH-?% acetic acid-H_2O (90:2:8) (gradient elution)	-	2.1 (LD-REMPI-TOFMS) & 20 (HPLC)	67 (HPLC) & 6.3 (LD-REMPI-TOFMS)	Sanchez et al. 2005

-	HPLC UV (λ_{det} = 217.2 and 305 nm)	-	-	-	-	Montsko et al. 2008

Table 2.1. Summary of analytical method validation parameters reported for *trans*-resveratrol (all using C18 column).

Key:

LOD = Limit of detection

LOQ = Limit of quantitation

HPLC-PDA = High performance liquid chromatography-photodiode array detection

TFA = Trifluoroacetic acid

ACN = Acetonitrile

MeOH = Methanol

EtOAc = Ethyl acetate

H_3PO_4 = Phosphoric acid

NaCl = Sodium chloride

H_2O = Water

TEA = Triethylamine

λ_{det} = wavelength of detection or quantification

LD-REMPI-TOFMS = Laser desorption resonance enhanced multiphoton ionization coupled with time-of-flight mass spectrometry

'-' = not reported

2.5. References

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CHAPTER THREE

METHODOLOGY

3.0. Chapter overview

This section describes how the method for *trans*-resveratrol analysis in the culture media and yeast was developed and the way the uptake studies were carried out.

3.1. Reagents and Chemicals

The *trans*-resveratrol primary standard (93.44%) was obtained from Lipa Pharmaceuticals (NSW, Australia). Analytical and reagent grade yeast nitrogen base, L-leucine, L-methionine, L-histidine, L-lysine monohydrate, uracil and ammonium sulfate were obtained from Sigma-Aldrich (MO, USA). D-glucose was obtained from Merck (VIC, Australia).

HPLC grade methanol was from Fisher Chemicals (NJ, USA) and the ethanol was from Sigma-Aldrich (USA). Analytical reagent grade chloroform, formic acid and acetonitrile were supplied by Ajax Finechem (Auckland, New Zealand). Analytical grade orthophosphoric acid (85%) was supplied by Biolab (VIC, Australia). The nitrogen gas used for solvent evaporation was supplied by Core Gas (NSW, Australia).

The purified water used for the media preparations, sample analysis and HPLC mobile phase was obtained from a MilliQ ultra high quality water system purchased from Millipore (NSW, Australia).

A summary of equipment used in this project is given in the section that follows.

3.2. Equipment

3.2.1. HPLC-PDA instrumentation

HPLC-PDA analysis was carried out on a Shimadzu Prominence system with a LC-20AT solvent delivery system, SPD-M20A detector and SIL-20AC auto sampler. Data acquisition and analysis was carried out through a Shimadzu Class VP 7.4 SP3-R1 software system using a LG 4300 Intel(R) Core 2, 1.80GHz CPU computer with a Windows XP operating system connected to a RICOH Aficio 3025 printer.

Separation was achieved on an Alltima C-18 (460 x 4.6 mm, particle size 5 µm) column from Alltech Associates (NSW, Australia) with a guard cartridge system supplied by Phenomenex (NSW, Australia).

3.2.2. Solid phase extraction (SPE) equipment

Analyte extraction by SPE was done with the aid of a SPE vacuum manifold with a pressure gauge and a capacity for 12 SPE columns supplied by Phenomenex (NSW, Australia).

The analyte was extracted from the supernatant using a C-18 (6 mL, 0.5 g) SPE column supplied by Supelco Bellefonte (PA, USA).

The SPE sample extracts were evaporated to dryness using a Savant DNA 120 SpeedVac concentrator by Thermo Fisher Scientific purchased from Biolab (NSW, Australia).

3.2.3. Balances

An Adams AFA-210LC analytical balance capable of measuring to 0.0001 g, purchased from Adams Equipment (NSW, Australia), was used for weighing the yeast cells and media components.

Trans-resveratrol used for preparation of the standard and stock solutions was weighed on a Sartorius SE-2 micro analytical balance supplied by Sartorius (QLD, Australia). The balance is capable of weighing to 7 decimal places of a gram.

3.2.4. Micro pipette

Micro pipettes in the ranges of 200 -1000 µL, 20 – 200 µL, and 2 – 20 µL were from Eppendorf AG (NSW, Australia).

3.2.5. Incubator and sterilizer

A Labnet 311DS shaker incubator by Labnet International (CA, USA), from Pacific Laboratory Products (NSW, Australia) was used for incubating the yeast for dose response studies.

A SterileMax steam sterilizer autoclave ST75920-33 from Barnstead International (IA, USA), was used for yeast media preparations and waste sterilisation.

3.2.6. Miscellaneous equipment

Greiner Bio-one CellStar cell culture flasks (Frickenhausen, Germany) (50 mL) were used for the yeast cell pre-culture and dose response reagent preparations.

A PowerSonic 420 ultrasonicator, set at the maximum setting, was used for analyte extraction and was supplied by Thermoline Scientific, Hawshin Technologies (South, Korea).

2 mL graduated microcentrifuge tubes were purchased from Neptune Scientific (San Diego, USA) and 15 mL high-clarity polypropylene conical centrifuge tubes were from Becton Dickinson and Company (NJ, USA).

Samples were spun down using an Eppendorf centrifuge model 5810R, from Eppendorf (Hamburg, Germany).

Two types of freezers – an Avita DD1-10 85L freezer from H. E. Group (Australia) and a ILS-DF8524E -86^{o}C freezer by Nuaire Inc. Ilshin Lab (MN, USA) were used for sample storage.

The samples were vortexed with BR-2000 Vortexer from Bio-Rad Laboratories (CA, USA).

To obtain the dry weight of the yeast cells, a Contherm Thermotec 2000 oven was used to dry the samples.

A Kyowa Unilux-12 light microscope (Tokyo, Japan) was used for yeast cell viability test analysis.

A Stericup and Steritop vacuum filtration system (0.22 µm pore size) from Millipore Corporation (NSW, Australia) was used for HPLC mobile phase and yeast media component filtration.

Microtest tissue culture plates (96 wells per plate) were purchased from Becton Dickinson and Company (NJ, USA).

Finally a FLUOStar OPTIMA plate reader from BMG Labtech (NSW, Australia) was used for measuring DPPH scavenging activity.

3.3. Experimental

3.3.1. Choice of solvent for dissolving trans-resveratrol

Trans-resveratrol is very soluble in solvents such as methanol, and ethanol but has low solubility in water (Sun et al. 2008), with solubility increasing with temperature.

For this project, acetonitrile, ethyl acetate, dimethyl sulfoxide, methanol, ethanol, water; and 50 and 80% methanol in water were trialled for dissolving *trans*-resveratrol. This was achieved by placing ~1.0 mg *trans*-resveratrol in a 10 mL volumetric flask, adding the solvent, sonicating for 10 min at 30^{o}C and then observing the solution against a bright light.

3.3.2. Standard and stock solution preparation

Trans-resveratrol was weighed (~1.07 mg) on the micro balance and quantitatively transferred to a volumetric flask (10 mL). The sample was dissolved in AR grade methanol, ultrasonicated for 15 min and the solution allowed to cool to room temperature before making up to volume with methanol. The solution was then vortexed for 2 min to ensure complete mixing. From the stock solution (100 µg/mL), serial dilutions were made to obtain seven calibration levels (2.50, 5.00, 15.00, 25.00, 50.00, 75.00 and 100.00 µg/mL).

Two different stock solutions of *trans*-resveratrol were used for the dose response studies. spiking ***Stock solution* 1** was prepared by weighing *trans*-resveratrol (1.341 mg or 1.253 mg purity corrected) using the micro balance and quantitatively transferring into a 2 mL Eppendorf centrifuge tube to which was added AR grade dimethyl sulfoxide (535.2 mL). The solution was mixed by vortexing (1 min) and ultrasonicating for 5 min to give a 2337.69 µg/mL (or 10234 µM) solution (purity corrected). From this stock, three spiking standards of 0.57, 1.14 and 2.28 µg/mL (or 2.5, 5.0 and 10 µM) were prepared.

Spiking ***Stock solution* 2** was prepared by dissolving *trans*-resveratrol (17.2842 mg or 16.150 mg purity corrected) in the same way as for spiking ***Stock solution* 1** to give a 29204 µg/mL (or 127950 µM) stock solution (purity corrected). From this stock, two spiking standards of 11.41 and 22.82 µg mL^{-1} (or 50.0 and 100.0 µM) were prepared.

3.3.3. Solvent optimisation for the extraction of trans-resveratrol from yeast cells

To determine the most efficient solvent for the extraction of *trans*-resveratrol from yeast cells, a 10.0 µM (2.28 µg/mL) *trans*-resveratrol standard was chosen and a dose response study carried out for 24 h in a 2% glucose synthetic media. Chloroform, methanol, ethanol, water and a 2:1 mixture of chloroform and water were tested (with five replicates per solvent) for their ability to extract the analyte from yeast cells.

A stock solution of *trans*-resveratrol was prepared as described for Stock solution 1 in section 3.3.2. A 10.0 mL aliquot of 2% glucose synthetic yeast media was transferred to a 50 mL CellStar cell culture flask. 5 replicates were prepared for each of the five extraction solvents to be tested. Yeast pre-culture (100 µL), prepared 12–16 h in advance, was added to each flask which was then vortexed, spiked with *trans*-resveratrol (10 µL, 10.0 µM),

vortexed again and incubated for 24 h in a Labnet shaker incubator set at 30^{o}C and 250 rpm (at 1.13 *g*). Following incubation, the content of each flask was transferred into pre-weighed 15 mL centrifuge tubes (kept on ice) and centrifuged for 5 min in a 5810R Eppendorf centrifuge set at 4^{o}C and 4000 rpm (at 18 *g*). The supernatant was then separated from the yeast pellet by decanting into another 15 mL centrifuge tube.

The yeast pellet was washed twice with ice-cold water and then lysed with the test solvent (10 mL) by vortexing until the pellet was well dispersed and then sonicating for 60 min at 30^{o}C. After sonicating the mixture was vortexed again for 2 min and centrifuged at 4000 rpm at room temperature for 5 min. The supernatant was then decanted into a new 15 mL centrifuge tube and evaporated to dryness with N_2 gas. The residue was then dissolved in AR grade methanol (200 µL) by vortexing and sonicating, and the solution filtered (if necessary) through a 0.45 µm PVDF syringe filter before analysis by HPLC-PDA.

The supernatant was cleaned up via C-18 SPE and the analyte concentration remaining in the supernatant, determined as described for the yeast sample.

The solvent which yielded the highest analyte recovery was selected as the solvent for *trans*-resveratrol extraction from yeast cells.

3.3.4. Trans-resveratrol extraction method optimization

Two extraction procedures were investigated for *trans*-resveratrol extraction. These are:

3.3.4.1. Acetonitrile–aqueous formic acid buffer extraction

A sample of *trans*-resveratrol (~1.07 mg) was weighed on the micro analytical balance into a 10 mL volumetric flask and dissolved in YPD media (yeast extract peptone dextrose

media; preparation as described in section 3.3.5.2.1) (10 mL) by vortexing for 1 min and sonicating at 30^{o}C for 10 min. Following sonication, the stock solution was allowed to cool to room temperature and three working standards (3.125, 1.56 and 0.78 µg/mL *trans*-resveratrol) were prepared, in triplicate, by serial dilution.

An aliquot (200 µL) of each standard was transferred into 2 mL centrifuge tubes and to each tube was added AR grade acetonitrile (200 µL), pH 2 formic acid buffer (50 µL) and the mixture vortexed for 1 min. To each mixture, solid NaCl was added to saturation and the mixture vortexed for 2 min and the mixture allowed to settle before the organic and aqueous layers were transferred to individual HPLC vials for analysis.

Given the difficulty of getting good precision and good recoveries with this method, the SPE method was later explored.

3.3.4.2. Solid phase extraction (SPE)

This cleanup method involves the use of reverse-phase Sep-Pak C-18 cartridges (6.0 mL, 0.50 g) in conjunction with a vacuum manifold. For testing this method, only one working standard (0.94 µg/mL *trans*-resveratrol) was used in triplicate. The stock preparation is the same as described for the acetonitrile–aqueous formic acid buffer extraction method. Using a 1000 µL micro pipette, 1 mL of the stock (107.0 µg/mL) was transferred into a 10 mL volumetric flask and made up to volume with methanol.

The SPE column was conditioned with AR grade acetonitrile (6x column volume) followed by 10 % acetonitrile in water (also 6x column volume) while ensuring that the column packing was kept moist and undisturbed throughout. A sample (1 mL) of the working standard was carefully added to the column and allowed to soak through by opening the vacuum tap slowly. The unwanted components were eluted with 10%

acetonitrile (5 mL) and the analyte eluted with two volumes of AR grade acetonitrile (2 mL) into a 2 mL microcentrifuge tube. The eluate was evaporated to dryness using a Savant DNA 120 SpeedVac concentrator and the residue dissolved in methanol (200 µL) by vortexing and sonication. The samples were then filtered through a syringe filter, were necessary, and analysed by HPLC-PDA.

This method was used for the entire project as it produced the best precision and recovery results.

3.3.5. Yeast media preparation

Both solid and liquid media are prepared containing different types and amounts of additives. Yeast can be grown in a solid or liquid media containing dextrous (glucose) as carbon source and ammonia salts which can provide nitrogen, phosphorous and trace metals. However the cells grow best in the presence of protein and yeast cell extract hydrolysates which provide amino acid, nucleotide precursors, vitamins and other metabolites which the cells would synthesize *do novo* (Ausubel et al. 1997).

3.3.5.1. Preparation of solid media

Preparing solid media for yeast growth is for the most part similar to preparing plates for bacteriological studies. For this project, the solid media was prepared from a 2% (20 g) concentration of agar and a 1% (10 g) yeast extract mixed in MilliQ water (900 mL) (Ausubel et al. 1997). A pellet (~0.1 g) of sodium hydroxide (NaOH) was added to raise the pH enough to prevent agar breakdown during autoclaving. Following autoclaving, the mixture was allowed to cool to room temperature before the filtered dextrose (20 g) solution prepared in MilliQ water (100 mL) was added. Prior to pouring, the mixture was

placed on a stirrer plate and mixed until homogeneity was achieved. After pouring, air bubbles were removed by passing the flame of a Bunsen burner over the surface of the molten agar. The plates were covered and left in the hood to set and cool before use.

3.3.5.2. Preparation of liquid media

For the purpose of this study, two types of liquid media were explored; these are:

3.3.5.2.1. Yeast extracts peptone dextrose (YPD) or rich media

The YPD media (or rich media) was prepared by mixing 10 g (for 1% concentration) of yeast extract and 20 g (for 2% concentration) of peptone in water (900 mL) and the mixture autoclaved at 121 ^{o}C for 2 h. 20 g of dextrose was prepared in a sterilised 100 mL Schott bottle by dissolving it in water (100 mL). Following autoclaving, the yeast extract and peptone mixture was cooled to room temperature before the filtered dextrose solution was added and the final mixture shaken well to ensure homogeneity before use (Ausubel et al. 1997).

Because of the complex nature of the YPD media however, the synthetic (also known as minimal media) was later employed.

3.3.5.2.2. Synthetic (or minimal) media

Unlike the YPD media, the exact quantity of each component of the synthetic media is known. Also, the synthetic media is mainly colourless, hence eliminating any interference due to background colour.

For 1 L of synthetic media, the preparation was done in two separate steps:

***Step1. Preparation of the autoclavable mixture*:** This was prepared by mixing 1.7 g of yeast nitrogen base with 5.0 g of ammonium sulphate in water (900 mL). This mixture was autoclaved at 121 ^{o}C for 2 h and allowed to cool to room temperature before use (Ausubel et al. 1997).

Step 2. Preparation of the dextrose-protein mixture: L-histidine (100 mg), L-leucine (300 mg), L-lysine (30 mg), L-methionine (20 mg), uracil (100 mg) and (for a 0.5% synthetic media) dextrose (10 g) were weighed (analytical balance) and dissolved in water (100 mL) by ultrasonication at room temperature for 10 min. Following sonication, the mixture was filtered through a 0.22 µm Stericup and Steritop filtration system and added to the autoclavable mixture from *Step 1* and vigorously shaken to ensure homogeneity before use (Ausubel et al. 1997).

3.3.5.3. Yeast strain storage and revival

The *S. cerevisiae* was originally stored at – 80 ^{o}C in 15% glycerol. Using this method of storage the yeast is reported to remain viable for up to 5 years. Another method of strain storage is done on slants consisting of YPD medium supplemented with potato starch and stored at 4 ^{o}C – with this method the yeast can be viable for 2 years (Ausubel et al. 1997).
The stored strains were revived often by collecting a small quantity (10 µL) and streaking it on an agar plate.

3.3.5.4. Growth in solid media

S. cerevisiae was grown in much the same way as bacteria. Cells from the -80 ^{o}C storage freezer were streaked on the surface of agar plates in a sterile environment (fumehood) using a sterile plate streaker and incubated at 30^{o}C in a well ventilated Labnet 311DS incubator for > 24 h to allow colonies to develop sufficiently for harvesting (Fig. 3.1).

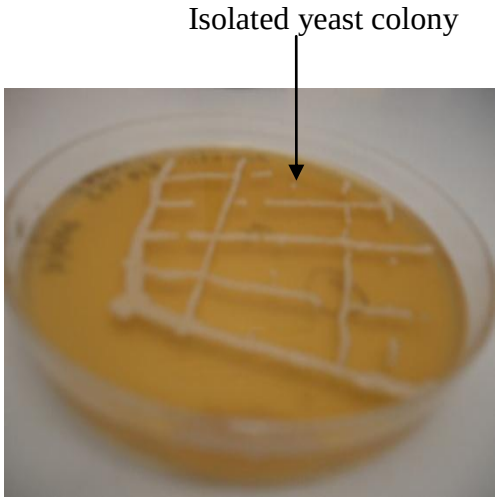

Figure 3.1. Fully developed *S. cerevisiae* yeast colonies after >24 h of incubation

3.3.5.5. Preparation of yeast cell culture

Saccharomyces cerevisiae grows well in liquid media at 30 ^{o}C with good aeration and with glucose as it carbon source. *S. cerevisiae* yeast culture was prepared in a rich (or 2% glucose synthetic) media.

To prepare the yeast culture (also referred to as yeast pre-culture), one isolated colony on the developed plate was selected and inoculated into the media (20 mL) contained in a 50 mL Greiner Bio-one CellStar cell culture flask. The mixture was then vortexed to disperse the cells and incubated at 30^{o}C in a Labnet 311DS shaker incubator for 12–17 h and then stored on ice prior to use.

3.3.5.6. Yeast cell viability test

In this project, the uptake study of *trans*-resveratrol by *S. cerevisiae* was carried out for up to the 20 day time point. Hence, the viability of the yeast cells up to the longest time point is important to the experiment. To ensure the yeast cells viability over the study time points, yeast viability tests were carried out at each time point by exposing a diluted aliquot (10 µL) of the yeast culture to methylene blue (10 µL) and viewing them under a light microscope. Viable cells appear normal with no staining but dead cells have their plasma membrane disrupted, allowing the dye to penetrate and stain the cytosolic material blue (Nurse et al. 1976).

3.3.5.7. Dose response studies of trans-resveratrol

Dose response studies were carried out over 1 h, 1 day, 4 days 10 days and 20 days, in two types of synthetic media (0.50 and 2.0% glucose), at five different concentrations (2.5, 5, 10, 50 and 100 µM or 1.11, 2.25, 4.46, 22.30 and 44.61 µg/mL) of *trans*-resveratrol. For each concentration, seven replicates (n = 7) were prepared. The media were prepared as described in section 3.3.5.2.2 and the spiking stock solutions and working standards prepared as described in section 3.3.2. Aliquots of 2.46, 4.92 and 9.84 µL of *trans*-resveratrol spiking ***Stock solution* 1** (2341.2 µg/mL) were added to 10 mL media containing 100 µL yeast pre-culture to give spiked media with 0.57, 1.14 and 2.26 µg/mL of *trans*-resveratrol respectively. Aliquots of 3.90 and 7.82 µL of *trans*-resveratrol spiking ***Stock solution* 2** (29204 µg/mL) were added to 10 mL media containing 100 µL yeast pre-culture to give spiked media with 11.4, and 22.8 µg/mL of *trans*-resveratrol respectively.

Each spiked media was prepared in a 50 mL CellStar cell culture flask under sterile conditions in the fume hood.

After addition of the yeast pre-culture (100 µL) to the media, the mixture was vortexed and incubated at 30 ^{o} C for 12–16 h to allow the cells to grow before spiking with *trans*-resveratrol.

After spiking the analyte, the mixture was vortexed and incubated for the respective time points after which the experiment was terminated as described in section 3.3.3. The approximate volume of the yeast pellet was determined by visual comparison with a known volume of water. The weight of the wet yeast pellet was determined prior to lyses by weighing the tube on an analytical balance and subtracting the weight of the pre-weighed empty tube. The yeast pellets were lysed and their content analysed as described in section 3.3.3. The dry weight of the yeast pellet was obtained by drying the pellet in a 60^{o}C oven for 24–48 h. After drying, the pellet was cooled in a desiccator and weighed to determine the dry weight of the yeast cells. The same procedure was used for the two media types. Details of calculations and raw data are presented in appendix A and B respectively.

HPLC-PDA analysis of the yeast extract was performed as described in section 3.3.3 and the supernatant analysed as described in section 3.3.4.2

3.3.6. HPLC-PDA conditions for trans-resveratrol analysis

For the HPLC-PDA analysis of *trans*-resveratrol, acidified water (0.10 % formic acid) and methanol was used as the mobile phase. Table 3.2 shows a summary of the mobile phase program for *trans*-resveratrol analysis.

