Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 11(1), 205-219, 2003

PRODUCTION OF FLAVORING COMPOUNDS THROUGH GENETICALLY ENGINEERED *SACCHAROMYCES CEREVISIAE* AND THEIR EVALUATION AS ANTIOXIDANT AND ANTIMICROBIAL

[16]

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ABSTRACT

Natural aroma compounds are of major interest to the flavor and fragrance industry. Biotransformation technology is useful to produce a novel active ingredients for the food and beverages. This work is aimed to produce a flavoring compound as eugenol which is the major constituent of clove essential oil by transforming the genetic material of clove bud into *Saccharomyces cerevisiae*. The transformants examined for their ability to produce eugenol as a biomarker of transformation process.The results indicated that 85% of transformants yeast isolates were able to produce eugenol. Eugenol (92%) followed by thymol (6%) and 2-phenylethyl alcohol (PEA) (0.09%), were represented in YEP broth (1) analyzed by GC/MS as the most potent aroma- active compounds of the transformants yeast. Carbon source in culture broth is consider as important factor in determining the amount and type of fragrance or flavor substances produced. The transformant extracts exhibited a potent scavenging activity on DPPH radicals. The radical scavenging activity of all extracts was significantly ($p>0.05$) depending on the carbon source in broth which decreased in the order of broth (1) (75%)> broth (2) (40%) > broth (3) (5%). Evaluation of cell free suspension (CFS) of transformants as antimicrobial was measured as the mycelium dry weight (MDW) of *Aspergillus flavus* and aflatoxin production. Data showed that the presence of either eugenol, phenylethyl alcohol or ethanol caused a reduction in MDW. The higher effect was recorded for CFS containing eugenol which reduced the MDW (33%) and aflatoxin production. The CFS containing PEA showed a lower activity. These results indicated the success of the transformation process for producing the volatile fragrance in CFS extracts which have antioxidant and antimicrobial activities and related to the phenolic content of these extracts.

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(Received October 20, 2002)
(Accepted November 27, 2002)

Keywords: Biotransformation, Eugenol, *Saccharomyces cerevisiae*, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Natural products (often referred to as secondary metabolites) are important in many aspects of life, imparting taste, aroma and color to most of our foods and providing a vast number of pharmacoactive chemicals used in medicine and agriculture. Although safe synthetic compounds are currently in use by industry and in modern agriculture, there is a strong international tendency to augment some of the synthetic chemicals with natural compounds that often are safer and friendly in concern to environmental aspects. Natural aroma compounds are of major interest to the flavor and fragrance industry. Due to the limited sources for natural aromas, there is a growing interest for developing alternative sources for natural aroma compounds, and in particular aromatic aldehydes and phenolic compounds (**Abraham and Berger, 1994**). Many essential oils contain compounds that resemble in their structure flavor and aroma compounds. Duplicating plant secondary metabolites in microbial systems (fermentation processes) leads to aroma compounds that are classified as natural by the European and US food legalization. This label represents a strong marketing advantage (**Berger and Krings, 1998**).

By using biotransformation technology the biotechnologists could manufactured a range of natural, novel active ingredients for the food and beverage, cosmetics and personal care markets, for example: flavours, fragrances, antioxidants and antimicrobials (**Shimoni *et al* 2000**). **Welsh (1994)**, reported that transferring the genetic material responsible for flavour production from a donor organism via an appropriate vector to the host, its metabolism may be directed a way from primary metabolism (growth and replication) to secondary metabolism, thereby increasing the potential for aroma production. The best way functionally to characterize a cloned gene is to put it into live cells and determine what effects the encoded gene product has on defined cellular processes. Similarly, to identify DNA elements required for the transcriptional control of a cloned gene, an appropriate reporter gene must be constructed and tested *in vivo* (**Gottesfeld *et al* 1997 and Smeets *et al* 2000**).

