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The Preliminary Study on the Role of 1-Hexene Monooxygenase in Delayed Fruit Ripening by Rhodococcus rhodochrous DAP 96253

Wenxin Jiang

THE PRELIMINARY STUDY ON THE ROLE OF 1-HEXENE MONOOXYGENASE IN DELAYED FRUIT RIPENING BY *RHODOCOCCUS RHODOCHROUS* DAP 96253

by

WENXIN JIANG

Under the Direction of George E. Pierce, PhD

ABSTRACT

Rhodococcus rhodochrous DAP 96253, a well-known industrial bacterium, had various 1-hexene monooxygenase (1-HMO) activities when grown on YEMEA plates supplemented with eight different carbohydrates. Besides, 1-HMO exhibited different storage temperature preferences. Lactose could induce the highest 1-HMO activity in *R. rhodochrous* DAP 96253 while the cells showed the lowest 1-HMO activity when trehalose was the supplement. The 1-HMO activity of *R. rhodochrous* DAP 96253 was not maintained when stored at 37°C as well as at 4°C and 25°C. Trehalose-induced 1-HMO activity of *R. rhodochrous* DAP 96253 was more stable from Day 0 to Day 21 at all these three temperatures, compared with the other seven carbohydrates.

Immobilization of enzymes can maintain enzyme activity longer, offer easier enzyme storage conditions and make some enzymes reusable, much research has been done in this area.

In this study, *R. rhodochrous* DAP 96253, grown on YEMEA plates supplemented by glucose and urea, was investigated using whole bananas as the inducer of 1-HMO activity and different immobilization methods to maintain this enzyme activity. It was shown that calcium-alginate polyvinyl alcohol (PVA) beads could maintain 1-HMO activity of *R. rhodochrous* DAP 96253 more stable than calcium-alginate beads. Whole bananas exhibited very obvious effects of inducing 1-HMO activity of *R. rhodochrous* DAP 96253.

A number of recent studies have clearly demonstrated that induced cells of *R. rhodochrous* DAP 96253 can prolong the shelf-life of post-harvested fruits. With USDA estimates of 40% of all harvested produce in the US not being consumed because of loss of quality, the ability to extend the period of ripeness of produce has great potential to improve the quality of nutrition. Modification or degradation of those signals (primary and secondary) associated with ripening in fruit or the perception of those signals represents a potential mode of action for delayed ripening by induced cells of *R. rhodochrous* DAP 96253. Ethylene and cyanide are the two primary signals in ripening. In this study, the role of 1-HMO from induced cells was investigated by time-course experiments focusing on 1-HMO activity and stability. In addition, fruit volatile organic compounds (VOCs) were detected and compared by GC-FID and GC/MS over the course of fruit ripening. The results show a correlation between 1-HMO activity and stability in delayed fruit ripening. It was further demonstrated that the presence of secondary signal fruit VOCs enhanced 1-HMO activity. Aromatic profiles of treated fruits, by GC-FID and GC/MS, show a consistent picture of VOCs associated with earlier fruit ripening stages.

INDEX WORDS: *Rhodococcus*, 1-HMO, Carbohydrates, Enzyme Immobilization, Delayed Fruit Ripening

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WENXIN JIANG

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WENXIN JIANG

Committee Chair: George E. Pierce

Committee: Sidney A. Crow

Eric S. Gilbert

Electronic Version Approved:

Office of Graduate Studies

College of Arts and Sciences

Georgia State University

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DEDICATION

To my family in China, whose spiritual strength always encourages me to chase my dreams endlessly!

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LIST OF ABBREVIATIONS

ACC……..1-Aminocyclopropane-1-carboxylic acid

AMO……Alkene monooxygenase

cdw……Cell dry weight

Co……Cobalt

ddiH_2O…….Double deionized water

G…….....Glucose

FAD……flavin adenine dinucleotide

FMN……..Flavin mononucleotide

HCN……..Hydrogen cyanide

1-Hexene Monooxygenase……1-HMO

HS-GC-FID……...Headspace-gas chromatography-flame ionization detector

HS-SPME-GC-FID…….Headspace-solid phase microextraction-gas chromatography-flame ionization detector

HS-SPME-GC/MS……...Headspace-solid phase microextraction-gas chromatography-mass spectrometry

ICL…….Isocitrate lyase

Met……...Methionine

MOs……Monooxygenases

NAD(P)H……...Nicotinamide adenine dinucleotide (phosphate) hydrogen

NBP…….4-(4-Nitrobenzyl)-pyridine

NHase…….Nitrile hydratase

P450s……Cytochrome P450 monooxygenases

PVA……..Polyvinyl alcohol

SAM……...S-adenosylmethionine

SEM……...Scanning electron microscopy

SMO……Styrene monooxygenase

TIC……..Total ion chromatogram

U……..Urea

VOCs……Volatile organic compounds

YEMEA……Yeast extract malt extract medium (agar)

1 INTRODUCTION

1.1 Rhodococcus

Rhodococci, which belong to the nocardioform actinomycetes, are aerobic, non-sporulating, non-mobile, Gram-positive bacteria which may form rudimentary branching mycelia from rod- or coccus-shaped cells (Goodfellow, 1989). The genus of *Rhodococcus* includes many species characterized by tremendous genetic and physiological diversity. Different *Rhodococcus* species can be isolated from both terrestrial and aquatic habitants and are considered to be environmentally ubiquitous (Martinkova *et al.*, 2009). The main components of *Rhodococcus* cell envelopes are fatty acids and mycolic acids with the cell walls characterized of chemotype IV (Sutcliffe *et al.*, 2010). Rhodococci produce many enzymes which are useful for industry, and they also can be used for biodegradation of many kinds of organic compounds based on their metabolic diversity (Bell *et al.*, 1998).

Many of the complex compounds metabolized by *Rhodococcus* are toxic synthetic molecules which are stable environmentally, for example, oxygenates, nitroaromatics and nitriles (Martinkova *et al.*, 2009). Since many *Rhodococcus* species can live in oligotrophic environments, it is also well suited for bioremediation. The decomposition of many pollutants by *Rhodococcus* can still be carried on even when commonly utilized and easily degradable carbon sources are available (Warhurst and Fewson, 1994). The aliphatic chains of mycolic acids, which exist in *Rhodococcus* cell walls, facilitate uptake that improves the degradation of hydrophobic pollutants (Neu, 1996). It has been found that *R. rhodochrous* ATCC 19067 can hydroxylate selected alkenes as well as oxidize various alkanes (Jurtshuk *et al.*, 1971). Furuhashi *et al.* (1981) suggested that *R. rhodochrous* B276 was able to produce chiral epoxypropane from some certain types of aliphatic alkenes. Degradation of alkenes into epoxides brings benefits to people in two

ways: alkenes will not contaminate our environments anymore; epoxides will be used in industrial and medical areas.

Immobilization of enzymes have been widely used in industry since it can maintain enzyme activity longer, offer easier enzyme storage conditions and make some enzyme preparations reusable. There are variable methods of immobilization. Entrapment is a popular immobilization method with the whole-cell enzymes enclosed in a polymeric and porous matrix, through which the substrates and products can flow to and from the cells. The commonly used materials for entrapment are alginate, agar, agarose, cellulose, polyacrylamide, etc (Tampion and Tampion, 1987). It has been shown that immobilized *Rhodococcus* sp. F92 can degrade petroleum products successfully and this result could be used to decrease the pollution caused by oil spill (Quek *et al.*, 2006). Immobilized *R. rhodochrous* M33 degraded acrylonitrile to acrylamide much better than free cells of strain M33 although nitrile hydratase (NHase) activity of these immobilized cells was lost quickly with increasing usage (Kim and Hyun, 2002).

1.2 Fruit Ripening

Fruits are essential for people's health since they are an important component of a balanced diet and they are also desirable because of their pleasant colors, flavors and tastes. Aroma and flavor are two leading determinant factors in fruit market value. Volatile organic compounds (VOCs) emitted from fruits can affect both aroma and flavor. The formation of VOCs may be from amino acids and fatty acids (Hui, 2006). The numbers and amounts of VOCs are indicators of fruit ripening (Goff and Klee, 2006). VOCs of fruits are usually composed of the following categories: esters, alcohols, aldehydes, ketones, lactones, and terpenoids, the profiles of which are affected by fruit cultivar, stage of ripening, analytical methods and some other factors as well (Berger *et al.*, 1986; Bruckner, 2008; Song and Forney, 2008).

It has been found that variation in aroma volatile production is one reason for the different flavors among tomato varieties (Brauss *et al.,* 1998). Among more than 400 volatile compounds which have been detected in tomato fruits, hexanal, hexenal, hexenol, 3-methylbutanal, 3-methylbutanol, methylnitrobutane and isobutylthiazole are the most relevant factors in the tomato fruit aroma (Hobson and Grierson, 1993). More than 150 VOCs belonging to several chemical classes have been detected in bananas, including esters, ketones, terpenes and aldehydes (Facundo *et al.*, 2013). Among these chemicals, isoamyl acetate, butyl acetate, ethyl acetate, butyl butanoate, isoamyl isobutanoate and 2-pentanone are the main VOCs which contribute to banana aroma (Salmon *et al.*, 1996; Jordan *et al.*, 2001 b).

The short ripening period and reduced post-harvest life of many fruits result in large economic losses (Prasanna *et al.,* 2007). There are many methods and techniques which have been developed to extend fruit post-harvest life and the subsequent ripening period. Because low temperatures can reduce the rate of enzymatic processes (the respiration rate, evolution of ethylene, etc.), the maintenance of a cold chain from harvest to market is commonly used despite the costs associated with refrigerator (Wills *et al.*, 1998). However, for some tropical fruits, such as bananas, low temperature results in chill injuries if the temperature is too low or the period in refrigerator is too long (Jiang *et al.,* 2004). Using silver ions or CO_2 as inhibitors of ethylene is another way to delay fruit ripening, but the cost is incredible (Yang and Hoffman, 1984).

Fruit ripening is a complex phenomenon which happens during the period between late growth/development and early senescence with changes in color, flavor, texture, and others (Kader, 1999). During this process, according to increased ethylene synthesis and an accompanying rise in the rate of respiration, fruits are usually divided into climacteric and non-climacteric. Climacteric fruits (e.g., bananas, tomatoes, apples, cantaloupe, etc.) are

characterized by a dramatic increase in ethylene and respiration rate at the beginning of ripening and continue with ripening after they are harvested, while non-climacteric fruits do not display these characteristics (Kader, 1999; Liu *et al.*, 2014).

Based on Yang cycle (Yang and Hoffman, 1984), Arc *et al.* (2013) proposed the pathway see below (Figure 1):

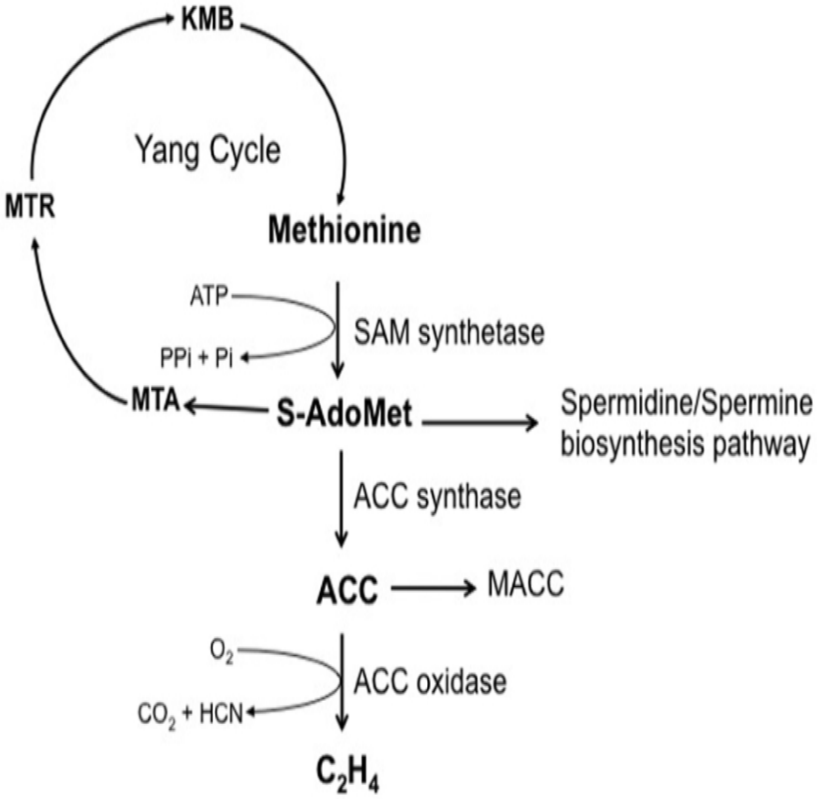


Figure 1 Pathway of ethylene biosynthesis in climacteric fruits.

During the ripening process, methionine (Met) is converted to *S*-adenosylmethionine (SAM) by *S*-adenosylmethionine synthetase, SAM in turn is converted to 1-aminocyclopropane-1-carboxylic acid (ACC), which yields ethylene, CO_2 and hydrogen cyanide (HCN) (three primary signals in fruit ripening) (Yang and Hoffman, 1984). The enzymes which catalyze the conversion of SAM to ACC and of ACC to ethylene are ACC synthase and ACC oxidase, respectively (Kende, 1993). Using antisense RNAs to ACC synthase and ACC oxidase can

decrease ethylene production and delay fruit ripening significantly (Hamilton *et al.*, 1990; Oeller *et al.*, 1991). Klee *et al.* (1991) expressed a bacterial ACC deaminase gene in tomatoes and removed ACC from the precursor pool, so ethylene synthesis and ripening were inhibited successfully.

There are two systems for ethylene regulation which have been found existing in plants. System 1 operates in normal vegetative growth which is an ethylene auto-inhibitory process. It functions by producing basal ethylene levels that are detected in all tissues including both climacteric and non-climacteric fruits. System 2 works only during the ripening of climacteric fruits and senescence of some petals when ethylene production is autocatalytic. The process of ripening usually starts from one region of a fruit followed by spreading to adjoining areas with ethylene diffusing from cell to cell and integrating this process throughout the whole fruit. By using gas chromatography and mass spectroscopy, many VOCs have been found, such as alcohols, aldehydes, esters, and so on (Alexander and Grierson, 2002).

1.3 Monooxygenases (MOs)

MOs are a class of enzymes which catalyze the insertion of one oxygen atom from a molecular oxygen (O_2) into an organic substrate. Based on the cofactor required, MOs have been divided into several families shown below (Torres Pazmino *et al.*, 2010):

1. Cytochrome P450 monooxygenases (P450s), which catalyze hydroxylation, are also called heme-dependent monooxygenases. P450s are especially present in eukaryotes (Nelson *et al.*, 1996).

2. Flavin-dependent monooxygenases are more common in prokaryotes. Either FMN or FAD is used as flavins which are not bound to these monooxygenases covalently. Most of these enzymes need NAD(P)H as a secondary coenzyme. Styrene monooxygenase (SMO) is a flavin-

dependent monooxygenase which is composed of a monooxygenase unit (StyA, [SMOA]) and a flavin oxidoreductase unit (StyB, [SMOB]). The coenzyme reduced FAD is generated by SMOB whose coenzyme is NAD(P)H. SMO has been reported to catalyze the epoxidation of the vinyl side-chain of styrene to *(S)*-styrene oxide (Figure 2) (Panke *et al.*, 1999; Hollmann *et al.*, 2003; van Berkel *et al.*, 2006):

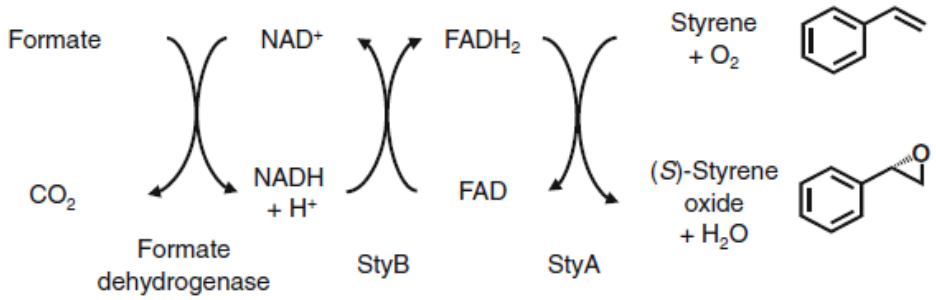


Figure 2 Pathway of epoxidation of styrene to *(S)*-styrene oxide.

Hartmans *et al.* (1990) reported SMO activity in the cell extracts of 11 bacterial isolates which were using styrene as the only source of carbon and energy. Toda *et al.* (2012) successfully expressed StyA and StyB-coding genes, which were from *Rhodococcus* sp. ST-5 and ST-10 in *Escherichia coli*.

3. Copper-dependent monooxygenases require copper ions for hydroxylation of their substrates (Levin *et al.*, 1960). Membrane-associated methane monooxygenase (pMMO) from *Methylococcus capsulatus* is an example of a copper-dependent MO (McGuirl and Dooley, 1999; Balasubramanian and Rosenzweig, 2007).

4. Non-heme iron-dependent monooxygenases need two iron atoms as their cofactor for the oxidative activity. They can catalyze hydroxylation and epoxidation reactions and are composed of three components: a monooxygenase, a reductase and a small regulatory protein (Wallar and Lipscomb, 1996; Leahy *et al.*, 2003). Alkene monooxygenase (AMO), soluble methane monooxygenase (sMMO) and toluene monooxygenase (TMO) are three main members

of this family (Gallagher *et al.*, 1997; Murrell *et al.*, 2000; Sazinsky *et al.*, 2004).

AMO has the ability to stereoselectively insert one oxygen atom from a molecular oxygen (O_2) across the alkene double-carbon bond producing the epoxide (*R*- and *S*-) with the remaining oxygen atom reduced to water through the addition of electrons provided by oxidation of NADH (Harayama *et al.*, 1992). The epoxides are an unstable chemical which can be degraded by carboxylation, hydrolysis or isomerization. Epoxide carboxylase in *R. rhodochrous* B-276 can catalyze the reaction of transferring epoxides to *β*-keto acids (Allen and Ensign, 1998). Mischitz *et al.* (1995) showed that the epoxide hydrolase of *Rhodococcus* sp. NCIMB 11216 catalyzed the hydrolysis of selected epoxides, yielding vicinal diols. In *Xanthobacter* strain Py2, propylene oxide conversion to acetone is accomplished by isomerization (Small and Ensign, 1995).

AMO from different bacteria can catalyze the epoxidation of a wide range of aliphatic alkenes (from C2 to C6) and chlorinated alkenes (van Ginkel *et al.*, 1987; Ensign *et al.*, 1992). This reaction is illustrated in Figure 3 (Small and Ensign, 1997):

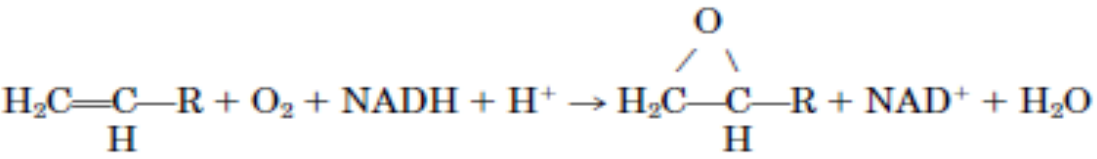


Figure 3 Epoxidation of alkenes to epoxides by AMO.

AMO from *R. rhodochrous* B-276 has been purified and found to consist of the following three components: a dimeric epoxygenase (consisting of 35- and 53-kDa subunits) which contains two non-heme iron atoms, a monomeric NADH-reductase (40-kDa) including FAD and a [2Fe-2S] cluster, and a monomeric coupling protein (14-kDa) which is a regulatory component with no cofactor (Miura and Dalton, 1995). Saeki and Furuhashi (1994) suggested that AMO was

encoded by *amoABCD* which was a four-gene operon and *amoA*, *amoC*, *amo D* and *amo B* encoding two subunits of epoxygenase, reductase and coupling protein, respectively.

1.4 Rationale

1. 1-HMO activity and stability in cells of *R. rhodochrous* DAP 96253

Carbon sources and storage temperatures are factors which can affect 1-HMO activity. Accordingly, *R. rhodochrous* DAP 96253 was grown on different media (YEMEA medium supplemented with different carbon sources) and 1-HMO activity in these cells under different storage conditions was determined.

Immobilization of whole cells is a useful tool in stabilizing enzymes. To maintain 1-HMO activity for long-term applications, *R. rhodochrous* DAP 96253 cells were immobilized and 1-HMO activity in the cells was enhanced by placing the immobilized cells in the proximity of ripening bananas.

AMO of *R. rhodochrous* DAP 96253 can degrade ethylene, indicating AMO may play a role in delayed fruit ripening. The relevance of 1-HMO activity and stability for delayed fruit ripening by *R. rhodochrous* DAP 96253 was explored. Based on previous research, *R. rhodochrous* DAP 96253 cells grown on YEMEA plates with various inducers (glucose, urea and cobalt chloride II) have shown different 1-HMO activities and capabilities of delaying fruit ripening. Additionally, it has been demonstrated that VOCs from fruits can influence 1-HMO activities in these cells as well. Time-course fruit tests by using these cells grown on YEMEA plates with various supplements aid in determining the relationship between 1-HMO activity/stability and delayed fruit ripening. Fruit colors are one main parameter telling us the ripening stage of bananas, so photos of different samples were taken for comparison. Fruit VOCs are another important parameter which stands for the fruit quality during ripening process, so

VOCs of different samples were evaluated using HS-SPME-GC-FID and HS-SPME-GC/MS methods.

2. Hypothesis for the role of 1-HMO in delayed fruit ripening by *R. rhodochrous* DAP 96253 (Figure 4):

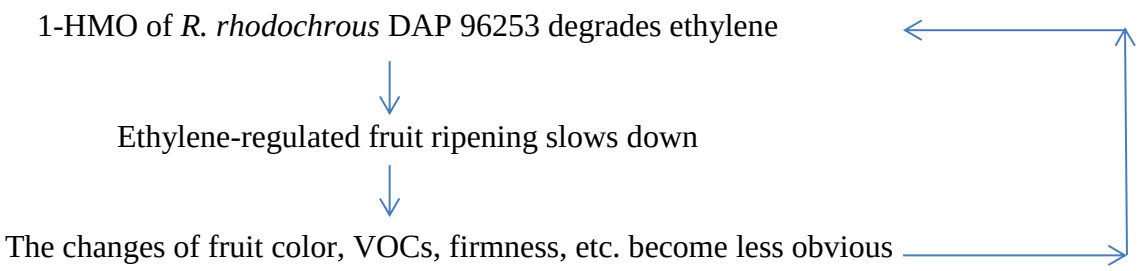


Figure 4 Hypothesis for delayed fruit ripening by *R. rhodochrous* DAP 96253.

1-HMO of *R. rhodochrous* DAP 96253 will assist in slowing down fruit ripening, and for selected fruits such as bananas, this reduction in ripening will become usually apparent. Fruit ripening stages also has a feedback on 1-HMO (Figure 4).

2 MATERIALS AND METHODS

2.1 Cell Cultivation

Cultures were started by inoculating a 1-ml glycerol stock of *R. rhodochrous* DAP 96253 into 75 mL of sterilized nutrient broth (8 $g \cdot L^{-1}$) in a 250-mL glass flask incubated at 30°C with shaking at 200 rpm for 2 days.

200 μL of the nutrient broth of *R. rhodochrous* DAP 96253 was inoculated onto each YEMEA plate supplemented with different extra components (depending on different experimental designs) and incubated at 30°C for 10 days before any experiment was set up.

2.2 Fruit Test

Two bunches of non-organic bananas (from Publix-a local grocery store) (with similar appearance, color, etc.) were obtained and split equally and put into a sealed 4.4-L polyester container (Rubbermaid). For each cell type, 20 mL of *R. rhodochrous* DAP 96253 cell suspension in M9 minimal medium (no glucose) (0.25 $g \cdot mL^{-1}$) was poured into a dish and placed between 2+2 bananas (each two were from two bunches, respectively) at room temperature. Controls consisting of bananas only and cells only were included.

On Day 0, 4, 7, 11 and 14, a. HS-SMPE-GC-FID or HS-SMPE-GC/MS was done to the bananas; b. 1.5 mL of different cell samples of *R. rhodochrous* DAP 96253 were taken out of the dishes and centrifuged at 13,200 rpm for 3 min and the supernatant was discarded. The cell pellets suspended into phosphate buffer were set up for 1-HMO assay; c. images of banannas+cells of *R. rhodochrous* DAP 96253 were taken.

2.3 Monooxygenase Assays

These two monooxygenase assays were generated from a modified NBP assay done by McClay *et al.* (2000). All the cell samples were set up as triplicates. The pyridine nitrogen of 4-

(4-nitrobenzyl)-pyridine (NBP) can open the epoxides, which have been formed from alkenes, by a nucleophilic attack. Then a purple chromophore will be formed after an organic base is added to NBP. These reactions are illustrated in Figure 5 (Cheung *et al.*, 2013):

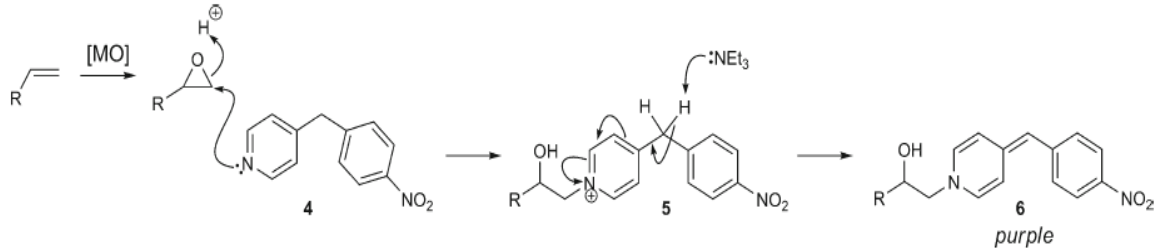


Figure 5 Principle of monooxygenase assays.

1-HMO assay: 10 mmol·L^{-1} of 1,2-epoxyhexane solution (3.01 μL of 1,2-epoxyhexane into 2.5 mL of acetone) was made as stock solution, then 1, 2, 3, 4 and 5 mmol·L^{-1} of 1,2-epoxyhexane solution was made by taking 100, 200, 300, 400 and 500 μL of the stock solution plus 900, 800, 700, 600 and 500 μL of acetone, correspondingly. Then 50 μL of each concentration of 1,2-epoxyhexane solution was transferred into outer vials with 5 mL of phosphate buffer (sodium salts, 50 mM, pH 7), respectively. 5 mL of *R. rhodochrous* strain DAP 96253 cell suspension (0.02 g·mL^{-1}) in phosphate buffer (sodium salts, 50 mM, pH 7.0) was transferred into a 40-ml amber glass vial (outer vial). 200 μmol of 1-hexene was added to the cell samples. 500 μL of 4-(4-nitrobenzyl) pyridine (NBP) solution (100 mmol·L^{-1}) in ethylene glycol was added to a 4-ml transparent glass vial (inner vial) and placed into the outer vial which was crimp-sealed with a Teflon-faced butyl rubber stopper finally. Blank (5 mL of phosphate buffer only) and negative control (no cell) were included. All the 2-vial sets were incubated at 30°C with shaking at 150 rpm for 24 h. Then 500 μL of triethylamine solution (1:1 v/v) in acetone was added to each inner vial and the absorbance at 600 nm was measured by BioPhotometer *plus* (Eppendorf) immediately.