Time (min)	Flow rate (mL/ min)	% 0.1% aqueous formic acid	% MeOH
5.00	1.00	70	30
15.00	1.00	30	70
15.01	1.00	0	100
20.00	1.00	0.0	100
20.01	1.00	70	30
30.00	stop	0.0	0.0

Table 3.1. Mobile phase composition for the HPLC-PDA analysis of *trans*-resveratrol.

The oven temperature was set at 30.0^{o}C and the injection volume at 10 µL. The system rinsing volume was 200 µL and the rinsing speed was 35 µL/s. The PDA acquired data from 190 nm to 600 nm with quantitation at 307 nm. The data sampling frequency was set at 1.5625 Hz.

The analytical column was an Alltima C-18 (4.6 x 150 mm, 5µm particle size, 100Å pore size) column. The acidified aqueous mobile phase was prepared by making up 1.0 mL of AR grade formic acid to 1L with water and sonicating for 5 min at room temperature.

3.3.7. Antioxidant activity test for trans-resveratrol

To test the antioxidant activity of *trans*-resveratrol, a sample (1.01 mg) of the substance was weighed into a 10 mL volumetric flask and dissolved in AR methanol by sonicating for 10 min and vortexing for 2 min. From this stock solution (~100 µg/mL), six working standards (2.5, 5.0, 10, 25, 50, and 100 µg/mL) were prepared by dilution with methanol.

DPPH solution (62.5 µM) was prepared by dissolving anhydrous DPPH (2.46 mg) in AR grade methanol (100 mL). As DPPH is light sensitive, the solution was wrapped in aluminium foil throughout the experiment.

The test samples were prepared by mixing 50 µL of the working standard with 150 µL of DPPH solution in a 96 well tissue plate (microtest tissue culture plate) and incubating in a Thermostar incubator at 30^{o}C and 250 rpm for 30.00 min. A control test sample consisting of DPPH (150 µL) and the solvent methanol (50 µL) was also set up.

Following incubation, the absorbance of the samples was measured at 517 nm (Balkir et al. 2011) using a microplate reader (FLUOStar OPTIMA plate reader).

3.4. Statistical interpretation of results

Following the HPLC-PDA analysis of samples, the results were processed using a GraphPad prism version 5.0 and R version 2.12.0 statistical analysis software. The results of the parameters examined in this project are presented in the result and discussion chapter.

3.5. References

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CHAPTER FOUR

RESULTS AND DISCUSSION

4.0. Chapter overview

In this chapter the results and discussion on the uptake of *trans*-resveratrol into *S.cerevisiae* is presented. The DPPH antioxidant test result is also presented.

4.1. Choice of solvent for trans-resveratrol solubility

Of the eight solvents tested, *trans*-resveratrol was found to dissolve readily and completely at room temperature in ACN, DMSO, and methanol but it is only partially soluble in ethyl acetate, ethanol, 50% and 80% MeOH in water and pure water. However upon sonication at 30^{o}C for 10 min, *trans*-resveratrol dissolved completely in all these solvents.

DMSO was used for dissolving *trans*-resveratrol for the spiking stock solution. For SPE extraction of *trans*-resveratrol from the yeast supernatant, ACN was used to elute the analyte from the column and MeOH was used to redissolve the dried *trans*-resveratrol extract extracted from the yeast cells and supernatant for HPLC analysis.

4.2. Standard solution preparation

The standard curve for *trans*-resveratrol was constructed from 7 calibration points. From the MeOH solution of *trans*-resveratrol (107.02 µg/mL) standard, dilutions were made to give calibration solutions in the range of 2.55 to 102.1 µg/mL. 10 µL of each solution was injected into the HPLC and the calibration curve deduced by processing of the data (table 4.1) obtained. A typical calibration curve is presented in figure. 4.1.

Concentration (µg/mL)	Peak area (x 10^6)
2.912	0.2327
6.092	0.4867
17.83	1.4244
33.76	2.6975
69.72	5.5708
105.34	8.4182
136.93	10.9415

Table 4.1: Calibration data obtained from the HPLC-PDA analysis of the *trans*-resveratrol standard.

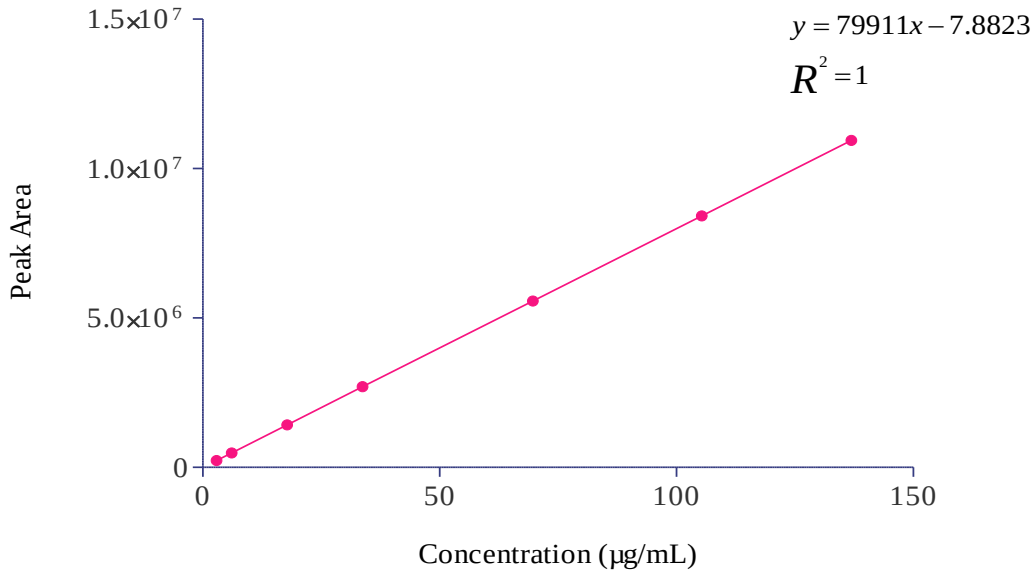

Figure 4.1: Calibration curve for *trans*-resveratrol in MeOH.

The calibration curve was obtained with the aid of GraphPad Prism 5.0 statistical software. The correlation coefficient (R^2) and equation of the linear regression of the standard curve were found to be 1 and $y = 79911x - 7.8823$ respectively.

4.3. Extraction solvent optimisation for the extraction of trans-resveratrol from yeast cells

The choice of extraction solvent for the extraction of *trans*-resveratrol in yeast cells is decided on the ability of the solvent to extract the analyte and the ease of evaporation of the solvent for later reconstitution. As the extraction process involves a substantial volume of solvent (~10 mL), the need to be able to evaporate it off is an important consideration.

The result of the extraction efficiency of the five solvents examined is presented in Figure 4.2.

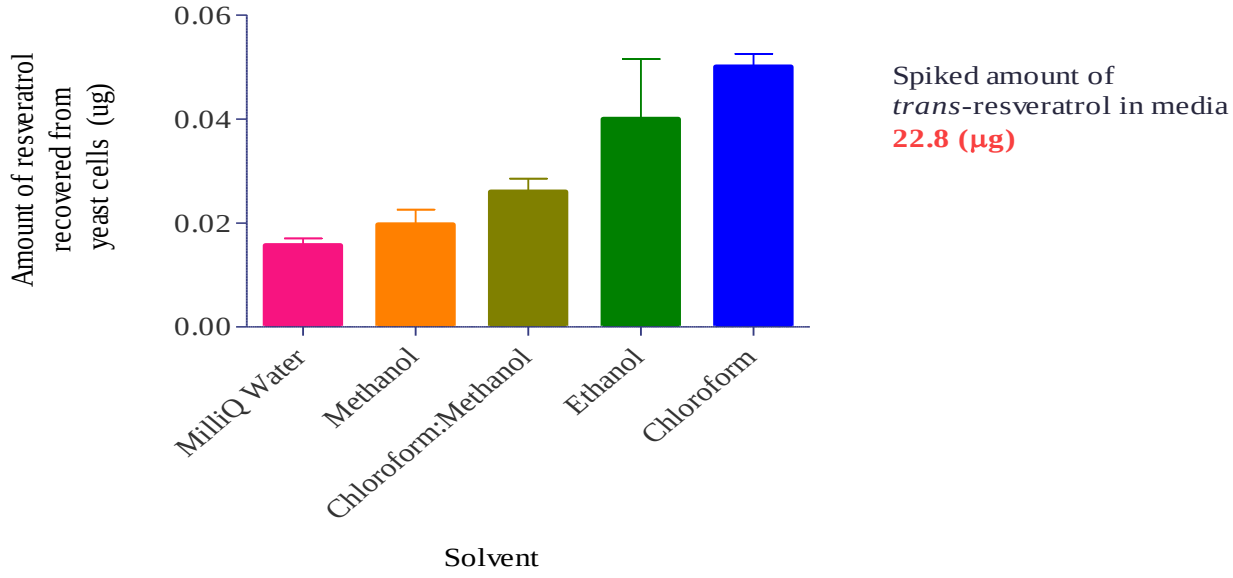

Figure 4.2: Comparison amount of *trans*-resveratrol extracted from yeast cells by different solvents.

Chloroform was found to be the most efficient solvent for *trans*-resveratrol extraction. This solvent is also relatively easy to evaporate with nitrogen gas. Ethanol was found to be the second best solvent for *trans*-resveratrol extraction but its lower volatility makes it less attractive.

The chloroform-methanol mixture was found to be very easy to evaporate compared to ethanol but less effective at extracting the analyte from the yeast cells. Water was the worst performer and most difficult to evaporate.

4.4. SPE extraction and clean up of trans-resveratrol in media

Of several extraction methods explored only the SPE method produced good precision and recovery results for *trans*-resveratrol extraction from the yeast media supernatant (table 4.2).

Replicate no.	Initial conc. (µg/mL)	Amount spiked (µg)	Recovered conc. (µg/mL)	Recovered amount (µg)	Recovery (%)
1	0.94	0.94	0.86	0.86	91.4
2	0.94	0.94	0.77	0.77	82.4
3	0.94	0.94	0.85	0.85	90.5
		Mean	**88.2**	**88.2**	**88.1**
		SD	0.05	0.05	4.95
		%RSD	**5.62**	**5.62**	**5.62**

Table 4.2: SPE clean up recoveries for *trans*-resveratrol in yeast media.

The median concentration (~10 µg/mL) of the five concentrations used for the dose response studies was examined in triplicate for extraction efficiency as outlined in section 3.3.4.2. The initial amount (at t = 0) of *trans*-resveratrol was 0.94 µg. 10 µL of each solution was analysed and recoveries for the triplicate determinations were found to be 91.4, 82.4 and 90.5% (mean = 88.1 ± 5.6% RSD).

4.4.1. Trans-resveratrol peak identity confirmation

To confirm the identity of the *trans*-resveratrol chromatographic peak the UV spectra of the sample and standard peaks are compared. To this end, the pure (93.44%) standard stock solution of the analyte was injected twice into the HPLC and the UV spectrum of the main peak obtained. Figures 4.3a-b and 4.4 are representative chromatograms and UV spectrum of *trans*-resveratrol respectively.

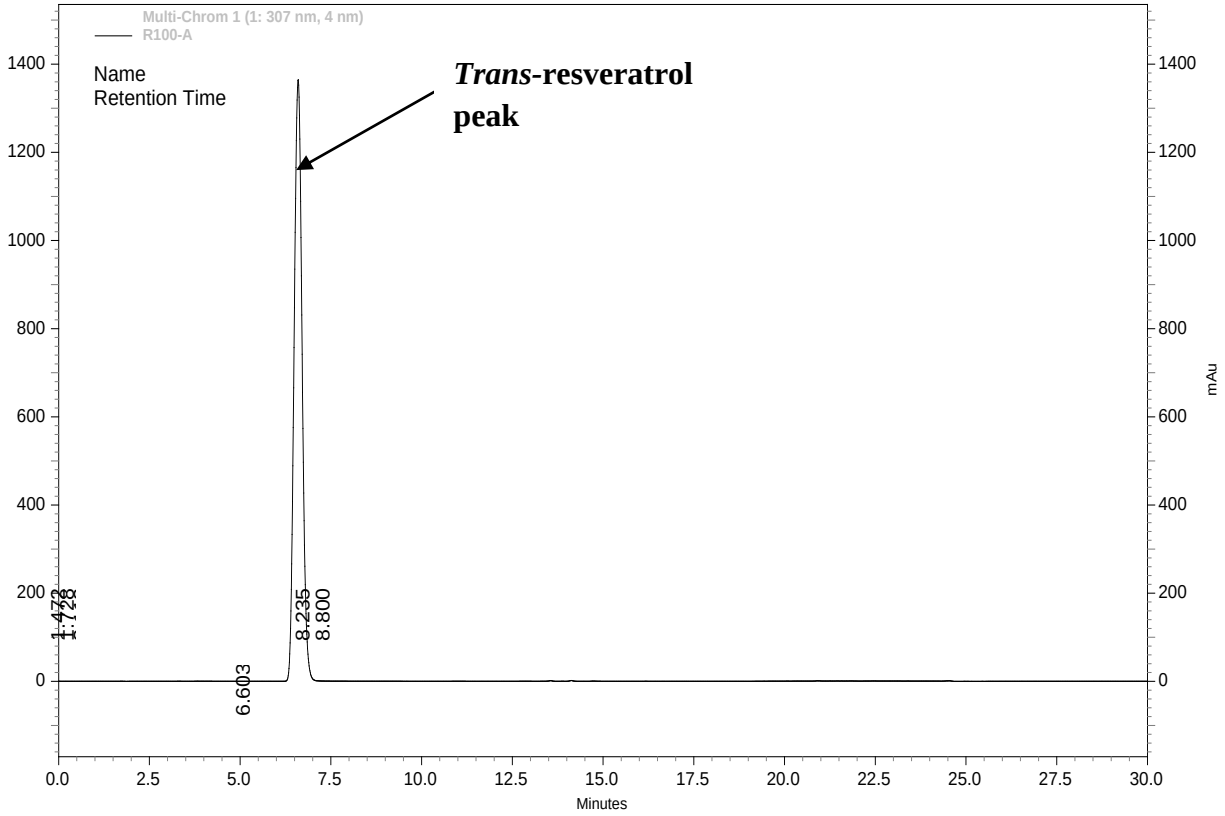

Figure 4.3a: Representative chromatogram showing *trans*-resveratrol standard peak at 22.48 µg/mL concentration in MeOH.

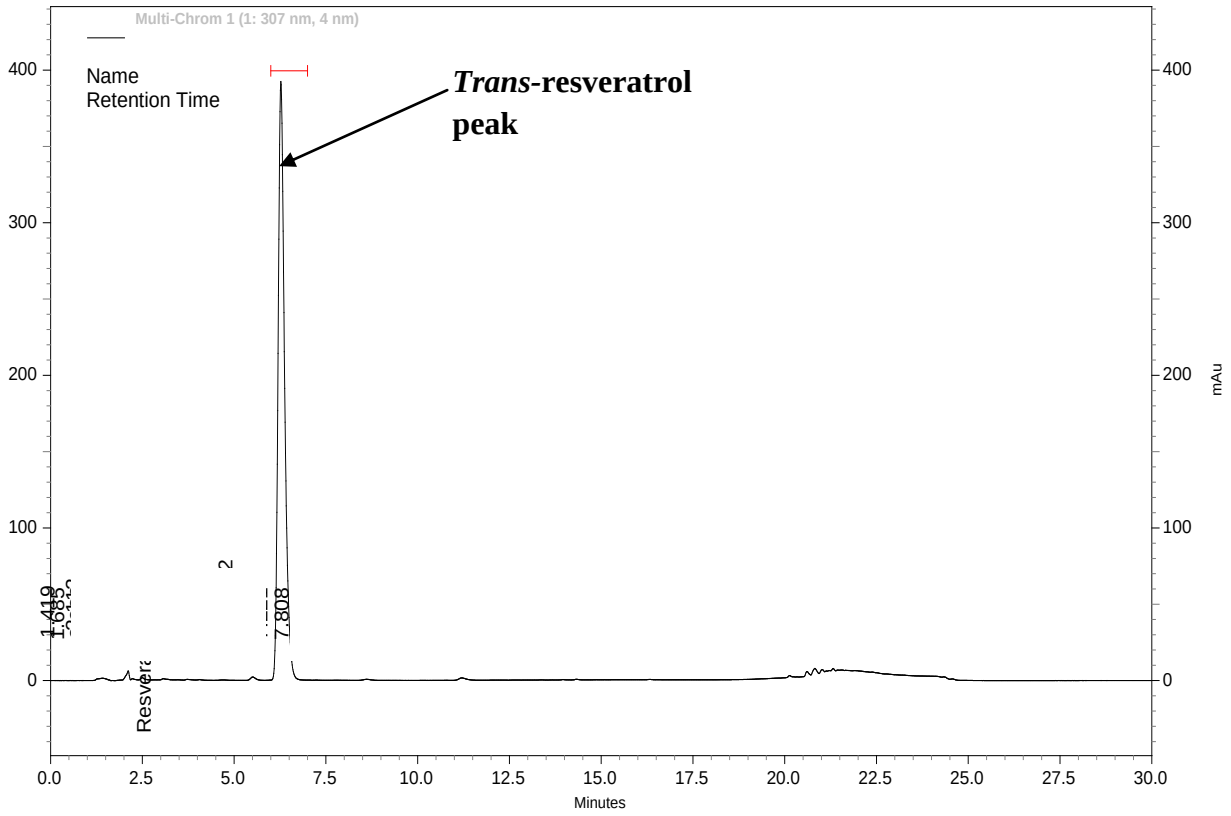

Figure 4.3b: Representative chromatogram showing *trans*-resveratrol sample peak at 22.82 µg/mL spike concentration in the yeast cell extract.

There is ~0.3 min (or 5%) shift in retention time between the sample and standard *trans*-resveratrol peaks and this level of shift has also been reported in the literature (Hanzlikova et al. 2004). Due to this shift, it is desirable to confirm the sample peak identity by comparing the UV spectrum of the standard and sample peaks. The UV spectrum of *trans*-resveratrol standard is shown in figure 4.4.

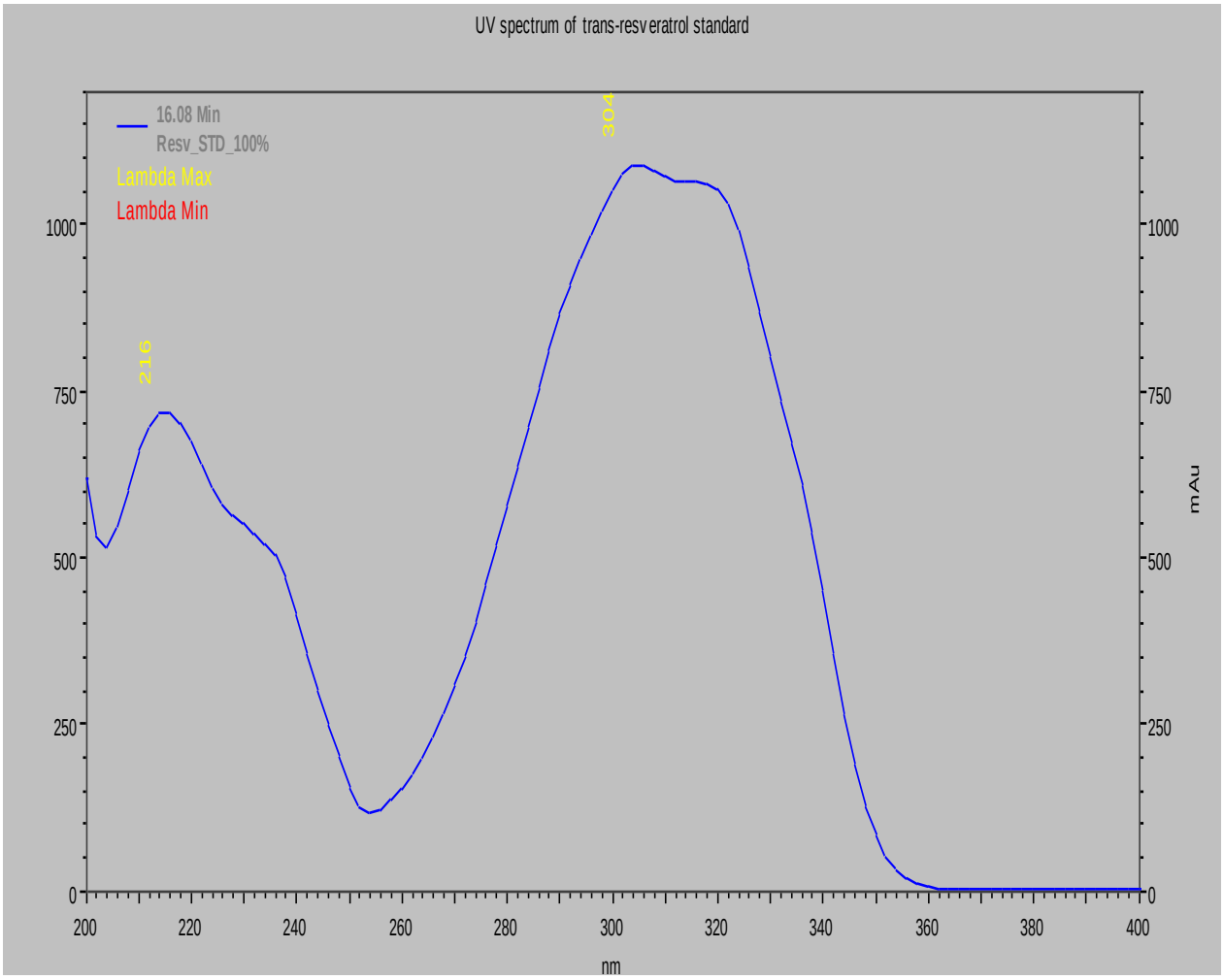

Figure 4.4: A representative UV spectrum of *trans*-resveratrol.