The aflatoxins are naturally occurring, fungal elaborated, toxic secondary metabolites and produced primarily by *Aspergillus flavus* and *A. parasiticus*. They are ubiquitous and have been found as natural contaminants in a variety of foods such as peanut butter, breakfast cereals, corn and corn meals, dairy products and other processed foods (**Daeschel, 1992**). Because of aflatoxins may significantly affect animal and human health, protection of food and feed stuffs from these contamination is a crucial need. Numerous chemicals have been shown to prevent the growth of *A . flavus* and aflatoxins biosynthesis including some essential oils or active components as eugenol (**Lee and Shibamoto, 2001**).

Eugenol (phenolic compound) occurs widely as a component of essential oils and is a major constituent of clove oil. It has been used since at least the nineteenth century, primarily as a flavoring agent, in a variety of foods and pharmaceutical

products, and as an analgesic in dental materials (**Dorman *et al* 2000**). *Saccharomyces cerevisiae* is a single-cell yeast which has a wide extensive use in food and beverage industries, and has a heterologus euokaryotic system to study the intracellular targeting proteins in different organelles (**Ting *et al* 1997**).

This work was undertaken to study the possibility for producing eugenol (flavoring compound) which is the major constituent in clove essential oil, via genetic transformation from clove plant to the yeast *Saccharomyces cerevisiae*. Also, to evaluate the functional properties of the new microbial products as antioxidant and antimycotic agent specially for *Aspergillums flavus* and inhibition of its aflatoxin production.

MATERIAL AND METHODS

Microorganisms

A diploid wild type *Saccharomyces cerevisiae* strain (recipient) was kindly provided by Egyptian Sugar and Distillation Company, Hawamdia, Egypt.
Mycotoxin producer isolate of *Asperighillus flavus* was supplied by Mycotoxin laboratory, National Research Center, Cairo, Egypt.

Media

The principal medium (Broth 1) (**Hafez *et al* 2000**): Yeast extract peptone (YEP) was used for yeast maintenance. It has the following composition: 5 g yeast extract, 3 g peptone,10 g glucose, distilled water 1000 mL, added 20 agar to prepare solid medium.

Modified media: Broth (2): resemble the above medium + 10 sucrose. Broth (3): as the principal medium except sucrose instead of glucose (10g/L).

Isolation of DNA

Donar DNA was isolated, purified and fragmented from clove buds (*Syzygium aromaticum L.*) by the method of **Hafez *et al* (2000).**

Transformation

Recipient cells (0.2 ml of $1x10^7$ to $3x10^6$ cells/ml) were mixed with different concentrations of eugenol and lithium acetate 0.3 % was used. After 5 to 10 min, 0.1 ml of the Donor DNA in 50 mM CaCl2 was added to the mixture. The latter was incubated at 0 ^{0}C for 15 min., the mixture was diluted 3 to 10 times after incubation and then plated and incubated at 30°C for selection of the transformants (**Diatchenko *et al* 1996**).

Isolation of volatile components

To extract extracellular volatile compounds, cells were removed from the suspension culture (48 h) by centrifugation for 10 min. A 200ml portion of cell-free suspension (CFS) of wild and transformed yeast, was adjusted at pH7.8 with 2% NaHCO3 solution and extracted three times with diethyl ether (20 mL). The volatile compounds were obtained by evaporation of the solvent on water bath at 40 ^{0}C, according to the methods described by **Drawert *et al* (1983) & Abraham and Berger (1994)**, and then the samples examined by GC/MS analysis.

Gas chromatography/Mass spectrometry analysis (GC/MS)

GC-MS analysis of the volatile components extracted from the filtrates of wild and transformed yeast (CFS), was performed on a Varian gas chromatograph interfaced to Finnigan SSQ 7000 mass selective detector (MSD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5. The oven temperature was programmed from 50°C for 3 min, isothermal, then heating by 7°C / min to 250°C, and isothermally for 5 min at 250°C. Injector temperature was 200°C and the volume injected was 0.5µL. Transition – line and ion source temperatures were 250°C and 150°C, respectively. Identifications were based on the comparison with the MS computer library (NIST–Software package, Finnigan), and with authentic components and published data (**Adams 1995**). The quantitative determination was carried out based on peak area integration.