SMO assay: 10 mmol·L^{-1} of styrene oxide solution (2.85 μL of styrene into 2.5 mL of acetone) was made as stock solution, then 1, 2, 3, 4 and 5 mmol·L^{-1} of styrene oxide solution was made by taking 100, 200, 300, 400 and 500 μL of the stock solution plus 900, 800, 700, 600 and 500 μL of acetone, correspondingly. Then 50 μL of each concentration of styrene oxide solution was transferred into outer vials with 5 mL of phosphate buffer (sodium salts, 50 mM, pH 7), respectively. 5 mL of *R. rhodochrous* strain DAP 96253 cell suspension (0.02 g·mL^{-1}) in phosphate buffer (sodium salts, 50 mM, pH 7.0) was transferred into a 40-ml amber glass vial (outer vial). 100 μmol of styrene was added to the cell samples. 500 μL of 4-(4-nitrobenzyl) pyridine (NBP) solution (100 mmol·L^{-1}) in ethylene glycol was added to a 4-ml transparent glass vial (inner vial) and placed into the outer vial which was crimp-sealed with a Teflon-faced butyl rubber stopper finally. Blank (5 mL of phosphate buffer only) and negative control (no cell) were included. All the 2-vial sets were incubated at 30°C with shaking at 150 rpm for 24 h. Then 500 μL of triethylamine solution (1:1 v/v) in acetone was added to each inner vial and the absorbance at 600 nm was measured by BioPhotometer *plus* (Eppendorf) immediately.

2.4 HS-SPME-GC-FID

The method of Facundo *et al.* (2013) was modified as follows: The Perkin Elmer Autosystem XL GC-FID was equipped with a DB-624 column capillary column 30 m in length and 317 μm in nominal diameter (J&W Scientific, Folsom, CA). The injector and detector were at 250°C, the helium flow rate was 4 ml/min and the oven was programmed as follows: 80°C for 2 min, from 80 to 180°C at 20°C/min, 180°C for 8 min. Attenuation was set to -5. Carboxen-polydimethylsiloxane (CAR/PDMS) fiber (75 μm) (Sigma-Aldrich, St Louis, MO) was inserted into these sealed 4.4-L polyester containers with bananas and cells of *R. rhodochrous* DAP 96253 at room temperature (25°C) for 15 min, then injected into the column for GC-FID test.

2.5 HS-SPME-GC/MS

The method of Jordan *et al.* (2001 a) was modified as follows: The Agilent Technologies 7890A/5977A GC/MSD was equipped with a HP-5ms Ultra Inert column capillary column 30 m in length and 250 μm in nominal diameter (J&W Scientific, Folsom, CA). The injection port and ionizing source were at 250°C, the helium flow rate was 1.5 ml/min and the oven was programmed as follows: 30°C for 1.5 min, then increased at 5°C/min to 160°C, and finally increased at 40°C/min to 200°C and held for 1.5 min. The split ratio was 20:1 and the split flow was 30 ml/min. The scan mode was set up from 20-200 m/z for mass spectrometer. Electron ionization (EI) was 70 ev. Mass spectra and total ion chromatograms (TICs) were recorded. Carboxen-polydimethylsiloxane (CAR/PDMS) fiber (75 μm) (Sigma-Aldrich, St Louis, MO) was inserted into these sealed 4.4-L polyester containers with bananas and cells of *R. rhodochrous* DAP 96253 at room temperature (25°C) for 30 min, then injected into the column for GC/MS test. Banana VOCs were identified by comparison of mass spectra from the fiber and the ones of NIST11 library.

2.6 Styrene and 1-Hexene Degradation Abilities of *R. rhodochrous* DAP 96253

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was set up for HS-GC-FID: 5 mL of *R. rhodochrous* strain DAP 96253 cell suspension (0.02 $g \cdot mL^{-1}$) in phosphate buffer (sodium salts, 50 mM, pH 7.0) was transferred into a 20-ml amber glass vial. 100 μmol of styrene or 200 μmol of 1-hexene were added to these cell samples in triplicates (triplicate controls with no cell were included too), separately. These samples were incubated at 30°C with shaking at 150 rpm for 24 h. The Perkin Elmer Autosystem XL GC-FID was equipped with a DB-624 column (J&W Scientific, Folsom, CA). The injector and detector were at 250°C,

the helium flow rate was 30 ml/min and the oven temperature was set up at 180°C for 5 min. Attenuation was set to -5. Direct headspace (10 μl) from different vials was injected into the column for GC-FID test.

2.7 SMO and 1-HMO Activities in Cells of *R. rhodochrous* DAP 96253

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was set up for SMO and 1-HMO assays.

2.8 Effects of selected carbohydrates and storage temperatures on 1-HMO activity and stability in cells of *R. rhodochrous* DAP 96253

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of one of the followings: glucose, fructose, galactose, trehalose, maltose, sucrose, lactose and maltodextrin, was set up for storage experiment. For each kind of carbohydrate supplement, 18 replicates of 0.4 g of cells were scraped and suspended in phosphate buffer (sodium salts, 50 mM, pH 7) (0.2 $g \cdot mL^{-1}$) and stored in separated 2-ml tubes at 4°C, 25°C and 37°C. On day 0, 7, 14, 21, 28, 35 and 42, each tube was centrifuged at 13,200 rpm for 3 min and the supernatant was discarded. The cell pellets were set up for 1-HMO assay.

2.9 Induction of 1-HMO activity in immobilized *R. rhodochrous* DAP 96253 cells stabilized in the presence of whole bananas

R. rhodochrous DAP 96253 was grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea. Two replicates of 2.5 g of cells were harvested from these plates and immobilized in calcium-alginate and calcium-alginate PVA, respectively. For calcium-alginate immobilization, 0.25 g of sodium alginate was dissolved in 10 mL of ddiH_2O which was heated to 100°C. After the solution was cooled down to 30°C, 10 mL of cell suspension in Tris-HCl

buffer (pH 7.0) (0.25 g/mL) was added and the mixture was cooled down to room temperature immediately. The mixture was added inoto a 0.5 M calcium chloride solution by dropwise to form beads which were about 4 mm in diameter. For calcium-alginate PVA method, 0.25 g of sodium alginate and 0.25 g of PVA were dissolved in 10 mL of ddiH_2O at 100°C. After the solution was cooled down to 30°C, 10 mL of cell suspension in Tris-HCl buffer (pH 7.0) (0.25 g/mL) was added and the mixture was cooled down to room temperature immediately. The mixture was dropped into a 0.5 M calcium chloride solution to form beads with about 4 mm diameter. Immobilization controls (no cell) were included. The weight ratio of immobilized cells and plate-scraped cells was 5:1. The cell samples were set up with bananas from two different bunches: a. calcium-alginate control, calcium-alginate immobilized cells, plate-harvested cells were transferred into three 50-ml tubes separately and placed between 1+1 bananas (one of upper hands from bunch 1 and one of lower hands from bunch 2) in a sealed 4.4-L polyester container for 3 days. b. calcium-alginate PVA control, calcium-alginate PVA immobilized cells, plate-harvested cells were transferred into three 50-ml tubes separately and placed between 1+1 bananas (one of lower hands from bunch 1 and one of upper hands from bunch 2) in a sealed 4.4-L polyester container for 3 days. The experiment also included control samples of each cell catalyst type described above placed in a 4.4-L polyester container with no banana. After the 3-day exposure, the plate-harvested cells (with or with no banana exposure) were immobilized in calcium-alginate and calcium-alginate PVA, respectively. All the immobilized samples (cells and controls) were stored in Tris-HCl buffer (pH 7.0) at 4°C. On week 0, 1, 2, 3, 4, 5 and 6, 0.5 g of each immobilized sample was set up for 1-HMO assay. One bead made by each immobilization method was sent for the scanning electron microscopy (SEM) test after this 6-week experiment was finished.

2.10 The correlation of delayed fruit ripening and 1-HMO activity and stability in cells of *R. rhodochrous* DAP 96253

a. Comparison of the effects of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening (set A)

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was suspended in M9 minimal medium (no glucose). 20 ml of GU and GCoU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) was set up for fruit test (tested by HS-SPME-GC-FID).

b. Comparison of the effects of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening (set B)

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was suspended in M9 minimal medium (no glucose). 20 ml of GU and GCoU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) was set up for fruit test (tested by HS-SPME-GC/MS).

c. Effects of ethylene- and propylene-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was suspended in M9 minimal medium (no glucose). 20 ml of GU and GCoU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) was transferred into the following 125-mL flasks, separately: flask A-with air only, flask B-with 100 ppm ethylene in air (Praxair, Norcross,

GA), flask C-with 100 ppm propylene in air (Praxair, Norcross, GA). These flasks were shaken at 80 rpm at room temperature for 48 hours. HS-GC-FID of the flasks was done to these cell samples after ethylene and propylene exposure. The Perkin Elmer Autosystem XL GC-FID was equipped with a DB-624 column (J&W Scientific, Folsom, CA). The injector and detector were at 250°C, the helium flow rate was 4 ml/min and the oven temperature was set up at 180°C for 5 min. Attenuation was set to -5. Direct headspace (500 μl) from different flasks was injected into the column for GC-FID test, which was followed by fruit test set up by these cell samples.

d. Effects of ethyl acetate-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was suspended in M9 minimal medium (no glucose). 20 ml of GU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) was exposed to 20 mL of 0%, 1% and 2% ethyl acetate in a 2.2-L sealed container for 24 hours, separately. These cell samples were set up for fruit test after ethyl acetate exposure.

e. The coordinative effects of organic red delicious apples on delayed fruit ripening by GU and GCoU plate cells of *R. rhodochrous* DAP 96253

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was suspended in M9 minimal medium (no glucose). 20 ml of GU and GCoU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) was set up for fruit test. Besides, there are two more samples for GU and GCoU plate cells: bananas+an organic red delicious apple+GU/GCoU plate cells, bananas+an organic red delicious apple.

f. Effects of peach juice-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea, 4 g/L of glucose+7.5 g/L of urea+30 ml (15 ml × 2) of peach juice (9 g of 5 × 5 × 5 cm peach pieces were suspended into 15 ml of ddiH_2O, then put into 50°C water bath for 10 min and vertexed for 10 min before filtered into the medium), 4 g/L of glucose+7.5 g/L of urea+60 ml (30 ml × 2) of peach juice (18 g of 5 × 5 × 5 cm peach pieces were suspended into 30 ml of ddiH_2O, then put into 50°C water bath for 10 min and vertexed for 10 min before filtered into the medium) and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$+30 ml (15 ml × 2) of peach juice (9 g of 5 × 5 × 5 cm peach pieces were suspended into 15 ml of ddiH_2O, then put into 50°C water bath for 10 min and vertexed for 10 min before filtered into the medium), 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$+60 ml (30 ml × 2) of peach juice (18 g of 5 × 5 × 5 cm peach pieces were suspended into 30 ml of ddiH_2O, then put into 50°C water bath for 10 min and vertexed for 10 min before filtered into the medium), was suspended in M9 minimal medium (no glucose). 20 ml of GU and GCoU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) with difference concentrations of peach juice was set up for fruit test.

g. Effects of whole banana and banana pulp-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

R. rhodochrous DAP 96253, grown on YEMEA plates supplemented with 4 g/L of glucose+7.5 g/L of urea and 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, was suspended in M9 minimal medium (no glucose). 20 ml of GU and GCoU plate cell suspension (0.25 g/mL in M9 minimal medium, no glucose) was exposed to: no banana, 2+2 whole bananas

(one of upper hands and one of lower hands from bunch 1 and 2, separately), banana pulp (made from one of upper hands and one of lower hands from bunch 1 and 2, separately) in a sealed 4.4-L polyester container for 72 hours, separately. These cell samples were set up for fruit test after banana exposure.

2.11 Mycelial Growth of *R. rhodochrous* DAP 96253

200 μL of the nutrient broth of *R. rhodochrous* DAP 96253 (from two flasks), which were started by two glycerol stocks made from the same culture on the same date, were inoculated onto each YEMEA plate supplemented with 4 g/L of glucose+7.5 g/L of urea or 4 g/L of glucose+7.5 g/L of urea+50 mg/L of $CoCl_2 \cdot 6H_2O$, respectively. After inoculation, one sterilized cover slide was inserted into each plate at a 40° angle in order to let the cells grow on the agar-slide border. Then these plates were incubated at 30°C for 10 days. After incubation, the cover slides were pulled out of the plates and observed by 40x10 microscope and 1-HMO assay was set up for the plate cells.

3 RESULTS

3.1 Styrene and 1-Hexene Degradation Abilities of *R. rhodochrous* DAP 96253

Table 1 HS-GC-FID of styrene degradation (at 30°C and 150 rpm for 24 h) by *R. rhodochrous* DAP 96253 grown on GU and GCoU plates.

	Retention Time (min)	Peak Area (μV·s)	Average Peak Area (μV·s)	Standard Deviation	Degradation Rate
Control 1	3.593	963629.51	749000	186000	N/A
Control 2	3.603	646393.69			
Control 3	3.603	637484.91			
GU 1	3.606	695150.08	677000	160000	9.6%
GU 2	3.609	826091.76			
GU 3	3.610	509977.25			
GCoU 1	3.610	401455.43	525000	114000	29.9%
GCoU 2	3.608	624475.54			
GCoU 3	3.606	550415.37			

Styrene degradation rate of GCoU plate cells is almost three times as much as that of GU plate cell (Table 1).

Table 2 HS-GC-FID of 1-hexene degradation (at 30°C and 150 rpm for 24 h) by *R. rhodochrous* DAP 96253 grown on GU and GCoU plates.

	Retention Time (min)	Peak Area (μV·s)	Average Peak Area (μV·s)	Standard Deviation	Degradation Rate
Control 1	2.444	11877380.62	11700000	337000	N/A
Control 2	2.437	12004271.05			
Control 3	2.448	11366932.21			
GU 1	2.503	4004188.81	5100000	955000	56.5%
GU 2	2.490	5640121.11			
GU 3	2.493	5675537.99			
GCoU 1	2.502	3970862.90	3860000	691000	67.1%
GCoU 2	2.492	3123748.28			
GCoU 3	2.496	4493561.38			

In Table 2, 1-hexene degradation rate of GCoU plate cells is slightly higher than that of GU plate cell.

3.2 SMO and 1-HMO Activities in cells of *R. rhodochrous* DAP 96253

Table 3 SMO and 1-HMO activities in cells of *R. rhodochrous* DAP 96253 grown on GU and GCoU plates.

	SMO*	Standard Deviation (SMO)	1-HMO**	Standard Deviation (1-HMO)
GU plate	3	0	12	2
GCoU plate	6	3	17	3

*units (nmol of styrene oxide · hour)/g (cdw).
**units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

SMO and 1-HMO activities of *R. rhodochrous* DAP 96253 grown on GCoU plates are both slightly higher than those of GU plates (Table 3).

3.3 Effects of selected carbohydrates and storage temperatures on 1-HMO activity and stability in cells of *R. rhodochrous* DAP 96253

a. Effects of three different storage temperatures on 1-HMO activity of *R. rhodochrous* DAP 96253 grown with different carbohydrate supplements.

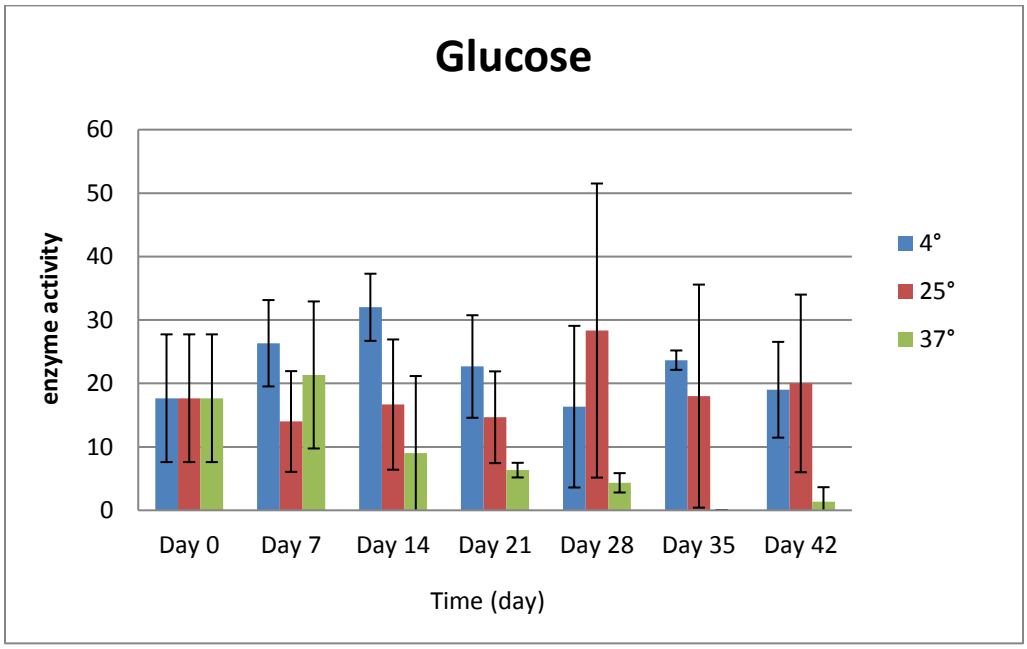


Figure 6 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with glucose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

After metabolizing glucose for 10 days, 1-HMO activity of *R. rhodochrous* DAP 96253 decreases with the increase of storage time at 37°C and is lower than those at 4°C and 25°C. At 4°C and 25°C, 1-HMO activities do not show any obvious change with the increase of storage time (Figure 6).

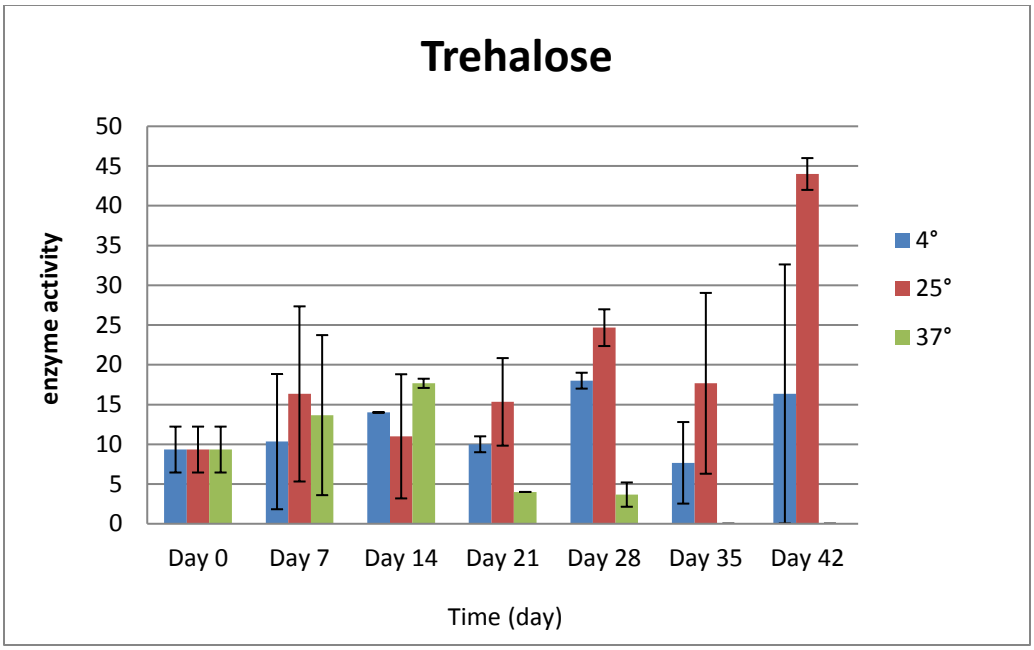


Figure 7 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with trehalose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

When supplemented with trehalose, 1-HMO activity of *R. rhodochrous* DAP 96253 at 25°C increases with the increase of storage time, the one at 4°C is essentially constant over the 42-day period, while the one at 37°C decreases from Day 14 (Figure 7).

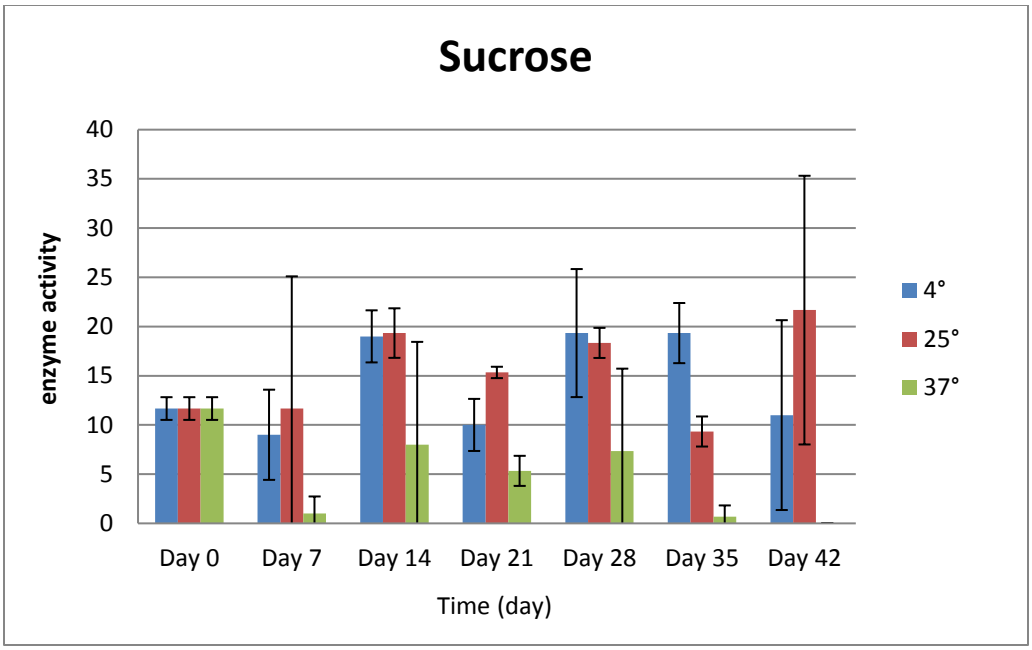


Figure 8 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with sucrose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

When supplemented with sucrose, *R. rhodochrous* DAP 96253 stored at 37°C results in a steady decrease of 1-HMO activity while 25°C increases 1-HMO activity (Figure 8).

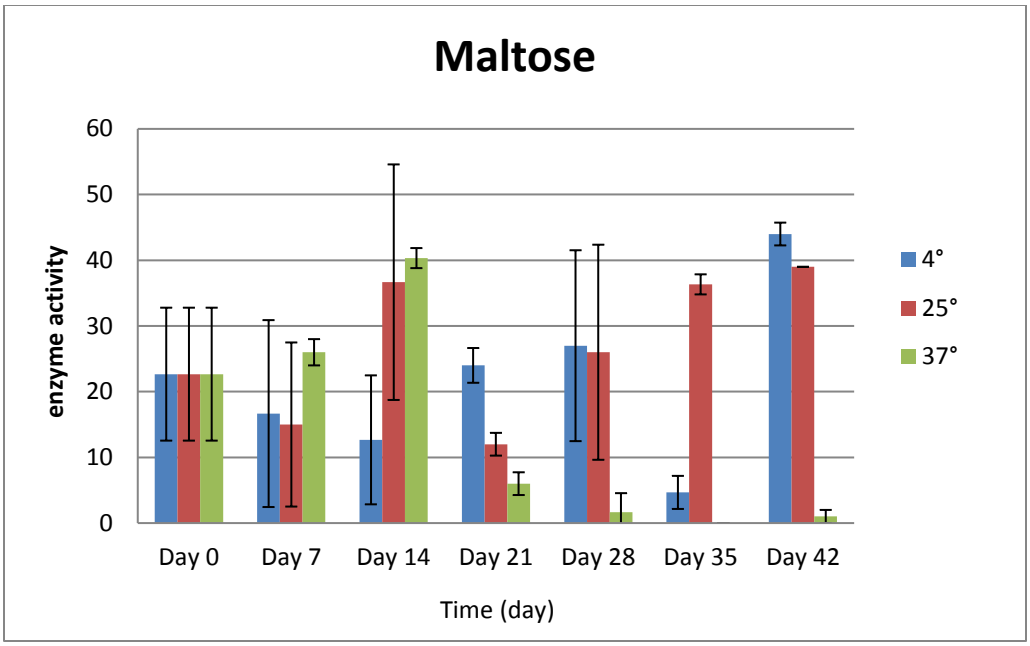


Figure 9 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with maltose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

For maltose, 4°C and 25°C induce 1-HMO activity of *R. rhodochrous* DAP 96253 while 1-HMO activity is not stable at 37°C (Figure 9).

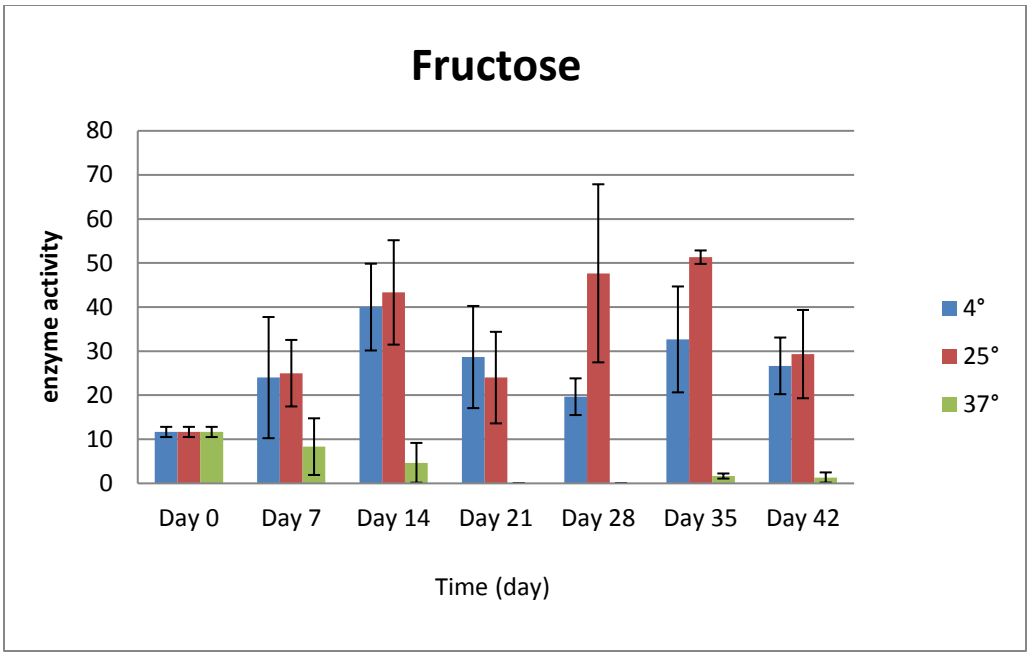


Figure 10 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with fructose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

When fructose is metabolized as a carbon source by *R. rhodochrous* DAP 96253, 25°C induces 1-HMO activity the most and 1-HMO activity is not stable at 37°C (Figure 10).