The λ_{max} at 216 and 304 nm is in agreement with those reported in the literature (Montsko et al. 2008).

The chromatograms and UV spectrum of *trans*-resveratrol in the standard solution, yeast cell extract, the media and experimental blank are compared (Figures 4.5a-b).

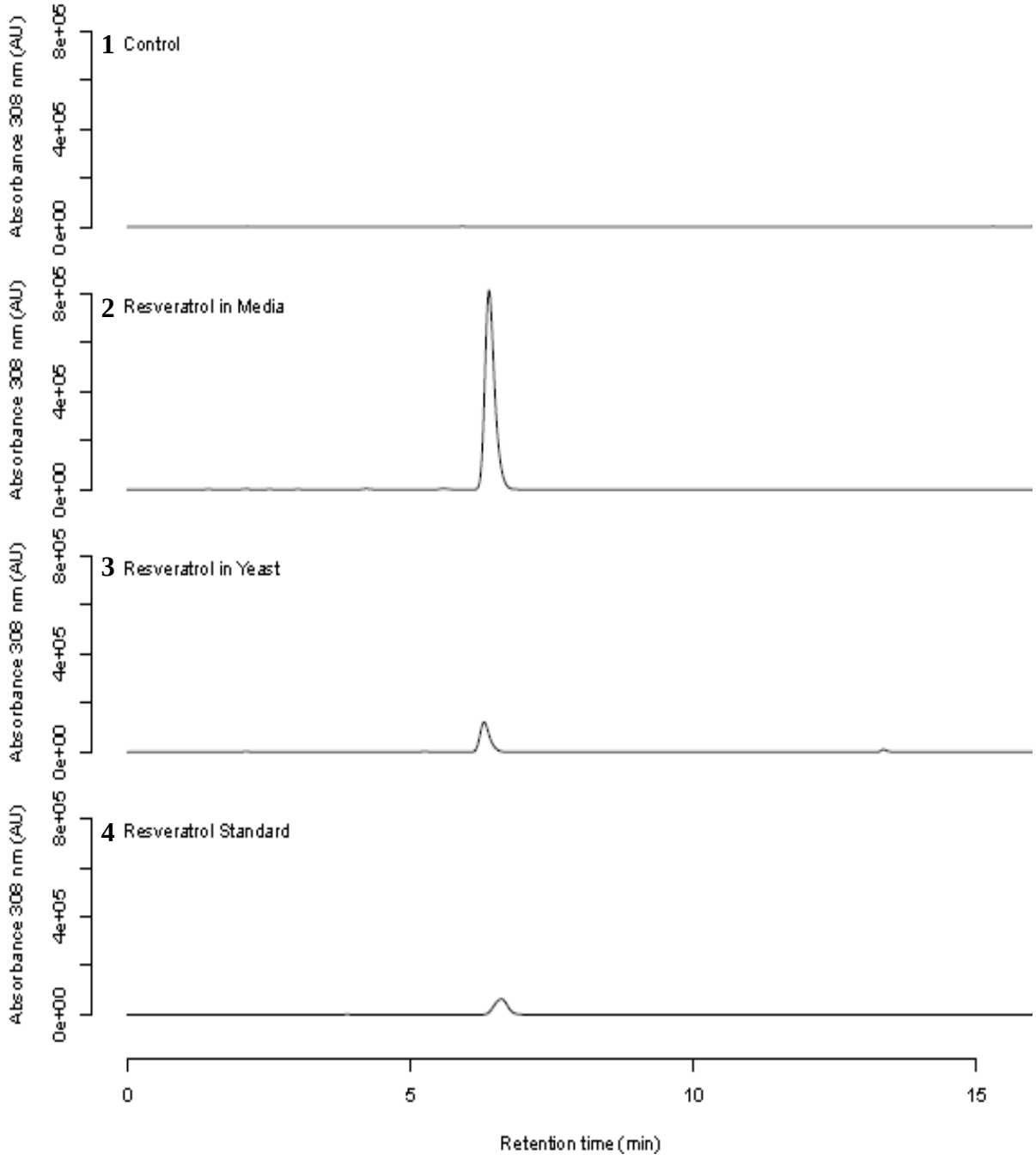

Figure 4.5a: Chromatograms of (1) experimental control, (2) *trans*-resveratrol in yeast media, (3) yeast extract, and standard solution in MeOH.

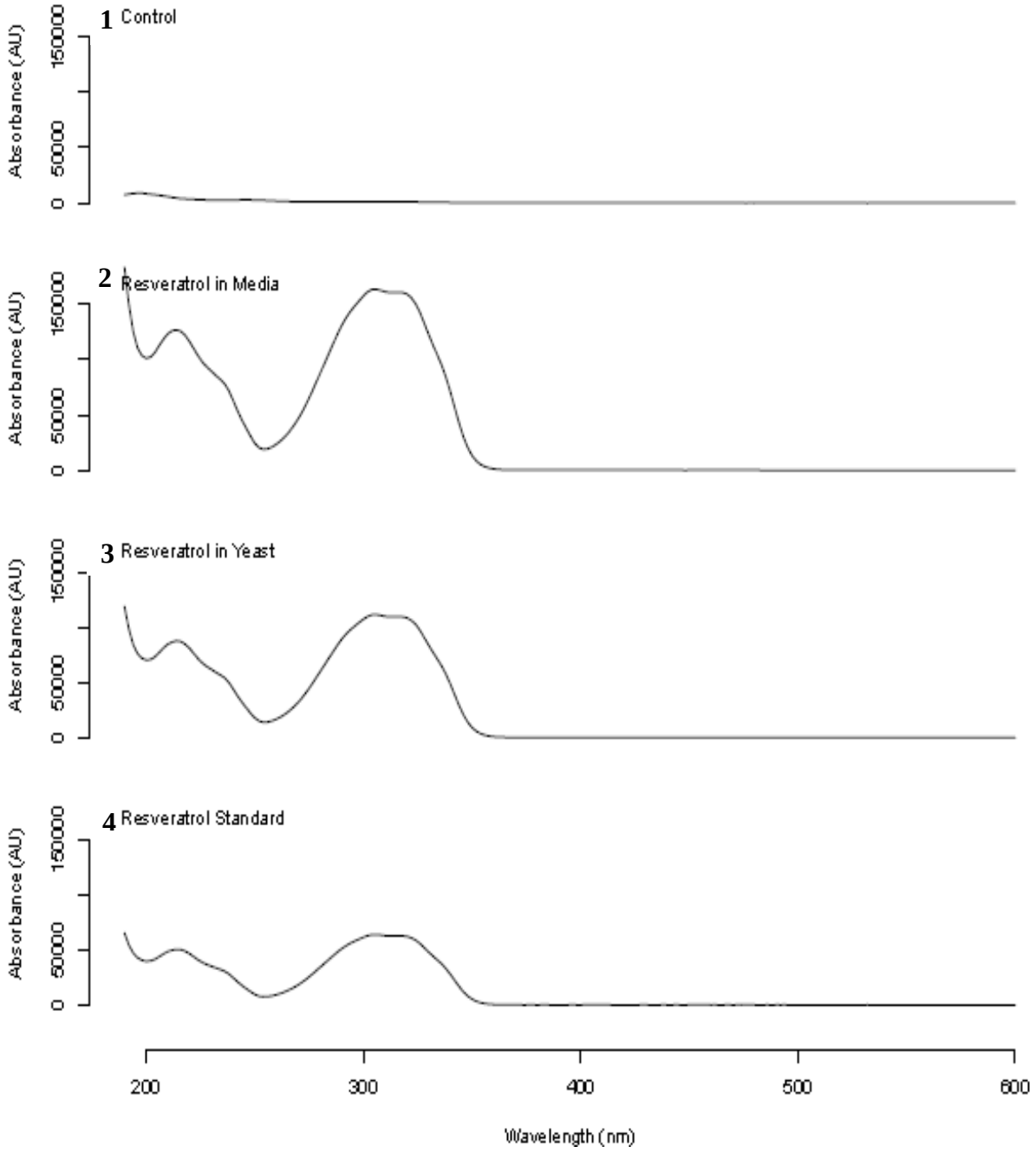

Figure 4.5b: Comparison of the UV spectrum of (1) experimental control, (2) *trans*-resveratrol in yeast media, (3) yeast extract, and (4) standard solution in MeOH.

As shown in Figure 4.5a, the *trans*-resveratrol peak in the standard share a common retention time and appear as a well resolved peak. Similarly Figure 4.5 shows that the UV-spectrum of the *trans*-resveratrol peak in the samples is comparable to that obtained for the standard.

4.5. Yeast cell viability test

Since this study covers quite a long time point (1 h-20 days), the viability of the *S. cerevisiae* yeast cells over this period was monitored (as outlined in section 3.3.5.6) to ensure that the cells were still alive at each experimental time point. This test was carried out for both media types studied and the result indicated that the cells remained viable at each time point with negligible non-viable cells observed.

4.6. Stability of trans-resveratrol in media

Knowledge of the stability of the analyte in the sample is important because of the relatively long time course of the study. A decreased concentration of *trans*-resveratrol in the media with time is assumed to be due to yeast uptake but it may also be due to analyte decomposition. To determine the stability of *trans*-resveratrol the highest concentration used for the dose response study (22.82 µg/mL) was spiked into 2% glucose synthetic media (10 mL) in triplicate and incubated under the same conditions as for the dose response study. 1 mL of the sample was then collected at each time point and analysed. The mean concentration of *trans*-resveratrol in the media was found to be 25.23 ± 2.34 µg/mL showing that *trans*-resveratrol is stable over the course of the study and a drop in concentration in the media is due to yeast uptake.

4.7. Dose response studies of trans-resveratrol

Following the establishment of the analytical method, a preliminary dose response study was conducted to determine *trans*-resveratrol uptake by *S.cerevisiae* yeast cells in 0.5% and 2% glucose synthetic media over 1 h, 1 day, 4 days and 10 days, with two replicates per concentration level studied. The results obtained were analysed and the uptake in both

media types was found to be significantly different. *Trans*-resveratrol uptake in the 2% glucose media was almost four times higher than that observed in the 0.5% glucose media.

To further examine this observation, the concentration of *trans*-resveratrol in the yeast cells as a function of initial dose concentration was examined for each time point in both media types. The results confirm the previous observation, namely that the uptake of *trans*-resveratrol increases with increase in glucose content of the media and the concentration in yeast also increases over time.

The standard dose response study of *trans*-resveratrol uptake into yeast cells was conducted over five time points (1 h, 1 day, 4 days, 10 days and 20 days). The expansion of the time point is to establish a point of inflexion (i.e., maximum uptake time point) for *trans*-resveratrol uptake into the yeast cells as it might be useful for future studies. The *trans*-resveratrol concentration reported in this study is also reported for the dry weight of the yeast cells - this avoids uncertainties introduced by difficulty in draining excess liquid from the yeast in a consistent way after centrifuging. Finally seven replicate experiments were carried out for each *trans*-resveratrol concentration and each time point examined to increase our confidence in the accuracy of our result. Besides the number of replicates, all other parameters are the same as for the preliminary study.

The result of the standard dose response study is presented in Figures 4.6a-f.

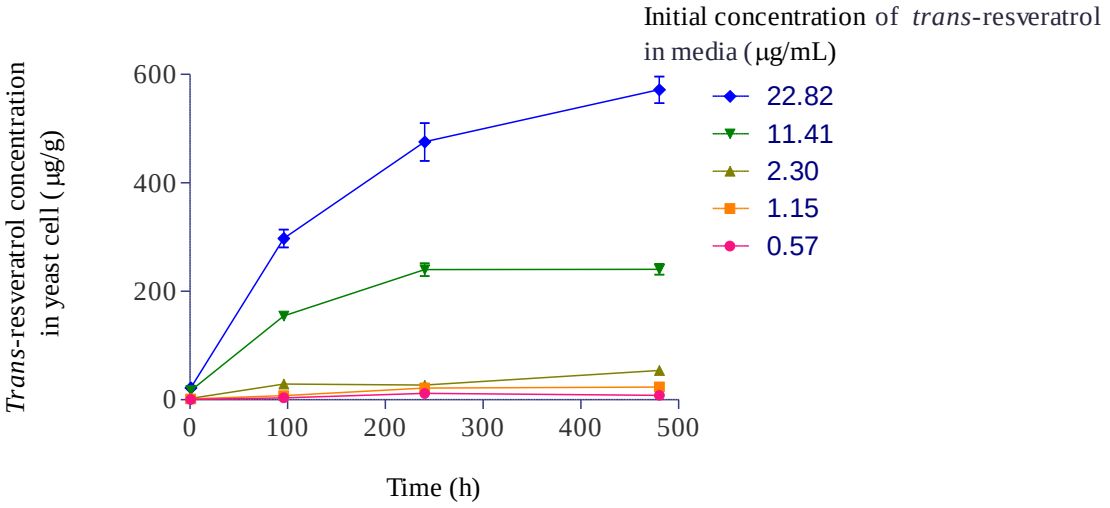

Figure 4.6a: *Trans*-resveratrol concentration in (wet) yeast cells grown in 0.5% glucose synthetic media

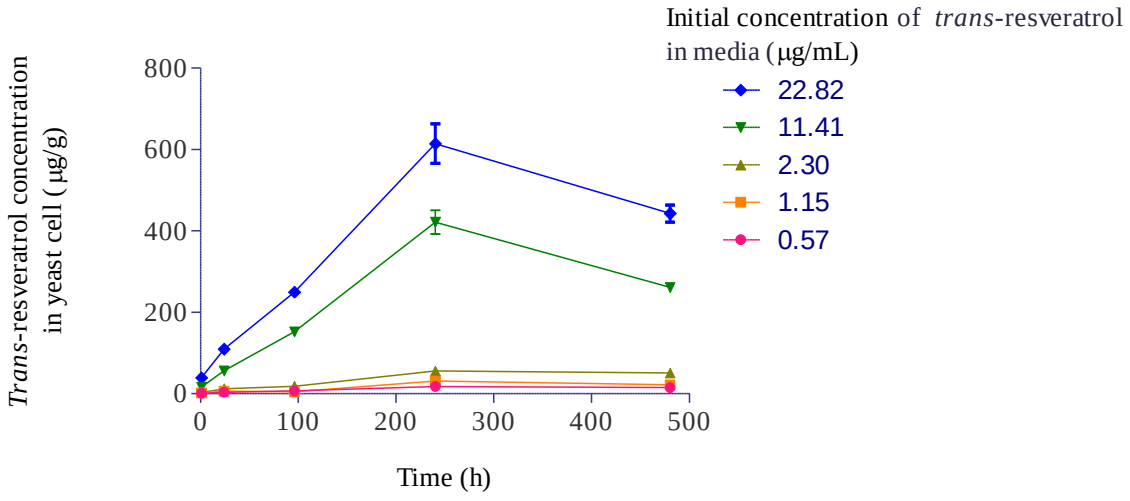

Figure 4.6b: *Trans*-resveratrol concentration in (wet)yeast cells grown in 2% glucose synthetic media

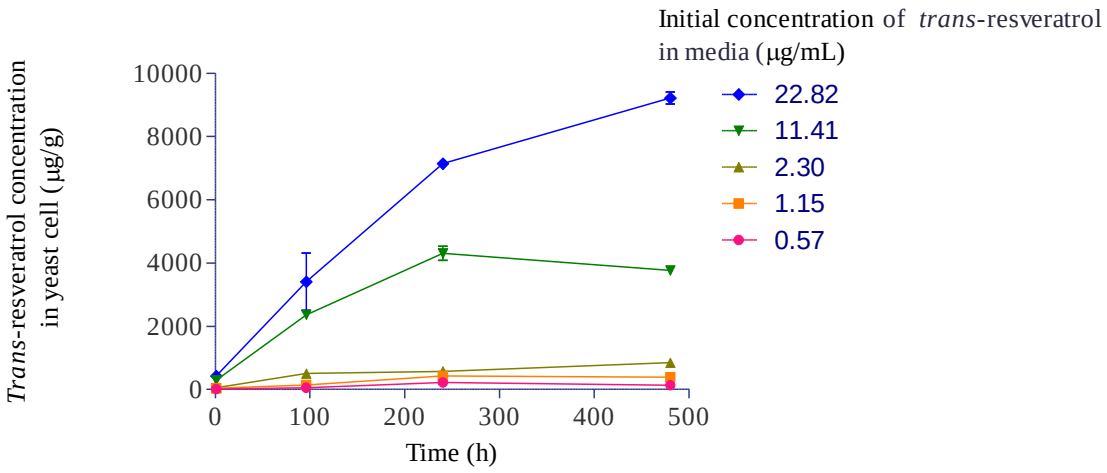

Figure 4.6c: *Trans*-resveratrol concentration in (dry) yeast cells grown in 0.5% glucose synthetic media.

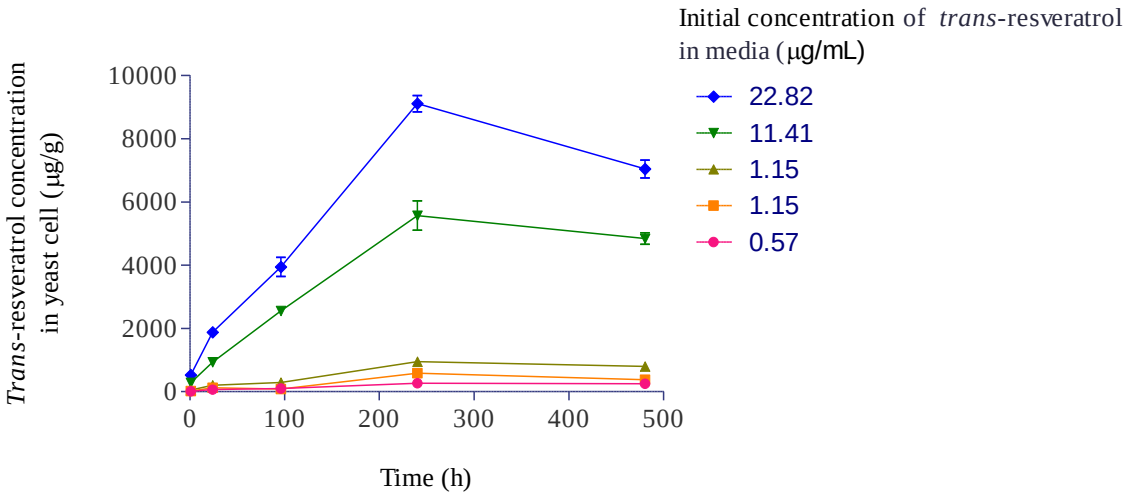

Figure 4.6d: *Trans*-resveratrol concentration in (dry) yeast cells grown in 2% glucose synthetic media.

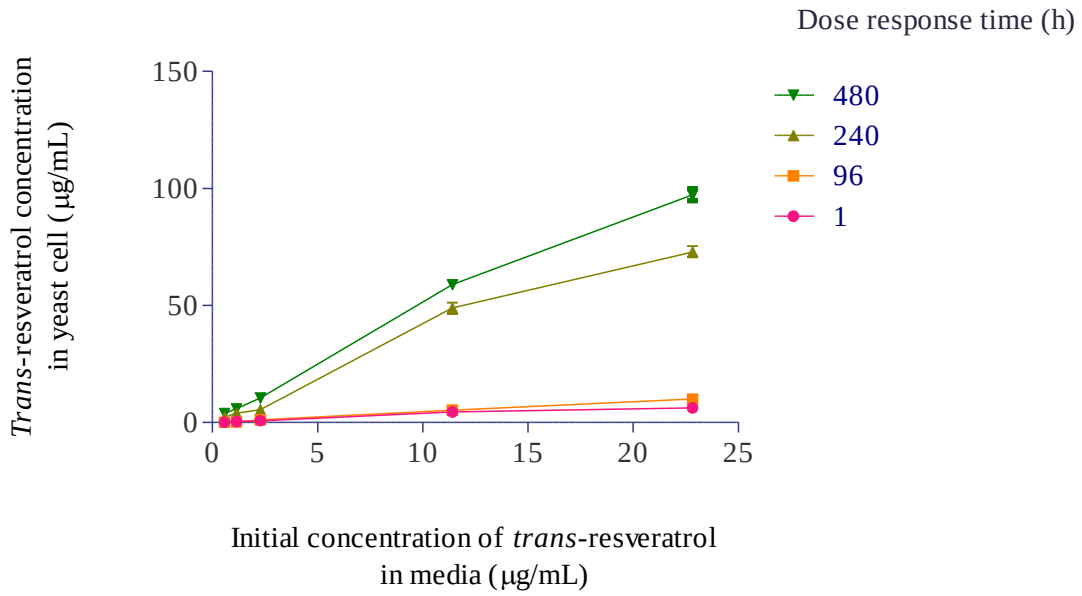

Figure 4.6e: *Trans*-resveratrol concentration in yeast cells grown in 0.5% glucose synthetic media.