Determination of total phenolic compounds

The total phenolic compounds present in the CFS volatile extracts of wild and transformed yeast were determined as described by **Singleton *et al.*(1999)** using Ciocalteu reactive reagent. The concentration of total phenolic compounds in tested CFS was determined by comparison with the absorbance of standard catechin at different concentrations.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The effect of volatile components in CFS extracts of wild and transformed yeast on DPPH radical was estimated according to **Hatano *et al* (1988)**. In this test, the percentage of DPPH reduction (antioxidant activity) of volatile CFS extracts of transformed yeast was compared to that of gallic acid, butylated hydroxyanisole (BHA) and cinnamic acid (negative control).

Antimicrobial Study

The antimicrobial activity of CFS extracts of transformed yeast was measured as the inhibition of *Aspergillus flavus* growth and its aflatoxin production according to the method of **Ali (1999)**. The level of inhibition relative to control was calculated as previously reported by **Deans and Svoboda (1990)**. Standard eugenol at different concentrations (zero, 100 and 200 ppm) were used as positive control to the activity of volatile CFS extracts of both wild and transformants.

Aflatoxin analysis

Aflatoxins in culture filtrate (after separation of mycelium) were extracted in an aliquot (10mL), clean up and then quantitatively assayed by using HPLC method as described by **AOAC (1995).**

HPLC procedure

HPLC analyses were performed as described by **Groopman *et al* (1985).**

RESULTS AND DISCUSSION

Twenty isolates were chosen and their CFS extracts were examined for their ability to produce eugenol as a biomarker

of transformation process. Volatiles of cell-free suspensions (CFS) of wild and transformed yeast strains were harvested after 48 h which obtained by direct solvent extraction and were subjected to GC/MS analysis. Table (1) showed that 17/20 of transformed yeast samples (85%) were efficient to produce eugenol through the biotransformation process indicating the genetic ability of the yeast transformants to produce these volatile compounds, which may be due to either one of the following reasons: First, is that a gene in a common pathway had been introduced through transformation which switched on the blocked pathway in yeast. Second, the introduction of regulatory gene (s) through transformation in different locations in the different yeast colonies (**Awad *et al* 1993**).

Table 1. The efficiency of yeast transformants *Saccharomyces cerevisiae* extracts to produce eugenol analyzed by GC-MS.

Trans. No.	Efficiency	Trans. No.	Efficiency	Trans. No.	Efficiency	Trans. No.	Efficiency
1	+	6	++++	11	+	16	+
2	++	7	_	12	++	17	++
3	++	8	++	13	+	18	++++
4	+++	9	+	14	-	19	+++
5	+++	10	++++	15	-	20	++

Trans: Transformants yeast
+ : 23 ± 2 (based on area percent)

On the other hand, 3/20 of transformed samples were not able to produce eugenol or any volatile compounds. The negative results may be due to the limited number of comptent cells in the population that can take up DNA very efficiently (**Rambosek and Leach, 1987**). From the above results, it was found that CFS of transformed yeast samples no 6, 10 and 18 were efficient to produce eugenol (> 90 %) in comparison with other samples (Table 1), and then subjected to further studies.

In GC / MS analysis (Table 2 and Figure 1) few volatile compounds were identified, however the profiles were quite different from that of the native clove essential oil. Eugenol (92 %) was the most abundant compound in the volatile CFS extract of the transformed

Table 2. The volatile components of yeast transformants grown on different carbon source

Identified components	Wild	Transformants		
		Broth (1)	Broth (2)	Broth (3)
Eugenol	Nil	92.0	59.5	7.0
Thymol	Nil	6.0	8.0	2.0
Phenylethanol	Nil	0.09	24.0	87.0

Broth (1) containing glucose as carbon source.
Broth (2) containing glucose + sucrose as carbon source.
Broth (3) containing sucrose as carbon source.