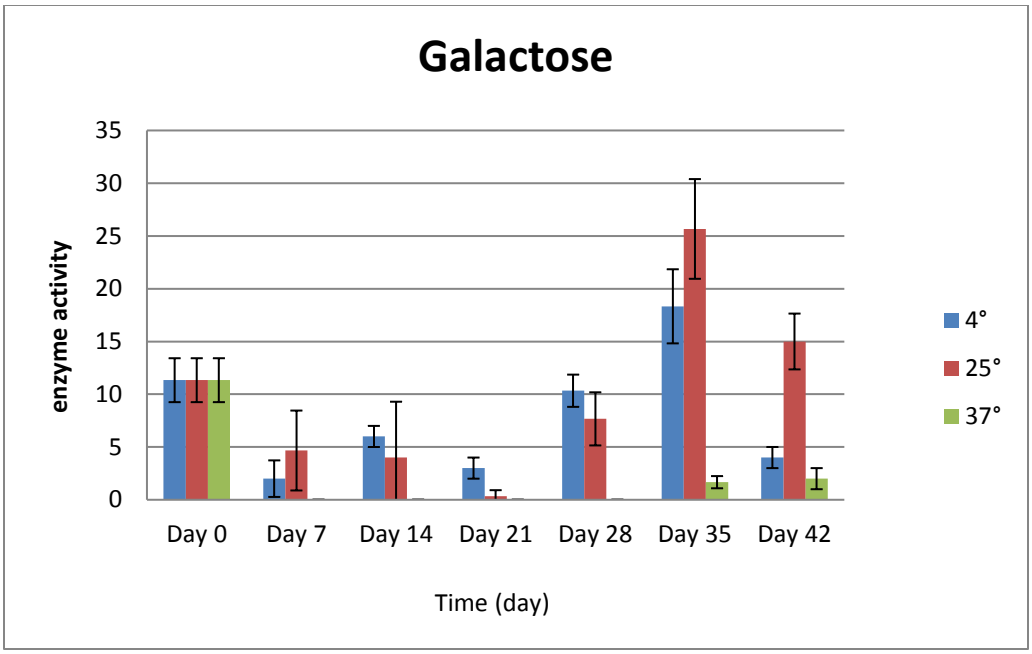


Figure 11 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with galactose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

For galactose, 1-HMO activity of *R. rhodochrous* DAP 96253 drops very fast at 37°C, the one at 25°C initially drops then goes up from Day 28, and the one at 4°C shows a similar trend as 25°C (Figure 11).

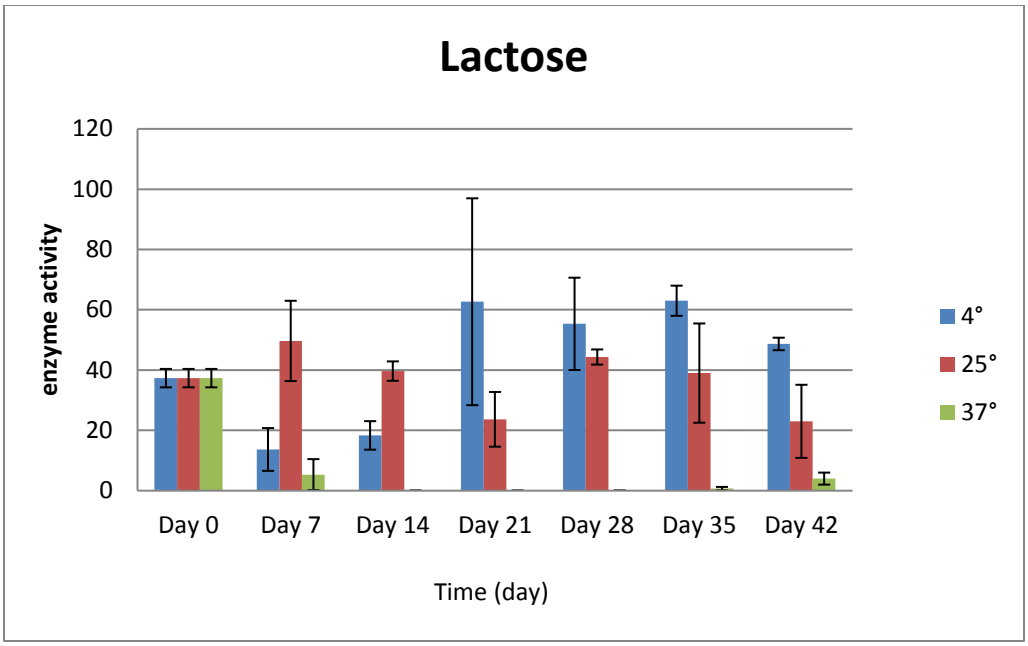


Figure 12 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with lactose. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

After the growth on YEMEA supplemented with lactose, 1-HMO activity of *R. rhodochrous* DAP 96253 is more stable at 4°C while unstable at 37°C (Figure 12).

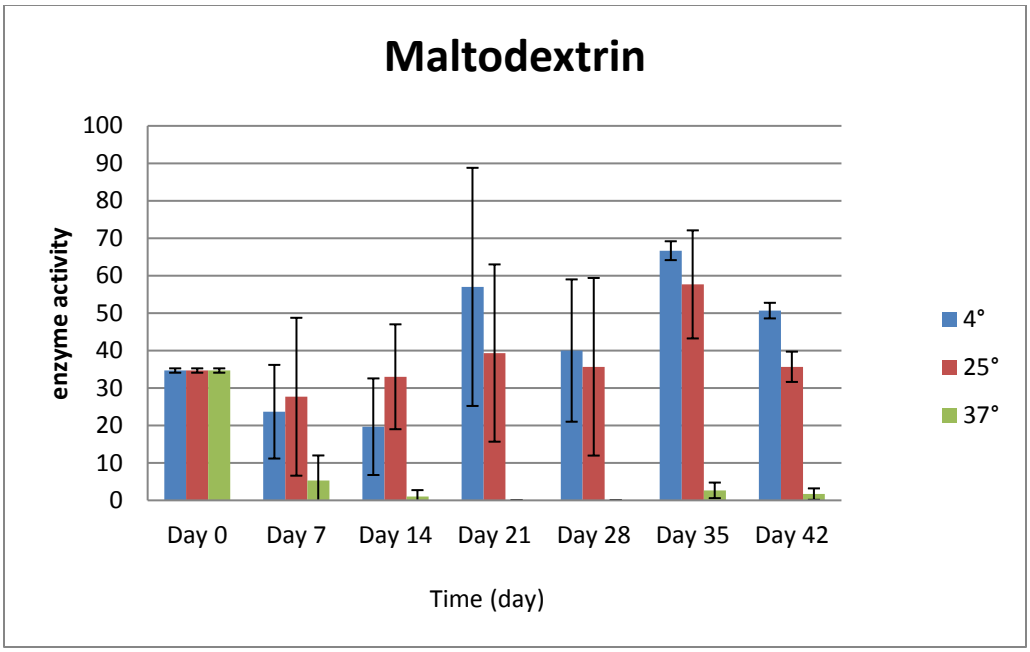


Figure 13 1-HMO activity of *R. rhodochrous* DAP 96253 grown on YEMEA medium supplemented with maltodextrin. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

For maltodextrin, 4°C and 25°C can induce 1-HMO activity of *R. rhodochrous* DAP 96253 related constantly, while 37°C decreases 1-HMO activity (Figure 13).

b. Effects of selected carbohydrates on 1-HMO activity of *R. rhodochrous* DAP 96253 at different storage temperatures.

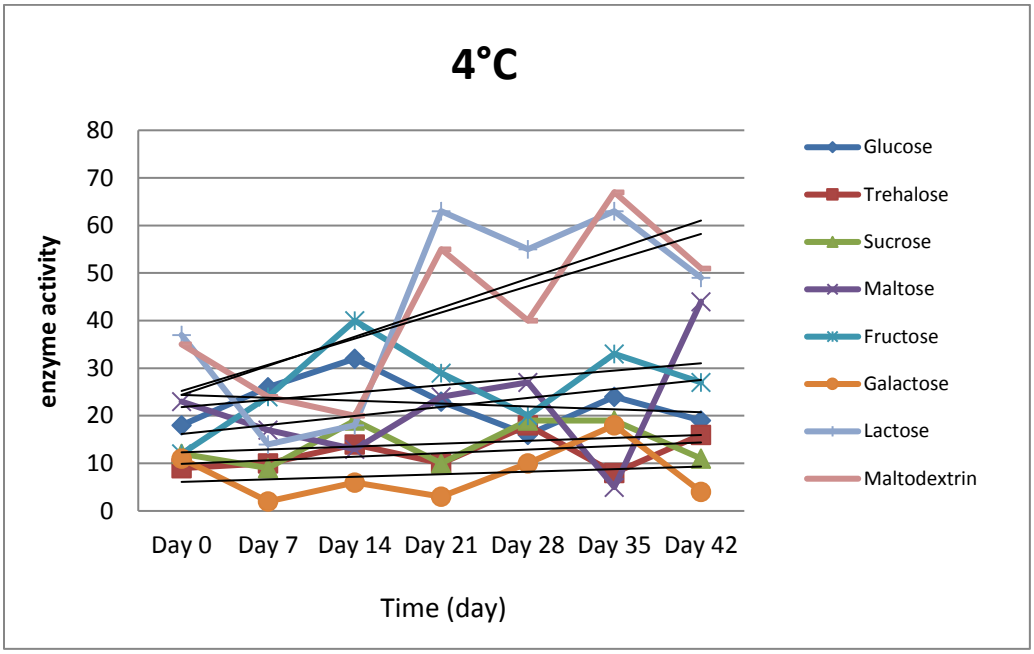


Figure 14 1-HMO activity of *R. rhodochrous* DAP 96253 (grown on YEMEA supplemented with different carbohydrates) stored at 4°C. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

At 4°C, *R. rhodochrous* DAP 96253 grown on YEMEA supplemented by lactose maintains the highest 1-HMO activity over the 42-day period, compared with the other carbohydrates. Maltodextrin can maintain 1-HMO activity better than the other six carbohydrates. Fructose is the third most effective carbohydrate for maintaining 1-HMO activity followed by glucose as the fourth best. Galactose is the least effective carbohydrate for inducing 1-HMO activity. Trehalose and sucrose perform similar ability of 1-HMO activity induction. Maltose shows dramatic changes in 1-HMO activity on Day 35 and 42 (Figure 14).

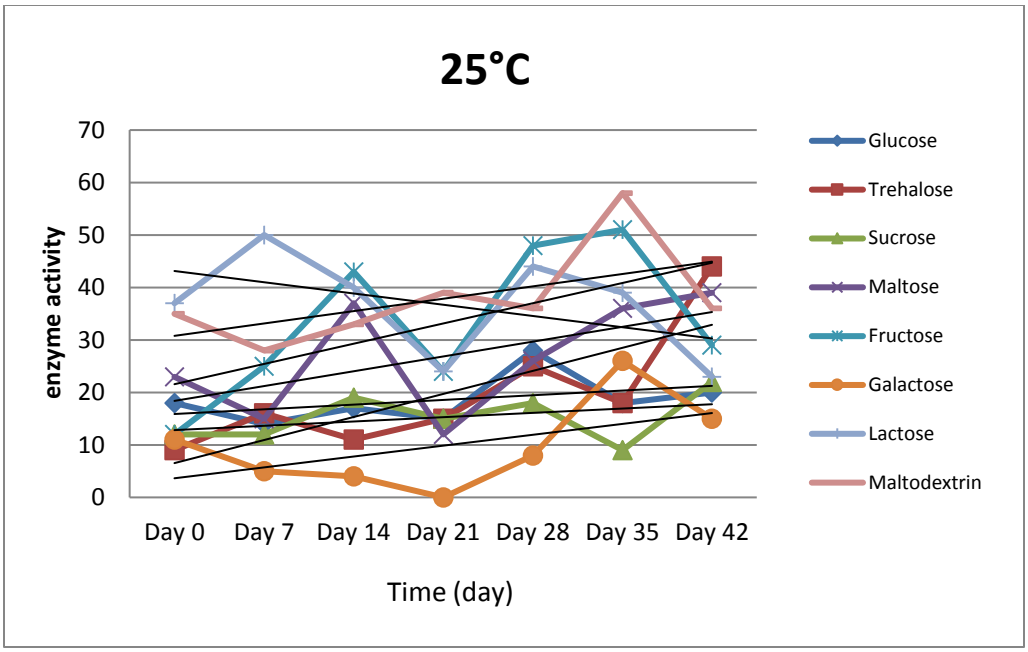


Figure 15 1-HMO activity of *R. rhodochrous* DAP 96253 (grown on YEMEA supplemented with different carbohydrates) stored at 25°C. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

As shown in Figure 15, for most of the carbohydrates, 1-HMO activity is more stable at 25°C compared with either 4°C or 37°C. Galactose induces 1-HMO activity in *R. rhodochrous* DAP 96253 the least, while maltodextrin, lactose and fructose keep 1-HMO activity at a higher level for most of the time although on Day 42 lactose cannot maintain 1-HMO activity as high as on Day 0.

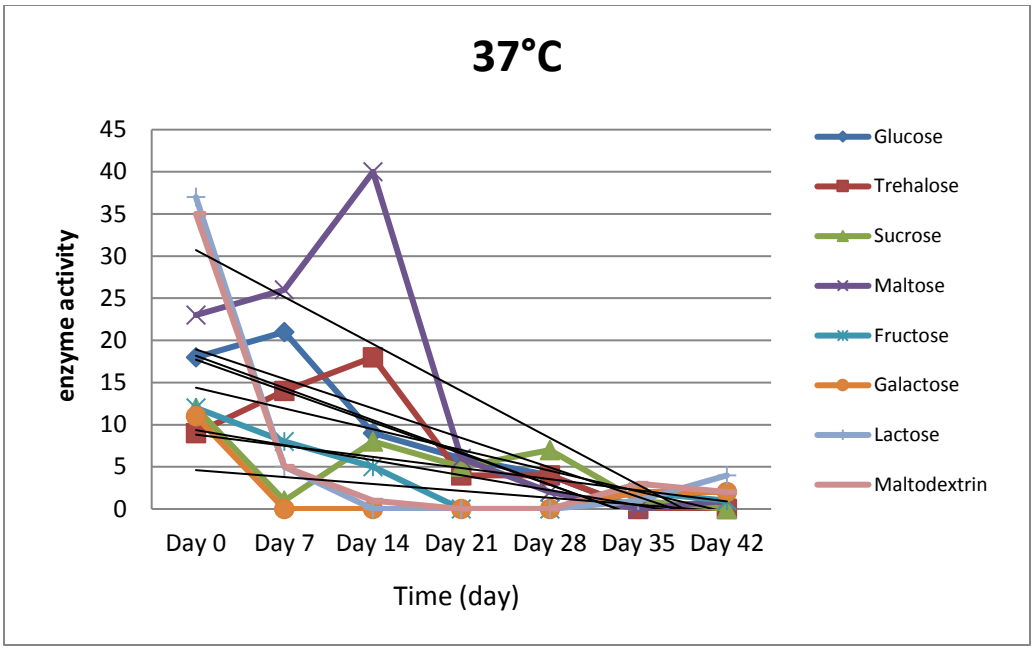


Figure 16 1-HMO activity of *R. rhodochrous* DAP 96253 (grown on YEMEA supplemented with different carbohydrates) stored at 37°C. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

In Figure 16, when stored at 37°C, 1-HMO activity of *R. rhodochrous* DAP 96253 decreases from Day 7 for lactose, maltodextrin, galactose and fructose. For maltose, it increases 1-HMO activity until Day 14 and dramatically decreases it from Day 21. Trehalose increases 1-HMO activity until Day 14 and decreases it from Day 21 but not as much as maltose. 1-HMO activity is induced by glucose until Day 7 and drops from Day 14. Sucrose decreases 1-HMO activity on Day 7 but increases it on Day 14 and then decreases it gradually till Day 42. For all supplements tested, 1-HMO activity was not stable when *R. rhodochrous* DAP 96253 was stored at 37°C.

c. 1-HMO stability of *R. rhodochrous* DAP 96253 at different storage temperatures.

Table 4 1-HMO activity of *R. rhodochrous* DAP 96253 grown with different carbohydrate supplements on Day 0.

Carbohydrate Name	1-HMO*	Standard Deviation
Glucose	18	10
Trehalose	9	3
Sucrose	12	1
Maltose	23	10
Fructose	12	1
Maltodextrin	35	1
Lactose	37	3
Galactose	11	2

*units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

On Day 0, 1-HMO activities of *R. rhodochrous* DAP 96253 grown with lactose and maltodextrin perform the highest and the ones with maltose and glucose are at a medium level, while the ones with sucrose, fructose, galactose and trehalose are the lowest (Table 4).

Table 5 Comparison of 1-HMO stability of *R. rhodochrous* DAP 96253 grown with different carbohydrate supplements and stored at 4°C.

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Glucose	100%	144%	178%	128%	89%	133%	106%
Trehalose	100%	111%	156%	111%	200%	89%	178%
Sucrose	100%	82%	158%	83%	158%	158%	92%
Maltose	100%	74%	57%	104%	117%	22%	191%
Fructose	100%	200%	333%	242%	167%	275%	225%
Maltodextrin	100%	69%	57%	157%	114%	191%	146%
Lactose	100%	38%	49%	170%	149%	170%	132%
Galactose	100%	18%	55%	27%	91%	164%	36%

At 4°C, 1-HMO activities of *R. rhodochrous* DAP 96253 grown with glucose, trehalose, maltose, fructose, maltodextrin and lactose are induced on Day 42 while the ones with sucrose and galactose are inhibited (Table 5). With the comparison of 1-HMO activities of *R. rhodochrous* DAP 96253 grown with different carbohydrate supplements on different storage time, it is obvious that the ones with all the other seven carbohydrates show relatively good stabilities (fructose is the best) with the exception of galactose (Table 5).

Table 6 Comparison of 1-HMO stability of *R. rhodochrous* DAP 96253 grown with different carbohydrate supplements and stored at 25°C.

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Glucose	100%	78%	94%	83%	156%	100%	111%
Trehalose	100%	178%	122%	167%	278%	200%	489%
Sucrose	100%	100%	158%	125%	150%	75%	183%
Maltose	100%	65%	161%	52%	113%	157%	170%
Fructose	100%	208%	358%	200%	400%	425%	242%
Maltodextrin	100%	80%	94%	111%	103%	166%	103%
Lactose	100%	135%	108%	65%	119%	105%	62%
Galactose	100%	45%	36%	0%	73%	236%	136%

At 25°C, 1-HMO activities of *R. rhodochrous* DAP 96253 grown with glucose, trehalose, sucrose, maltose, fructose, maltodextrin and galactose are induced on Day 42 (with trehalose and fructose showing superior results) while the one with lactose is inhibited (Table 6). 1-HMO stability of *R. rhodochrous* DAP 96253 grown with lactose is the worst while the ones with the

other seven carbohydrates are relatively good (trehalose is the best) (Table 6).

Table 7 Comparison of 1-HMO stability of *R. rhodochrous* DAP 96253 grown with different carbohydrate supplements and stored at 37°C.

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Glucose	100%	117%	50%	33%	22%	0%	6%
Trehalose	100%	156%	200%	44%	44%	0%	0%
Sucrose	100%	8%	67%	42%	58%	8%	0%
Maltose	100%	113%	174%	26%	9%	0%	4%
Fructose	100%	67%	42%	0%	0%	17%	8%
Maltodextrin	100%	14%	3%	0%	0%	9%	6%
Lactose	100%	14%	0%	0%	0%	3%	11%
Galactose	100%	0%	0%	0%	0%	18%	18%

At 37°C, 1-HMO activities of *R. rhodochrous* DAP 96253 grown with all the carbohydrates are inhibited from Day 21 quickly (Table 7). They drop to very low levels with 18% as the highest and trehalose and sucrose drop to 0 on Day 42 (Table 7).

3.4 Induction of 1-HMO activity in immobilized *R. rhodochrous* DAP 96253 cells stabilized in the presence of whole bananas

a. Effects of two different immobilization methods on 1-HMO activity of *R. rhodochrous* DAP 96253 cells

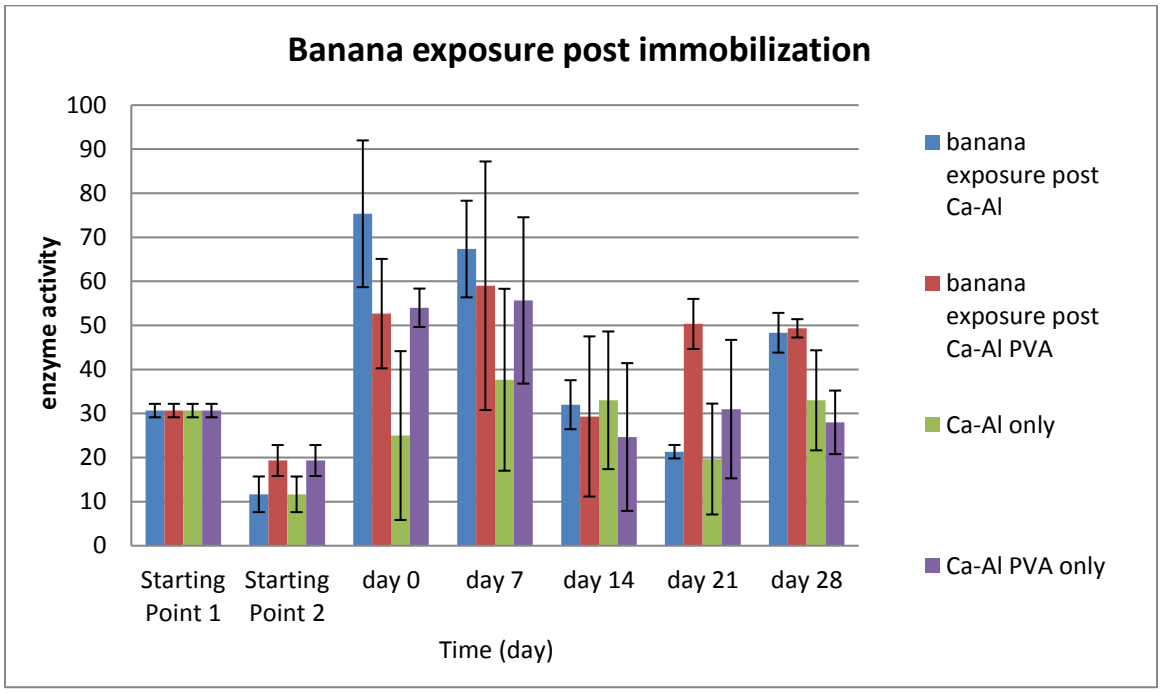


Figure 17 1-HMO activity of *R. rhodochrous* DAP 96253 cells which were immobilized before banana exposure. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw). Note: starting point 1-plate cell, starting point 2-immoblized cell before banana exposure, day 0-immoblized cell after banana exposure.

After immobilization, 1-HMO activity of *R. rhodochrous* DAP 96253 cells decreases. After banana exposure, calcium-alginate immobilized cells maintain higher 1-HMO activity compared with non-banana exposed cells. For calcium-alginate PVA immobilized cells, no matter with or without banana exposure, they have similar 1-HMO activity until Day 21 when banana-exposed cells show more 1-HMO activity. With banana exposure, 1-HMO activity in calcium-alginate PVA immobilized cells is more stable than that in calcium-alginate immobilized cells. With no banana exposure, 1-HMO activity in calcium-alginate PVA immobilized cells is higher than that

in calcium-alginate immobilized cells (Figure 17).

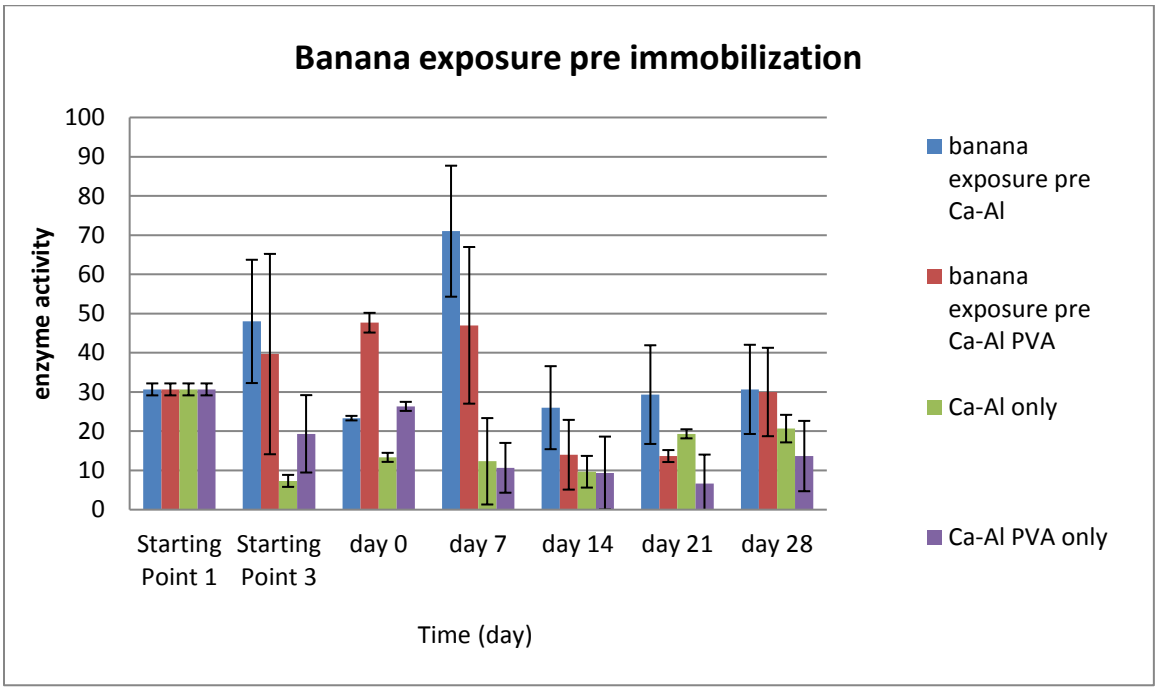


Figure 18 1-HMO activity of *R. rhodochrous* DAP 96253 cells which were immobilized after banana exposure. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw). Note: starting point 1-plate cell, starting point 3-plate cell after banana exposure, day 0-immoblized cell from banana exposed plate cell.