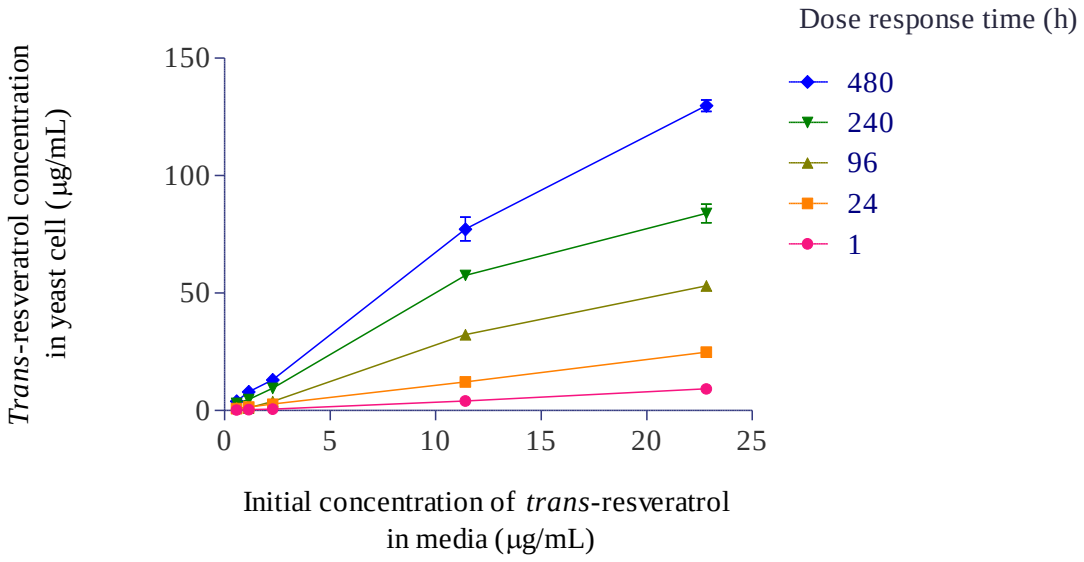

Figure 4.6f: *Trans*-resveratrol concentration in yeast cells grown in 2% glucose synthetic media.

Figure 4.6a compares the uptake of *trans*-resveratrol (in wet weight of yeast cells) as a function of time in 0.5% and 2% glucose synthetic yeast media (Figure 4.6b). As observed in the preliminary studies, the uptake of the analyte increases with the increasing glucose and analyte concentration in the media. However the uptake of *trans*-resveratrol peaked at 10 days in the 2% glucose synthetic media whereas in the 0.5% synthetic media, the uptake continued to increase steadily over time. Lin et al. (2000) suggested that the steady increase in *trans*-resveratrol uptake in 0.5% glucose synthetic media could be a result of replicative lifespan increase in the number of the yeast cells. A replicative lifespan of yeast cells is defined as the number of daughter cells an actively dividing mother cell can produce (Piper et al. 2006) and this has been reported to be greatly influenced by calorie restriction and hence the steady uptake of the analyte as new cells are continuously being produced.

Figures 4.6c-d present *trans*-resveratrol uptake (as dry weight of yeast cells) as a function of time, in both media types. As expected, the uptake trend is the same as that observed for the wet weight results.

Figure 4.6e gives the concentration of *trans*-resveratrol in the yeast cells grown in 0.5% synthetic yeast media versus the initial concentration of *trans*-resveratrol in the media. Figure 4.6f shows the concentration of *trans*-resveratrol in the yeast cells versus initial media concentration of the analyte for yeast cells grown in 2% synthetic yeast media. In both cases, the concentration of the analyte in yeast increases steadily with time.

Unlike in the preliminary study, there is less than a two-fold difference in *trans*-resveratrol uptake between yeast cells grown in 0.5% and 2% glucose synthetic media. In the preliminary studies, an increase in glucose content of the media was observed as having an

influence on uptake. This particular study shows that with or without calorie restriction, *trans*-resveratrol uptake remains approximately the same. Results from the yeast viability test (section 4.4) in the higher glucose content media (2% glucose) indicate that glucose content may have affected the chronological lifespan of the yeast cells - hence there is a steady increase in uptake but there is no significant difference in uptake between the high and low glucose media types.

4.8. DPPH scavenging activity of trans-resveratrol

One of the most researched aspects of *trans*-resveratrol is its antioxidant potential (Balkir et al. 2012). In this study, the antioxidant activity of *trans*-resveratrol was determined via a DPPH scavenging assay as described in section 3.3.7. The antioxidant activity is determined by the change in colour of a methanolic solution of the DPPH when *trans*-resveratrol solution is added. The result of the investigation is presented in figure 4.7.

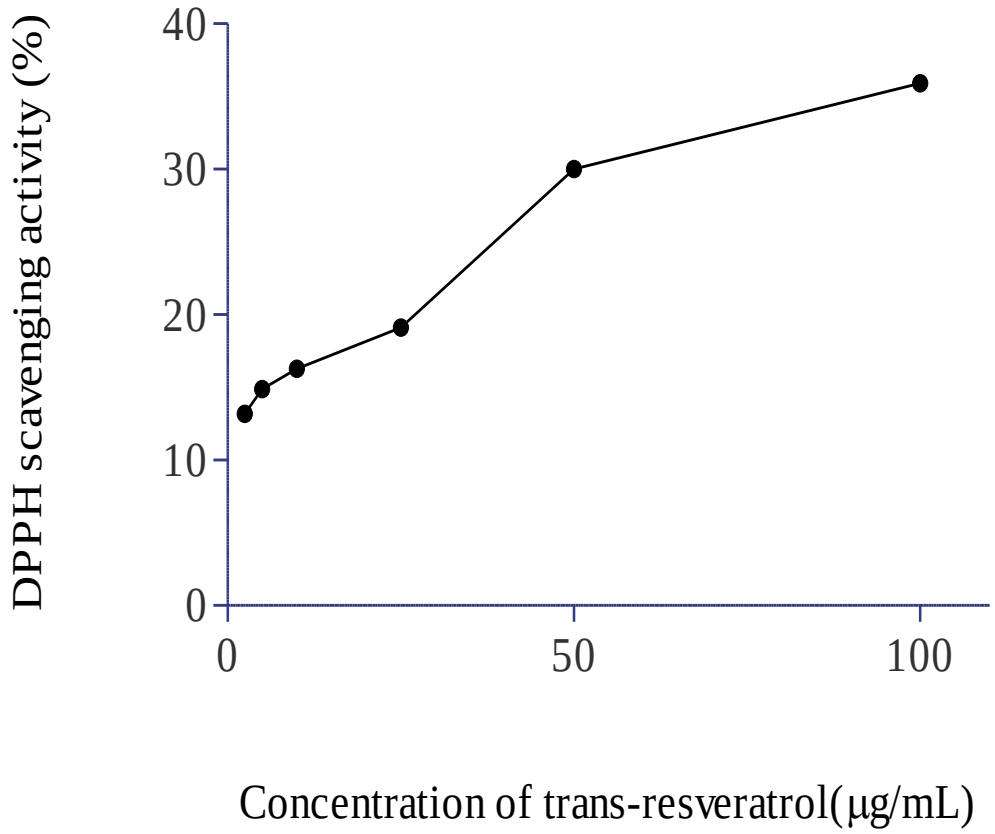

Figure 4.7: Antioxidant activity study of *trans*-resveratrol using the DPPH scavenging activity test.

The results show that the DPPH scavenging activity of *trans*-resveratrol is high and that the activity is concentration dependent. The results confirm that *trans*-resveratrol has high antioxidant activity.

4.9. References

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Piper, P.W, Harris, N.L, and MacLean, M, 2006, 'Preadaptation to efficient respiratory maintenance is essential both for maximal longevity and the retention of replicative potential in chronologically ageing yeast', *Mech. Ageing Dev.* 127, pp.733-740.

CHAPTER FIVE

CONCLUSION

5.0. Chapter overview

This chapter presents the conclusion of the findings of this study by giving a brief description of what was found and possible directions for future investigations.

5.1. Conclusions

Trans-resveratrol is one of the most sought after natural compounds to help ameliorate the effects of ageing and improve longevity. There have been many publications on the health benefits of *trans*-resveratrol including its anti-cancer, antioxidant, anti-inflammatory, neuroprotective, antiviral, cardio-protective properties and longevity effects, to name a few (Gulcin. 2010).

What has not been explored about this compound however is how much of it is taken up by the cells over time and what conditions favour this uptake. By using *S. cerevisiae* yeast cells as the simplest representation of eukaryotic cells, this study is the first to quantify the uptake of *trans*-resveratrol under different growth conditions.

The method used for the preparation of samples is new and easy to vary between large and small scale preparation and analysis (section 3.3.5.7).

The SPE cleanup method for the growth media worked effectively for the extraction of *trans*-resveratrol from the yeast supernatant. The method can separate out > 90% of the analyte with just one elution and the columns can be reused (provided they are kept moist after every extraction) at least 5 times. The only disadvantage with this method is that it may be time consuming especially when a large number of samples are involved, unless a SPE vacuum manifold is used.

The synthetic yeast media was found to be better for carrying out *trans*-resveratrol uptake studies in yeast cells because of its well defined chemical composition. Also, the uptake

results for both the calorie restricted (0.5% glucose) and non-calorie restricted (2% glucose) media demonstrated the effect of calorie availability (Kaeberlein et al. 2007) on uptake and the replicative life span of the yeast cells (Rockenfeller et al. 2008). The use of different *trans*-resveratrol concentrations in the media at different time points also showed the influence of time and concentration on uptake.

The optimum solvent for the extraction of *trans*-resveratrol from yeast cells was found to be analytical grade chloroform.

The stability testing of *trans*-resveratrol over each time point studied showed that the compound is quite stable and that the change in concentration in the media is due to yeast uptake rather than decomposition. The low concentration of *trans*-resveratrol following the dose response results however indicate that the compound is significantly metabolised *in vivo* (Walle et al. 2004).

The analysis of *trans*-resveratrol by HPLC-PDA is reliable and suited for this type of analysis, confirming other reports (He et al, 2006, Juan et al.2010 and Rudolf et al. 2005). The gradient mobile phase of MeOH and 0.1% aqueous formic acid successfully resolved the *trans*-resveratrol peak. The Alltech Alltima C 18 (150 mm x 4.6 mm, 5um) stationary phase performance was not adversely affected by the mobile phase composition and a reproducible retention time of 6.44 ± 0.33 min was observed.

Identity confirmation of the analyte was achieved by comparing the UV spectrum of the sample and standard which matched closely.

The LOQ of *trans*-resveratrol in the matrices used in this study was found to be 0.57 µg/mL.

The anti-oxidative ability of *trans*-resveratrol determined by the DPPH assay confirmed numerous claims that this compound is a strong antioxidant (Balkir et al. 2011 and Pandey et al. 2010).

The findings of this work are that *trans*-resveratrol uptake into yeast cells is significantly affected by calorie restriction and that uptake steadily increases over time when there is calorie restriction. By comparison, in a non-restricted calorie medium, the uptake peaks at 10 days beyond which the uptake of the analyte substantially falls. In addition, *trans*-resveratrol uptake was also found to be concentration dependent - the higher the concentration of the analyte, the higher the uptake. Finally, due to the metabolism of *trans*-resveratrol *in vivo*, it is difficult to quantify the actual amount taken up by the cells.

5.2. Future work and recommendation

The work reported in this thesis can be expanded to include the examination of the uptake mechanism of the yeast cells and the inter-laboratory testing of samples to improve method ruggedness.

Trans-resveratrol can be explored to find a link between *trans*-resveratrol uptake (in conjunction with calorie restriction) and the longevity of *S.cerevisiae* yeast cells and other eukaryotic cell lines.

To make the analyte identity confirmation more robust, LC-MS/MS analysis of the sample can also be done. A future study could include the measurement of the metabolites of *trans*-resveratrol following dose response. The identities of the metabolites have to be identified and a method for their determination developed. This may involve an enzyme digestion to free up the conjugated analyte.

The testing of *trans*-resveratrol from food sources such as grapes and berry could be examined to see if there is any difference in uptake compared to the using the pure compound.

5.3. References

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APPENDIX A

A.1. Calculations for stock and working standard solutions preparation.

Analyte: *trans*-resveratrol

Stock 1:

Mass of impure *trans*-resveratrol weighed = 1.341 mg

% purity of *trans*-resveratrol used = 93.44

Hence, mass of pure resveratrol in 1.341 mg weighed =

$$\frac{93.44}{100}\ x\ 1.341\ mg = 1.253\ mg\ (dissolved\ in\ 535.2\ \mu L\ of\ DMSO).$$

Given molar mass of *trans*-resveratrol = 228.25 g,

No. of moles of pure *trans*-resveratrol used = $\frac{1.253\ x\ 10^{-3}}{228.25} = 5.4896\ x\ 10^{\cdot 6}\ moles.$

Volume of solvent used = 535.2 $\mu L = 0.5352\ mL\ or\ 5.352\ x\ 10^{-4}\ L$

Thus, concentration (conc.) of stock solution = $\frac{5.4896\ x\ 10^{\cdot 6}\ moles}{5.352\ x\ 10^{-4}\ L}$ = 0.0102571 M = 10257 µM

or 2341.18 µg/mL. From this stock, the working standard solutions were prepared thus:

Working standards:

Required conc. of working standards = 2.5, 5 and 10 µM (or 0.57, 1.14 and 2.28 µg/mL)

Given final volume of working standard = 10100 µL,

Vol. of stock required (for 2.5 µM) = $\frac{2.5\ \mu M\ x\ 10100\ \mu L}{10257 \mu M}$ = 2.46 µL and 4.92 µL and 9.84 µL for 5 and 10 µM respectively.

Amount of analyte in working standard

Given stock concentration = 10257 µM or 2341.18 µg/mL 2341.18 µg in 1000 µL

Thus 2.46 x 2341.18 /1000 µg = 5.76 µg and hence, 4.92 µL = 11.52 µg and 9.84 = 23.04 µg respectively.

Similarly, for

Stock 2:

Mass of pure *trans*-resveratrol used = 16.150 mg; from this the stock conc. was found to be 29204.34 µg/mL (or 127950 µM).

APPENDIX B

B.1. Raw data summary for trans-resveratrol analysis in yeast cells grown in 0.5% glucose synthetic media.

Table B.1. *Trans*- resveratrol dose response result data summary for 1 h time point in 0.5% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells g		µg resv/g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. -2.5 µM _0.5%_1H_R1	0.57	2.5	5.70	*2.233*	*0.207*	*2.440*	*0.377*	*0.015*	*0.549*	*13.80*	*42.81*	3.63
Resv. -2.5 µM _0.5%_1H_R2	0.57	2.5	5.70	*2.445*	*0.195*	*2.640*	*0.392*	*0.015*	*0.497*	*13.27*	*46.32*	3.42
Resv. -2.5 µM _0.5%_1H_R3	0.57	2.5	5.70	*2.402*	*0.156*	*2.558*	*0.262*	0.016	*0.595*	*9.87*	*44.88*	2.74
Resv. -2.5 µM _0.5%_1H_R4	0.57	2.5	5.70	*3.029*	*0.155*	*3.184*	*0.430*	*0.015*	*0.360*	*10.33*	*55.85*	2.72
Resv. -2.5 µM _0.5%_1H_R5	0.57	2.5	5.70	*2.533*	*0.168*	*2.701*	*0.368*	*0.015*	*0.457*	*11.35*	*47.38*	2.95
Resv. -2.5 µM _0.5%_1H_R6	0.57	2.5	5.70	*2.419*	*0.335*	*2.754*	*0.394*	*0.015*	*0.849*	23.10	*48.32*	5.88
Resv. -2.5 µM _0.5%_1H_R7	0.57	2.5	5.70	4.360	0.267	*4.627*	0.178	*0.015*	1.497	*18.29*	81.17	4.68
			Average	2.510	0.203	2.713	0.371	0.015	0.551	12.819	47.59	
			SD	0.272	0.068	0.256	0.057	0.000	0.167	3.101	4.486	
			%RSD	10.84	33.64	9.43	15.44	1.399	30.26	24.19	9.426	
Resv._5 µM _0.5%_1H_R1	1.14	5.0	11.43	*6.721*	*0.738*	*7.459*	0.239	*0.014*	*3.089*	*51.25*	*65.26*	6.46
Resv._5 µM _0.5%_1H_R2	1.14	5.0	11.43	*5.535*	*0.413*	*5.948*	*0.272*	*0.014*	*1.521*	*28.88*	*52.04*	3.61
Resv._5 µM _0.5%_1H_R3	1.14	5.0	11.43	*5.033*	*0.473*	*5.506*	*0.298*	*0.015*	*1.588*	*31.53*	48.17	4.14
Resv._5 µM _0.5%_1H_R4	1.14	5.0	11.43	*6.676*	*0.347*	*7.023*	*0.258*	*0.016*	*1.346*	21.96	*61.45*	3.04
Resv._5 µM _0.5%_1H_R5	1.14	5.0	11.43	*6.025*	*0.514*	*6.539*	*0.277*	0.013	*1.854*	*41.12*	*57.21*	4.50
Resv._5 µM _0.5%_1H_R6	1.14	5.0	11.43	4.790	0.844	*5.634*	*0.242*	*0.016*	3.489	*54.45*	*49.29*	7.38
Resv._5 µM _0.5%_1H_R7	1.14	5.0	11.43	*5.652*	*0.384*	*6.036*	*0.255*	*0.015*	*1.505*	*25.10*	*52.81*	3.36
			Average	5.940	0.478	6.351	0.267	0.015	1.817	38.7	56.34	
			SD	0.668	0.141	0.788	0.020	0.001	0.645	12.2	6.12	
			%RSD	11.24	29.45	12.41	7.36	4.003	35.48	31.51	10.86	
Resv. _10 µM _0.5%_1H_R1	2.28	10.0	22.84	*0.076*	0.481	*0.557*	*0.249*	*0.016*	1.929	*30.83*	*2.439*	2.11
Resv. _10 µM _0.5%_1H_R2	2.28	10.0	22.84	*0.095*	*0.762*	*0.857*	0.220	*0.016*	*3.462*	*48.85*	*3.752*	3.34
Resv. _10 µM _0.5%_1H_R3	2.28	10.0	22.84	*0.078*	*0.719*	*0.797*	*0.260*	*0.017*	*2.765*	*42.54*	*3.491*	3.15
Resv. _10 µM _0.5%_1H_R4	2.28	10.0	22.84	*0.098*	0.000	*0.098*	*0.311*	*0.017*	0.000	*0.00*	0.429	0.00
Resv. _10 µM _0.5%_1H_R5	2.28	10.0	22.84	0.129	*1.169*	*1.298*	*0.364*	0.014	*3.209*	86.59	5.682	5.12
Resv. _10 µM _0.5%_1H_R6	2.28	10.0	22.84	*0.057*	*0.893*	*0.950*	*0.284*	*0.015*	*3.150*	*57.99*	*4.159*	3.91
Resv. _10 µM _0.5%_1H_R7	2.28	10.0	22.84	*0.076*	*0.839*	*0.915*	*0.307*	*0.015*	*2.730*	*57.07*	*4.006*	3.67
			Average	0.080	0.876	0.760	0.296	0.016	3.063	47.457	3.570	
			SD	0.015	0.177	0.404	0.042	0.001	0.311	11.24	0.681	
			%RSD	18.63	20.18	53.17	14.04	5.142	10.16	23.69	19.09	
Resv. _50 µM _0.5%_1H_R1	11.41	50.0	113.90	0.000	*3.602*	*3.602*	*0.232*	*0.016*	*15.52*	*230.9*	*3.162*	3.16
Resv. _50 µM _0.5%_1H_R2	11.41	50.0	113.90	0.000	6.835	*6.835*	*0.284*	*0.016*	24.07	435.4	6.001	6.00
Resv. _50 µM _0.5%_1H_R3	11.41	50.0	113.90	0.000	*4.324*	*4.324*	*0.322*	*0.015*	*13.42*	*296.2*	*3.796*	3.80
Resv. _50 µM _0.5%_1H_R4	11.41	50.0	113.90	0.000	*4.671*	*4.671*	*0.297*	*0.015*	*15.72*	*305.3*	*4.101*	4.10
Resv. _50 µM _0.5%_1H_R5	11.41	50.0	113.90	0.000	*4.445*	*4.445*	*0.223*	*0.015*	*19.98*	*296.3*	*3.903*	3.90
Resv. _50 µM _0.5%_1H_R6	11.41	50.0	113.90	0.000	*4.752*	*4.752*	*0.232*	*0.015*	*20.52*	*316.8*	*4.172*	4.17
Resv. _50 µM _0.5%_1H_R7	11.41	50.0	113.90	0.000	*5.519*	*5.519*	*0.298*	*0.016*	*18.51*	*353.8*	*4.845*	4.85
			Average	0.000	4.552	4.772	0.227	0.015	17.28	299.879	3.997	
			SD	0.000	0.625	1.090	0.040	0.000	2.818	40.015	0.549	
			%RSD	#DIV/0!	13.74	22.85	17.63	2.672	16.31	13.34	13.74	
Resv. _100 µM _0.5%_1H_R1	22.82	100.0	228.4	0.000	*6.664*	*6.664*	*0.272*	*0.016*	*24.48*	*427.2*	*2.918*	2.92
Resv. _100 µM _0.5%_1H_R2	22.82	100.0	228.4	0.000	4.813	*4.813*	*0.268*	*0.016*	*17.99*	*310.5*	*2.107*	2.11
Resv. _100 µM _0.5%_1H_R3	22.82	100.0	228.4	0.000	*6.385*	*6.385*	*0.294*	*0.014*	*21.71*	*473.0*	*2.796*	2.80
Resv. _100 µM _0.5%_1H_R4	22.82	100.0	228.4	0.000	*6.658*	*6.658*	*0.318*	*0.015*	*20.95*	*456.0*	*2.915*	2.92
Resv. _100 µM _0.5%_1H_R5	22.82	100.0	228.4	0.000	*7.380*	*7.380*	*0.307*	*0.015*	*24.02*	*495.3*	*3.231*	3.23
Resv. _100 µM _0.5%_1H_R6	22.82	100.0	228.4	0.000	*5.830*	*5.830*	0.253	0.014	*23.07*	*404.9*	*2.553*	2.55
Resv. _100 µM _0.5%_1H_R7	22.82	100.0	228.4	0.000	*5.235*	*5.235*	*0.316*	*0.016*	*16.56*	*327.2*	*2.292*	2.29
			Average	0.000	6.138	6.288	0.296	0.015	21.25	413.4	2.688	
			SD	0.000	0.744	0.879	0.022	0.001	3.01	71.13	0.392	
			%RSD	#DIV/0!	12.12	13.98	7.389	5.977	14.15	17.20	14.60	