Saccharomyces cerevisiae, followed by thymol (6%) and 2-phenyl ethyl alcohol (PEA) (0.09%), which represent the most potent aroma-active compounds of the broth culture (Fig 1. B). The above results suggest that DNA transformation of clove bud (donor) to the yeast cells (host) could enhance the development of flavoring compounds (eugenol, thymol, 2-phenyl ethyl alcohol) although their flavoring characteristics and concentrations were different from that of the original clove essential oil.

A low concentration of volatile components (50 mg / L) was obtained from the CFS of the transformed *Saccharomyces cerevisiae*. The low yield of the volatile compounds produced via such process may be explained on the basis that many of the desired secondary metabolites may limit the biosynthesis either by inhibiting steps in the metabolic pathway leading to the metabolite or by disrupting the cell. Finally, the metabolites often produced in low concentration, such that large fermenter volumes may be required to obtain commercial scale quantities of the desired flavoring substances (**Dornenburg and Knorr, 1995**). Many microbiologically mediated fragrance and aroma syntheses produce low amounts of the secondary metabolites, as these metabolites inhibit cell or pathway activity (end-product inhibition) as reported by **Dornenburg and Knorr, 1987**.

By changing the composition of the nutrient medium (sucrose instead of glucose) of the grown transformed *Saccharomyces cerevisiae*, it was found that transformant isolates grown on YEP medium containing only glucose as carbon source (broth 1) showed a higher concentration of eugenol (92±5%) compared to other volatiles as thymol and 2-PEA (6±0.3 and 0.09 ± 0.009%, respectively). On the other hand, by

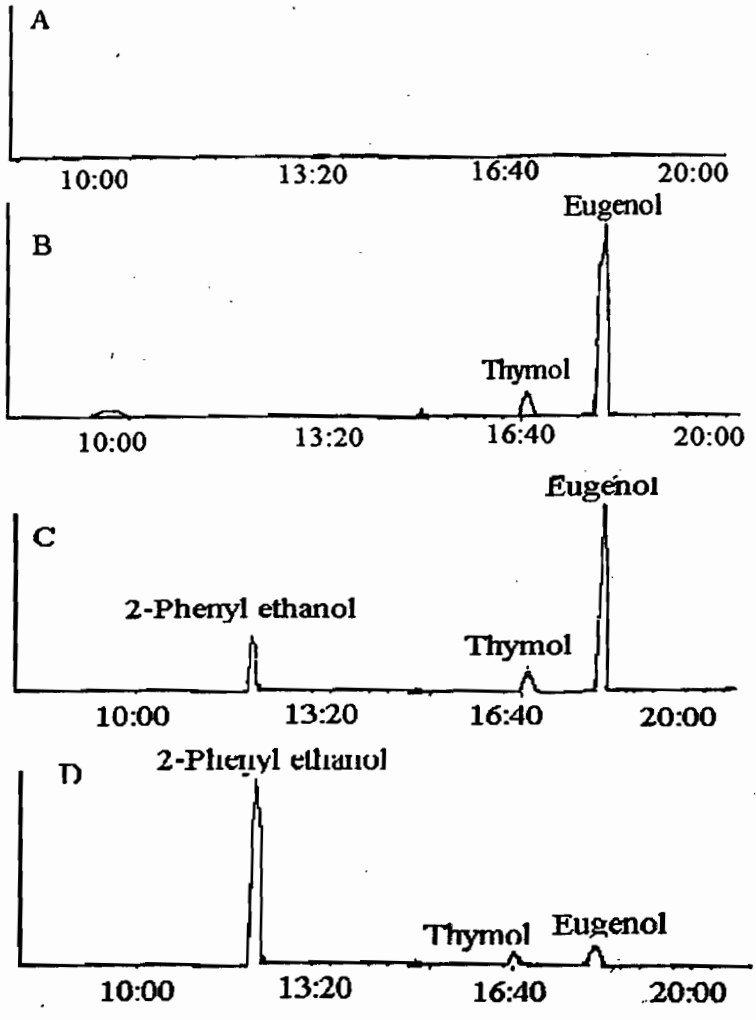

Figure (1):GC-MS chromatogram of volatile components isolated from wild and transformed culture free cells (CFS) of *Saccharomyces cerevisiae*