After banana exposure, 1-HMO activity in *R. rhodochrous* DAP 96253 has increased while that in *R. rhodochrous* DAP 96253 with no banana exposure has decreased. *R. rhodochrous* DAP 96253 cells maintain higher 1-HMO activity after immobilization except for the calcium-alginate immobilized ones which are from banana-exposed cells. Calcium-alginate PVA immobilized cells show more stable 1-HMO activity than calcium-alginate immobilized ones (Figure 18).

b. Effects of pre and post banana exposure on 1-HMO activity of *R. rhodochrous* DAP 96253 cells

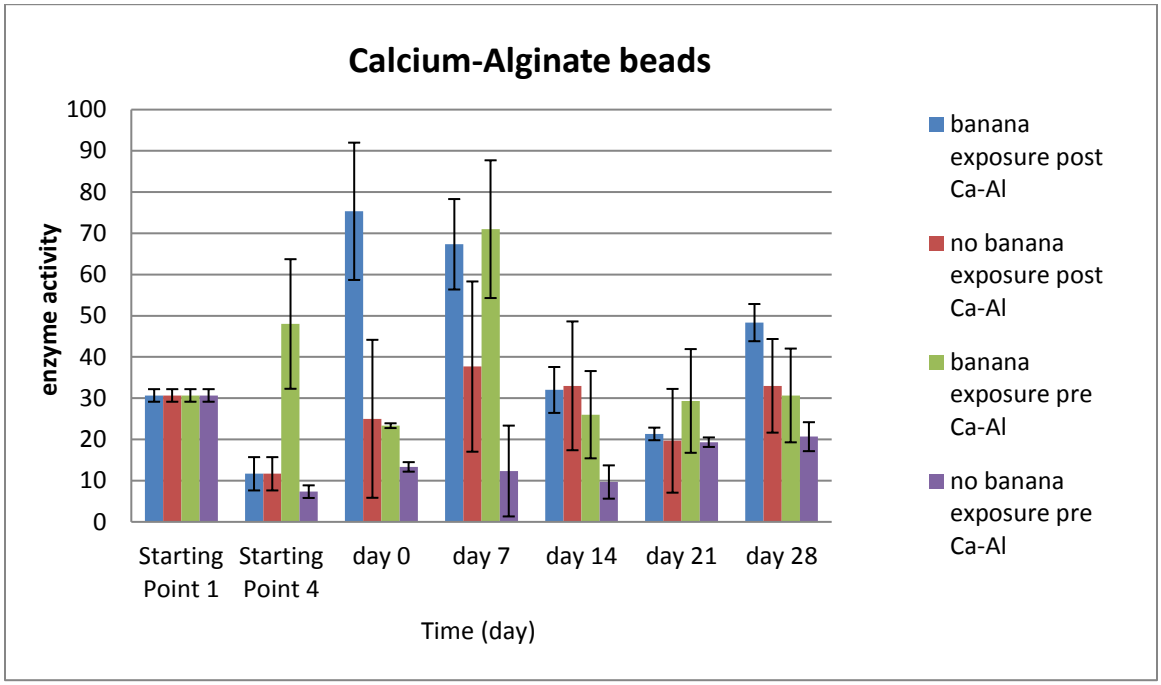


Figure 19 1-HMO activity of calcium-alginate immobilized *R. rhodochrous* DAP 96253 cells. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw). Note: starting point 1-plate cell, starting point 4-plate cell post banana exposure, or calcium-alginate immobilized cell pre banana exposure, day 0-calcium-alginate immobilized cell from banana exposed plate cell, or immobilized cell after banana exposure.

For calcium-alginate immobilization, no matter whether the cells are exposed to bananas or not, 1-HMO activity in *R. rhodochrous* DAP 96253 is higher when the cells are immobilized pre banana exposure. The cells with no banana exposure maintain more stable but lower 1-HMO activity than the cells with banana exposure (Figure 19).

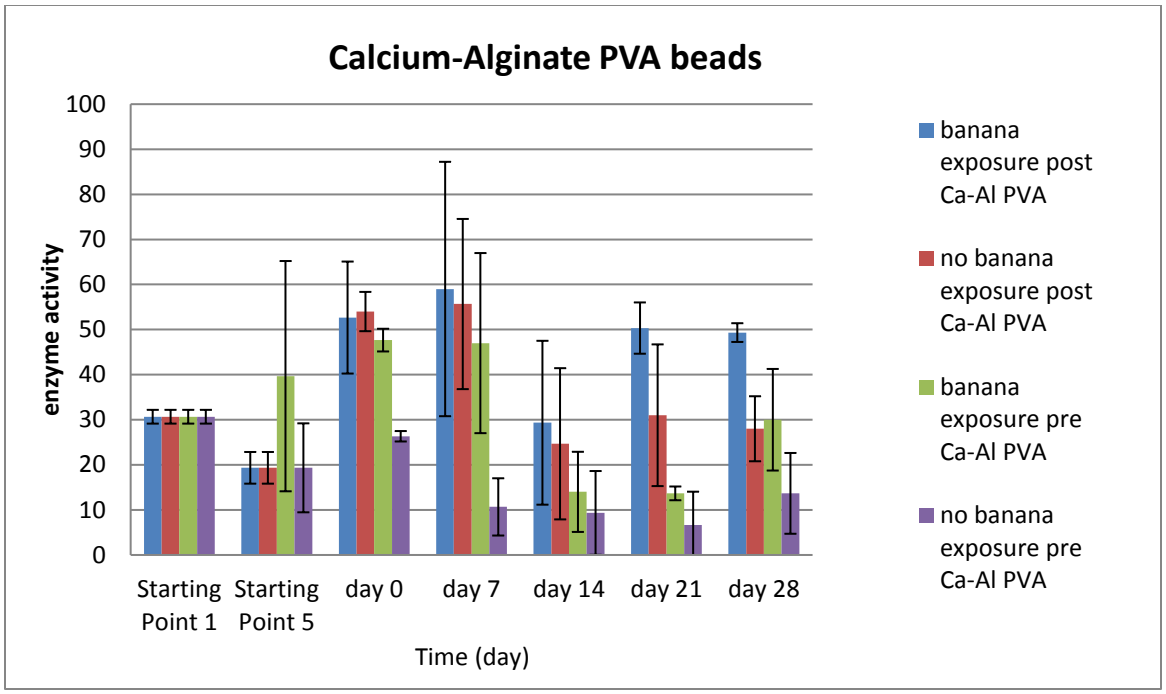


Figure 20 1-HMO activity of calcium-alginate PVA immobilized *R. rhodochrous* DAP 96253 cells. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw). Note: starting point 1-plate cell, starting point 5- plate cell post banana exposure, or calcium-alginate PVA immobilized cell pre banana exposure, day 0-calcium-alginate PVA immobilized cell from banana exposed plate cell, or immobilized cell after banana exposure.

When the cells are treated by calcium-alginate PVA immobilization method, 1-HMO activity in *R. rhodochrous* DAP 96253 is higher when they are immobilized first before banana exposure. By using the same method, banana exposure induces higher 1-HMO activity. However, calcium-alginate PVA immobilized cells with no banana exposure show higher 1-HMO activity than those immobilized cells which are exposed to bananas before immobilization (Figure 20).

c. SEM of calcium-alginate (PVA) bead of immobilized *R. rhodochrous* DAP 96253 cells

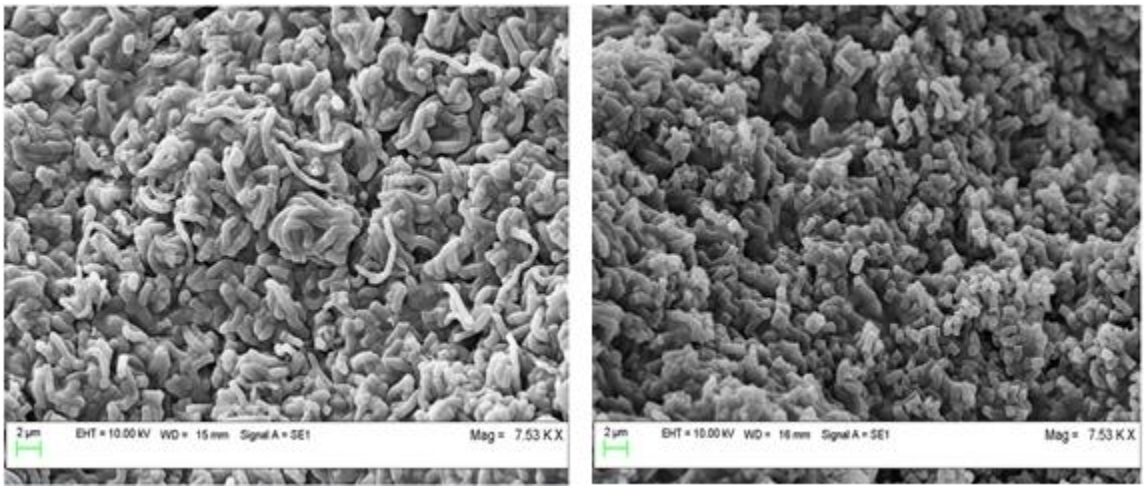


Figure 21 Photomicrographs of interior of calcium-alginate bead (left) and calcium-alginate PVA bead (right).

In Figure 21, the cells of *R. rhodochrous* DAP 96253 in calcium-alginate bead develop much more mycelial growth than the ones in calcium-alginate PVA bead.

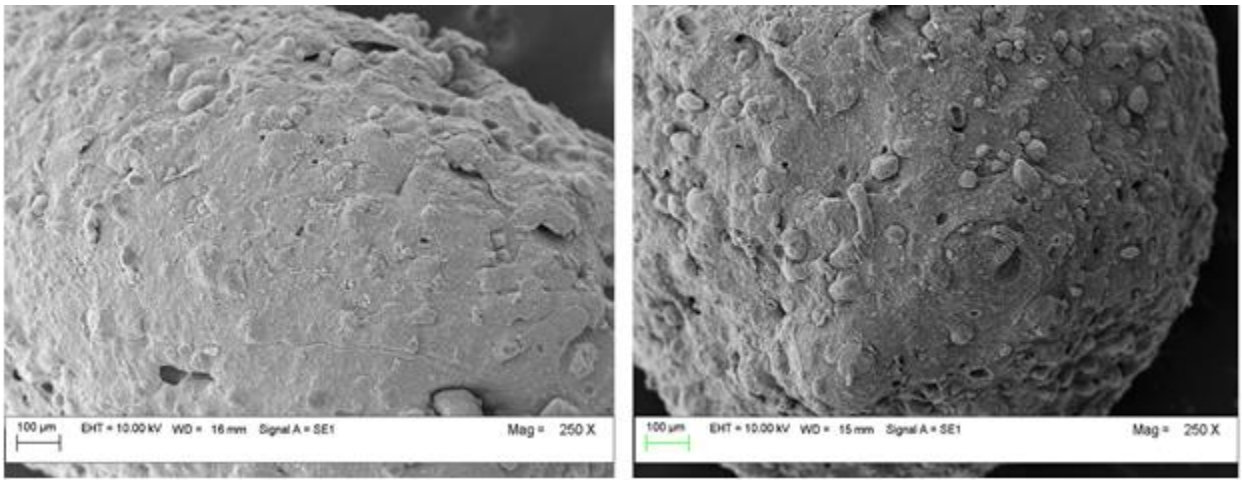


Figure 22 Photomicrographs of exterior of calcium-alginate bead (left) and calcium-alginate PVA bead (right).

The external appearance of calcium-alginate bead is smoother than that of calcium-alginate PVA bead (Figure 22).

d. Photos of calcium-alginate (PVA) beads of immobilized *R. rhodochrous* DAP 96253 cells

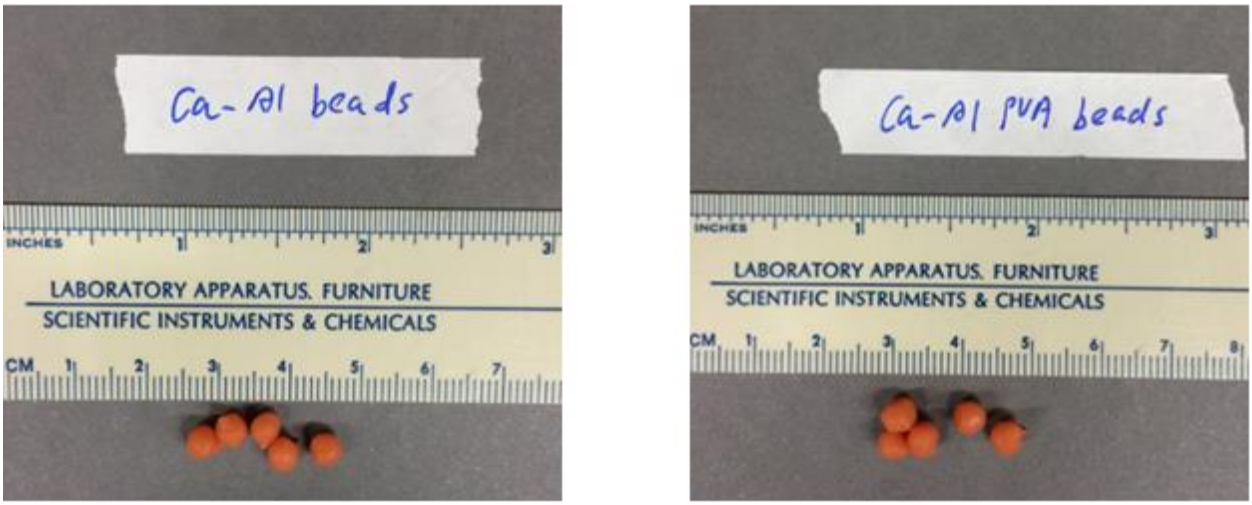


Figure 23 Photos of calcium-alginate beads (left) and calcium-alginate PVA beads (right).

These beads were made by dropwise. Calcium-alginate PVA beads are not as elastic as calcium-alginate beads by touching (Figure 23).

3.5 The correlation of delayed fruit ripening and 1-HMO activity and stability in cells of *R. rhodochrous* DAP 96253

a. Comparison of the effects of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening (set A)

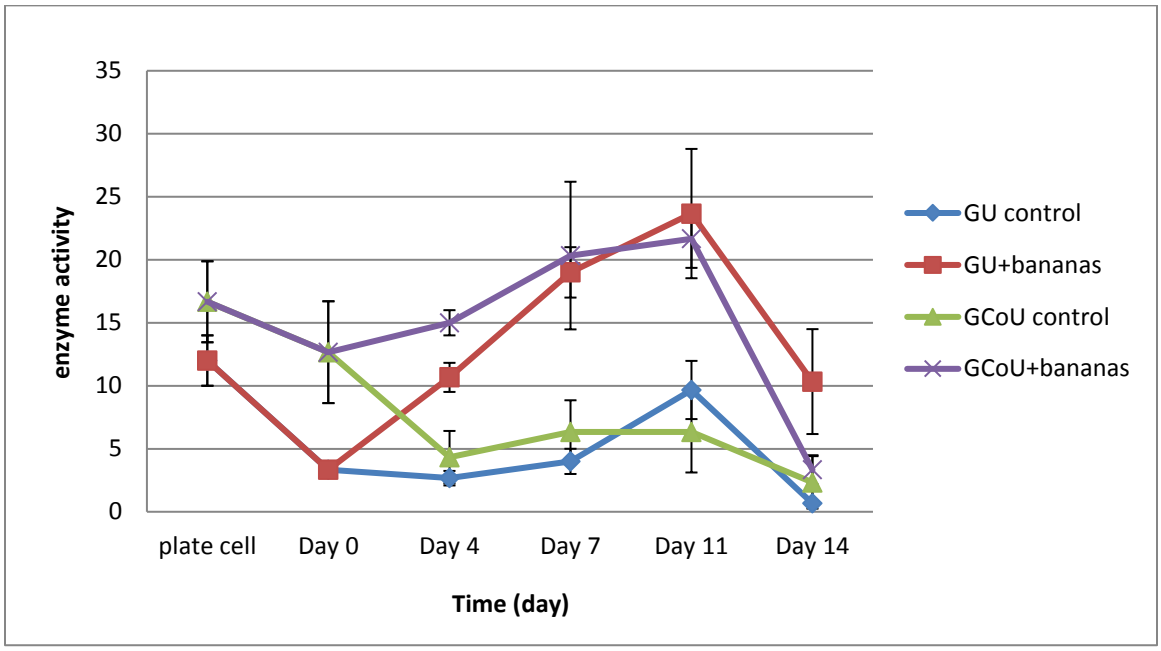
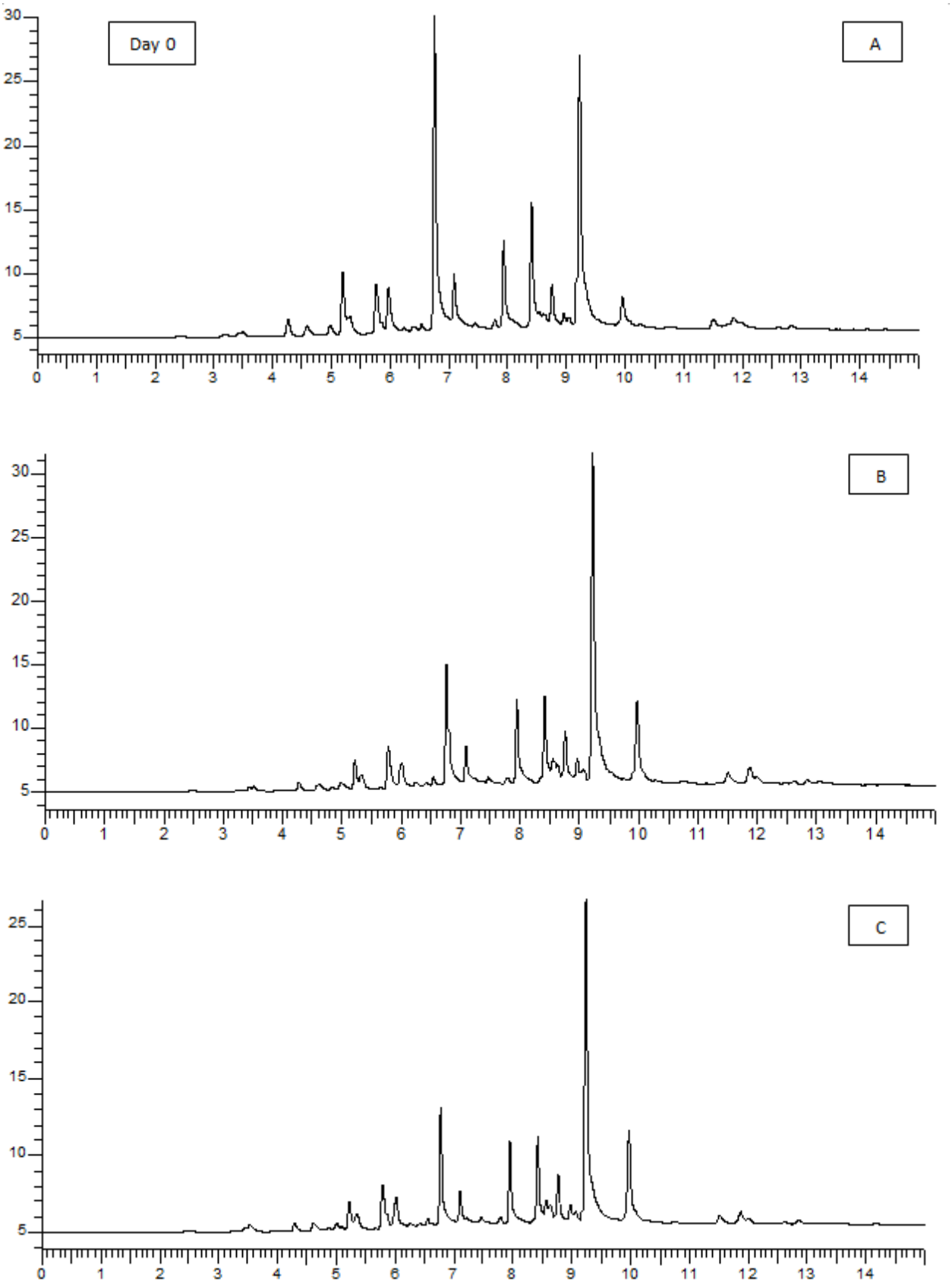


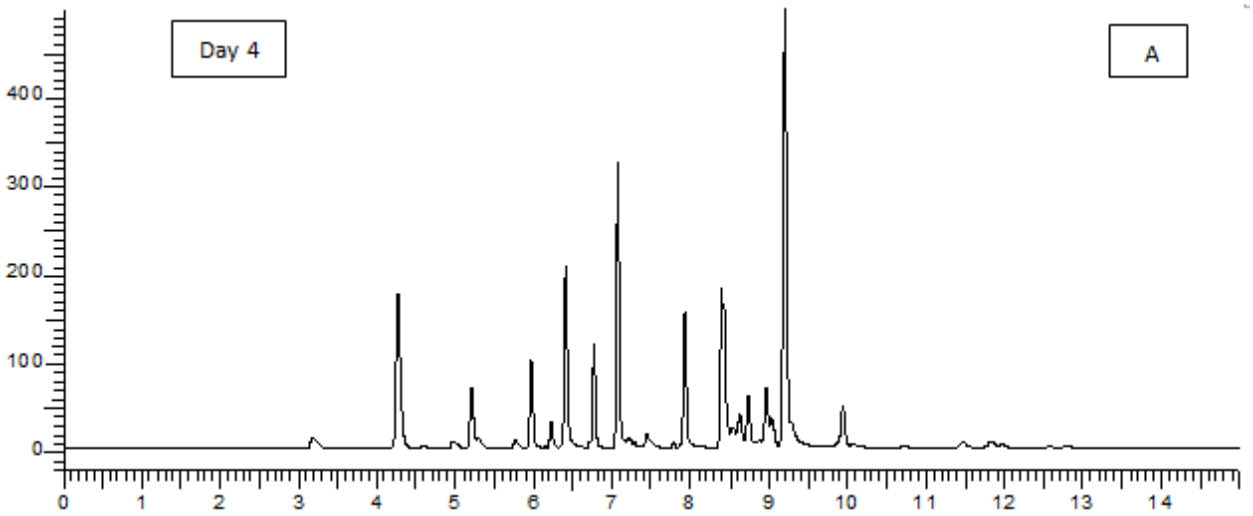
Figure 24 1-HMO activity of *R. rhodochrous* DAP 96253 grown on GU and GCoU plates (set A). *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

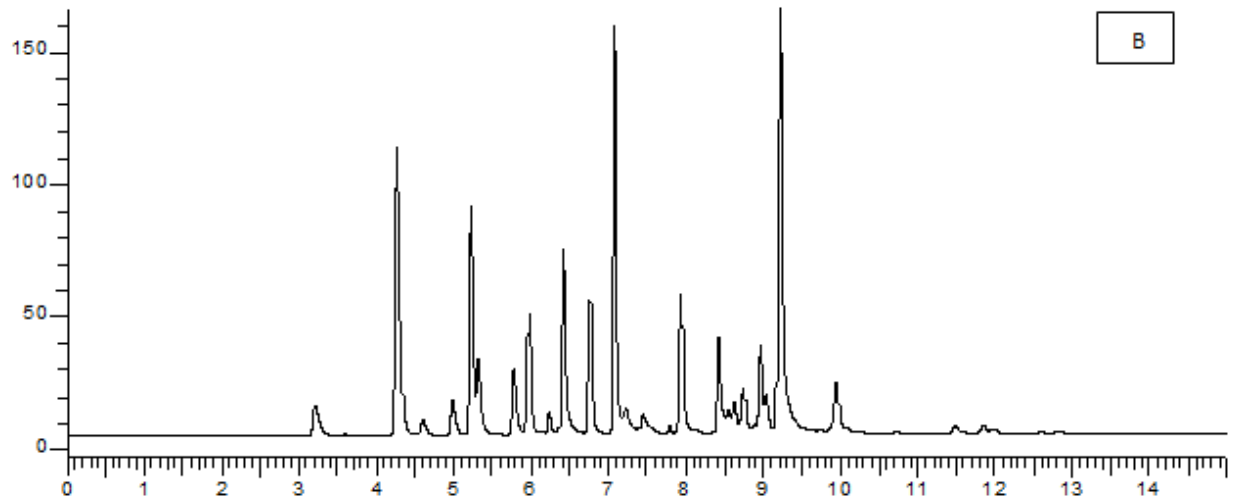
The cells exposed to bananas show much higher 1-HMO activity than controls. 1-HMO in GCoU plate cells are higher than that in GU plate cells except from Day 11 (Figure 24).

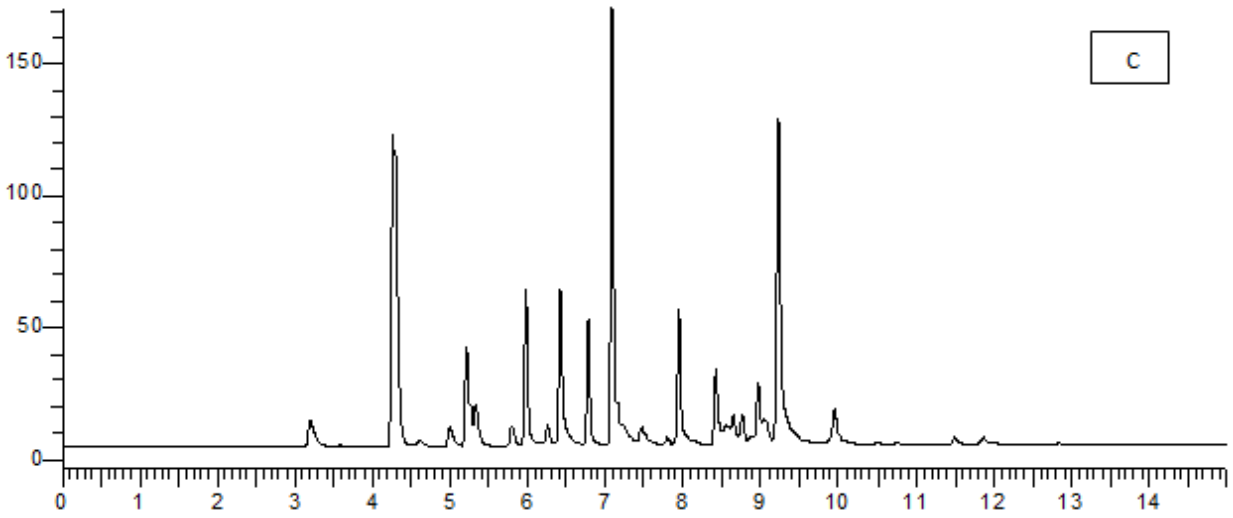
Figure 25 Banana test of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 (set A). A. control; B. GU plate; C. GCoU plate.

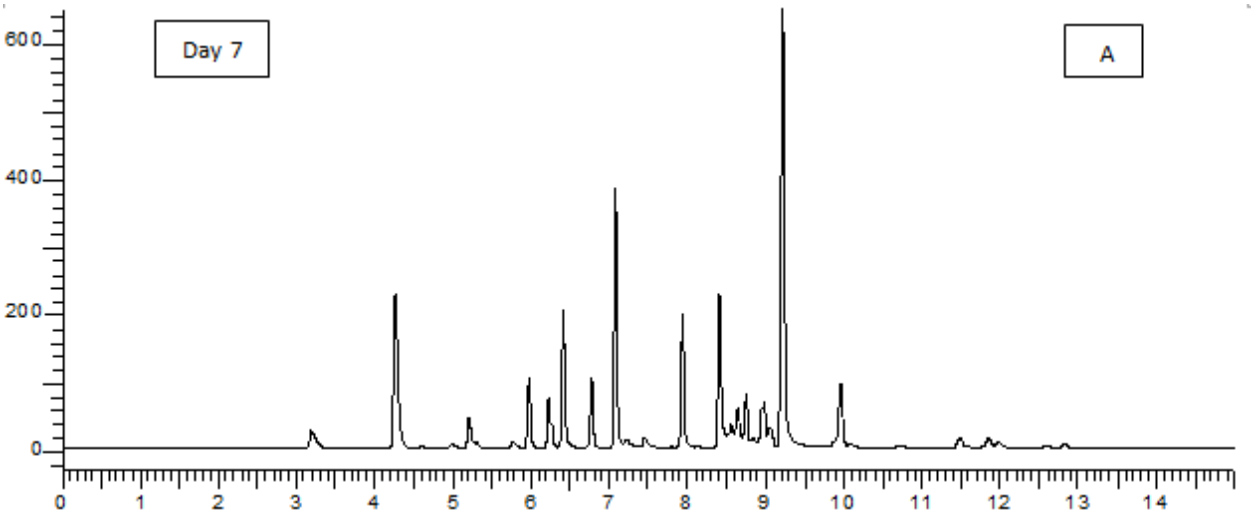
In Figure 25, GCoU plate cells of *R. rhodochrous* DAP 96253 can work better than GU plate cells, especially on Day 4 and 7.