Table B.2. *Trans*- resveratrol dose response result data summary for 4 days time point in 0.5% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered Amount (µg)		Total recovered amount	Weight of cells		µg resv/g of yeast (µg/g)		Percentage Recovery	percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _0.5%_4D_R1	0.57	2.50	5.77	4.084	0.135	4.219	0.177	0.011	3.828	62.59	82.49	2.34
Resv. _2.5 µM _0.5%_4D_R2	0.57	2.50	5.77	6.080	0.173	6.253	0.202	0.011	4.271	81.39	120.3	2.99
Resv. _2.5 µM _0.5%_4D_R3	0.57	2.50	5.77	6.360	0.114	6.474	0.191	0.011	2.995	50.94	120.1	1.98
Resv. _2.5 µM _0.5%_4D_R4	0.57	2.50	5.77	4.713	0.165	4.878	0.197	0.013	4.191	63.02	95.99	2.86
Resv. _2.5 µM _0.5%_4D_R5	0.57	2.50	5.77	3.211	0.058	3.269	0.227	0.011	1.280	26.14	60.68	1.01
Resv. _2.5 µM _0.5%_4D_R6	0.57	2.50	5.77	3.742	0.110	3.853	0.093	0.011	5.904	50.59	74.42	1.91
Resv. _2.5 µM _0.5%_4D_R7	0.57	2.50	5.77	4.103	0.000	4.103	0.194	0.010	0.000	0.00	71.11	0.00
			Average	4.322	0.139	4.824	0.191	0.011	4.238	61.707	94.1	
			SD	0.992	0.029	1.303	0.009	0.000	1.060	12.545	21.99	
			%RSD	22.95	20.49	27.02	4.924	3.06	25.01	20.33	23.38	
Resv. _5 µM _0.5%_4D_R1	1.14	5.00	11.54	14.43	0.191	14.62	0.183	0.011	5.24	87.81	133.3	1.66
Resv. _5 µM _0.5%_4D_R2	1.14	5.00	11.54	13.95	0.478	14.42	0.173	0.010	13.82	236.5	141.5	4.14
Resv. _5 µM _0.5%_4D_R3	1.14	5.00	11.54	11.99	0.349	12.34	0.191	0.012	9.115	141.8	119.0	3.02
Resv. _5 µM _0.5%_4D_R4	1.14	5.00	11.54	16.89	0.202	17.09	0.160	0.011	6.332	93.57	155.1	1.75
Resv. _5 µM _0.5%_4D_R5	1.14	5.00	11.54	7.186	0.145	7.331	0.134	0.011	5.429	64.86	68.56	1.26
Resv. _5 µM _0.5%_4D_R6	1.14	5.00	11.54	8.066	0.218	8.284	0.139	0.010	7.828	108.8	79.33	1.89
Resv. _5 µM _0.5%_4D_R7	1.14	5.00	11.54	6.931	0.636	7.567	0.151	0.014	21.07	224.1	87.63	5.51
			Average	12.08	0.264	12.35	0.16	0.01	6.788	148.76	119.32	
			SD	3.80	0.125	3.838	0.02	0.00	1.655	65.98	30.25	
			%RSD	31.44	47.40	31.08	12.17	7.70	24.38	44.35	25.35	
Resv. _10 µM _0.5%_4D_R1	2.28	10.00	23.08	24.37	0.866	25.23	0.217	0.010	19.93	428.7	124.3	3.75
Resv. _10 µM _0.5%_4D_R2	2.28	10.00	23.08	28.82	1.106	29.92	0.187	0.010	29.62	564.3	148.8	4.79
Resv. _10 µM _0.5%_4D_R3	2.28	10.00	23.08	28.44	0.900	29.34	0.160	0.010	28.09	436.9	142.7	3.90
Resv. _10 µM _0.5%_4D_R4	2.28	10.00	23.08	25.57	1.313	26.89	0.218	0.012	30.18	551.6	139.2	5.69
Resv. _10 µM _0.5%_4D_R5	2.28	10.00	23.08	29.03	1.154	30.18	0.229	0.011	25.17	515.2	150.8	5.00
Resv. _10 µM _0.5%_4D_R6	2.28	10.00	23.08	22.09	1.153	23.25	0.181	0.011	31.81	524.3	120.7	5.00
Resv. _10 µM _0.5%_4D_R7	2.28	10.00	23.08	24.80	1.125	25.93	0.188	0.014	29.97	401.9	131.8	4.88
			Average	25.68	1.125	27.92	0.192	0.011	29.14	503.5	137.8	
			SD	2.563	0.133	2.164	0.022	0.001	2.280	57.65	12.57	
			%RSD	9.979	11.79	7.752	11.56	7.357	7.823	11.45	9.121	
Resv. _50 µM _0.5%_4D_R1	11.41	50.00	115.2	98.72	1.808	100.52	0.176	0.011	51.30	860.9	93.55	1.57
Resv. _50 µM _0.5%_4D_R2	11.41	50.00	115.2	94.46	5.421	99.88	0.169	0.011	160.00	2420	105.5	4.71
Resv. _50 µM _0.5%_4D_R3	11.41	50.00	115.2	107.0	5.098	112.06	0.179	0.011	142.50	2339	115.0	4.43
Resv. _50 µM _0.5%_4D_R4	11.41	50.00	115.2	101.0	5.741	106.78	0.173	0.012	166.13	2373	112.6	4.98
Resv. _50 µM _0.5%_4D_R5	11.41	50.00	115.2	92.49	4.881	97.37	0.158	0.011	154.74	2239	101.5	4.24
Resv. _50 µM _0.5%_4D_R6	11.41	50.00	115.2	113.7	5.271	118.95	0.173	0.012	152.26	2178	121.6	4.58
Resv. _50 µM _0.5%_4D_R7	11.41	50.00	115.2	98.08	5.443	103.53	0.178	0.010	152.97	2642	108.8	4.73
			Average	98.62	5.309	105.93	0.175	0.011	152.50	2365	110.8	
			SD	5.111	0.299	8.328	0.004	0.001	6.358	162.1	7.150	
			%RSD	5.183	5.630	7.86	2.054	5.943	4.169	6.854	6.451	
Resv. _100 µM _0.5%_4D_R1	22.82	100.00	230.4	1062	9.10	1071	0.174	0.010	261.7	4737	480.6	3.95
Resv. _100 µM _0.5%_4D_R2	22.82	100.00	230.4	1128	10.41	1138	0.153	0.011	340.4	4864	512.1	4.52
Resv. _100 µM _0.5%_4D_R3	22.82	100.00	230.4	1055	12.26	1067	0.148	0.010	414.9	6257	484.5	5.32
Resv. _100 µM _0.5%_4D_R4	22.82	100.00	230.4	1085	11.85	1097	0.215	0.097	275.7	610.8	496.8	5.14
Resv. _100 µM _0.5%_4D_R5	22.82	100.00	230.4	1122	9.01	1131	0.182	0.091	248.0	495.2	506.7	3.91
Resv. _100 µM _0.5%_4D_R6	22.82	100.00	230.4	1084	10.31	1094	0.157	0.010	328.9	5003	492.8	4.47
Resv. _100 µM _0.5%_4D_R7	22.82	100.00	230.4	1208	9.664	1217	0.146	0.010	330.3	4737	545.2	4.20
			Average	1089	10.06	1100	0.160	0.024	297.5	3408	495.6	
			SD	30.18	1.055	29.68	0.015	0.033	40.27	2214	12.27	
			%RSD	2.771	10.49	2.699	9.104	139.9	13.53	64.96	2.476	

Table B.3. *Trans*- resveratrol dose response result data summary for 10 days time point in 0.5% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		Resveratrol/g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _0.5%_10D_R1	0.57	2.50	5.78	***0.108***	***2.142***	***2.250***	***0.189***	0.009	***11.36***	***230.3***	***38.92***	37.06
Resv. _2.5 µM _0.5%_10D_R2	0.57	2.50	5.78	***0.104***	***2.257***	***2.361***	***0.200***	***0.009***	***11.27***	***245.3***	***40.85***	39.05
Resv. _2.5 µM _0.5%_10D_R3	0.57	2.50	5.78	***0.114***	2.904	3.019	***0.218***	***0.009***	***13.32***	30.90	***52.23***	50.25
Resv. _2.5 µM _0.5%_10D_R4	0.57	2.50	5.78	***0.153***	***2.598***	***2.751***	0.178	***0.009***	14.62	27.94	***47.59***	44.95
Resv. _2.5 µM _0.5%_10D_R5	0.57	2.50	5.78	***0.084***	***2.355***	***2.439***	***0.201***	***0.011***	***11.72***	***218.1***	***42.19***	40.75
Resv. _2.5 µM _0.5%_10D_R6	0.57	2.50	5.78	0.155	***2.354***	***2.509***	***0.201***	***0.010***	***11.72***	***230.8***	***43.41***	40.72
Resv. _2.5 µM _0.5%_10D_R7	0.57	2.50	5.78	***0.082***	***2.098***	***2.180***	***0.190***	***0.011***	***11.06***	***194.3***	37.72	36.30
			Average	**0.107**	**2.301**	**2.415**	**0.193**	**0.010**	**11.959**	**223.7**	**44.20**	
			SD	**0.026**	**0.180**	**0.204**	**0.009**	**0.001**	**1.330**	**19.10**	**4.892**	
			%RSD	**23.99**	**7.835**	**8.440**	**4.879**	**7.514**	**11.121**	**8.538**	**11.07**	
Resv. _5 µM _0.5%_10D_R1	1.15	5.00	11.59	***0.197***	***4.119***	***4.316***	0.215	***0.010***	***19.14***	***396.1***	***37.24***	35.54
Resv. _5 µM _0.5%_10D_R2	1.15	5.00	11.59	***0.189***	***3.215***	***3.405***	***0.199***	***0.011***	16.19	306.2	29.38	27.74
Resv. _5 µM _0.5%_10D_R3	1.15	5.00	11.59	0.228	5.025	5.253	***0.212***	***0.010***	***23.72***	***497.5***	***45.33***	43.36
Resv. _5 µM _0.5%_10D_R4	1.15	5.00	11.59	***0.191***	***4.882***	***5.073***	***0.194***	***0.010***	***25.14***	***469.4***	***43.77***	42.12
Resv. _5 µM _0.5%_10D_R5	1.15	5.00	11.59	***0.209***	***3.870***	***4.079***	***0.201***	***0.011***	***19.26***	***361.7***	***35.19***	33.39
Resv. _5 µM _0.5%_10D_R6	1.15	5.00	11.59	***0.211***	***4.409***	***4.620***	***0.213***	***0.010***	***20.70***	***423.9***	***39.86***	38.04
Resv. _5 µM _0.5%_10D_R7	1.15	5.00	11.59	***0.215***	***4.239***	***4.453***	***0.195***	***0.010***	***21.69***	***411.5***	***38.42***	36.57
			Average	**0.202**	**4.122**	**4.324**	**0.202**	**0.010**	**21.61**	**426.7**	**39.97**	
			SD	**0.011**	**0.558**	**0.560**	**0.008**	**0.000**	**2.421**	**49.48**	**3.894**	
			%RSD	**5.378**	**13.544**	**12.961**	**4.049**	**1.923**	**11.20**	**11.60**	**9.743**	
Resv. _10 µM _0.5%_10D_R1	2.30	10.00	23.19	***0.536***	***1.839***	***2.375***	***0.096***	0.005	***19.11***	***367.7***	***10.24***	7.93
Resv. _10 µM _0.5%_10D_R2	2.30	10.00	23.19	0.432	***6.760***	***7.192***	***0.142***	***0.010***	47.54	***656.3***	***31.01***	29.15
Resv. _10 µM _0.5%_10D_R3	2.30	10.00	23.19	***0.444***	***7.003***	***7.447***	***0.180***	***0.010***	***38.97***	***679.9***	***32.11***	30.20
Resv. _10 µM _0.5%_10D_R4	2.30	10.00	23.19	***0.565***	***5.978***	***6.543***	***0.187***	***0.010***	***31.95***	***574.8***	***28.21***	25.78
Resv. _10 µM _0.5%_10D_R5	2.30	10.00	23.19	***0.533***	***6.470***	***7.003***	***0.212***	***0.010***	***30.50***	***640.6***	***30.20***	27.90
Resv. _10 µM _0.5%_10D_R6	2.30	10.00	23.19	***0.621***	***5.041***	***5.662***	***0.217***	***0.010***	***23.20***	***494.2***	***24.42***	21.74
Resv. _10 µM _0.5%_10D_R7	2.30	10.00	23.19	***0.592***	1.726	2.318	0.088	***0.006***	***19.70***	308.2	10.00	7.44
			Average	**0.549**	**5.515**	**6.037**	**0.172**	**0.009**	**27.24**	**568.9**	**25.99**	
			SD	**0.065**	**1.930**	**1.901**	**0.046**	**0.002**	**11.02**	**119.3**	**8.291**	
			%RSD	**11.83**	**35.00**	**31.48**	**26.68**	**20.09**	**40.44**	**20.97**	**31.90**	
Resv. _50 µM _0.5%_10D_R1	11.41	50.00	115.05	***6.027***	***57.50***	***63.53***	***0.179***	***0.011***	320.7	5374	55.22	49.98
Resv. _50 µM _0.5%_10D_R2	11.41	50.00	115.05	***5.569***	38.16	43.72	0.172	***0.011***	***221.6***	***3566***	***38.00***	33.16
Resv. _50 µM _0.5%_10D_R3	11.41	50.00	115.05	***5.402***	***41.93***	***47.34***	***0.205***	***0.011***	***204.3***	***3812***	***41.14***	36.45
Resv. _50 µM _0.5%_10D_R4	11.41	50.00	115.05	***6.347***	***49.67***	***56.02***	***0.207***	***0.011***	***239.7***	***4731***	***48.69***	43.17
Resv. _50 µM _0.5%_10D_R5	11.41	50.00	115.05	***5.311***	***45.80***	***51.11***	***0.194***	***0.011***	***236.1***	***4362***	***44.43***	39.81
Resv. _50 µM _0.5%_10D_R6	11.41	50.00	115.05	***5.940***	***44.73***	***50.67***	***0.180***	0.010	***248.8***	***4301***	***44.04***	38.88
Resv. _50 µM _0.5%_10D_R7	11.41	50.00	115.05	4.798	***53.63***	***58.43***	***0.185***	***0.011***	***289.6***	***5060***	***50.79***	46.62
			Average	**5.766**	**48.88**	**54.52**	**0.19**	**0.011**	**240.0**	**4305**	**44.52**	
			SD	**0.404**	**5.874**	**5.946**	**0.012**	**0.000**	**28.88**	**555.6**	**4.707**	
			%RSD	**7.00**	**12.02**	**10.91**	**6.46**	**1.75**	**12.03**	**12.90**	**10.57**	
Resv. _100 µM _0.5%_10D_R1	22.82	100.00	230.39	***11.15***	***76.25***	***87.40***	***0.165***	***0.010***	***462.1***	7550	***37.94***	33.10
Resv. _100 µM _0.5%_10D_R2	22.82	100.00	230.39	9.488	50.95	***60.44***	***0.185***	***0.010***	275.6	4899	26.23	22.12
Resv. _100 µM _0.5%_10D_R3	22.82	100.00	230.39	11.82	***78.01***	***89.83***	***0.168***	0.012	***464.4***	***6611***	***38.99***	33.86
Resv. _100 µM _0.5%_10D_R4	22.82	100.00	230.39	***10.84***	79.77	***90.61***	***0.144***	***0.011***	***552.4***	***7318***	***39.33***	34.62
Resv. _100 µM _0.5%_10D_R5	22.82	100.00	230.39	***10.04***	***69.67***	***79.71***	0.119	***0.010***	***587.9***	***7257***	***34.60***	30.24
Resv. _100 µM _0.5%_10D_R6	22.82	100.00	230.39	***10.23***	***64.63***	***74.86***	***0.185***	0.009	349.2	***7181***	32.49	28.05
Resv. _100 µM _0.5%_10D_R7	22.82	100.00	230.39	***11.40***	***69.62***	***81.02***	***0.159***	***0.010***	***437.9***	***6893***	***35.17***	30.22
			Average	**10.79**	**68.48**	**79.01**	**0.159**	**0.010**	**444.2**	**6756**	**34.93**	
			SD	**0.874**	**10.12**	**10.69**	**0.025**	**0.001**	**118.5**	**1064**	**5.031**	
			%RSD	**8.100**	**14.78**	**13.53**	**15.96**	**9.552**	**26.68**	**15.75**	**14.40**	

Table B.4. *Trans*- resveratrol dose response result data summary for 20 days time point in 0.5% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		µg resv./g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _0.5%_20D_R1	0.54	2.50	5.78	*0.057*	*2.976*	*3.03*	*0.240*	*0.012*	*11.45*	**223.3**	*52.48*	51.49
Resv. _2.5 µM _0.5%_20D_R2	0.54	2.50	5.78	*0.191*	*4.679*	4.87	*0.194*	*0.013*	*4.695*	68.93	*84.26*	80.95
Resv. _2.5 µM _0.5%_20D_R3	0.54	2.50	5.78	*0.125*	*4.413*	*4.54*	*0.213*	*0.014*	*5.821*	**89.98**	*78.52*	76.35
Resv. _2.5 µM _0.5%_20D_R4	0.54	2.50	5.78	*0.068*	*4.593*	*4.66*	*0.205*	*0.014*	*5.450*	**80.54**	*80.63*	79.46
Resv. _2.5 µM _0.5%_20D_R5	0.54	2.50	5.78	*0.097*	*3.648*	*3.75*	*0.200*	*0.014*	*10.17*	**147.5**	*64.79*	63.11
Resv. _2.5 µM _0.5%_20D_R6	0.54	2.50	5.78	*0.114*	*3.135*	*3.25*	*0.201*	*0.013*	*12.63*	**197.8**	*56.20*	54.23
			Average	**0.109**	**3.907**	*4.02*	**0.209**	**0.013**	**8.370**	**134.7**	**69.48**	
			SD	**0.048**	**0.755**	**0.781**	**0.017**	**0.001**	**3.446**	**65.20**	**13.50**	
			%RSD	**44.32**	**19.33**	**19.44**	**7.928**	**4.896**	**41.18**	**48.41**	**19.44**	
Resv. _5 µM _0.5%_20D_R1	1.08	5.00	11.59	*0.231*	*6.312*	*6.54*	*0.215*	0.014	**23.52**	**360.5**	*56.46*	
Resv. _5 µM _0.5%_20D_R2	1.08	5.00	11.59	*0.229*	*5.799*	6.03	*0.235*	0.014	*23.67*	**412.0**	*52.01*	50.04
Resv. _5 µM _0.5%_20D_R3	1.08	5.00	11.59	*0.238*	*6.120*	*6.36*	*0.230*	0.014	**22.78**	**381.9**	*54.86*	52.81
Resv. _5 µM _0.5%_20D_R4	1.08	5.00	11.59	*0.243*	*6.275*	*6.52*	*0.202*	0.015	**25.14**	**345.0**	*56.24*	54.14
Resv. _5 µM _0.5%_20D_R5	1.08	5.00	11.59	0.268	*6.100*	*6.37*	*0.231*	0.014	**22.61**	**386.8**	*54.95*	52.63
Resv. _5 µM _0.5%_20D_R6	1.08	5.00	11.59	*0.219*	*5.250*	*5.47*	*0.235*	0.015	*26.03*	*419.2*	*47.19*	45.30
Resv. _5 µM _0.5%_20D_R7	1.08	5.00	11.59	*0.231*	7.040	*7.27*	0.263	0.014	16.42	308.5	62.74	60.74
			Average	**0.23**	**5.976**	**6.214**	**0.225**	**0.014**	**23.96**	**384.2**	**53.62**	
			SD	**0.008**	**0.399**	**0.409**	**0.013**	**0.001**	**1.358**	**28.67**	**3.526**	
			%RSD	**3.526**	**6.680**	**6.576**	**6.008**	**3.833**	**5.669**	**7.462**	**6.576**	
Resv. _10 µM _0.5%_20D_R1	2.15	10.00	23.19	0.861	*11.07*	*11.93*	*0.225*	*0.015*	*50.02*	*771.2*	*51.45*	
Resv. _10 µM _0.5%_20D_R2	2.15	10.00	23.19	*0.398*	*11.93*	12.32	*0.212*	*0.015*	*51.15*	*744.2*	*53.15*	51.43
Resv. _10 µM _0.5%_20D_R3	2.15	10.00	23.19	*0.295*	*10.66*	*10.96*	*0.223*	*0.015*	*54.77*	*837.7*	*47.26*	45.99
Resv. _10 µM _0.5%_20D_R4	2.15	10.00	23.19	*0.382*	*9.762*	*10.14*	*0.208*	*0.014*	*62.63*	*959.3*	43.74	42.10
Resv. _10 µM _0.5%_20D_R5	2.15	10.00	23.19	*0.183*	*10.29*	*10.47*	*0.239*	*0.014*	*53.28*	*883.3*	*45.15*	44.36
Resv. _10 µM _0.5%_20D_R6	2.15	10.00	23.19	*0.174*	13.036	*13.21*	*0.225*	*0.014*	44.44	707.8	*56.96*	56.21
Resv. _10 µM _0.5%_20D_R7	2.15	10.00	23.19	*0.336*	*9.969*	*10.30*	0.240	0.015	*53.69*	*882.5*	*44.44*	42.99
			Average	**0.295**	**10.61**	**11.40**	**0.222**	**0.014**	**52.54**	**824.7**	**50.79**	
			SD	**0.097**	**0.798**	**1.269**	**0.011**	**0.000**	**5.962**	**97.88**	**4.940**	
			%RSD	**32.90**	**7.517**	**11.14**	**4.837**	**2.808**	**11.35**	**11.87**	**9.73**	
Resv. _50 µM _0.5%_20D_R1	11.41	50.00	115.30	*4.373*	*59.29*	*63.66*	*0.203*	**0.014**	*254.3*	*3742*	*55.21*	
Resv. _50 µM _0.5%_20D_R2	11.41	50.00	115.30	*3.503*	*54.26*	57.77	*0.220*	**0.014**	*261.2*	*4169*	*50.10*	47.06
Resv. _50 µM _0.5%_20D_R3	11.41	50.00	115.30	*3.020*	42.35	*45.37*	*0.224*	**0.015**	312.7	4789	39.35	36.73
Resv. _50 µM _0.5%_20D_R4	11.41	50.00	115.30	*3.812*	*57.30*	*61.12*	*0.225*	**0.014**	*240.4*	**3926**	*53.01*	49.70
Resv. _50 µM _0.5%_20D_R5	11.41	50.00	115.30	*3.941*	*57.46*	*61.40*	*0.215*	**0.014**	*250.5*	*3963*	*53.25*	49.83
Resv. _50 µM _0.5%_20D_R6	11.41	50.00	115.30	*4.737*	*66.00*	*70.74*	*0.231*	**0.014**	*193.1*	*3229*	*61.35*	57.24
Resv. _50 µM _0.5%_20D_R7	11.41	50.00	115.30	6.024	*59.50*	*65.52*	*0.203*	**0.014**	*245.3*	*3555*	*56.83*	51.60
			Average	**3.898**	**58.97**	**63.37**	**0.216**	**0.014**	**240.8**	**3764**	**54.96**	
			SD	**0.611**	**3.925**	**4.458**	**0.012**	**0.000**	**24.44**	**334.7**	**3.867**	
			%RSD	**15.67**	**6.656**	**7.035**	**5.328**	**2.514**	**10.15**	**8.893**	**7.035**	
Resv. _100 µM _0.5%_20D_R1	22.82	100.00	230.50	*11.04*	*101.0*	*112.04*	*0.226*	*0.013*	*523.3*	*9113*	*48.61*	
Resv. _100 µM _0.5%_20D_R2	22.82	100.00	230.50	7.962	*85.56*	93.52	*0.200*	*0.014*	685.2	*9926*	*40.57*	37.12
Resv. _100 µM _0.5%_20D_R3	22.82	100.00	230.50	*9.571*	*101.1*	*110.67*	*0.198*	*0.013*	*604.9*	*9078*	*48.01*	43.86
Resv. _100 µM _0.5%_20D_R4	22.82	100.00	230.50	*9.058*	79.12	*88.18*	*0.225*	*0.014*	*633.7*	10313	38.26	34.33
Resv. _100 µM _0.5%_20D_R5	22.82	100.00	230.50	*10.98*	*100.8*	*111.74*	0.250	*0.014*	*475.8*	*8544*	*48.48*	43.71
Resv. _100 µM _0.5%_20D_R6	22.82	100.00	230.50	*9.895*	*100.8*	*110.67*	*0.207*	*0.013*	*579.4*	*9078*	*48.01*	43.72
Resv. _100 µM _0.5%_20D_R7	22.82	100.00	230.50	*10.18*	*94.66*	*104.84*	*0.205*	*0.013*	*612.4*	*9519*	*45.49*	41.07
			Average	**10.12**	**97.31**	**107.2**	**0.210**	**0.014**	**571.6**	**9210**	**46.53**	
			SD	**0.783**	**6.274**	**7.219**	**0.012**	**0.000**	**60.33**	**468.1**	**3.132**	
			%RSD	**7.736**	**6.448**	**6.731**	**5.862**	**2.581**	**10.55**	**5.083**	**6.731**	