A: wild yeast B: Yeast transformed on broth (1)
C: Yeast transformed on broth (2) D: Yeast transformed on broth (3)

replacing glucose by sucrose (100 %) to the YEP medium (broth 3), a significant elevation in the 2- PEA concentration (87±4%) with its mild rose–like aroma which considered to improve the flavour of distilled beverages (**Beltiz and Grosch, 1987**), followed by eugenol and thymol (7 + 0.2 and 2 + 0.3 %, respectively) (Table 2).

These results indicated that carbon source in culture is considered as an important factor in determining the amount and type of fragrance or flavor substances produced. Our results are in good harmony with those obtained by **Monory *et al* (1994),** they noticed that seed culture generation, pH, fermentation time, temperature were also affected on flavor profile. Generally, it is assumed that many volatile metabolites useful in flavors and fragrances are the result of secondary metabolites and are found at their maximum levels after peak cell growth. Also, carbon and nitrogen sources influence the production of aroma substances.

Jiang (1995) reported the production of short-chain esters, alcohols and phenyl ethyl derivatives by different *Kluyvermyces lactis* using different carbon / nitrogen sources. Moreover, modifications of culture conditions, especially the choice of nitrogen and carbon sources, have often been reported to influence, at least quantitatively the composition of the fungi odorous profiles (**Gallois *et al* 1990**).

From all the above results we can concluded that the variation in the nutrient media composition (type of carbon source) greatly influence the aroma profile obtained from the transformed yeast and directed them to the formation of different compounds as eugenol and 2-phenyl ethyl alcohol.

The bioactivity of the volatile compounds in CFS

Antioxidant activity (DPPH)

It is well known that free radicals play an important role in autoxidation of unsaturated lipids in foodstuffs (**Kaur and Perkins, 1991**). For example, oxidation of muscle cholesterol may be intiated by free radicals generated during the oxidation of polyunsaturated fatty acids (**Hoelscher *et al* 1988**). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as a free radical to evaluate antioxidative activity of some natural sources (**Yen and Chen, 1995**). On the other hand, antioxidants are believed to intercept the free-radical of chain of oxidation and to contribute hydrogen from the phenolic hydroxyl groups themselves, thereby forming stable free radicals which do not initiate or propagate further oxidation of lipids (**Dziezak, 1986 & Lee and Shibamoto, 2001**).

In the present work, the scavenging effects of the volatile compounds in CFS extracts of wild and transformed yeast grown on different media composition (sucrose instead of glucose) are shown in Figure 2. All the tested volatile CFS extracts showed a remarkable activity by inhibiting the DPPH radical. The scavenging activity of all extracts on inhibition of the DPPH radical was related to the chemical components of these extracts as well as their phenolic content (Figure 2). The scavenging effects of all extracts significantly ($P<0.05$) decreased in the order of transformant yeast (broth 1)> transformants (broth 3)> transformants (broth 2) > wild (75 %, 40 %, 5 % and zero %), respectively. These results indicating that the transformant volatile