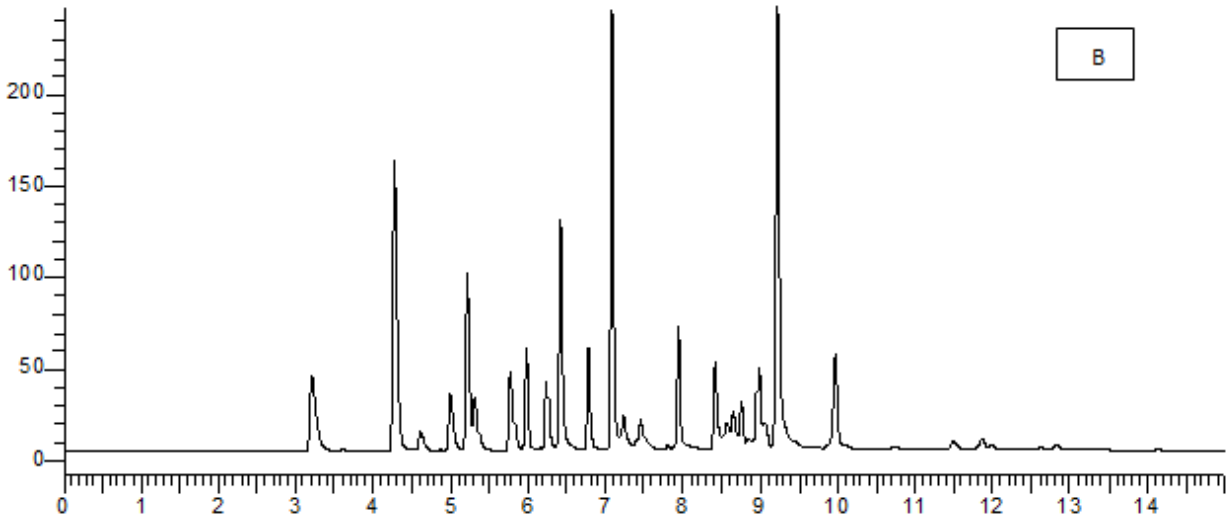

Day 0
A
B
C

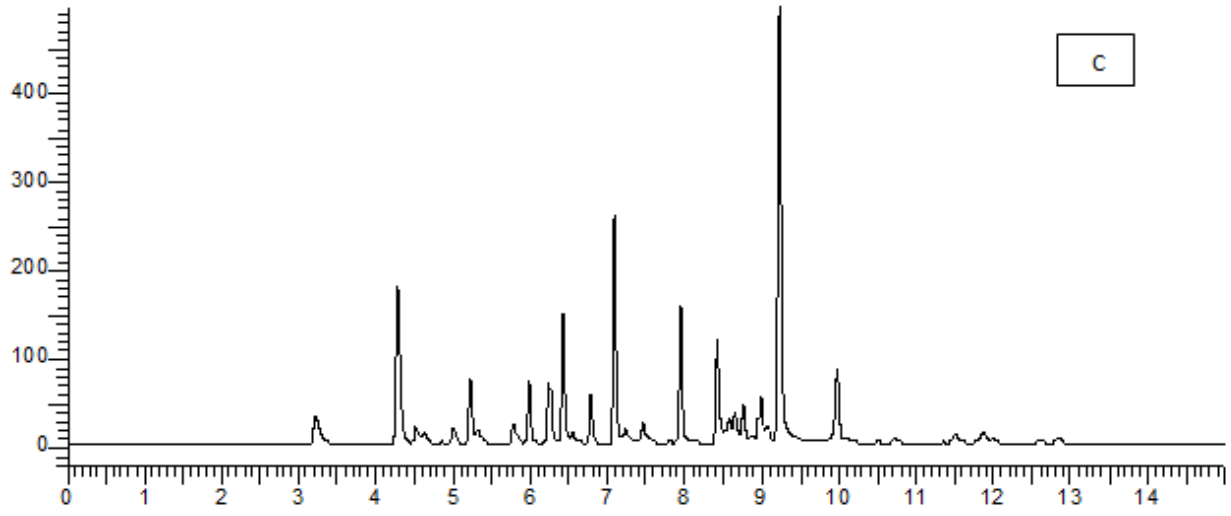

Day 4
A
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

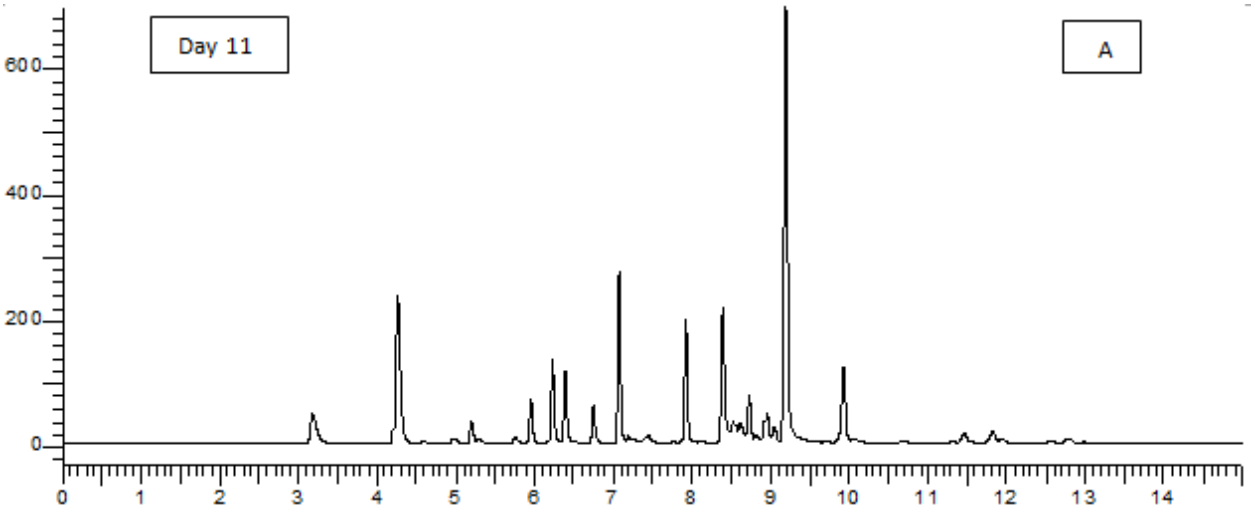

B
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

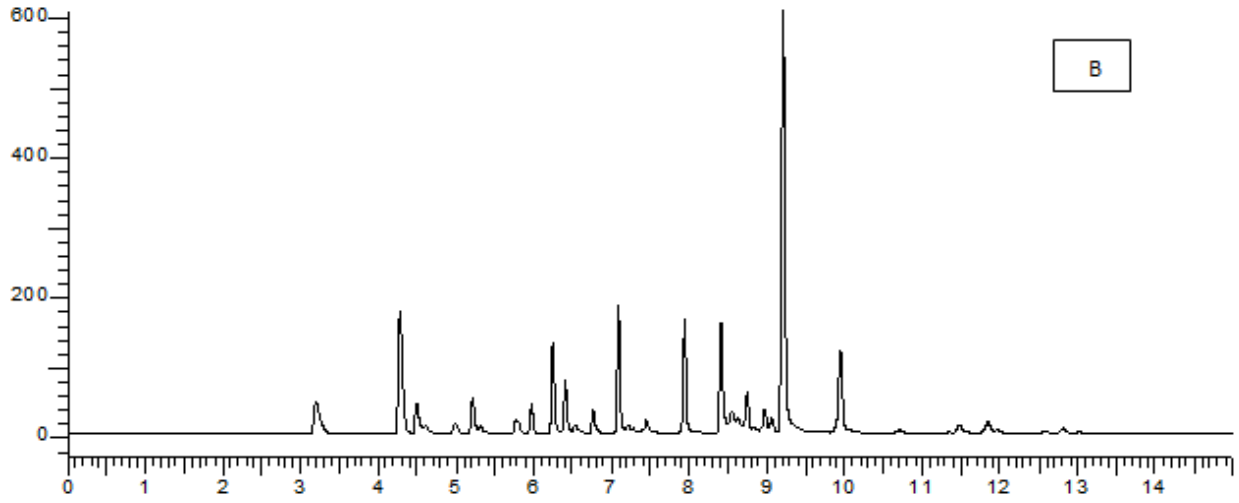

C
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

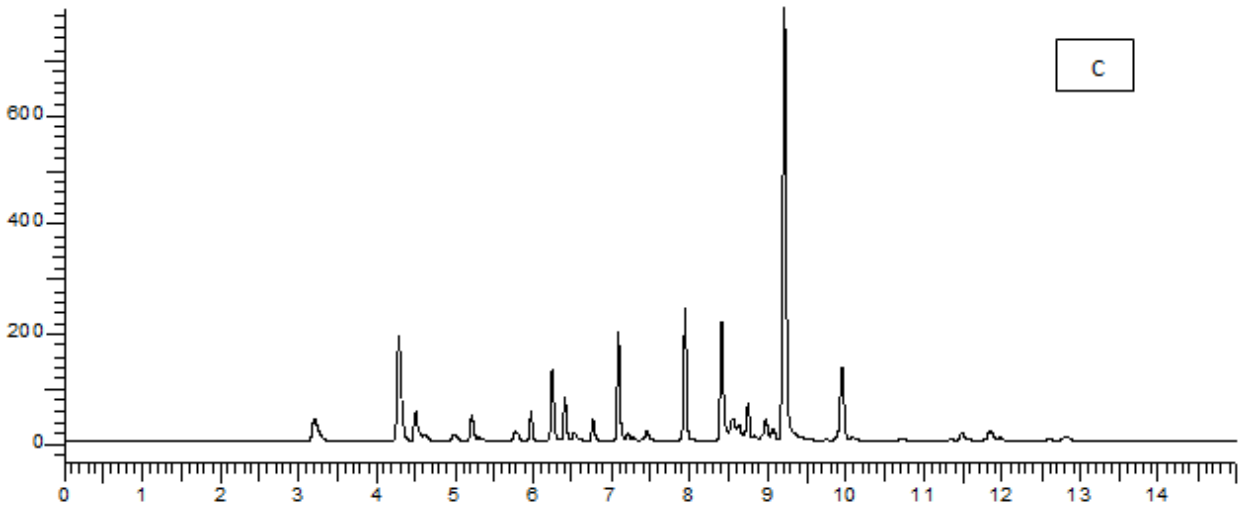

Day 7
A
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14


B
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14


C
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14


Day 11
A
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14


B
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14


C
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

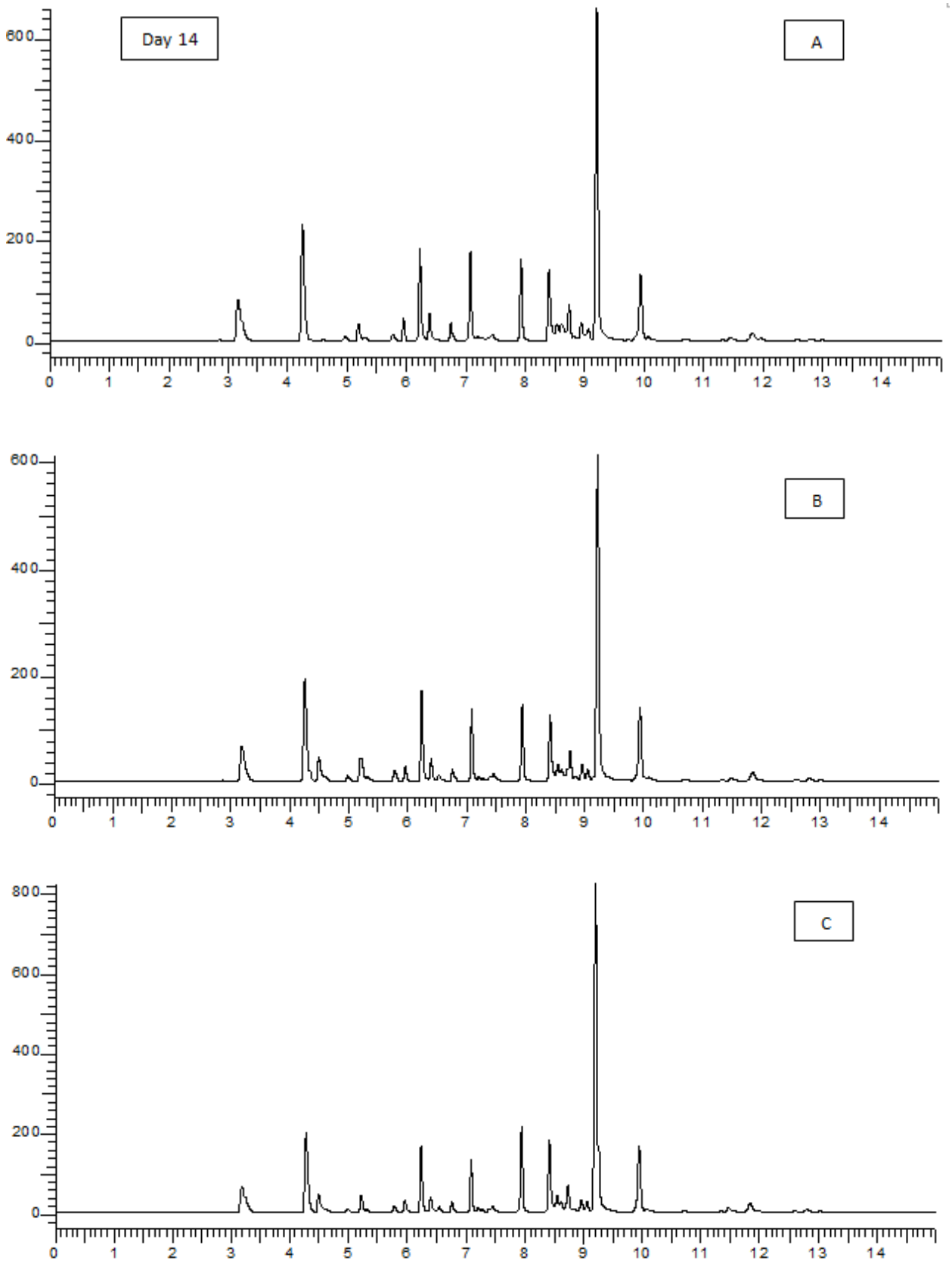

Day 14
A
B
C
600
400
200
0
800
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 26 HS-SPME-GC-FID of banana test of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 (set A). A. control; B. GU plate; C. GCoU plate.

In Figure 26, on Day 4 and 7, banana VOCs from the samples with GU and GCoU plate cells of *R. rhodochrous* DAP 96253 are considerably lower much fewer than control. The banana VOCs seen for all three samples appear to be quite similar, but with levels reduced for the ones with GU and GCoU plate cells.

b. Comparison of the effects of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening (set B)

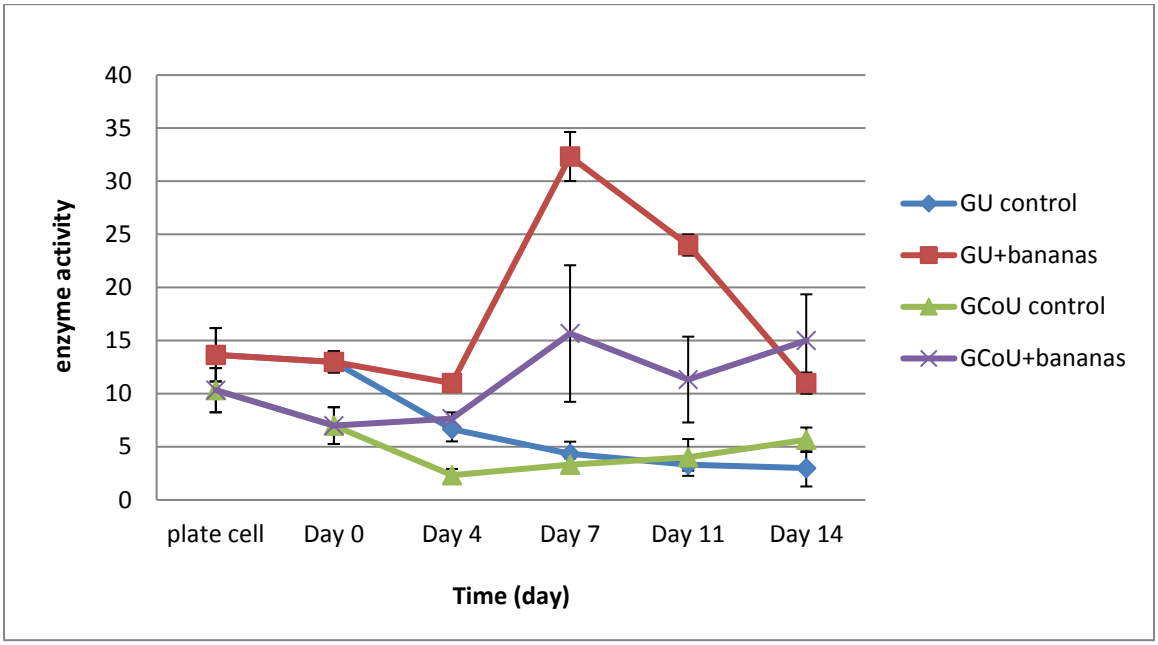


Figure 27 1-HMO activity of *R. rhodochrous* DAP 96253 grown on GU and GCoU plates (set B). *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

In Figure 27, 1-HMO activity in *R. rhodochrous* DAP 96253 cells from GU is higher than that in cells from GCoU except on Day 14. When cells are exposed to bananas, 1-HMO activity increases dramatically from Day 4, especially for GU.

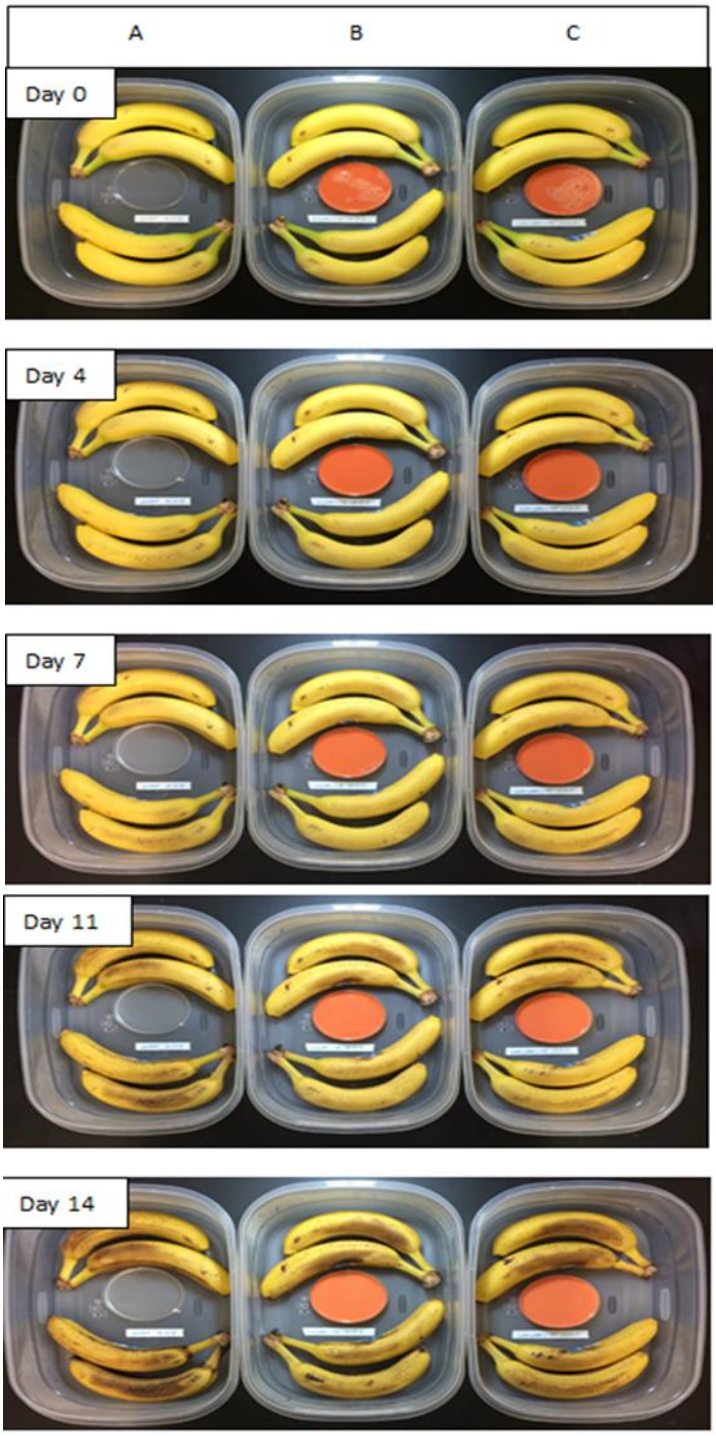
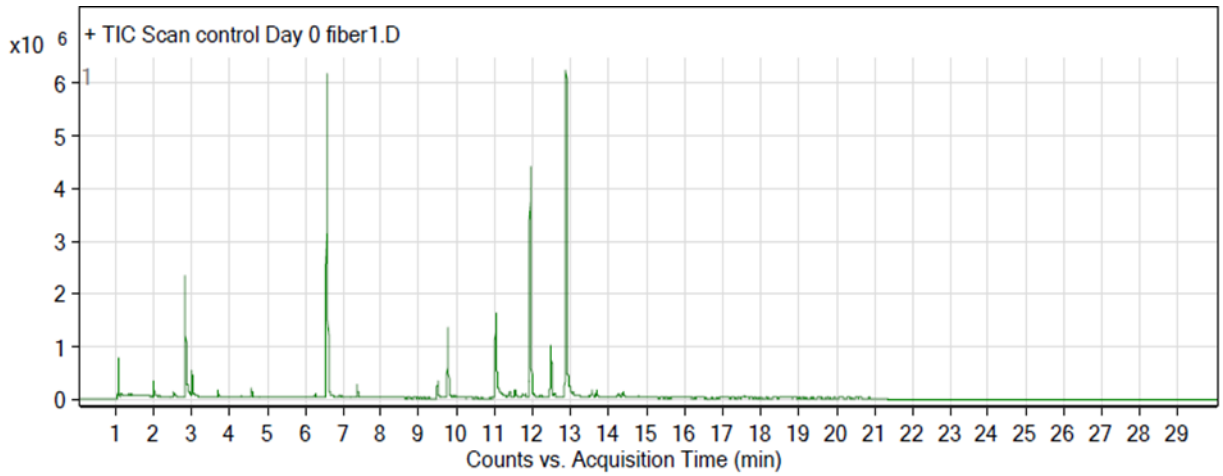
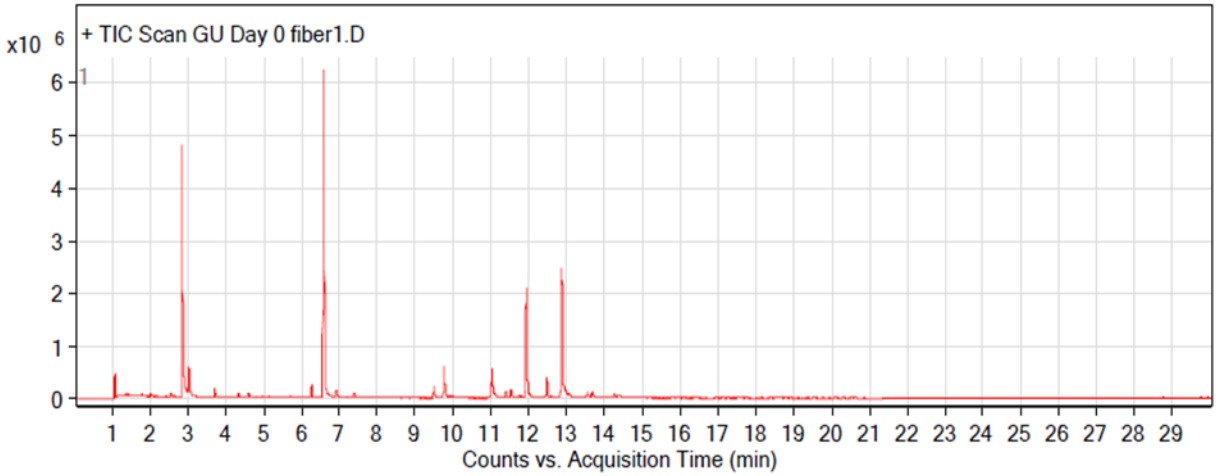
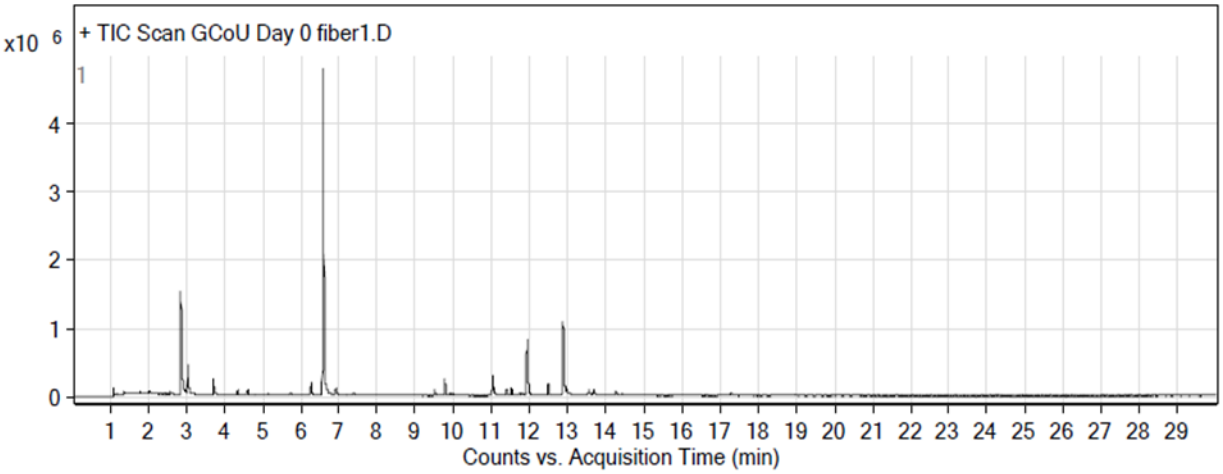


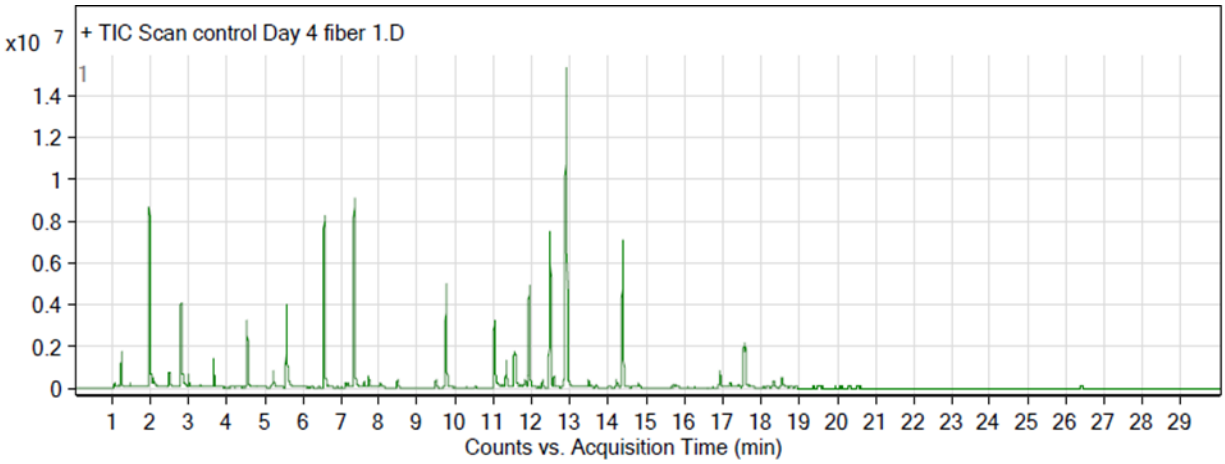
Figure 28 Banana test of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 (set B). A. control; B. GU plate; C. GCoU plate.