B.2. Raw data summary for trans-resveratrol analysis in yeast cells grown in 2% glucose synthetic media.

Table B.5. *Trans*- resveratrol dose response result data summary for 1h time point in 2% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		µg resv./g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. - 2.5 µM _2%_1H_R1	0.57	2.50	5.70	***0.247***	***0.195***	***0.44***	0.202	***0.015***	***0.965***	***13.00***	***7.754***	3.42
Resv. - 2.5 µM _2%_1H_R2	0.57	2.50	5.70	***0.250***	***0.193***	0.44	0.262	0.017	***0.738***	***11.70***	***7.775***	3.39
Resv. - 2.5 µM _2%_1H_R3	0.57	2.50	5.70	***0.319***	***0.213***	***0.53***	***0.255***	***0.016***	***0.836***	***13.23***	***9.330***	3.74
Resv. - 2.5 µM _2%_1H_R4	0.57	2.50	5.70	***0.193***	***0.325***	***0.52***	***0.248***	***0.016***	***1.313***	***20.44***	***9.088***	5.70
Resv. - 2.5 µM _2%_1H_R5	0.57	2.50	5.70	***0.267***	***0.199***	***0.47***	***0.251***	***0.016***	***0.794***	***12.36***	***8.168***	3.49
Resv. - 2.5 µM _2%_1H_R6	0.57	2.50	5.70	***0.252***	***0.197***	***0.45***	***0.230***	***0.015***	***0.858***	***13.22***	***7.877***	3.46
Resv. - 2.5 µM _2%_1H_R7	0.57	2.50	5.70	0.440	0.113	***0.55***	***0.233***	***0.015***	0.486	7.533	9.709	1.98
			Average	**0.255**	**0.220**	**0.475**	**0.238**	**0.016**	**0.917**	**13.99**	**8.332**	
			SD	**0.040**	**0.052**	**0.040**	**0.022**	**0.001**	**0.208**	**3.215**	**0.699**	
			%RSD	**15.85**	**23.49**	**8.390**	**9.042**	**3.806**	**22.67**	**22.98**	**8.390**	
Resv. - 5 µM _2%_1H_R1	1.14	5.00	11.43	***0.715***	***0.365***	***1.08***	***0.247***	0.016	***1.477***	***22.96***	***9.449***	3.19
Resv. - 5 µM _2%_1H_R2	1.14	5.00	11.43	***0.589***	***0.316***	0.90	***0.261***	0.017	***1.212***	***19.04***	***7.916***	2.76
Resv. - 5 µM _2%_1H_R3	1.14	5.00	11.43	***0.535***	***0.312***	***0.85***	***0.238***	0.017	***1.313***	***18.35***	***7.414***	2.73
Resv. - 5 µM _2%_1H_R4	1.14	5.00	11.43	0.742	***0.378***	***1.12***	***0.237***	0.016	***1.595***	***23.05***	***9.797***	3.31
Resv. - 5 µM _2%_1H_R5	1.14	5.00	11.43	***0.661***	***0.385***	***1.05***	***0.264***	0.017	***1.459***	***23.26***	***9.153***	3.37
Resv. - 5 µM _2%_1H_R6	1.14	5.00	11.43	***0.641***	0.542	***1.18***	0.272	0.017	1.991	32.26	10.35	4.74
Resv. - 5 µM _2%_1H_R7	1.14	5.00	11.43	***0.628***	***0.303***	***0.93***	***0.243***	0.016	***1.245***	***18.94***	***8.145***	2.65
			Average	**0.63**	**0.343**	**0.988**	**0.248**	**0.016**	**1.383**	**20.932**	**8.646**	
			SD	**0.062**	**0.037**	**0.109**	**0.012**	**0.000**	**0.150**	**2.376**	**0.952**	
			%RSD	**9.800**	**10.72**	**11.008**	**4.637**	**2.486**	**10.86**	**11.35**	**11.01**	
Resv. - 10 µM _2%_1H_R1	2.28	10.00	22.84	***0.208***	***0.674***	***0.88***	***0.239***	***0.015***	***2.820***	***46.16***	***3.859***	2.95
Resv. - 10 µM _2%_1H_R2	2.28	10.00	22.84	0.009	5.125	5.13	***0.252***	0.015	20.35	341.7	22.48	22.44
Resv. - 10 µM _2%_1H_R3	2.28	10.00	22.84	***0.190***	***0.604***	***0.79***	0.263	***0.015***	***2.301***	***41.37***	***3.478***	2.64
Resv. - 10 µM _2%_1H_R4	2.28	10.00	22.84	***0.220***	***0.569***	***0.79***	***0.196***	***0.015***	***2.910***	***38.45***	***3.452***	2.49
Resv. - 10 µM _2%_1H_R5	2.28	10.00	22.84	***0.217***	***0.426***	***0.64***	***0.196***	***0.013***	***2.179***	***33.28***	***2.815***	1.87
Resv. - 10 µM _2%_1H_R6	2.28	10.00	22.84	***0.174***	***0.710***	***0.88***	***0.205***	***0.013***	3.467	55.04	***3.868***	3.11
Resv. - 10 µM _2%_1H_R7	2.28	10.00	22.84	***0.253***	***0.565***	***0.82***	***0.229***	***0.015***	***2.472***	***38.18***	***3.580***	2.47
			Average	**0.210**	**0.591**	**0.80**	**0.219**	**0.01**	**2.69**	**42.08**	**3.51**	
			SD	**0.027**	**0.100**	**0.088**	**0.024**	**0.001**	**0.475**	**7.629**	**0.385**	
			%RSD	**12.90**	**16.84**	**10.985**	**10.95**	**6.817**	**17.66**	**18.13**	**10.98**	
Resv. - 50 µM _2%_1H_R1	11.41	50.00	113.90	***0.022***	***4.126***	***4.15***	***0.227***	***0.014***	***18.17***	***290.6***	***3.642***	3.62
Resv. - 50 µM _2%_1H_R2	11.41	50.00	113.90	0.011	***3.459***	3.47	0.272	***0.014***	***12.70***	***247.1***	***3.046***	3.04
Resv. - 50 µM _2%_1H_R3	11.41	50.00	113.90	***0.025***	1.071	***1.10***	***0.246***	***0.013***	4.348	81.14	0.962	0.94
Resv. - 50 µM _2%_1H_R4	11.41	50.00	113.90	***0.013***	***4.164***	***4.18***	***0.246***	***0.014***	***16.91***	***306.2***	***3.668***	3.66
Resv. - 50 µM _2%_1H_R5	11.41	50.00	113.90	***0.026***	***3.914***	***3.94***	***0.229***	***0.015***	***17.07***	***268.1***	***3.459***	3.44
Resv. - 50 µM _2%_1H_R6	11.41	50.00	113.90	***0.027***	***3.705***	***3.73***	***0.254***	***0.014***	***14.62***	***264.6***	***3.277***	3.25
Resv. - 50 µM _2%_1H_R7	11.41	50.00	113.90	***0.029***	***4.916***	***4.95***	***0.253***	0.017	***19.40***	***296.1***	***4.342***	4.32
			Average	**0.024**	**4.047**	**4.1**	**0.243**	**0.014**	**16.5**	**278.8**	**3.572**	
			SD	**0.006**	**0.501**	**0.505**	**0.012**	**0.000**	**2.437**	**22.41**	**0.443**	
			%RSD	**23.74**	**12.38**	**12.411**	**4.804**	**3.477**	**14.79**	**8.038**	**12.41**	
Resv. -100 µM _2%_1H_R1	22.82	100.00	228.38	***0.101***	***7.385***	***7.49***	***0.256***	***0.016***	***28.86***	***450.3***	***3.278***	3.23
Resv. -100 µM _2%_1H_R2	22.82	100.00	228.38	***0.087***	***11.33***	11.42	***0.224***	0.017	***50.67***	***678.7***	***5.001***	4.96
Resv. -100 µM _2%_1H_R3	22.82	100.00	228.38	***0.115***	***7.370***	***7.49***	0.189	***0.015***	***39.10***	***504.8***	***3.277***	3.23
Resv. -100 µM _2%_1H_R4	22.82	100.00	228.38	***0.054***	***11.70***	***11.76***	***0.268***	***0.015***	***43.68***	801.5	5.147	5.12
Resv. -100 µM _2%_1H_R5	22.82	100.00	228.38	***0.062***	***5.924***	***5.99***	***0.286***	***0.016***	20.69	***363.4***	***2.621***	2.59
Resv. -100 µM _2%_1H_R6	22.82	100.00	228.38	***0.100***	***8.683***	***8.78***	***0.236***	***0.014***	***36.78***	***607.2***	***3.846***	3.80
Resv. -100 µM _2%_1H_R7	22.82	100.00	228.38	0.054	***8.355***	***8.41***	***0.238***	***0.015***	***35.09***	***568.4***	***3.682***	3.66
			Average	**0.087**	**8.897**	**8.3**	**0.251**	**0.015**	**39.03**	**528.8**	**3.618**	
			SD	**0.024**	**2.248**	**1.825**	**0.023**	**0.001**	**7.498**	**113.4**	**0.799**	
			%RSD	**27.55**	**25.27**	**22.092**	**9.227**	**6.203**	**19.21**	**21.44**	**22.09**	

Table B.6. *Trans*-resveratrol dose response result data summary for 1 day time point in 2% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		µg resv./g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _2%_24H_R1	0.57	2.50	0.57	0.036	*0.774*	*0.810*	*0.201*	*0.014*	*3.857*	*57.33*	*142.1*	135.79
Resv. _2.5 µM _2%_24H_R2	0.57	2.50	0.57	0.000	*0.728*	*0.728*	*0.214*	0.009	*3.408*	*80.89*	*127.7*	127.72
Resv. _2.5 µM _2%_24H_R3	0.57	2.50	0.57	0.000	*0.641*	0.641	0.251	*0.014*	*2.550*	47.13	*112.5*	112.46
Resv. _2.5 µM _2%_24H_R4	0.57	2.50	0.57	0.000	0.939	*0.939*	*0.196*	*0.014*	4.796	*67.07*	*164.7*	164.74
Resv. _2.5 µM _2%_24H_R5	0.57	2.50	0.57	0.000	*0.802*	*0.802*	*0.226*	*0.013*	*3.552*	*62.66*	*140.7*	140.70
Resv. _2.5 µM _2%_24H_R6	0.57	2.50	0.57	0.000	*0.679*	*0.679*	*0.212*	*0.014*	*3.198*	*48.85*	*119.1*	119.12
Resv. _2.5 µM _2%_24H_R7	0.57	2.50	0.57	0.020	*0.921*	*0.941*	*0.236*	*0.014*	*3.908*	*68.22*	*165.1*	161.58
			Average	#REF!	0.758	0.816	0.214	0.014	3.412	64.17	143.2	
			SD	#REF!	0.100	0.107	0.015	0.000	0.500	10.85	18.81	
			%RSD	#REF!	13.15	13.13	6.998	3.122	14.67	16.90	13.13	
Resv. _5 µM _2%_24H_R1	1.14	5.00	1.14	*0.019*	*1.537*	*1.556*	*0.221*	0.011	*6.958*	*141.0*	*136.5*	134.82
Resv. _5 µM _2%_24H_R2	1.14	5.00	1.14	*0.019*	*1.351*	*1.370*	*0.241*	*0.012*	5.601	*115.5*	*120.2*	118.51
Resv. _5 µM _2%_24H_R3	1.14	5.00	1.14	0.206	*1.402*	1.608	*0.224*	*0.013*	*6.270*	*107.8*	*141.0*	122.98
Resv. _5 µM _2%_24H_R4	1.14	5.00	1.14	*0.019*	*1.526*	*1.545*	*0.211*	*0.012*	*7.219*	*123.1*	*135.6*	133.86
Resv. _5 µM _2%_24H_R5	1.14	5.00	1.14	*0.028*	1.245	*1.273*	*0.211*	*0.013*	*5.889*	92.91	111.6	109.21
Resv. _5 µM _2%_24H_R6	1.14	5.00	1.14	*0.007*	*1.467*	*1.474*	0.249	*0.012*	*5.884*	*119.3*	*129.3*	128.68
Resv. _5 µM _2%_24H_R7	1.14	5.00	1.14	0.000	*1.575*	*1.575*	*0.238*	*0.013*	*6.618*	*125.0*	*138.2*	138.16
			Average	0.015	1.476	1.465	0.224	0.013	6.47	121.9	133.4	
			SD	0.010	0.086	0.121	0.013	0.001	0.555	11.15	7.571	
			%RSD	65.49	5.843	8.235	5.700	4.685	8.57	9.146	5.674	
Resv. _10 µM _2%_24H_R1	2.28	10.00	2.28	*0.155*	*2.672*	*2.827*	*0.213*	*0.014*	*12.57*	*195.0*	*124.0*	117.19
Resv. _10 µM _2%_24H_R2	2.28	10.00	2.28	*0.122*	*2.699*	*2.821*	*0.226*	*0.013*	*11.96*	*202.9*	*123.7*	118.38
Resv. _10 µM _2%_24H_R3	2.28	10.00	2.28	*0.104*	*2.876*	*2.980*	0.191	*0.013*	15.03	*226.5*	*130.7*	126.14
Resv. _10 µM _2%_24H_R4	2.28	10.00	2.28	*0.093*	*2.819*	*2.912*	*0.211*	0.012	*13.34*	*231.1*	*127.7*	123.64
Resv. _10 µM _2%_24H_R5	2.28	10.00	2.28	*0.082*	3.222	3.304	*0.220*	*0.013*	*14.62*	247.8	*144.9*	141.32
Resv. _10 µM _2%_24H_R6	2.28	10.00	2.28	0.039	*2.519*	*2.558*	*0.259*	*0.014*	*9.730*	*182.5*	*112.2*	110.48
Resv. _10 µM _2%_24H_R7	2.28	10.00	2.28	*0.118*	*2.489*	*2.607*	*0.224*	*0.014*	*11.10*	*183.0*	*114.4*	109.17
			Average	0.112	2.679	2.784	0.225	0.013	12.22	206.3	125.0	
			SD	0.026	0.155	0.167	0.017	0.000	1.712	25.99	11.90	
			%RSD	22.93	5.796	6.016	7.717	3.239	14.01	12.60	9.524	
Resv. _50 µM _2%_24H_R1	11.41	50.00	11.41	*1.820*	*13.59*	*15.41*	*0.241*	*0.012*	*56.52*	*1096*	*135.1*	119.14
Resv. _50 µM _2%_24H_R2	11.41	50.00	11.41	*1.492*	*11.10*	*12.59*	0.182	*0.013*	*61.13*	*853.5*	*110.3*	97.24
Resv. _50 µM _2%_24H_R3	11.41	50.00	11.41	*3.099*	*9.510*	*12.61*	*0.203*	*0.014*	*46.96*	*699.3*	*110.5*	83.35
Resv. _50 µM _2%_24H_R4	11.41	50.00	11.41	*1.619*	*10.53*	*12.15*	*0.229*	*0.014*	*45.97*	*774.0*	*106.4*	92.25
Resv. _50 µM _2%_24H_R5	11.41	50.00	11.41	5.670	*12.33*	*18.00*	*0.234*	*0.013*	*52.59*	*948.3*	*157.7*	108.05
Resv. _50 µM _2%_24H_R6	11.41	50.00	11.41	*0.993*	*15.94*	*16.93*	*0.216*	*0.013*	*73.66*	*1226*	*148.4*	139.69
Resv. _50 µM _2%_24H_R7	11.41	50.00	11.41	*2.482*	5.238	7.72	*0.217*	0.011	24.17	476.2	67.66	45.91
			Average	1.917	12.165	14.61	0.223	0.013	56.14	932.9	128.1	
			SD	0.755	2.332	2.518	0.014	0.000	10.32	199.5	22.06	
			%RSD	39.37	19.17	17.23	6.256	3.448	18.38	21.39	17.23	
Resv. _100 µM _2%_24H_R1	22.82	100.00	22.82	18.49	*22.16*	*40.65*	*0.229*	0.014	*96.98*	*1594*	178.2	97.11
Resv. _100 µM _2%_24H_R2	22.82	100.00	22.82	*6.950*	18.43	25.38	*0.239*	*0.014*	77.14	1326	*111.2*	80.76
Resv. _100 µM _2%_24H_R3	22.82	100.00	22.82	*9.021*	27.59	*36.61*	*0.231*	*0.013*	*119.3*	*2123*	*160.4*	120.92
Resv. _100 µM _2%_24H_R4	22.82	100.00	22.82	0.608	*23.26*	*23.87*	*0.210*	*0.013*	*110.7*	*1803*	*104.6*	101.93
Resv. _100 µM _2%_24H_R5	22.82	100.00	22.82	0.814	*25.93*	*26.74*	0.241	*0.013*	*107.4*	*1935*	*117.2*	113.62
Resv. _100 µM _2%_24H_R6	22.82	100.00	22.82	*8.633*	*25.91*	*34.54*	*0.224*	*0.013*	*115.9*	*2008*	*151.4*	113.53
Resv. _100 µM _2%_24H_R7	22.82	100.00	22.82	*8.261*	*23.68*	*31.95*	*0.223*	*0.013*	*106.4*	*1836*	*140.0*	103.79
			Average	8.216	24.76	32.39	0.226	0.013	109.5	1883	130.8	
			SD	0.899	2.043	6.253	0.010	0.000	7.865	183.2	22.99	
			%RSD	10.94	8.253	19.30	4.299	3.101	7.186	9.726	17.57	

Table B.7. *Trans*- resveratrol dose response result data summary for 4 days time point in 2% glucose synthetic yeast media.