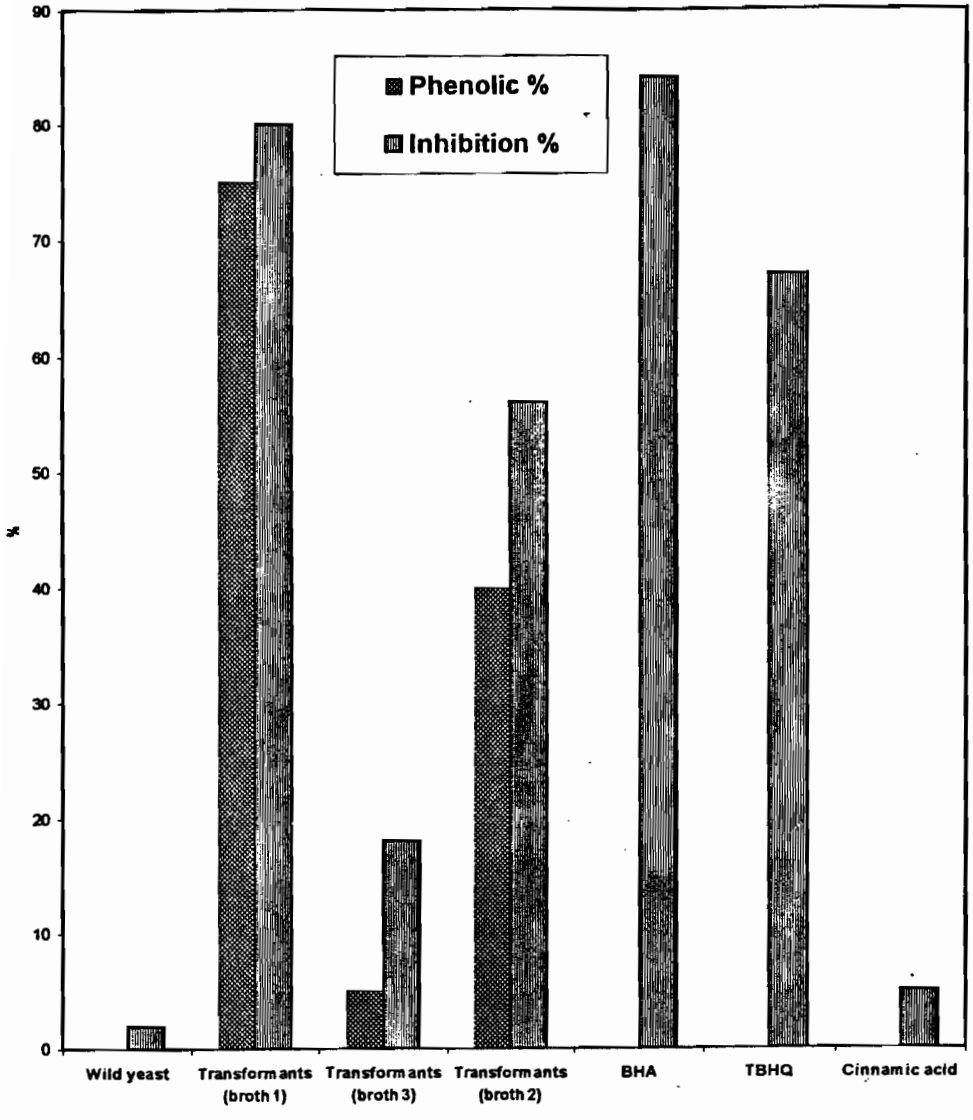

Figure 2 : The relationship between the radical scavenging activity on DPPH radicals and total phenolic content of transformed *Saccharomyces cerevisiae*

extracts exhibited a potent scavenging activity on DPPH radicals depending on the chemical composition (Fig 1) and the phenolic content. Varying degrees of antioxidative activity were exhibited by the volatile CFS extracts of transformed *Saccharomyces cerevisiae* grown on YEP and other modified media. Even though the antioxidative activity of clove buds has been reported several times and related to the presence of phenolic compounds as eugenol (**Farag *et al* 1989 and Satoh *et al* 1998**), where phenolic groups play an important role in the antioxidative activity (**Huang and Frankel, 1997**). The antioxidative activity of an aroma extract of transformed yeast has not been investigated prior to the present study. The present study suggests that the antioxidative activities of volatile CFS extracts of the transformant yeast grown on different media compositions are due in part to the contribution of aroma chemicals as eugenol, thymol and 2-phenylethyl alcohol. The presence of various aroma chemicals was explain the improvement of food stability. Moreover, ingestion of these compounds was useful in prevent *in vivo* oxidation damage, such as lipid peroxidation, which is associated with many diseases, including cancer, atherosclerosis, diabetes and immune difficiency.