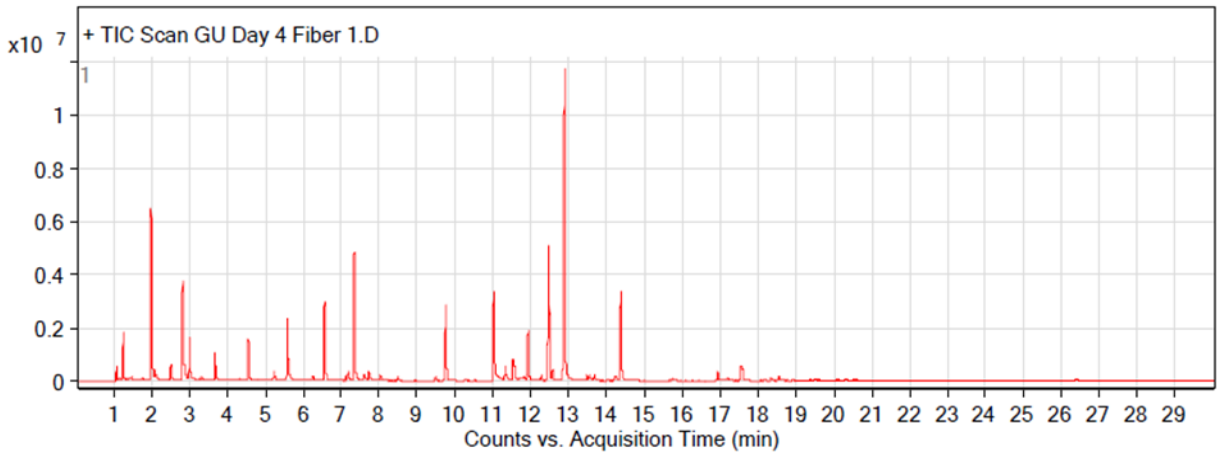
In Figure 28, GU plate cells of *R. rhodochrous* DAP 96253 can work better than GCoU plate cells, especially on Day 7, 11 and 14.

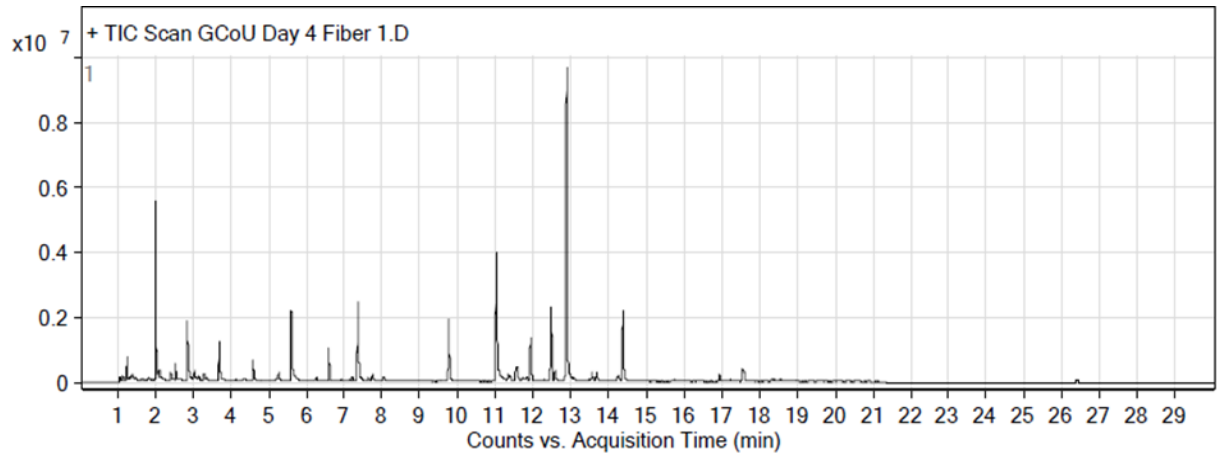

x10 6
+ TIC Scan control Day 0 fiber1.D
1
0 1 2 3 4 5 6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)

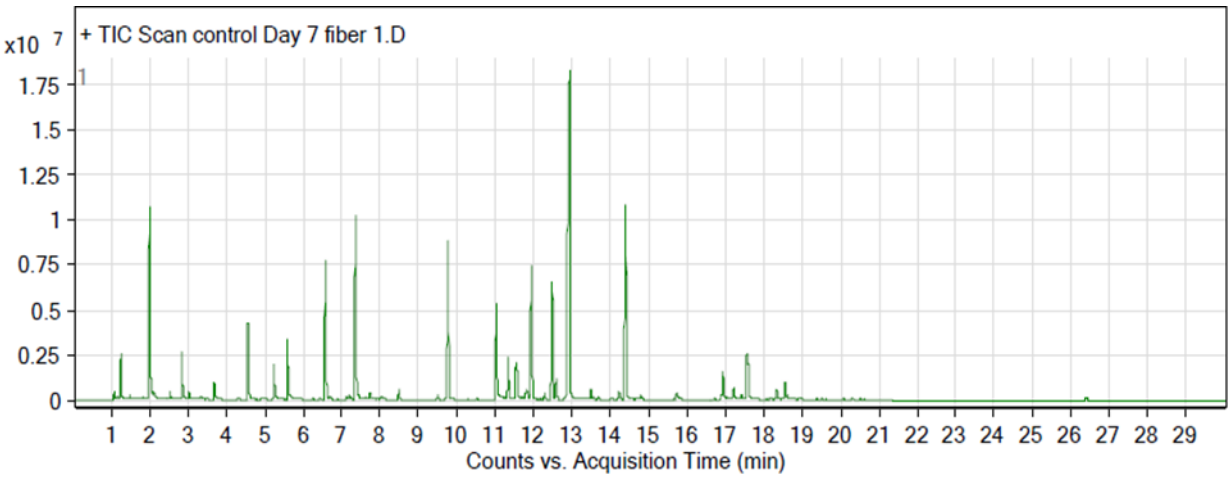

x10 6
+ TIC Scan GU Day 0 fiber1.D
1
0 1 2 3 4 5 6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)

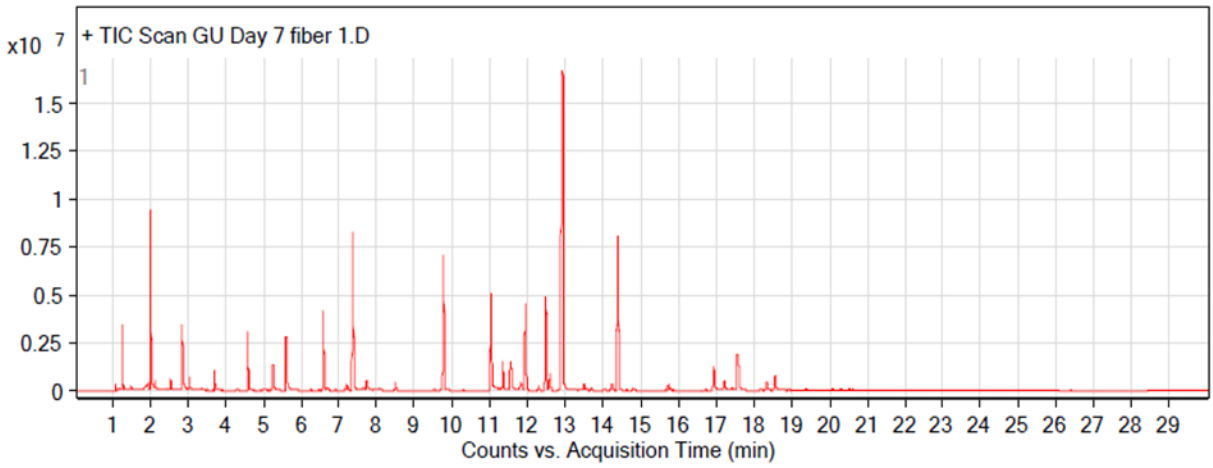

x10 6
+ TIC Scan GCoU Day 0 fiber1.D
1
0 1 2 3 4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)

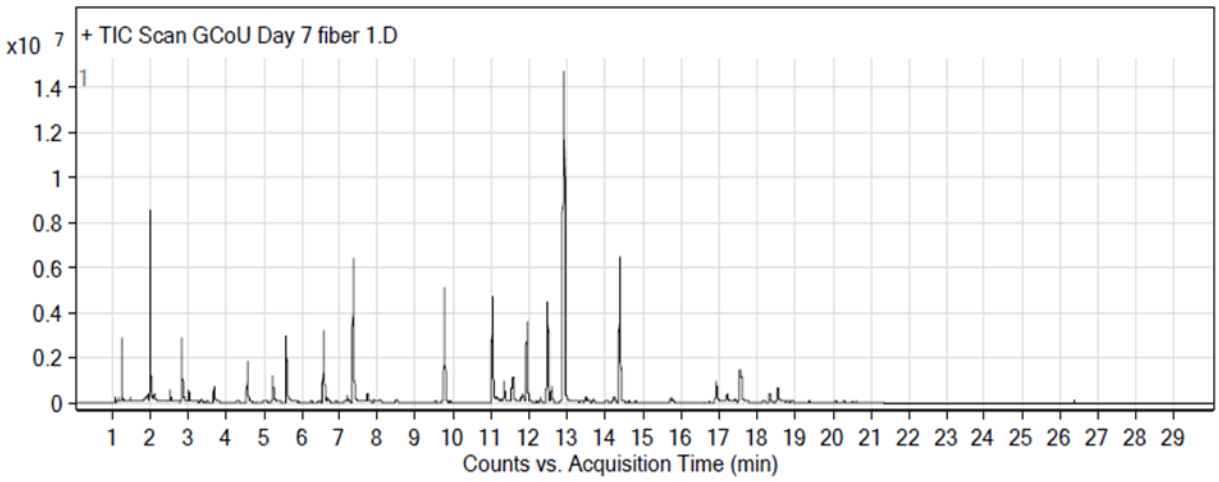

+ TIC Scan control Day 4 fiber 1.D
x10 7
1
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)

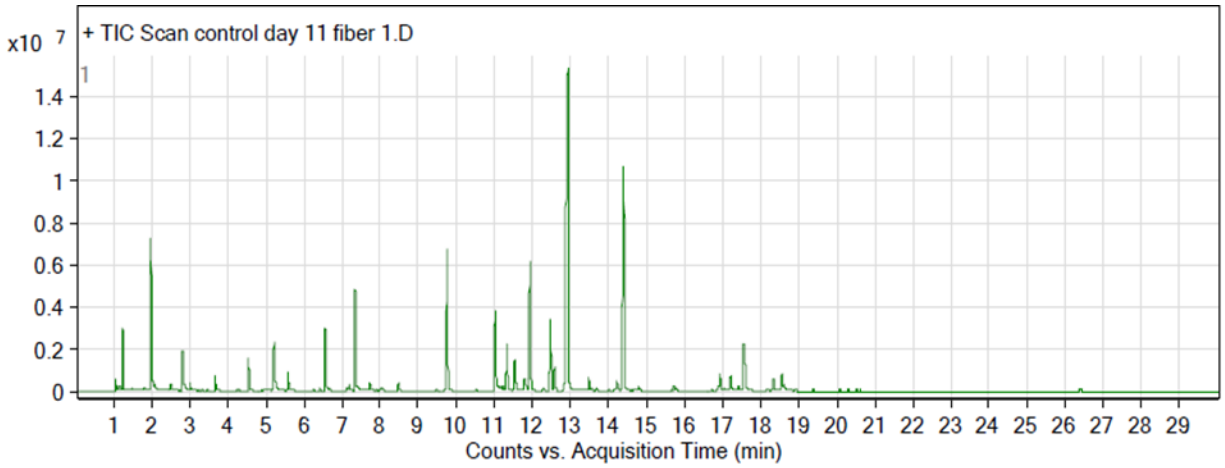

+ TIC Scan GU Day 4 Fiber 1.D
x10 7
1
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)

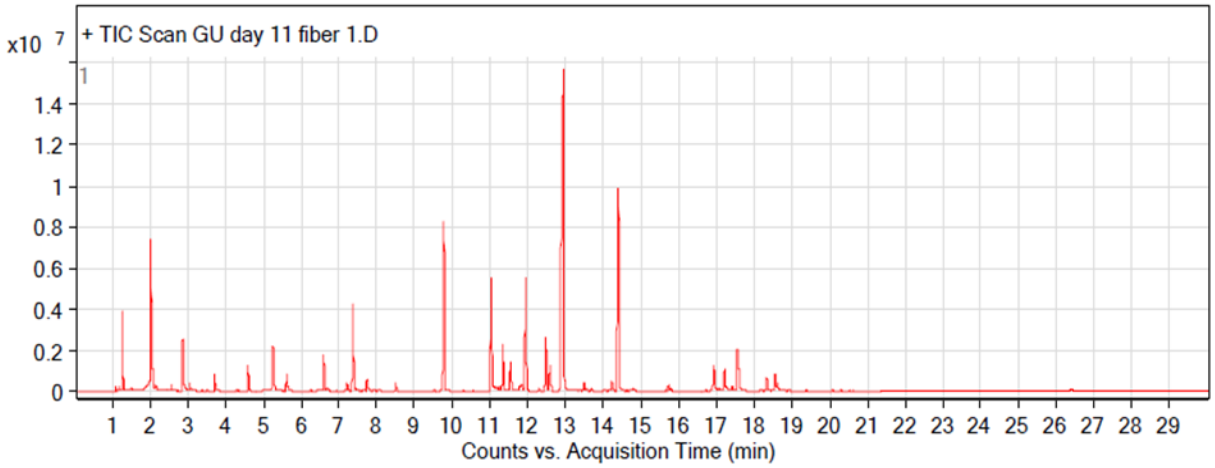

+ TIC Scan GCoU Day 4 Fiber 1.D
x10 7
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)

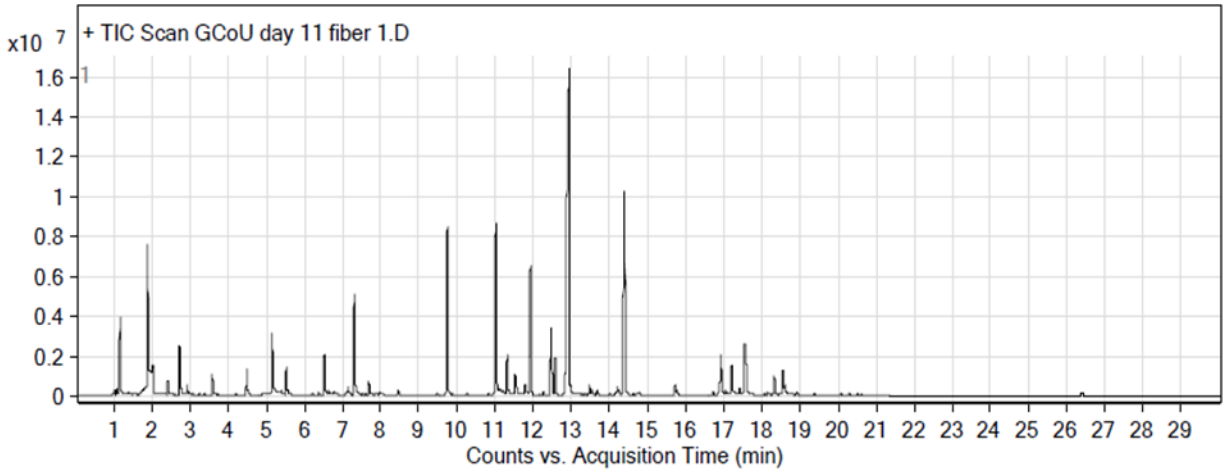

+ TIC Scan control Day 7 fiber 1.D
x10 7
1.75
1.5
1.25
1
0.75
0.5
0.25
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)


+ TIC Scan GU Day 7 fiber 1.D
x10 7
1.5
1.25
1
0.75
0.5
0.25
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)


+ TIC Scan GCoU Day 7 fiber 1.D
x10 7
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Counts vs. Acquisition Time (min)


+ TIC Scan control day 11 fiber 1.D
x10 7
Counts vs. Acquisition Time (min)


+ TIC Scan GU day 11 fiber 1.D
x10 7
Counts vs. Acquisition Time (min)


+ TIC Scan GCoU day 11 fiber 1.D
x10 7
Counts vs. Acquisition Time (min)

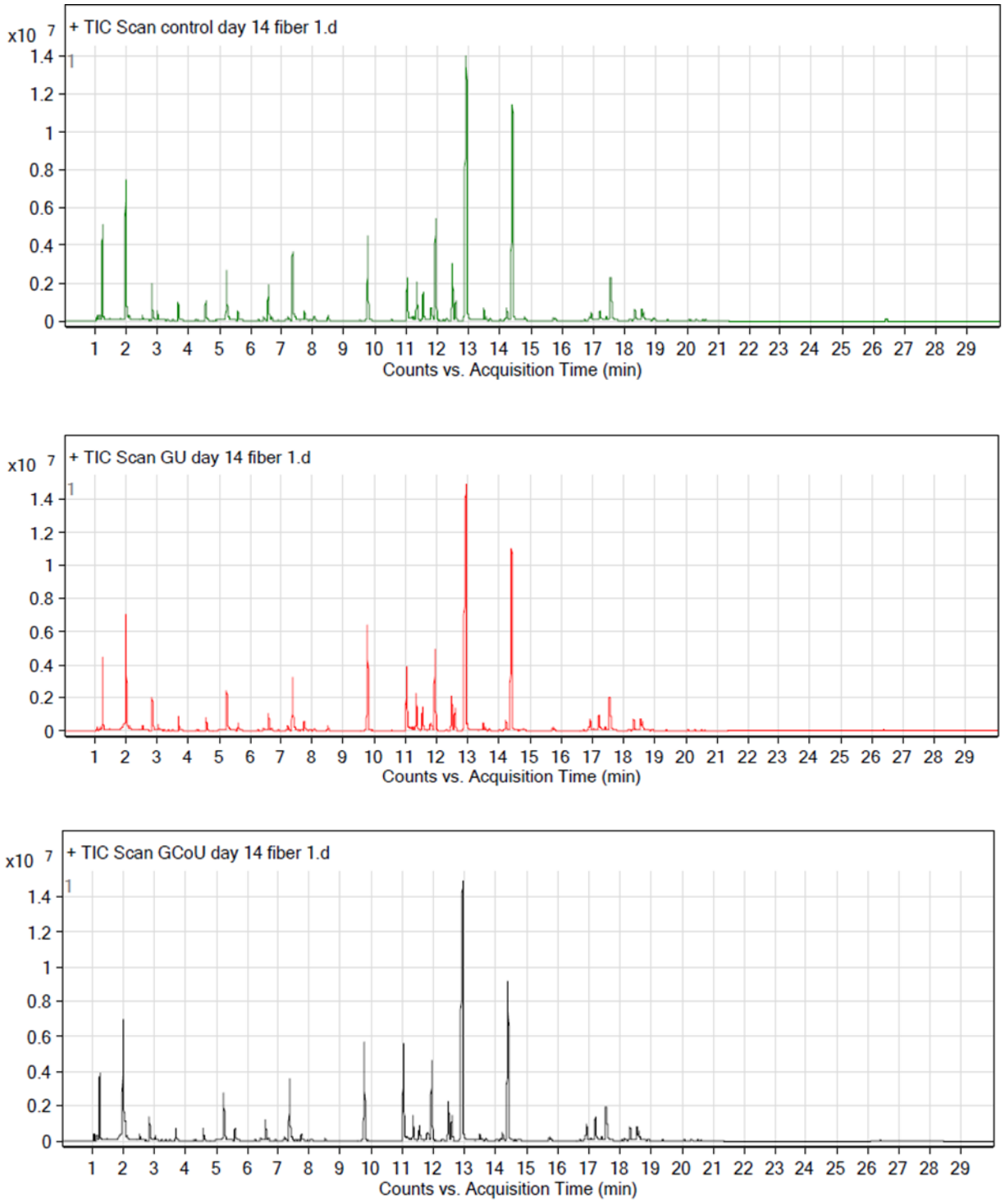
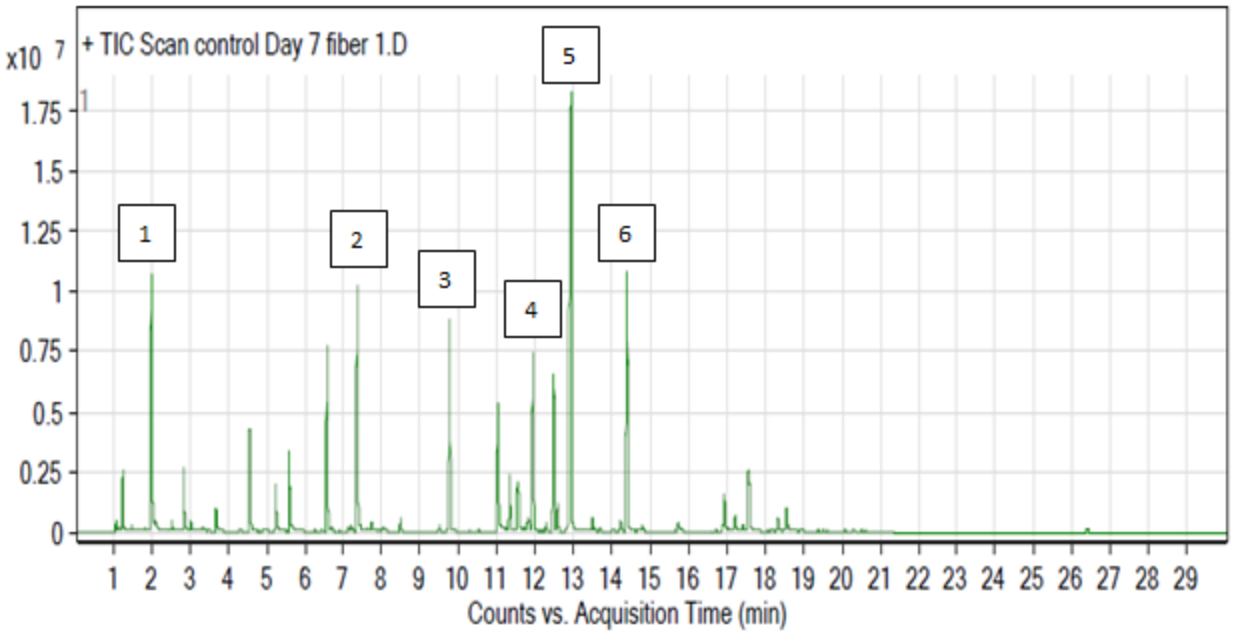


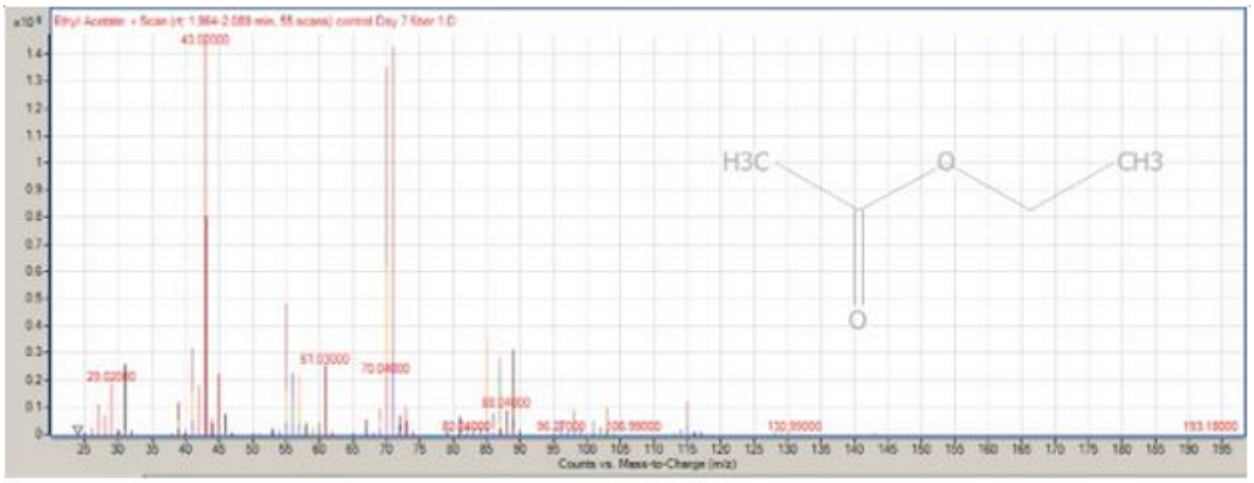
Figure 29 HS-SPME-GC/MS of banana test of GU and GCoU plate cells of *R. rhodochrous* DAP 96253 (set B).

In Figure 29, we can find consistence of banana VOCs and the results in Figure 27 on Day 4 and Day 7.