	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		µg resveratrol/g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _2%_4D_R1	0.57	2.50	5.77	***0.070***	6.720	6.790	***0.220***	0.013	30.56	516.9	117.7	116.46
Resv. _2.5 µM _2%_4D_R2	0.57	2.50	5.77	***0.092***	***3.180***	***3.272***	***0.216***	***0.012***	***14.76***	***265.0***	***56.71***	55.11
Resv. _2.5 µM _2%_4D_R3	0.57	2.50	5.77	***0.083***	***0.680***	***0.763***	0.057	***0.010***	***11.93***	***68.00***	***13.22***	11.79
Resv. _2.5 µM _2%_4D_R4	0.57	2.50	5.77	***0.079***	***1.020***	***1.099***	***0.209***	***0.012***	***4.878***	***85.00***	***19.05***	17.68
Resv. _2.5 µM _2%_4D_R5	0.57	2.50	5.77	0.048	***0.800***	***0.848***	***0.212***	***0.013***	***3.781***	***61.54***	***14.71***	13.86
Resv. _2.5 µM _2%_4D_R6	0.57	2.50	5.77	***0.077***	***0.710***	***0.787***	***0.218***	***0.013***	***3.263***	***54.62***	***13.64***	12.31
Resv. _2.5 µM _2%_4D_R7	0.57	2.50	5.77	***0.078***	***0.730***	***0.808***	***0.227***	***0.012***	***3.216***	***60.83***	***14.01***	12.65
			Average	**0.083**	**1.187**	**1.263**	**0.217**	**0.012**	**6.971**	**99.16**	**21.89**	
			SD	**0.007**	**0.984**	**0.992**	**0.006**	**0.001**	**5.052**	**81.91**	**17.19**	
			%RSD	**8.396**	**82.94**	**78.53**	**2.934**	**9.129**	**72.48**	**82.60**	**78.53**	
Resv. _5 µM _2%_4D_R1	1.14	5.00	11.54	***0.287***	2.120	***2.407***	***0.194***	0.012	10.94	176.7	***20.86***	18.37
Resv. _5 µM _2%_4D_R2	1.14	5.00	11.54	***0.312***	***0.970***	***1.282***	***0.221***	***0.013***	***4.383***	***74.62***	***11.11***	8.41
Resv. _5 µM _2%_4D_R3	1.14	5.00	11.54	***0.101***	***1.240***	***1.341***	***0.223***	***0.013***	***5.571***	***95.38***	***11.62***	10.75
Resv. _5 µM _2%_4D_R4	1.14	5.00	11.54	***0.168***	***0.550***	***0.718***	0.171	***0.013***	***3.209***	***42.31***	***6.22***	4.77
Resv. _5 µM _2%_4D_R5	1.14	5.00	11.54	***0.238***	***0.920***	***1.158***	***0.215***	***0.013***	***4.289***	***70.77***	***10.04***	7.97
Resv. _5 µM _2%_4D_R6	1.14	5.00	11.54	1.576	***1.600***	3.176	***0.228***	***0.013***	7.024	123.1	27.52	13.86
Resv. _5 µM _2%_4D_R7	1.14	5.00	11.54	***0.305***	***0.750***	***1.055***	***0.215***	***0.013***	***3.484***	***57.69***	***9.142***	6.50
			Average	**0.235**	**1.005**	**1.327**	**0.216**	**0.013**	**4.660**	**77.31**	**11.50**	
			SD	**0.085**	**0.371**	**0.573**	**0.012**	**0.000**	**1.423**	**28.57**	**4.966**	
			%RSD	**36.03**	**36.96**	**43.19**	**5.506**	**0.000**	**30.53**	**36.96**	**43.19**	
Resv. _10 µM _2%_4D_R1	2.28	10.00	23.08	***1.433***	6.200	7.633	0.230	***0.014***	26.98	442.9	33.07	26.86
Resv. _10 µM _2%_4D_R2	2.28	10.00	23.08	***1.058***	***2.090***	***3.148***	***0.203***	***0.013***	***10.30***	***160.8***	***13.64***	9.06
Resv. _10 µM _2%_4D_R3	2.28	10.00	23.08	***1.625***	***3.120***	***4.745***	***0.206***	***0.013***	***15.17***	***240.0***	***20.56***	13.52
Resv. _10 µM _2%_4D_R4	2.28	10.00	23.08	***0.955***	***4.250***	***5.205***	***0.214***	***0.013***	***19.90***	***326.9***	***22.55***	18.41
Resv. _10 µM _2%_4D_R5	2.28	10.00	23.08	0.484	***4.160***	***4.644***	***0.216***	***0.014***	***19.29***	***297.1***	***20.12***	18.02
Resv. _10 µM _2%_4D_R6	2.28	10.00	23.08	***0.786***	***5.160***	***5.946***	***0.224***	***0.013***	***23.04***	***396.9***	***25.76***	22.36
Resv. _10 µM _2%_4D_R7	2.28	10.00	23.08	***0.899***	***4.240***	***5.139***	***0.208***	***0.014***	***20.37***	***302.9***	***22.26***	18.37
			Average	**1.126**	**3.837**	**4.804**	**0.212**	**0.013**	**18.01**	**287.4**	**20.82**	
			SD	**0.330**	**1.073**	**0.933**	**0.008**	**0.001**	**4.55**	**80.22**	**4.041**	
			%RSD	**29.32**	**27.96**	**19.41**	**3.63**	**3.873**	**25.27**	**27.91**	**19.41**	
Resv. _50 µM _2%_4D_R1	11.41	50.00	115.18	18.44	43.92	***62.36***	***0.215***	***0.014***	204.0	***3137***	***54.14***	38.13
Resv. _50 µM _2%_4D_R2	11.41	50.00	115.18	***10.72***	***31.88***	42.60	0.229	***0.014***	***139.4***	***2277***	***36.98***	27.68
Resv. _50 µM _2%_4D_R3	11.41	50.00	115.18	***13.97***	***33.53***	***47.50***	***0.213***	***0.014***	***157.6***	***2395***	41.24	29.11
Resv. _50 µM _2%_4D_R4	11.41	50.00	115.18	***9.760***	***27.89***	***37.65***	***0.214***	***0.014***	***130.2***	1992	***32.69***	24.21
Resv. _50 µM _2%_4D_R5	11.41	50.00	115.18	***13.04***	***33.84***	***46.88***	***0.205***	***0.014***	***164.9***	***2417***	***40.70***	29.38
Resv. _50 µM _2%_4D_R6	11.41	50.00	115.18	***14.19***	***35.38***	***49.57***	***0.194***	***0.014***	***182.0***	***2527***	***43.04***	30.72
Resv. _50 µM _2%_4D_R7	11.41	50.00	115.18	***12.43***	***31.09***	***43.52***	***0.221***	0.012	***140.7***	***2591***	***37.79***	26.99
			Average	**12.35**	**32.27**	**44.62**	**0.210**	**0.014**	**152.5**	**2367**	**38.74**	
			SD	**1.782**	**2.625**	**4.285**	**0.009**	**0.000**	**19.28**	**213.4**	**3.720**	
			%RSD	**14.43**	**8.135**	**9.603**	**4.453**	**0.000**	**12.65**	**9.016**	**9.603**	
Resv. _100 µM _2%_4D_R1	22.82	100.00	230.36	***33.72***	***59.49***	***93.21***	***0.202***	0.011	***293.9***	5408	***40.46***	25.82
Resv. _100 µM _2%_4D_R2	22.82	100.00	230.36	***18.86***	***49.39***	68.24	0.229	***0.014***	***215.4***	***3528***	29.62	21.44
Resv. _100 µM _2%_4D_R3	22.82	100.00	230.36	***23.86***	***48.93***	***72.79***	***0.200***	***0.014***	***245.3***	***3495***	***31.60***	21.24
Resv. _100 µM _2%_4D_R4	22.82	100.00	230.36	43.43	***56.75***	***100.2***	***0.218***	***0.014***	***259.9***	***4053***	***43.49***	24.63
Resv. _100 µM _2%_4D_R5	22.82	100.00	230.36	***38.94***	***50.33***	***89.27***	***0.215***	***0.014***	***234.4***	***3595***	***38.75***	21.85
Resv. _100 µM _2%_4D_R6	22.82	100.00	230.36	***21.12***	***53.68***	***74.80***	***0.215***	***0.015***	***250.1***	***3579***	***32.47***	23.30
Resv. _100 µM _2%_4D_R7	22.82	100.00	230.36	***43.03***	32.27	***75.30***	***0.212***	***0.014***	152.2	2305	***32.69***	14.01
			Average	**29.92**	**53.09**	**84.26**	**0.210**	**0.014**	**249.8**	**3943**	**36.58**	
			SD	**10.04**	**4.320**	**11.49**	**0.008**	**0.001**	**26.43**	**746.4**	**4.987**	
			%RSD	**33.57**	**8.137**	**13.63**	**3.583**	**9.997**	**10.58**	**18.93**	**13.63**	

Table B.8. *Trans-r*esveratrol dose response result data summary for 10 days time point in 2% glucose synthetic yeast media.

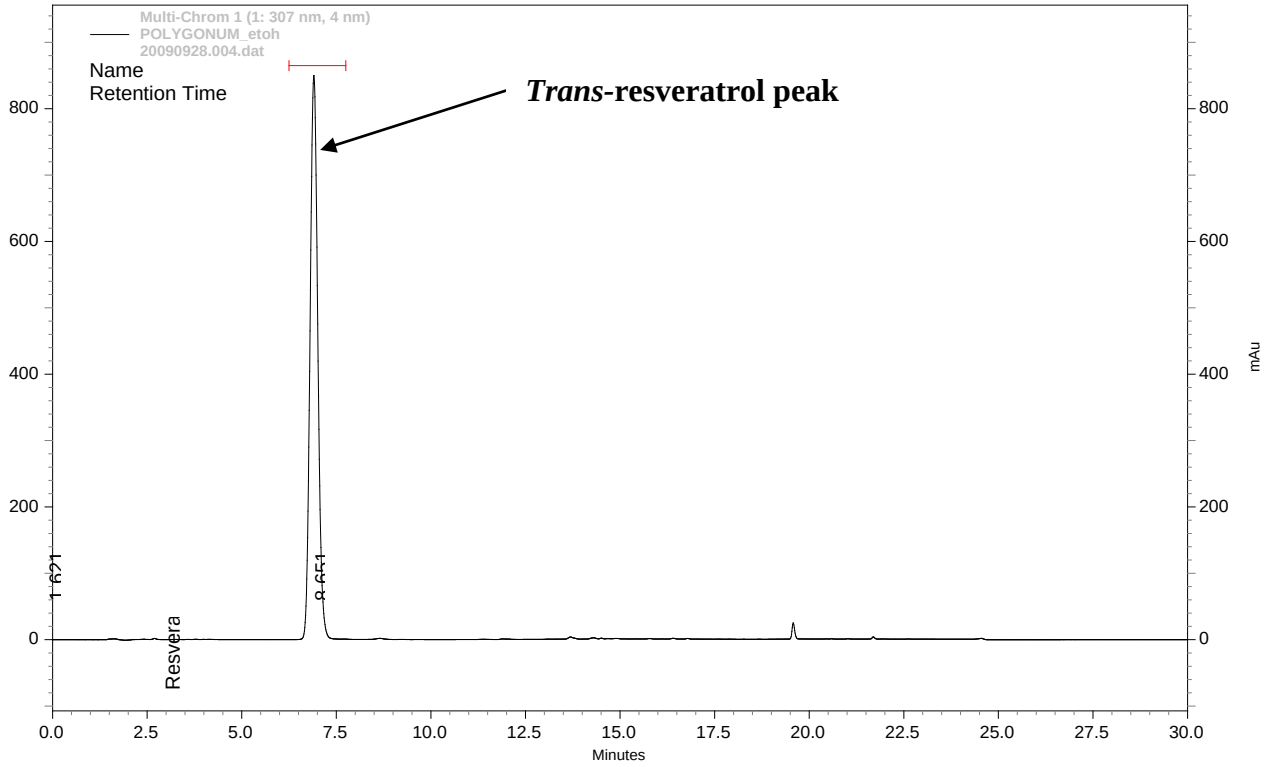
	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		µg resveratrol/g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _2%_10D_R1	0.57	2.50	5.78	*0.397*	*3.936*	*4.333*	*0.269*	**0.016**	*14.64*	*246.0*	*74.97*	68.10
Resv. _2.5 µM _2%_10D_R2	0.57	2.50	5.78	*0.487*	*3.150*	*3.637*	0.357	0.017	8.83	185.3	62.92	54.50
Resv. _2.5 µM _2%_10D_R3	0.57	2.50	5.78	*0.546*	*5.361*	*5.907*	*0.281*	*0.014*	*19.10*	*382.9*	*102.2*	92.75
Resv. _2.5 µM _2%_10D_R4	0.57	2.50	5.78	*0.427*	*3.254*	*3.681*	*0.232*	*0.014*	*14.02*	*232.4*	*63.68*	56.29
Resv. _2.5 µM _2%_10D_R5	0.57	2.50	5.78	0.316	*3.364*	*3.680*	*0.238*	*0.014*	*14.16*	*240.3*	*63.67*	58.20
Resv. _2.5 µM _2%_10D_R6	0.57	2.50	5.78	*0.396*	*4.313*	*4.710*	*0.219*	*0.015*	*19.73*	*287.6*	*81.49*	74.63
Resv. _2.5 µM _2%_10D_R7	0.57	2.50	5.78	*0.576*	6.456	7.032	*0.268*	*0.014*	*24.11*	461.2	121.7	111.70
			Average	**0.471**	**3.896**	**4.325**	**0.251**	**0.015**	**17.63**	**308.4**	**84.61**	
			SD	**0.077**	**0.846**	**0.889**	**0.025**	**0.001**	**4.063**	**93.42**	**23.07**	
			%RSD	**16.38**	**21.70**	**20.57**	**9.883**	**5.770**	**23.05**	**30.29**	**27.27**	
Resv. _5 µM _2%_10D_R1	1.15	5.00	11.59	*0.655*	*7.725*	*8.38*	0.151	*0.013*	51.19	*594.2*	*72.30*	66.65
Resv. _5 µM _2%_10D_R2	1.15	5.00	11.59	*0.636*	*7.406*	*8.04*	*0.228*	*0.014*	*32.56*	*529.0*	*69.39*	63.90
Resv. _5 µM _2%_10D_R3	1.15	5.00	11.59	*0.736*	*8.657*	*9.39*	*0.277*	*0.015*	*31.25*	*577.1*	*81.04*	74.69
Resv. _5 µM _2%_10D_R4	1.15	5.00	11.59	*0.611*	*7.901*	*8.51*	*0.259*	*0.013*	*30.53*	*607.8*	*73.44*	68.17
Resv. _5 µM _2%_10D_R5	1.15	5.00	11.59	0.523	6.454	6.98	*0.280*	*0.015*	23.06	430.2	*60.19*	55.68
Resv. _5 µM _2%_10D_R6	1.15	5.00	11.59	*0.698*	*8.455*	*9.15*	*0.207*	*0.014*	*40.77*	*603.9*	*78.98*	72.95
Resv. _5 µM _2%_10D_R7	1.15	5.00	11.59	*0.654*	*7.635*	*8.29*	*0.262*	*0.013*	*29.19*	*587.3*	*71.52*	65.87
			Average	**0.665**	**7.963**	**8.628**	**0.252**	**0.014**	**35.91**	**583.2**	**74.44**	
			SD	**0.045**	**0.490**	**0.528**	**0.029**	**0.001**	**8.535**	**28.79**	**4.554**	
			%RSD	**6.741**	**6.156**	**6.117**	**11.405**	**6.389**	**23.76**	**4.936**	**6.117**	
Resv. _10 µM _2%_10D_R1	2.3	10.00	23.19	*1.538*	*11.85*	*13.38*	0.198	*0.014*	*59.98*	*846.1*	*57.71*	51.08
Resv. _10 µM _2%_10D_R2	2.3	10.00	23.19	*1.193*	*11.12*	*12.32*	*0.224*	*0.014*	*49.60*	*794.6*	*53.11*	47.97
Resv. _10 µM _2%_10D_R3	2.3	10.00	23.19	*1.493*	*13.22*	*14.71*	*0.289*	*0.014*	*45.75*	*944.3*	*63.45*	57.01
Resv. _10 µM _2%_10D_R4	2.3	10.00	23.19	*1.798*	16.26	18.06	*0.219*	*0.014*	74.42	1161	77.87	70.12
Resv. _10 µM _2%_10D_R5	2.3	10.00	23.19	0.392	*15.09*	*15.48*	*0.257*	*0.014*	*58.66*	*1078*	*66.77*	65.08
Resv. _10 µM _2%_10D_R6	2.3	10.00	23.19	*0.957*	*13.74*	*14.70*	*0.210*	*0.014*	*65.39*	*981.4*	*63.37*	59.25
Resv. _10 µM _2%_10D_R7	2.3	10.00	23.19	*1.844*	*13.55*	*15.39*	*0.242*	0.013	*56.05*	*1042*	*66.37*	58.42
			Average	**1.471**	**13.09**	**14.33**	**0.240**	**0.014**	**55.90**	**947.8**	**61.80**	
			SD	**0.344**	**1.419**	**1.240**	**0.029**	**0.000**	**7.172**	**110.3**	**5.347**	
			%RSD	**23.422**	**10.84**	**8.652**	**12.22**	**0.000**	**12.83**	**11.64**	**8.652**	
Resv. _50 µM _2%_10D_R1	11.41	50.00	115.05	*1.538*	110.30	111.83	*0.147*	*0.014*	751.3	7878	*97.20*	95.87
Resv. _50 µM _2%_10D_R2	11.41	50.00	115.05	*1.044*	*77.46*	*78.50*	0.230	*0.014*	*336.8*	*5533*	*68.23*	67.32
Resv. _50 µM _2%_10D_R3	11.41	50.00	115.05	*1.381*	*60.95*	*62.33*	*0.167*	*0.015*	*364.1*	*4063*	54.18	52.98
Resv. _50 µM _2%_10D_R4	11.41	50.00	115.05	*1.753*	*94.40*	*96.15*	*0.198*	*0.015*	*476.5*	*6293*	*83.57*	82.05
Resv. _50 µM _2%_10D_R5	11.41	50.00	115.05	0.387	*73.95*	*74.33*	*0.174*	*0.014*	*424.7*	*5282*	*64.61*	64.27
Resv. _50 µM _2%_10D_R6	11.41	50.00	115.05	*0.957*	*68.47*	*69.42*	*0.130*	*0.014*	*528.7*	*4890*	*60.34*	59.51
Resv. _50 µM _2%_10D_R7	11.41	50.00	115.05	*1.568*	*88.03*	*89.60*	*0.221*	0.012	*397.9*	*7336*	*77.88*	76.51
			Average	**1.373**	**77.21**	**78.39**	**0.173**	**0.014**	**421.5**	**5566**	**75.31**	
			SD	**0.314**	**12.36**	**12.62**	**0.033**	**0.001**	**71.50**	**1136**	**13.73**	
			%RSD	**22.83**	**16.01**	**16.09**	**19.31**	**3.603**	**16.96**	**20.42**	**18.24**	
Resv. _100 µM _2%_10D_R1	22.82	100.00	230.39	30.95	135.9	166.86	*0.206*	*0.015*	*661.4*	**9061**	*72.42*	58.99
Resv. _100 µM _2%_10D_R2	22.82	100.00	230.39	*50.23*	*132.7*	*182.93*	*0.169*	*0.014*	784.7	**9478**	*79.40*	57.60
Resv. _100 µM _2%_10D_R3	22.82	100.00	230.39	*38.08*	*117.7*	*155.76*	*0.232*	*0.015*	*507.3*	**7846**	67.61	51.08
Resv. _100 µM _2%_10D_R4	22.82	100.00	230.39	*51.16*	*111.4*	*162.57*	*0.241*	0.027	461.7	4126	*70.56*	48.36
Resv. _100 µM _2%_10D_R5	22.82	100.00	230.39	*47.27*	*132.0*	*179.27*	*0.195*	*0.014*	*676.6*	*9429*	*77.81*	57.30
Resv. _100 µM _2%_10D_R6	22.82	100.00	230.39	*40.96*	*132.0*	*172.93*	0.152	*0.014*	867.1	*9426*	*75.06*	57.28
Resv. _100 µM _2%_10D_R7	22.82	100.00	230.39	*45.47*	*131.5*	*177.01*	*0.221*	*0.014*	*595.0*	*9396*	*76.83*	57.10
			Average	**45.53**	**126.22**	**171.75**	**0.208**	**0.014**	**682.0**	**9106**	**75.35**	
			SD	**5.162**	**9.263**	**10.49**	**0.032**	**0.001**	**129.1**	**635.5**	**3.353**	
			%RSD	**11.34**	**7.339**	**6.109**	**15.43**	**3.603**	**18.93**	**6.979**	**4.450**	

Table B.9. *Trans*- resveratrol dose response result data summary for 20 days time point in 2% glucose synthetic yeast media.