Antimicrobial test

Evaluation of volatile CFS extracts of transformed *Saccharomyces cerevisiae as* antimicrobial was measured by determining the potency of these extracts to inhibit the mycelium dry weight (MDW) of *Aspergillus flavus* and its aflatoxin production. Data in Table (3) showed that the presence of volatile compounds in transformed CFS extracts as eugenol, 2-phenyethyl alcohol or ethanol caused a reduction in the MDW. The reduction is dependent on the presence of active components and their concentrations, which produced during fermentation of transformant yeast extracts. The reduction reached 33 % for CFS extract containing eugenol (broth 1), meanwhile, CFS extract containing 2-PEA (broth 3) reduced the MDW by 27.3 %, which is quite near from that obtained by standard eugenol (50 ppm) indicating that the transformants growth reached its maximum at 50 ppm. So, it is important to find technology to immobilize the transformant cells to produce volatile compounds economically. Data in Table (4) showed that standard eugenol at concentration (50 ppm) reduced the MDW by 27.3% and completely inhibits the aflatoxin production (100%). Also, the aflatoxin production was inhibited when *A. flavus* grown on CFS extract containing components as eugenol and 2-PEA. These results indicated the presence of active components in CFS that have antimicrobial activity. Our results are in good agreement with those obtained by **Wilson *et al* (1997)**, who reported that eugenol as the main component of clove essential oil had higher antimicrobial activity when tested against several fungal species. Regarding 2-PEA caused lower percentage of reduction in fungal growth and aflatoxin production. This activity related to the structure of each component. The strong antimicrobial activity of eugenol is due to the presence of phenolic group. However, 2- phenylethanol does not have a phenolic group, therefore its activity may be due to its phenolic nature (Fig 1).

Table 3. Effect of volatile extracts of yeast transformant on the growth and aflatoxin production of *A.flavus*

*Cultures	Mycelium dry weight (mg/100ml)	Inhibition %	Aflatoxin (µg/L)	Inhibition %
Wild	1500	16.0	27.0	23.0
Broth(1)	1200	33.0	0.0	100.0
Broth(2)	1300	27.0	0.0	100.0
Broth(3)	1300	27.3	0.0	100.0
Control	1800	0.0	35.0	0.0

*Wild is culture of *A. Flavus* in YEPG, broth, broth1= culture containing glucose as carbon source, broth 2 culture containing sucrose as carbon source., broth 3 culture containing glucose + sucrose as carbon source and control is culture of *A. Flavus* in YEPG broth

Table 4. Effect of standard eugenol on the growth and aflatoxin production of *A. flavus*

Eugenol concentration ppm	Mycelium dry weight (MDW)	Inhibition %	Aflatoxin Ug/L	Inhibition %
25	1.7	5.55	0.0	100.0
50	1.3	27.3	0.0	100.0
100	0.0	100	0.0	100.0
Control	1.8	0.0	35.0	0.0

CONCLUSION

From the above results, it is concluded that the biotransformation of the genetic material of clove bud into *Saccharomyces cerevisiae* cells could produce flavoring substances such as eugenol and 2-pheny ethyl alcohol. Although, there are some limitations of the biotechnological production of flavors via microorganisms such as low product yields, substrate/product toxicity, long fermentation times, organism morphology, product recovery due to low concentration in aqueous medium as well as products mixes, i.e., not pure chemicals.

Yet, the main advantage of such technique; all naturals will get much higher price, uniqueness may justify price, e.g., sterioisomers high flavor impact may reduce needs to where cost is not as much as an issue. Therefore, it is of great importance to find new natural flavor sources participating partially in covering the shortage of plant flavor consumed or produced in Egypt. Further investigations are also required to assay the bioactivity as antioxidant effect *in vivo* and to evaluate its relevance to human health for preventing some chronic diseases.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(١) ، ٢٠٥ -٢١٩ ، ٢٠٠٣

إنتاج مركبات نكهة باستخدام عزلة من سكارواميسيس سرفيسيا مهندسة وراثيا وتقييمها كمانعة للأكسدة ومضادة للميكروبات

[١٦]