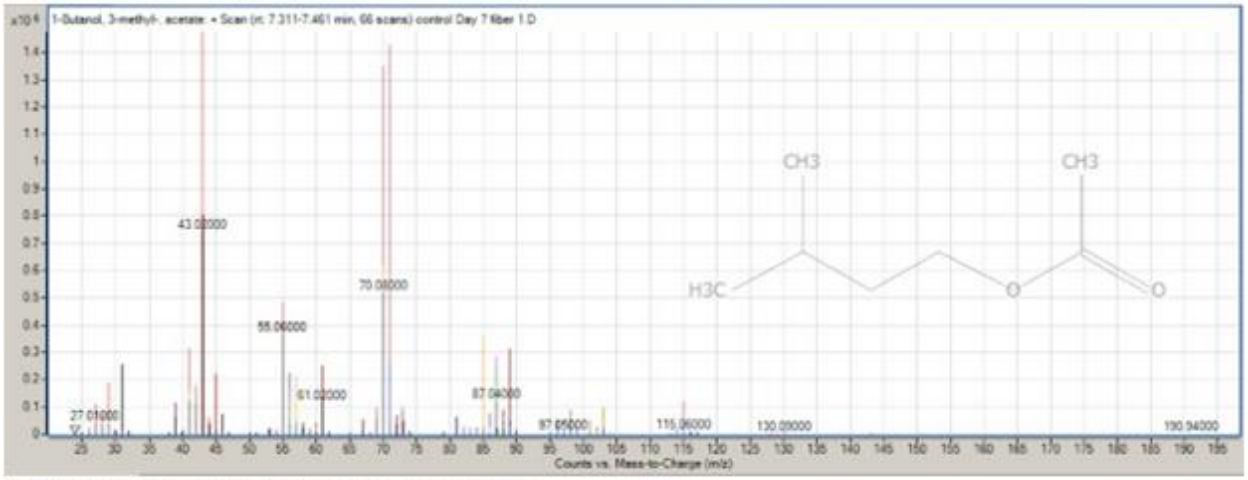


1. Ethyl acetate
2. 1-Butanol, 3-methyl-, acetate
3. Butanoic acid, 2-methylpropyl ester
4. Butanoic acid, 1-methylbutyl ester
5. Butanoic acid, 3-methylbutyl ester
6. Butanoic acid, 3-methyl-, 3-methylbutyl ester

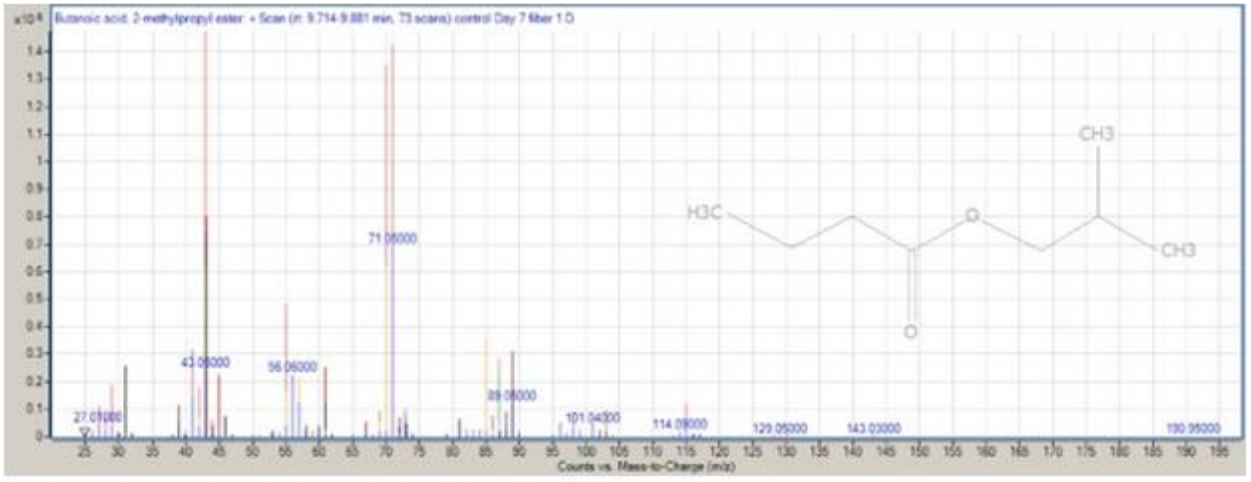
Figure 30 Peak labels of six VOCs in GC/MS chromatogram of banana control on day 7 (set B).



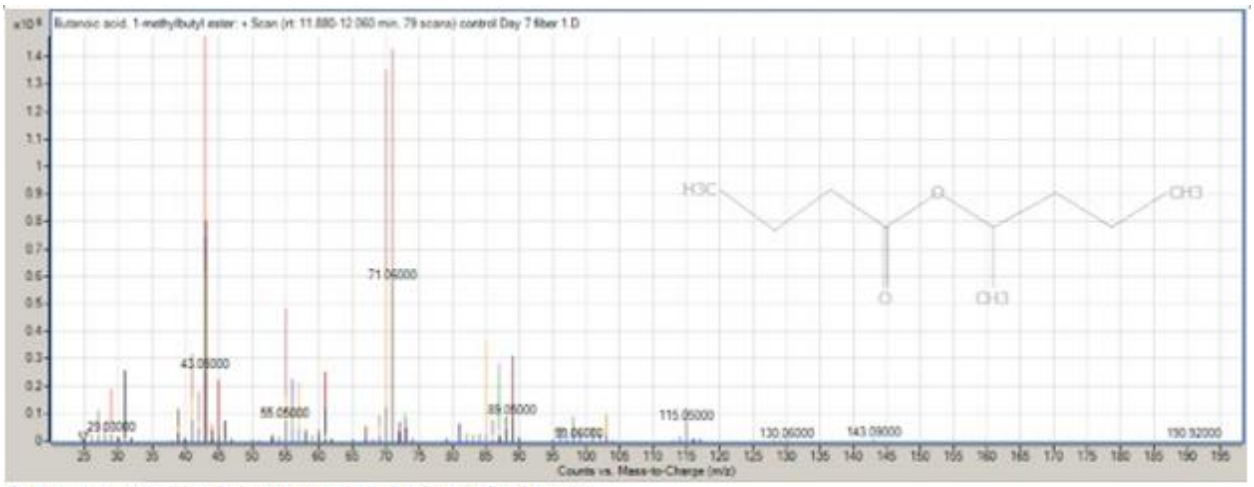
1. Mass spectrum of ethyl acetate



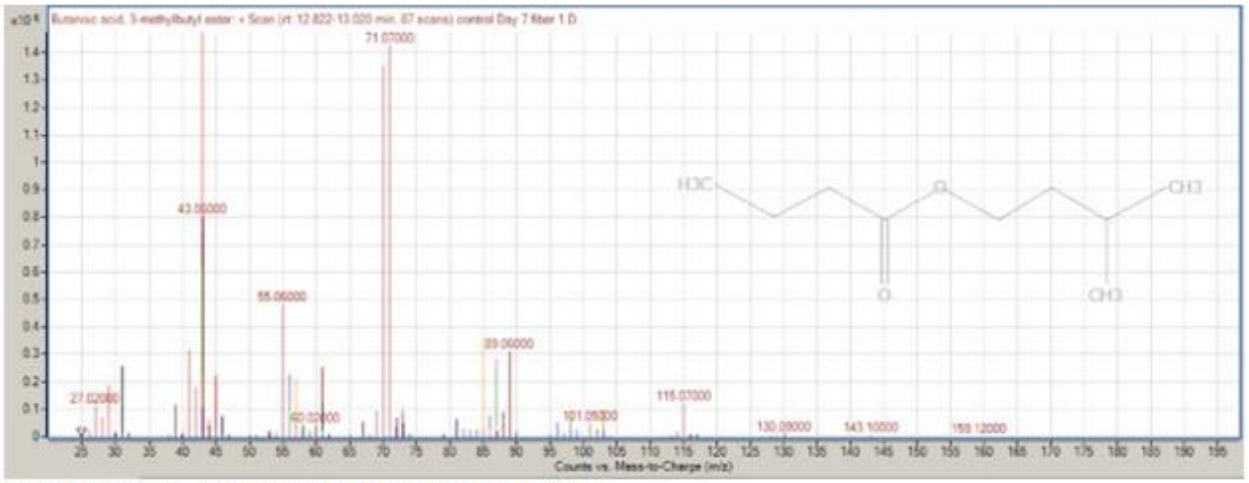
2. Mass spectrum of 1-butanol, 3-methyl-, acetate



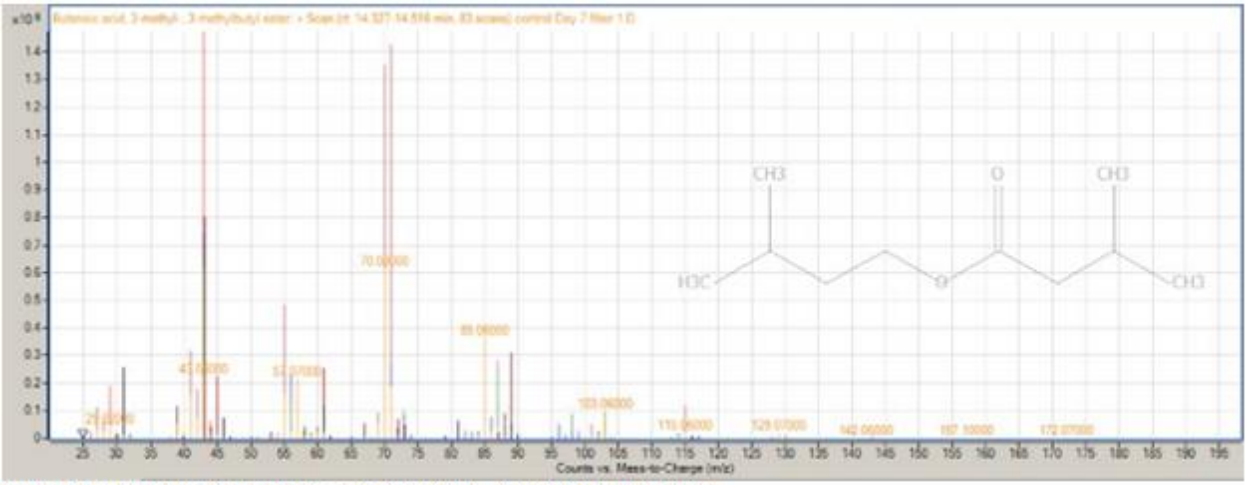
3. Mass spectrum of butanoic acid, 2-methylpropyl ester



4. Mass spectrum of butanoic acid, 1-methylbutyl ester



5. Mass spectrum of butanoic acid, 3-methylbutyl ester



6. Mass spectrum of butanoic acid, 3-methyl-, 3-methylbutyl ester

Figure 31 Mass spectrums of six VOCs in banana control day 7 (set B).

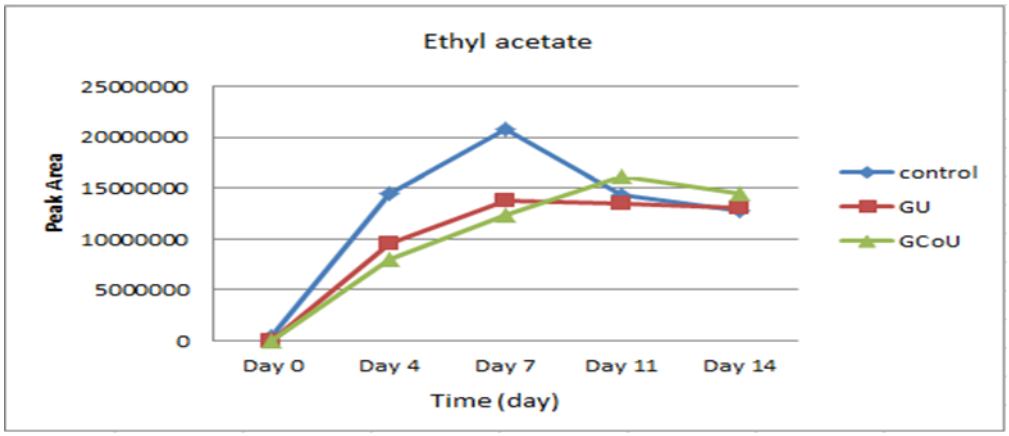


Figure 32 Comparison of peak 1 area in whole bananas from 3 samples (set B).

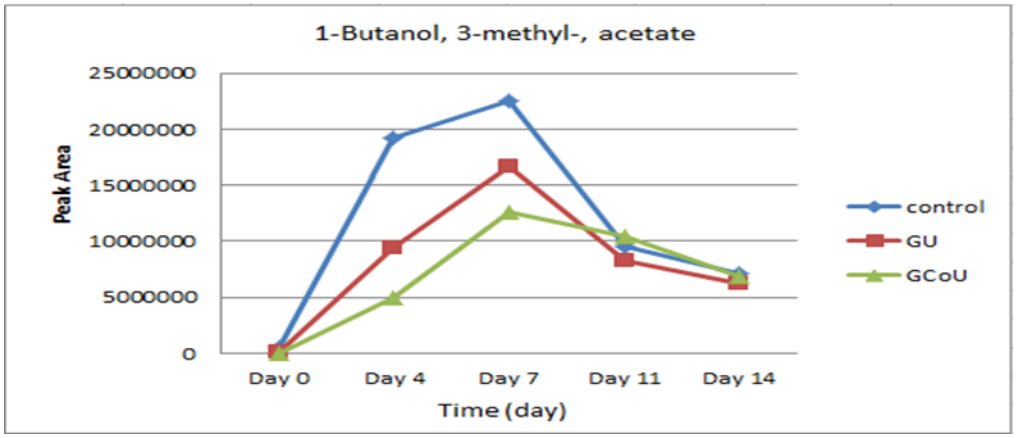


Figure 33 Comparison of peak 2 area in whole bananas from 3 samples (set B).

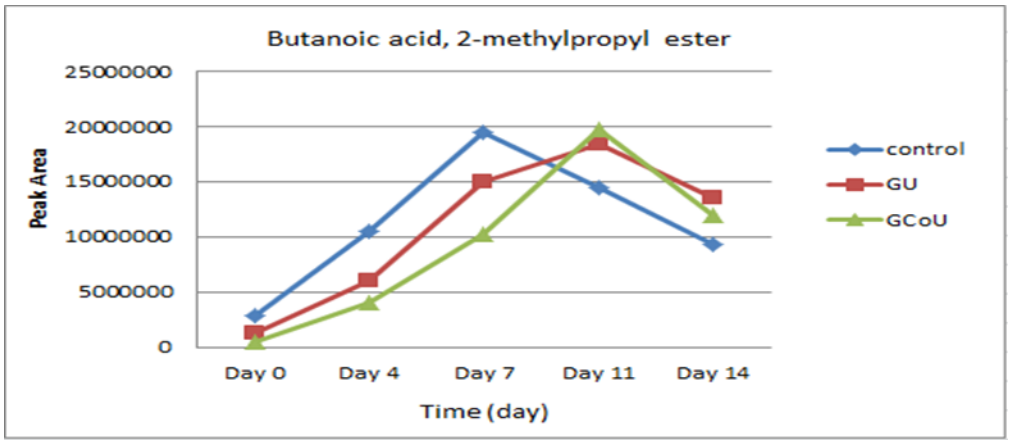


Figure 34 Comparison of peak 3 area in whole bananas from 3 samples (set B).

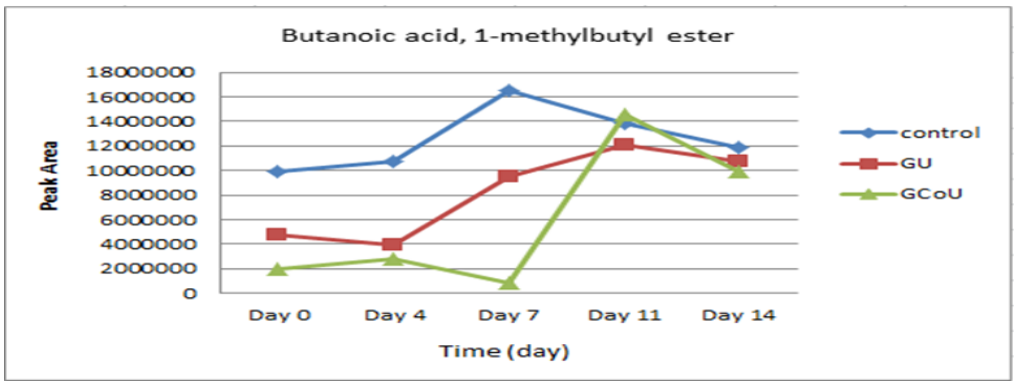


Figure 35 Comparison of peak 4 area in whole bananas from 3 samples (set B).

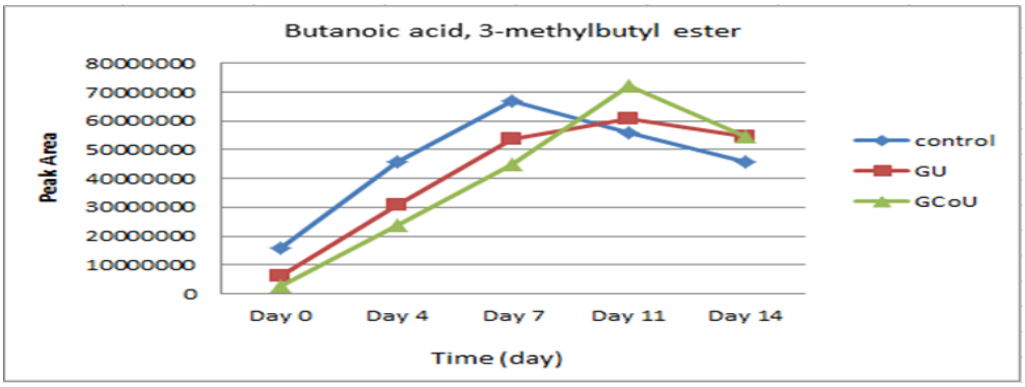


Figure 36 Comparison of peak 5 area in whole bananas from 3 samples (set B).

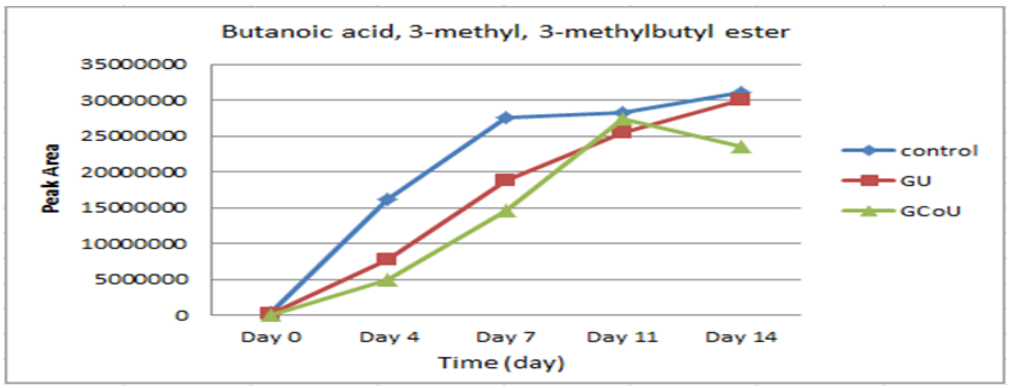


Figure 37 Comparison of peak 6 area in whole bananas from 3 samples (set B).

From Figure 32-37, it is very interesting to see that these six VOCs from control are the most while those from GCoU are the least from Day 0 to Day 7.

c. Effects of ethylene- and propylene-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

Table 8 HS-GC-FID of ethylene and propylene degradation by *R. rhodochrous* DAP 96253 (GU plate) in M9 minimal medium (no glucose) for 48 hours.

	Retention Time (min)	Peak area (μV·s)	Degradation Rate
Ethylene control	2.246	39556.10	N/A
GU in M9 with ethylene 1	2.273	35208.93	10.99%
GU in M9 with ethylene 2	2.256	32299.43	18.35%
Propylene control	2.312	57491.71	N/A
GU in M9 with propylene 1	2.311	53599.02	6.77%
GU in M9 with propylene 2	2.308	55608.94	3.27%

After 48 hours, GU plate cells of *R. rhodochrous* DAP 96253 in M9 minimal medium (no glucose) have shown higher degradation rate of ethylene than that of propylene (Table 8).

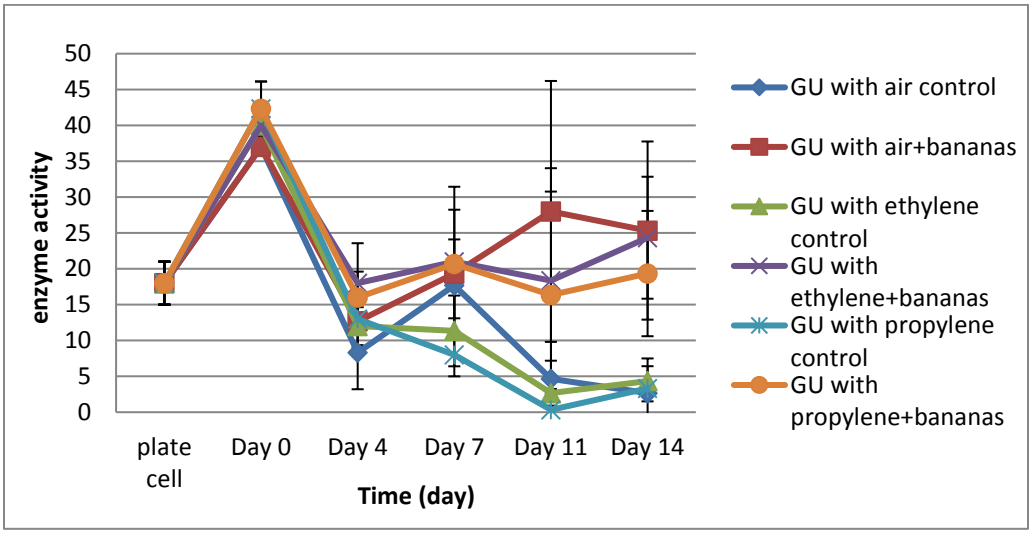
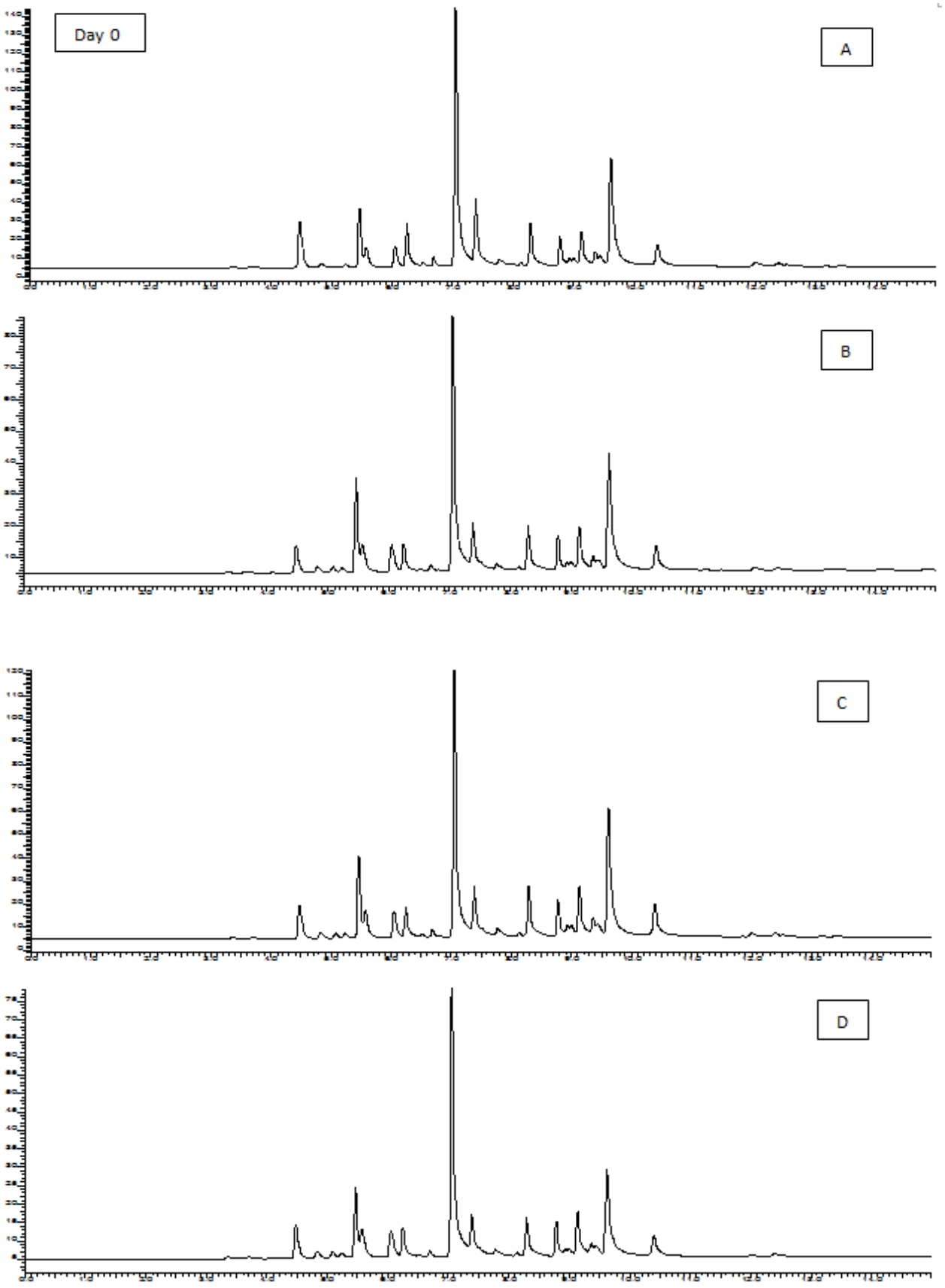


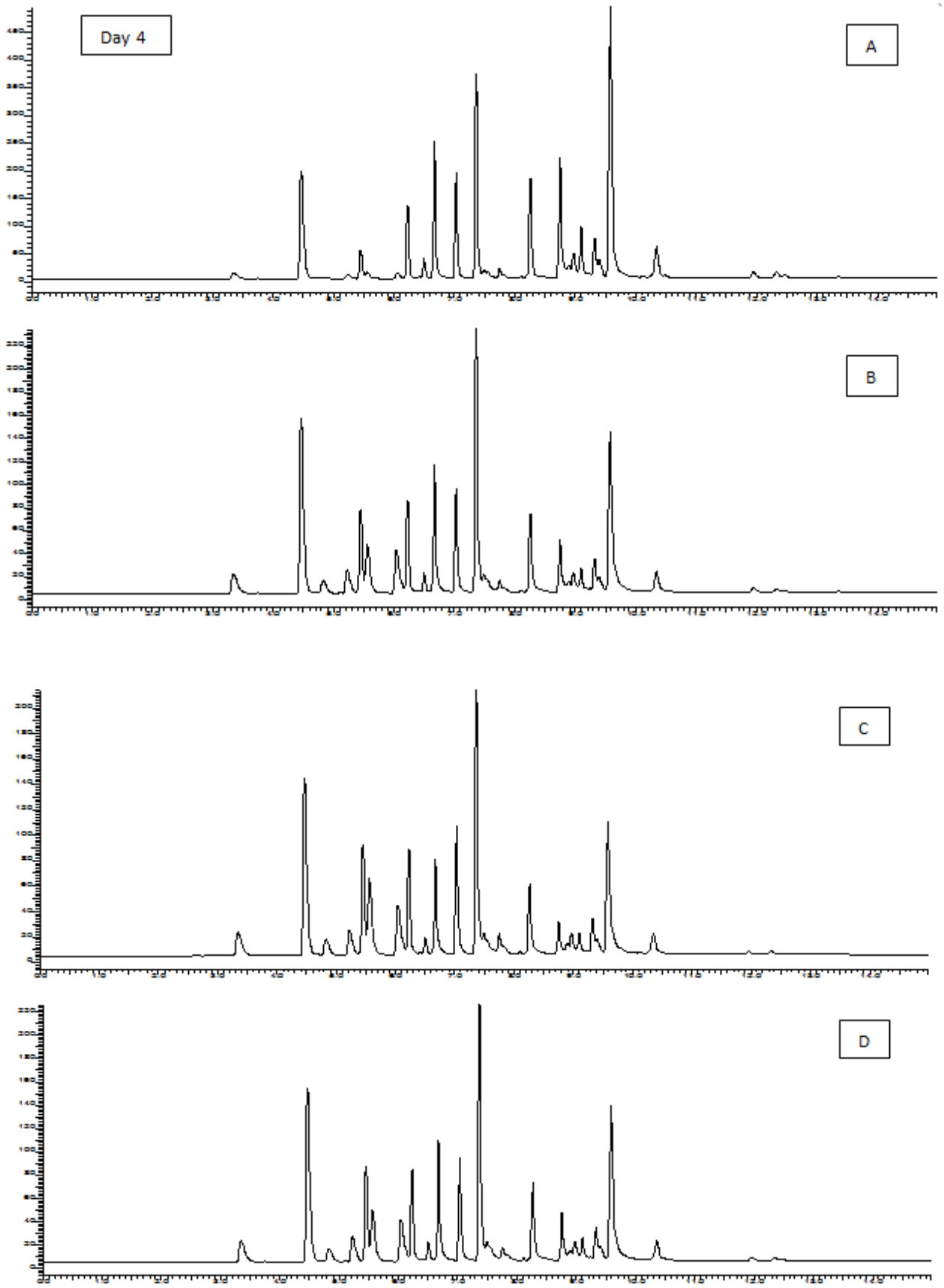
Figure 38 1-HMO activity of *R. rhodochrous* DAP 96253 (GU plate) with ethylene and propylene induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