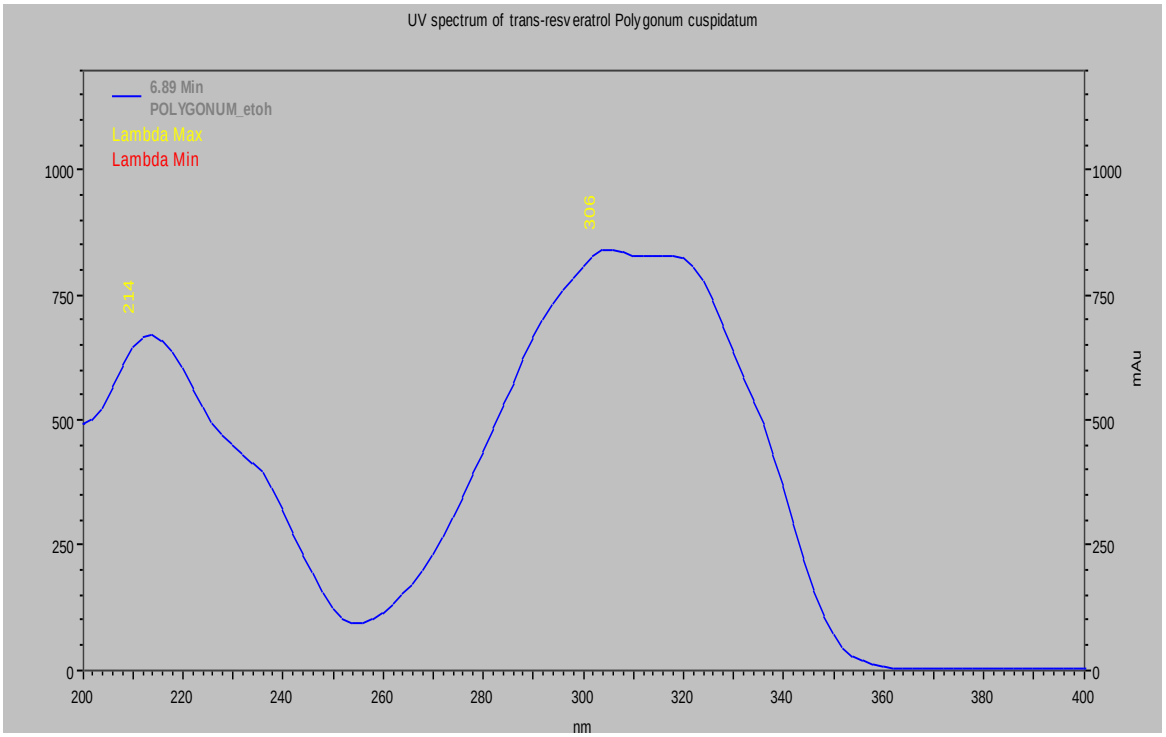
	Initial Concentration		Initial amount at t = 0	Recovered amount (µg)		Total recovered amount	Weight of cells		µg resveratrol/g of yeast (µg/g)		Percentage Recovery	Percentage uptake
Sample ID	µg/mL	µM	µg	Media	Yeast	µg	Wet	Dry	Wet	Dry	(%)	(%)
Resv. _2.5 µM _2%_20D_R1	0.54	2.50	5.78	0.00	3.397	3.397	0.225	0.012	15.07	290.3	58.77	58.77
Resv. _2.5 µM _2%_20D_R2	0.54	2.50	5.78	0.00	3.597	3.597	0.210	0.013	17.14	281.0	62.23	62.23
Resv. _2.5 µM _2%_20D_R3	0.54	2.50	5.78	0.00	3.325	3.325	0.211	0.012	15.79	284.2	57.53	57.53
Resv. _2.5 µM _2%_20D_R4	0.54	2.50	5.78	0.00	2.910	2.910	0.203	0.011	14.31	255.3	50.35	50.35
Resv. _2.5 µM _2%_20D_R5	0.54	2.50	5.78	0.00	2.535	2.535	0.188	0.013	13.46	199.6	43.86	43.86
Resv. _2.5 µM _2%_20D_R6	0.54	2.50	5.78	0.00	2.382	2.382	0.183	0.013	13.05	189.0	41.21	41.21
Resv. _2.5 µM _2%_20D_R7	0.54	2.50	5.78	0.00	6.077	6.077	0.195	0.012	31.20	528.4	105.1	105.14
			Average	0.00	3.024	3.024	0.20	0.012	14.80	249.9	52.32	
			SD	0.000	0.494	0.494	0.012	0.001	1.524	44.81	8.554	
			%RSD	#DIV/0!	16.35	16.35	5.846	4.867	10.30	17.93	16.35	
Resv. _5 µM _2%_20D_R1	1.08	5.00	11.59	0.00	5.748	5.748	0.206	0.013	27.89	456.2	49.59	49.59
Resv. _5 µM _2%_20D_R2	1.08	5.00	11.59	0.00	4.745	4.745	0.217	0.013	21.88	379.6	40.94	40.94
Resv. _5 µM _2%_20D_R3	1.08	5.00	11.59	0.00	4.094	4.094	0.208	0.012	19.72	332.8	35.32	35.32
Resv. _5 µM _2%_20D_R4	1.08	5.00	11.59	0.00	5.158	5.158	0.248	0.012	20.77	422.8	44.50	44.50
Resv. _5 µM _2%_20D_R5	1.08	5.00	11.59	0.00	3.941	3.941	0.225	0.012	17.54	328.4	34.00	34.00
Resv. _5 µM _2%_20D_R6	1.08	5.00	11.59	0.00	4.482	4.482	0.221	0.012	20.31	373.5	38.67	38.67
Resv. _5 µM _2%_20D_R7	1.08	5.00	11.59	0.00	6.702	6.702	0.192	0.012	34.87	577.8	57.83	57.83
			Average	0.00	4.695	4.695	0.221	0.012	21.35	382.2	40.51	
			SD	0.000	0.678	0.678	0.015	0.000	3.511	50.07	5.853	
			%RSD	#DIV/0!	14.45	14.45	6.951	2.561	16.44	13.10	14.45	
Resv. _10 µM _2%_20D_R1	2.15	10.00	23.19	0.00	8.539	8.539	0.194	0.012	43.97	688.6	36.82	36.82
Resv. _10 µM _2%_20D_R2	2.15	10.00	23.19	0.00	8.686	8.686	0.186	0.011	46.75	768.7	37.46	37.46
Resv. _10 µM _2%_20D_R3	2.15	10.00	23.19	0.00	12.32	12.32	0.207	0.012	59.51	1018	53.12	53.12
Resv. _10 µM _2%_20D_R4	2.15	10.00	23.19	0.00	9.782	9.782	0.209	0.011	46.92	858.1	42.18	42.18
Resv. _10 µM _2%_20D_R5	2.15	10.00	23.19	0.00	9.882	9.882	0.208	0.012	47.49	803.4	42.61	42.61
Resv. _10 µM _2%_20D_R6	2.15	10.00	23.19	0.00	9.233	9.233	0.182	0.012	50.81	769.4	39.81	39.81
Resv. _10 µM _2%_20D_R7	2.15	10.00	23.19	0.00	10.83	10.83	0.197	0.012	54.90	887.4	46.68	46.68
			Average	0.00	9.491	9.491	0.200	0.012	48.47	795.9	40.93	
			SD	0.000	0.853	0.853	0.009	0.000	3.832	71.05	3.680	
			%RSD	#DIV/0!	8.990	8.990	4.633	3.267	7.905	8.926	8.990	
Resv. _50 µM _2%_20D_R1	11.41	50.00	115.3	2.013	47.66	49.68	0.185	0.012	258.2	3939	43.09	41.34
Resv. _50 µM _2%_20D_R2	11.41	50.00	115.3	1.092	61.06	62.15	0.228	0.012	267.9	5219	53.90	52.96
Resv. _50 µM _2%_20D_R3	11.41	50.00	115.3	0.448	64.66	65.11	0.181	0.012	356.6	5257	56.47	56.08
Resv. _50 µM _2%_20D_R4	11.41	50.00	115.3	1.624	50.41	52.03	0.198	0.013	254.5	4033	45.13	43.72
Resv. _50 µM _2%_20D_R5	11.41	50.00	115.3	2.382	57.10	59.48	0.227	0.012	251.1	4681	51.59	49.53
Resv. _50 µM _2%_20D_R6	11.41	50.00	115.3	5.825	54.25	60.07	0.199	0.011	272.6	4844	52.10	47.05
Resv. _50 µM _2%_20D_R7	11.41	50.00	115.3	17.34	58.16	75.50	0.222	0.012	262.1	4971	65.48	50.44
			Average	2.587	54.77	59.82	0.210	0.012	261.1	4614	51.88	
			SD	1.872	5.026	9.111	0.018	0.000	8.150	518.4	7.902	
			%RSD	72.36	9.175	15.23	8.716	3.481	3.122	11.23	15.23	
Resv. _100 µM _2%_20D_R1	22.82	100.00	230.5	5.457	108.9	114.3	0.188	0.012	580.6	9305	49.60	47.23
Resv. _100 µM _2%_20D_R2	22.82	100.00	230.5	15.18	77.99	93.17	0.198	0.013	393.7	6190	40.42	33.83
Resv. _100 µM _2%_20D_R3	22.82	100.00	230.5	17.33	89.41	106.7	0.186	0.013	480.2	7153	46.31	38.79
Resv. _100 µM _2%_20D_R4	22.82	100.00	230.5	8.03	91.60	99.63	0.201	0.012	455.5	7697	43.22	39.74
Resv. _100 µM _2%_20D_R5	22.82	100.00	230.5	12.28	70.76	83.04	0.179	0.011	394.6	6433	36.03	30.70
Resv. _100 µM _2%_20D_R6	22.82	100.00	230.5	17.91	95.36	113.3	0.183	0.012	520.5	7946	49.14	41.37
Resv. _100 µM _2%_20D_R7	22.82	100.00	230.5	23.75	78.19	101.9	0.189	0.012	413.1	6799	44.23	33.92
			Average	13.77	87.13	103.2	0.191	0.012	459.7	7395	45.49	
			SD	6.678	14.08	11.47	0.007	0.000	76.29	1164	3.557	
			%RSD	48.50	16.16	11.12	3.714	3.629	16.60	15.74	7.821	

APPENDIX C

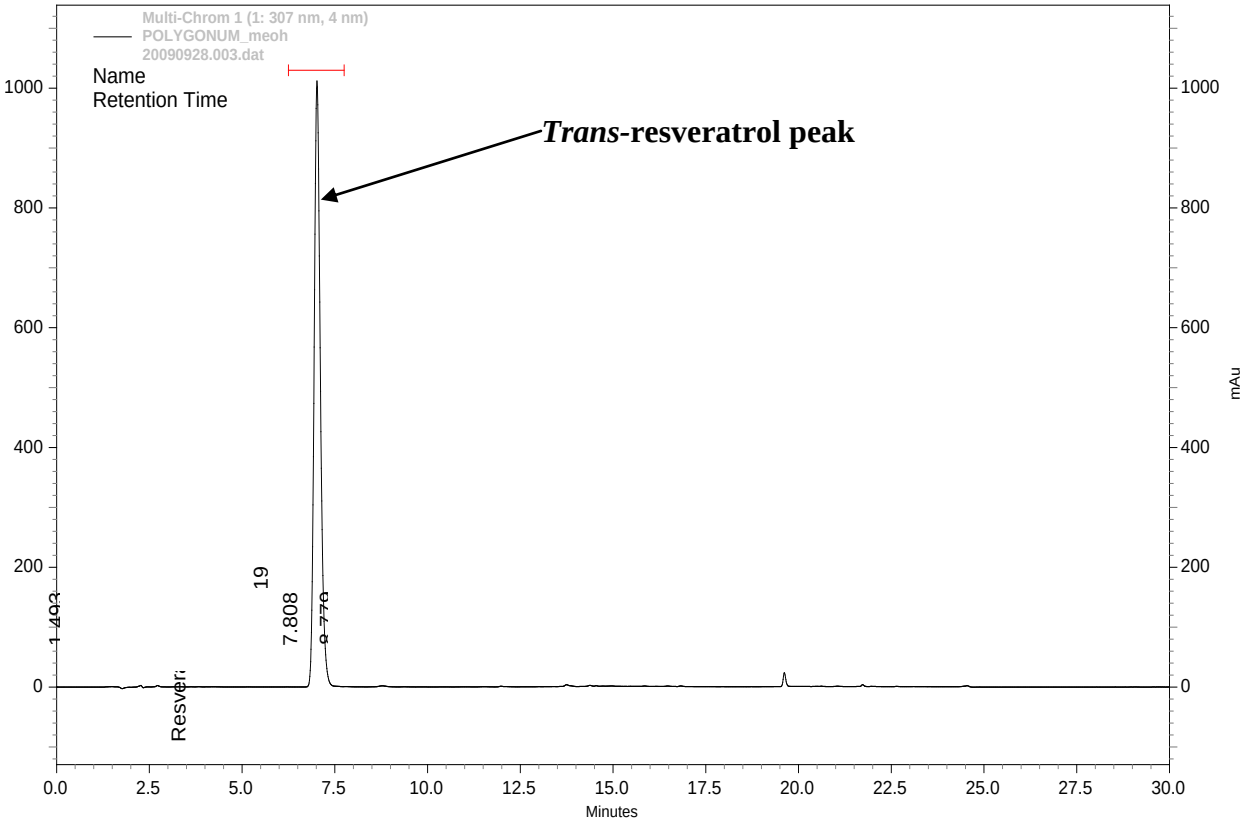
C. Comparison of the chromatograms and UV spectrum of trans-resveratrol in ethanolic, methanolic and DMF solutions of Polygonum cuspidatum.



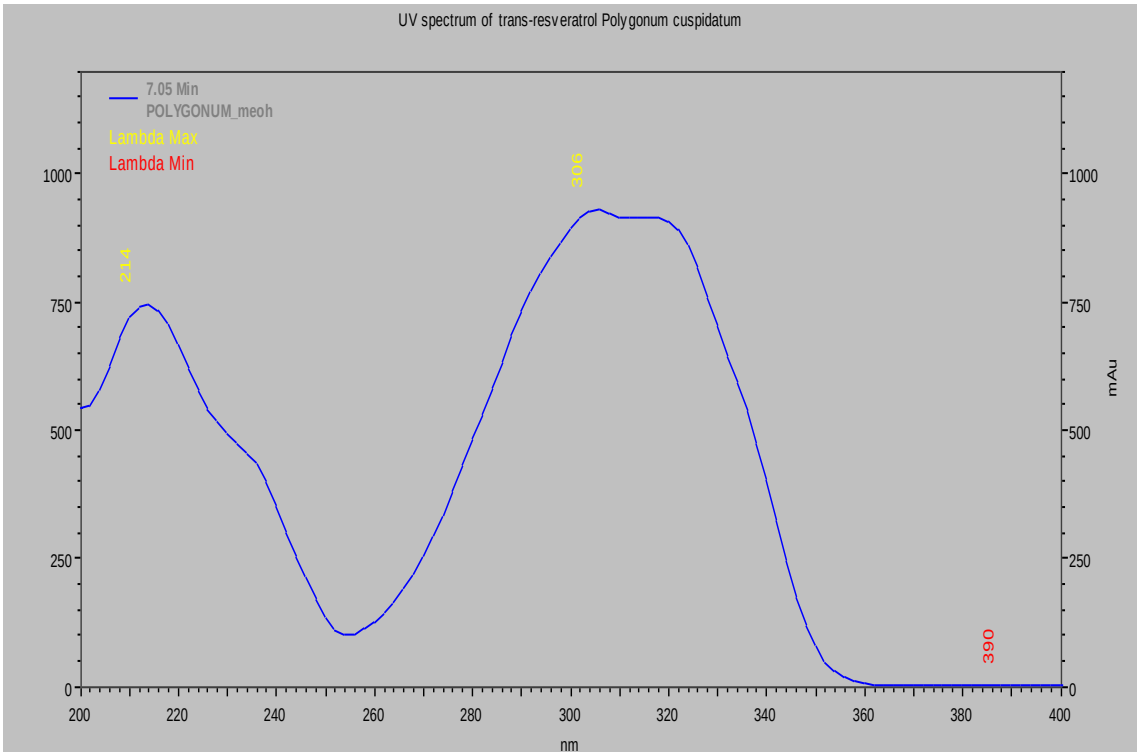
C1. Chromatogram of *trans*-resveratrol in an ethanolic solution of *Polygonum cuspidatum.*



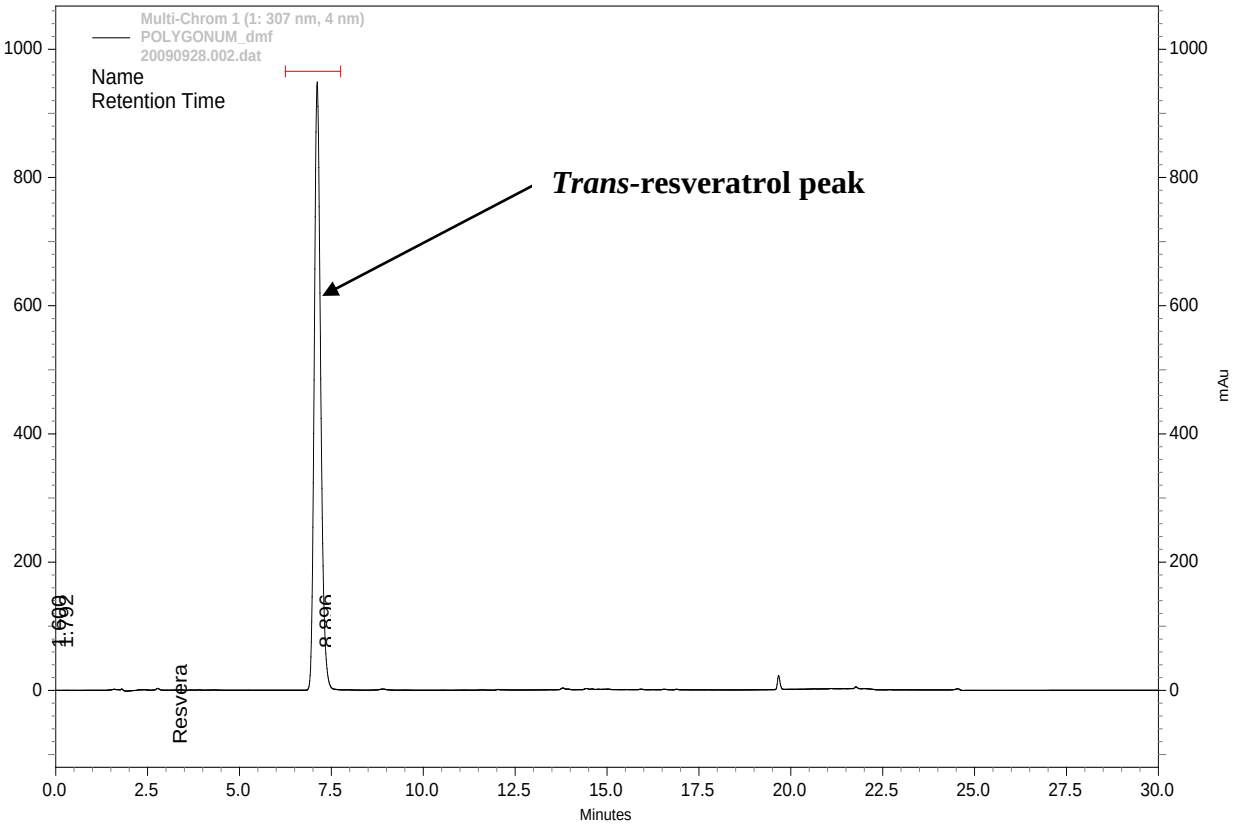
C2. UV spectrum of *trans*-resveratrol in an ethanolic solution of *Polygonum cuspidatum*.



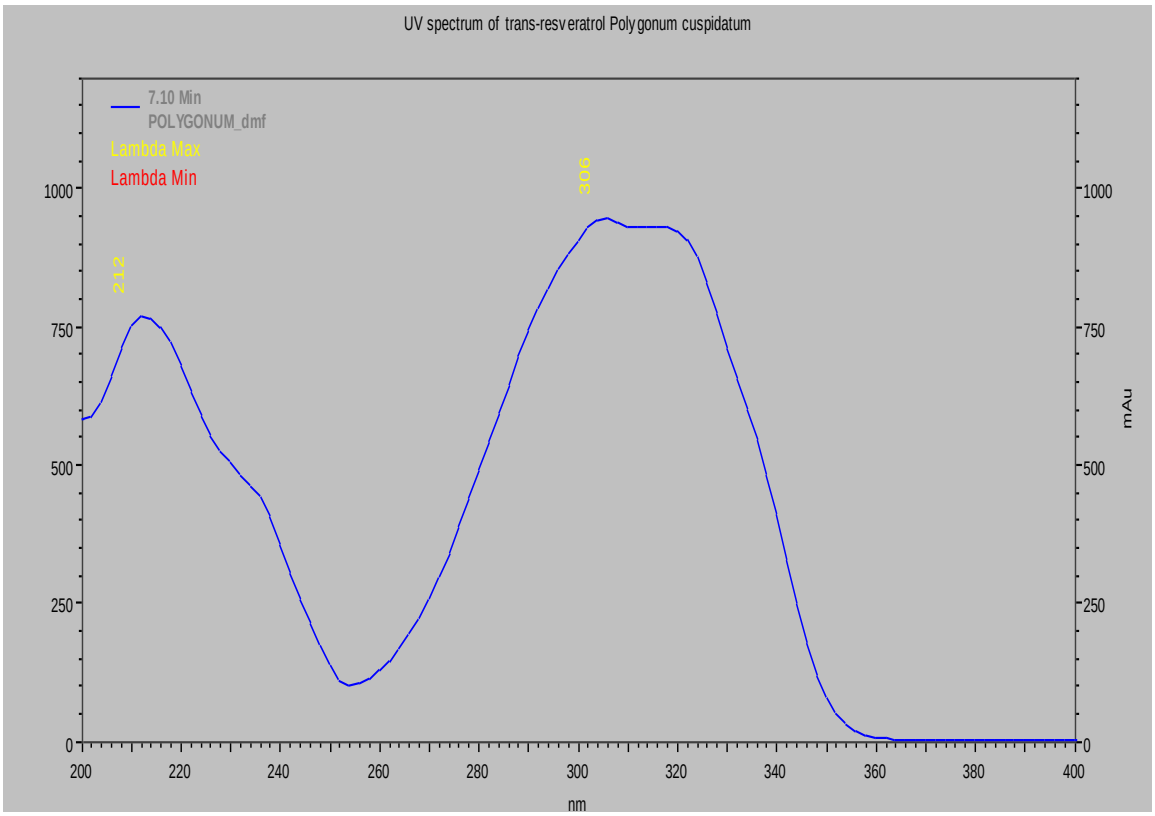
C3. Chromatogram of *trans*-resveratrol in a methanolic solution of *P. cuspidatum*.



C4. UV spectrum of *trans*-resveratrol in a methanolic solution of *P. cuspidatum*.



C5. Chromatogram of *trans*-resveratrol in a DMF solution of *P. cuspidatum*.



C6. UV spectrum of *trans*-resveratrol in a DMF solution of *P. cuspidatum*.