سهير السيد على[١] - نيفين أبو سريع[٢] - خالد فاروق المصري[٣]

١- سموم وملوثات الغذاء - المركز القومي للبحوث - الدقى- القاهرة - مصر

٢- قسم الوراثة الميكروبية - المركز القومي للبحوث - الدقى- القاهرة - مصر

٣- قسم كيمياء مكسبات الطعم والرائحة - المركز القومي للبحوث - الدقى- القاهرة - مصر

تعتبر مركبات النكهة الطبيعية لها أهمية كبيرة في صناعة مكسبات الطعم والرائحة. وتستخدم إحدى طرق التقنيات الحيوية وخاصة النقل الحيوي Biotransformation لانتاج مركبات جديدة مكسبه للطعم والرائحة وذات نشاط حيوى.

ويهدف البحث إلى إنتاج مركبات نكهة مثل اليوجينول وهو المركب الأساسي للزيت الطيار لنبات القرنفل باستخدام طريقة النقل الحيوي للمادة الوراثية من نبات القرنفل إلى خلية الخميرة من نوع *Sccharomyces cerevisiae* وكانت النتائج كالآتي:

- تم نقل المادة الوراثية من نبات القرنفل إلى خلية الخميرة.
- تم اختبار العزلات الجديدة من الخميرة لمدى قدرتها على إنتاج اليوجينول ودلت النتائج على أن ٨٥% من العزلات الجديدة لها القدرة على إنتاج اليوجينول مع وجود اختلاف في تركيز المادة

المتحصل عليها من العزلات دلالة على كفاءة عملية نقل المادة الوراثية . وأمكن تقدير تركيب المستخلص الناتج من نمو بعض العزلات فى البيئة الاساسية YEP باستخدام GC/MS ووجد ان المستخلص يحتوى على ٩٢% يوجينول و٦% ثيمول و ٠,٠٩% فينيل الايثانول.

- وقد وجد انه باستبدال سكر الجلوكوز بالسكروز كمصدر للكربون فى بيئة YEP التي تنمو عليها الخميرة المعدلة وراثيا أن تركيز المركبات الطيارة في المستخلص للخميرة المعدلة اختلف باختلاف نسبة السكروز إلى الجلوكوز.
- تم تقييم المستخلص العطري للخميرة المعدلة وراثيا كمواد مضادة للأكسدة ووجد انه له قدره على تثبيط تكون الشقوق الحرة وانه مضاد للأكسدة وتختلف قدرته أيضا باختلاف نوع السكر فى البيئة YEP فقد وجد ان وجود الجلوكوز وحده أعطى أعلى تأثير كمضاد للأكسدة ٧٥% يليه وجود الجلوكوز+ السكروز ثم السكروز وحده ٥٠%.
- كما تمت دراسة تأثير راشح الخميرة المعدلة وراثيا كمضاد للميكروبات ووجد أن له تأثير مثبط لنمو فطر *Aspergillus flavus* وكذلك قدرته على إنتاج السموم aflatoxins وأن هذا التأثير يختلف أيضا باختلاف نوع السكر كمصدر للكربون. وقد يعزى قدرة الراشح كمانع للأكسدة ومضاد لنمو وافراز السموم من فطر *Aspergillus flavus* الى وجود مادة اليوجينول فى البيئة حيث انه مركب فينولى أما البيئات المحتوية على مادة الفينيل ايثانول فقد أعطت تأثيراً أقل.

نستخلص من الدراسة نجاح عملية نقل المادة الوراثية من القرنفل إلى الخميرة وانتاج عزلات جديدة منها ذات أهمية حيوية أعلى من العزلات الأصلية فى مجالات أوسع للاستخدام كمانعة للأكسدة ومضادة للميكروبات . وانه بتغيير مصدر الكربون فى بيئة النمو يمكن الحصول على العديد من المركبات الطيارة الطبيعية ذات الأهمية الحيوية العالية ويمكن استخدام مصدر كربوني رخيص يعظم الاستفادة من عزلات الخميرة المعدلة وراثياً.

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