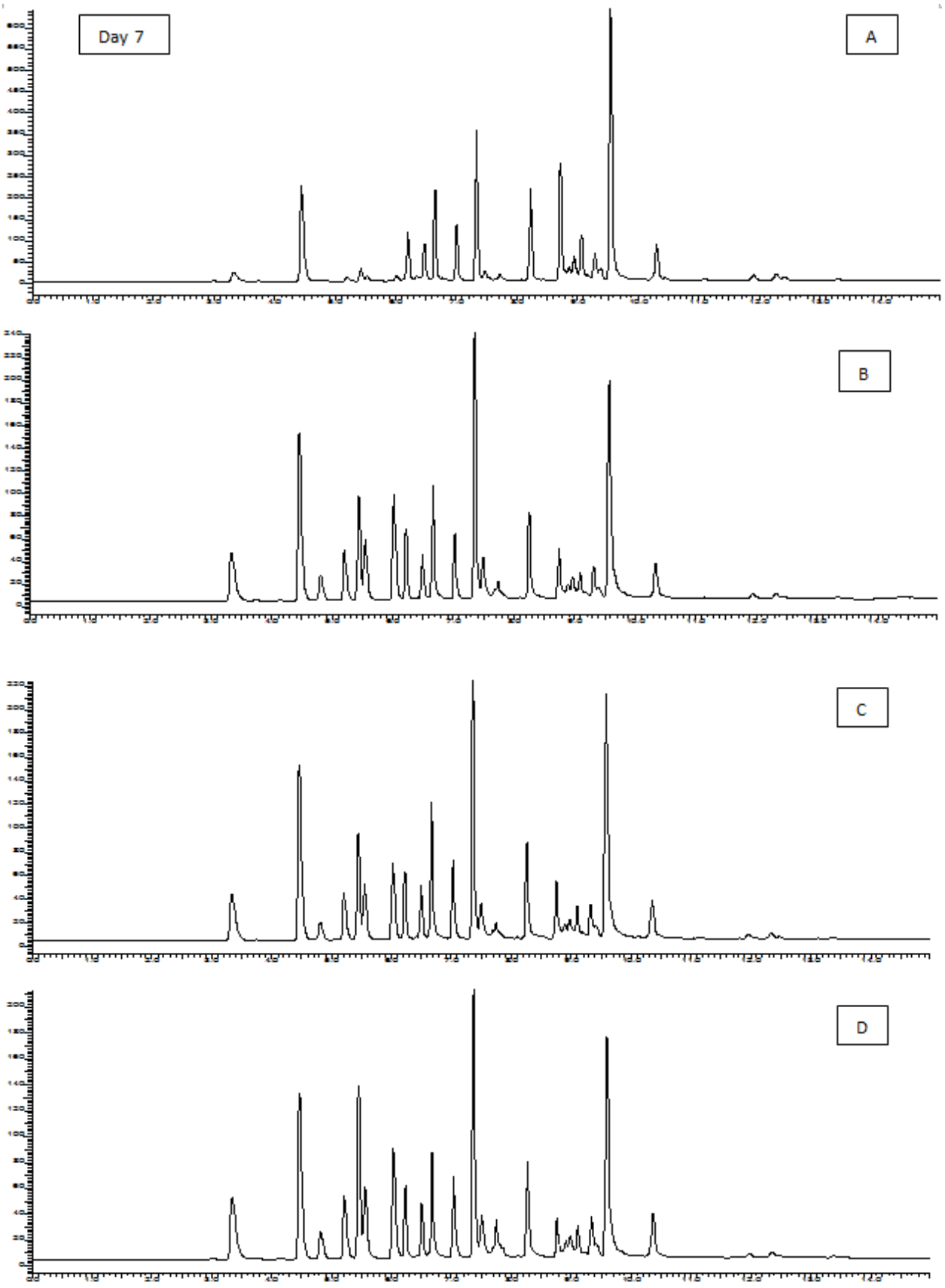
In Figure 38, 1-HMO activities of all the samples drop dramatically from Day 0 to Day 4. The cells exposed to bananas maintain higher 1-HMO activities compared with the ones with no banana exposure. The cells with propylene treatment show the lowest 1-HMO activity while the ones exposed to air show the highest 1-HMO activity.

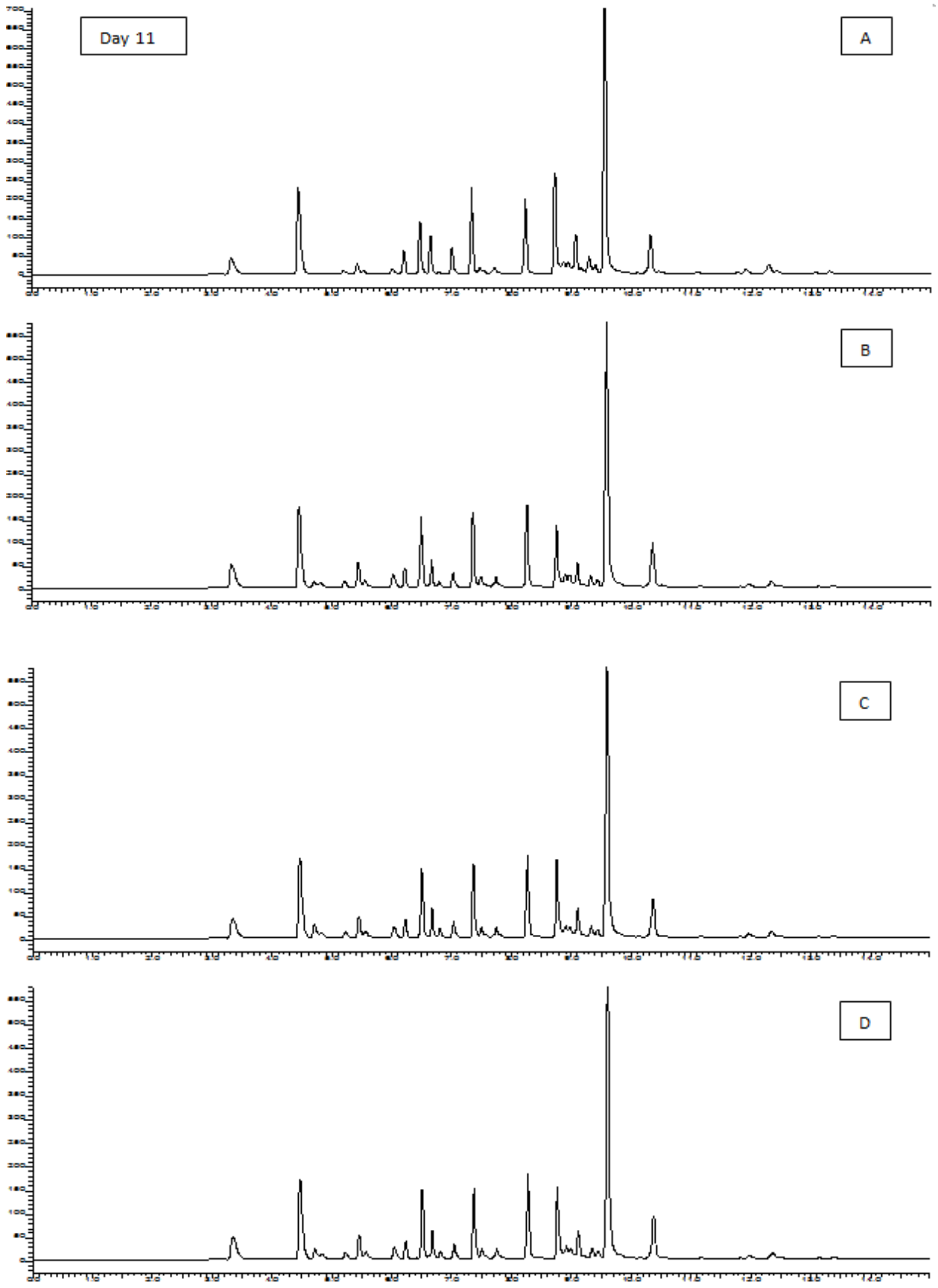
Figure 39 Banana test of ethylene and propylene-induced GU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GU plate; C. GU plate with 100 ppm ethylene; D. GU plate with 100 ppm propylene.

In Figure 39, GU plate cells exposed to air can delay banana ripening the best. Ethylene treatment cancelled the delayed banana ripening effect GU plate cells and propylene treatment partially cancelled the delayed banana ripening effect of GU plate cells.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D


Day 11
A
B
C
D

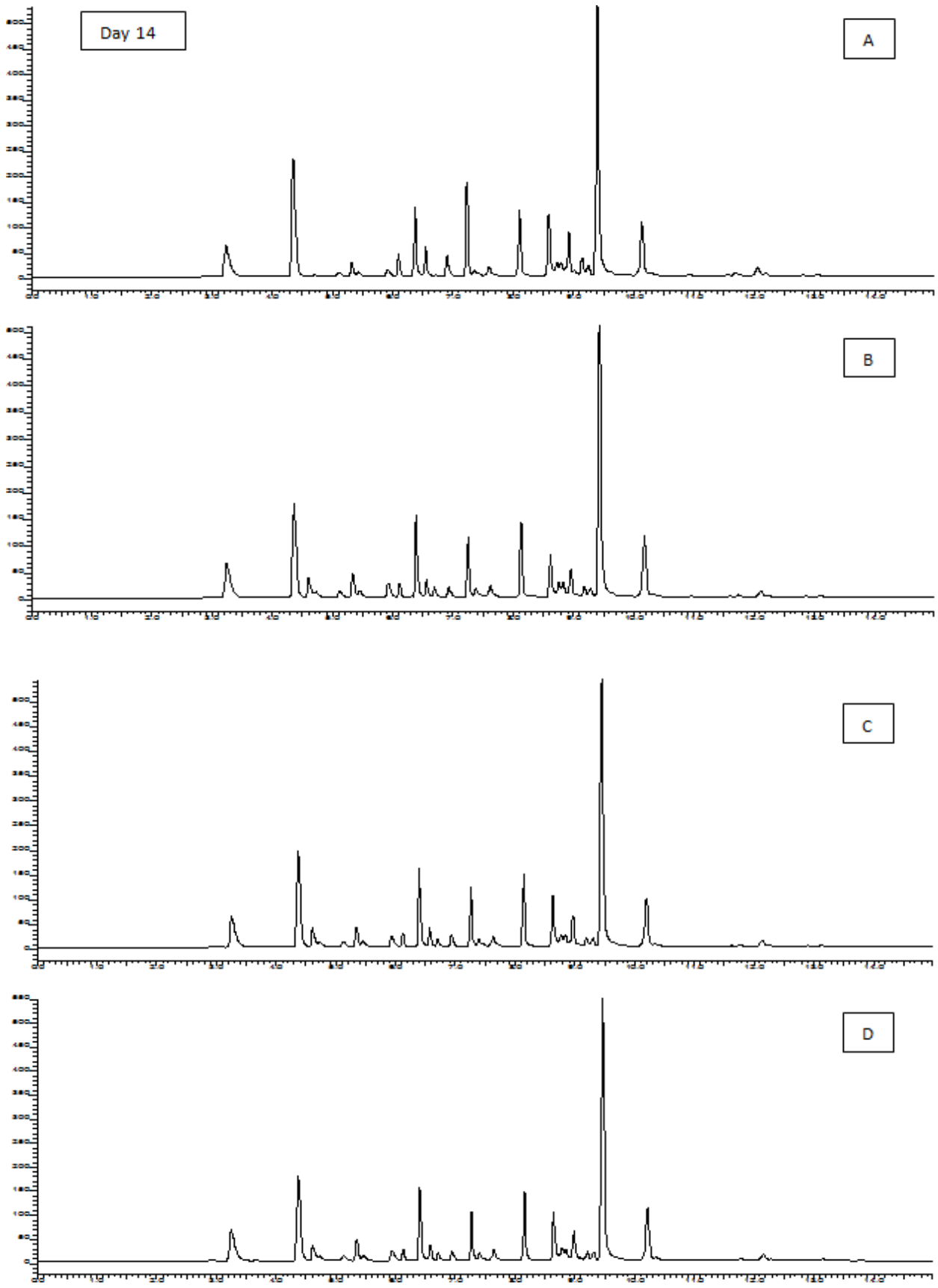

Day 14
A
B
C
D

Figure 40 HS-SPME-GC-FID of banana test of ethylene and propylene-induced *R. rhodochrous* DAP 96253 (GU plate). A. control; B. GU plate; C. GU plate with 100 ppm ethylene; D. GU plate with 100 ppm propylene.

From Day 4, banana VOCs from the control are more than those from the containers with GU cells, which is consistent with the banana photos (Figure 40).

Table 9 HS-GC-FID of ethylene and propylene degradation by *R. rhodochrous* DAP 96253 (GCoU plate) in M9 minimal medium (no glucose) for 48 hours.

	Retention time (min)	Peak area (μV·s)	Degradation Rate
Ethylene control	2.484	31079.47	N/A
GCoU in M9 with ethylene 1	2.501	16299.75	47.55%
GCoU in M9 with ethylene 2	2.498	18412.28	40.76%
Propylene control	2.532	46774.17	N/A
GCoU in M9 with propylene 1	2.533	35018.09	25.13%
GCoU in M9 with propylene 2	2.543	34112.26	27.07%

After 48 hours, GCoU plate cells of *R. rhodochrous* DAP 96253 in M9 minimal medium (no glucose) show much higher degradation rate of ethylene than that of propylene (Table 9).

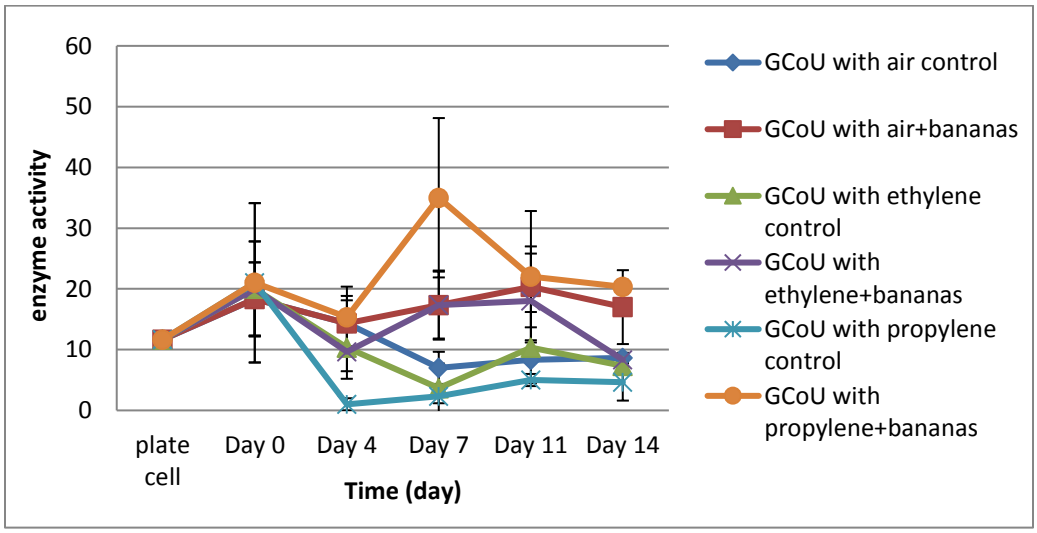


Figure 41 1-HMO activity of *R. rhodochrous* DAP 96253 (GCoU plate) with ethylene and propylene induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

In Figure 41, the cells exposed to propylene and bananas show the highest 1-HMO activity while the ones exposed to propylene with no banana exposure show the lowest 1-HMO activity. No matter with or with no banana exposure, the cells exposed to air maintain higher 1-HMO activity than those exposed to ethylene.

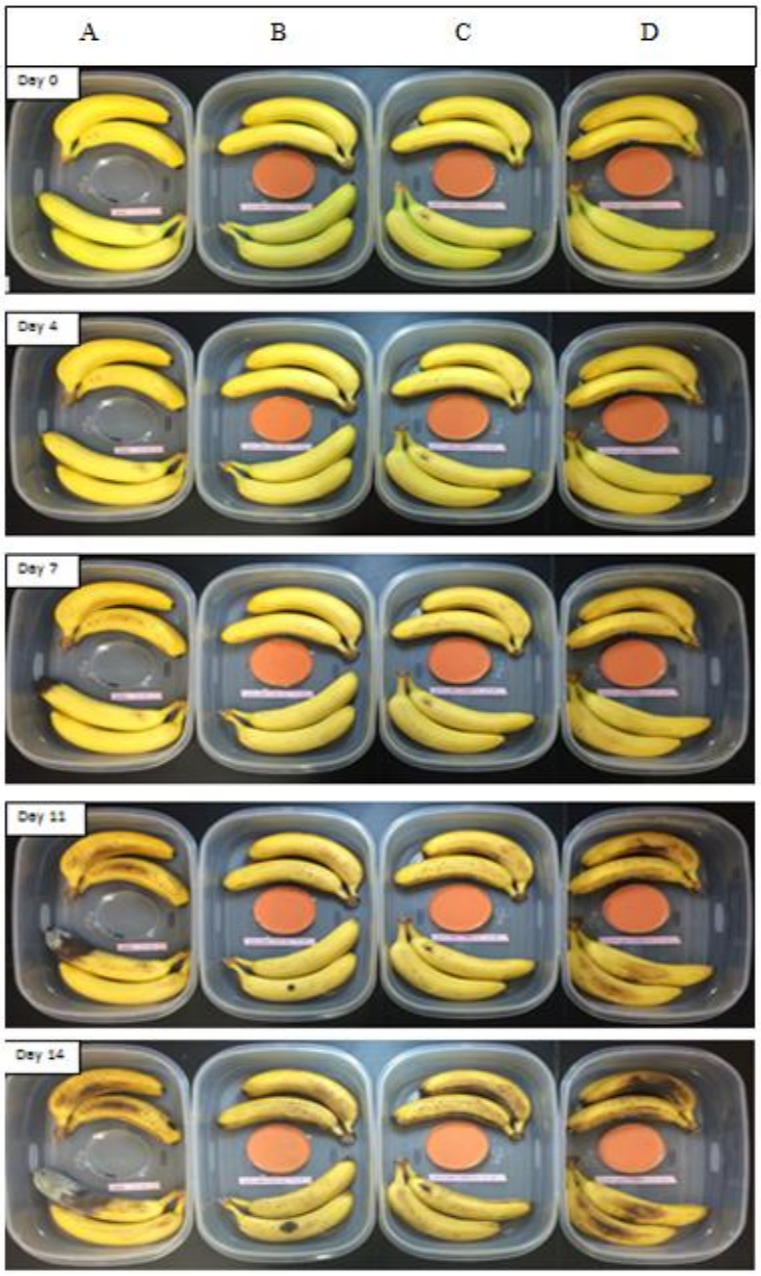
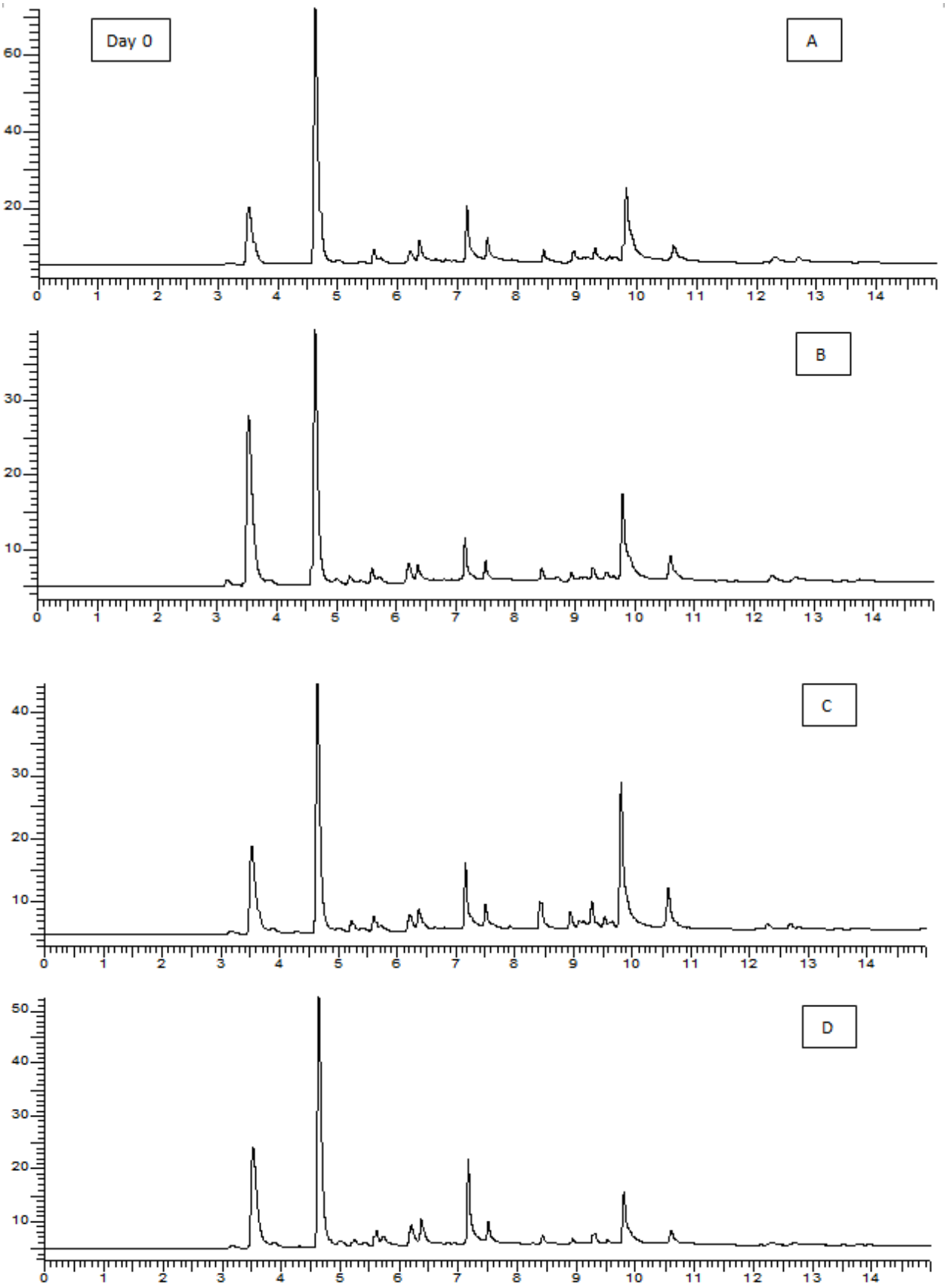
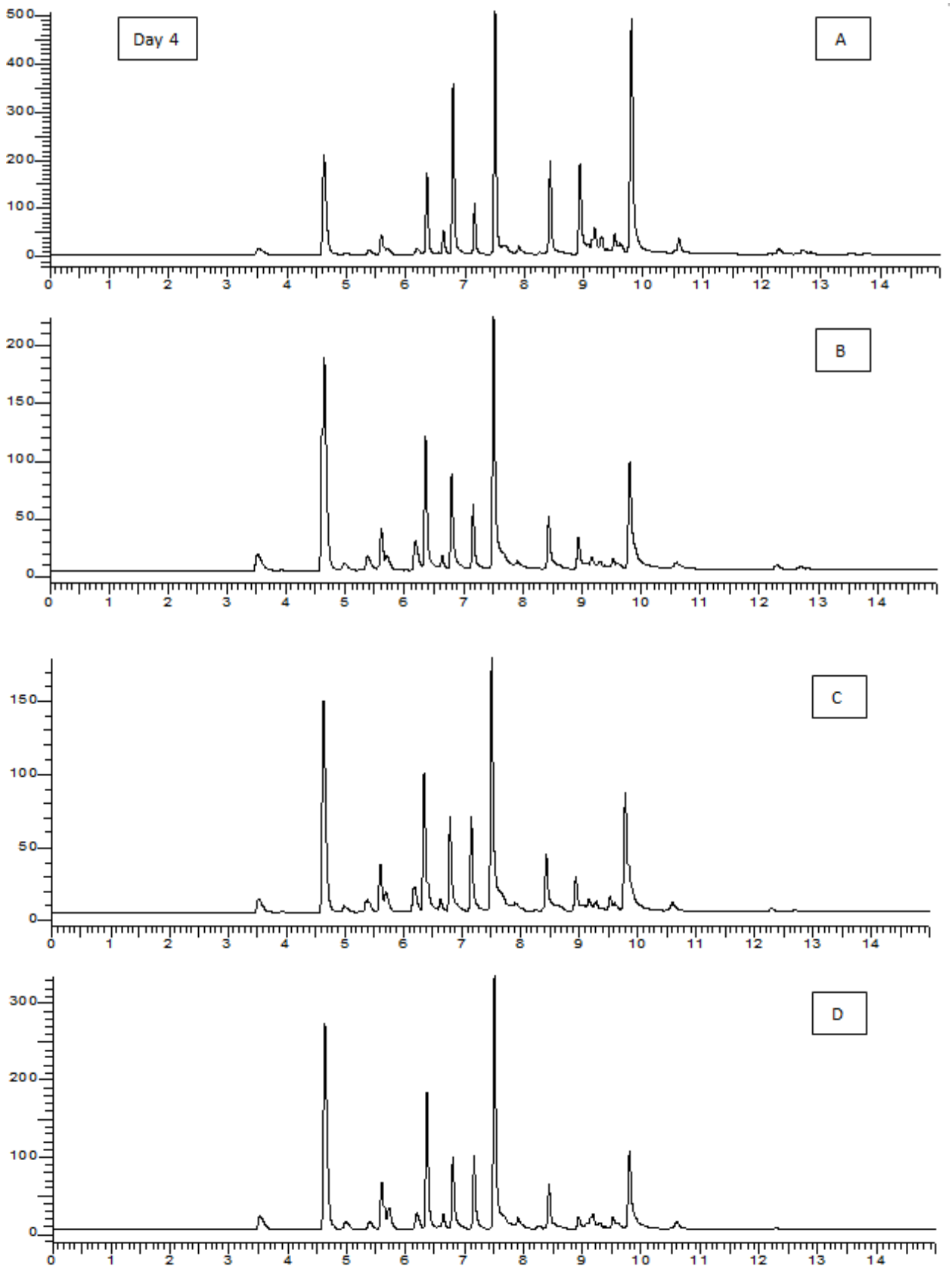
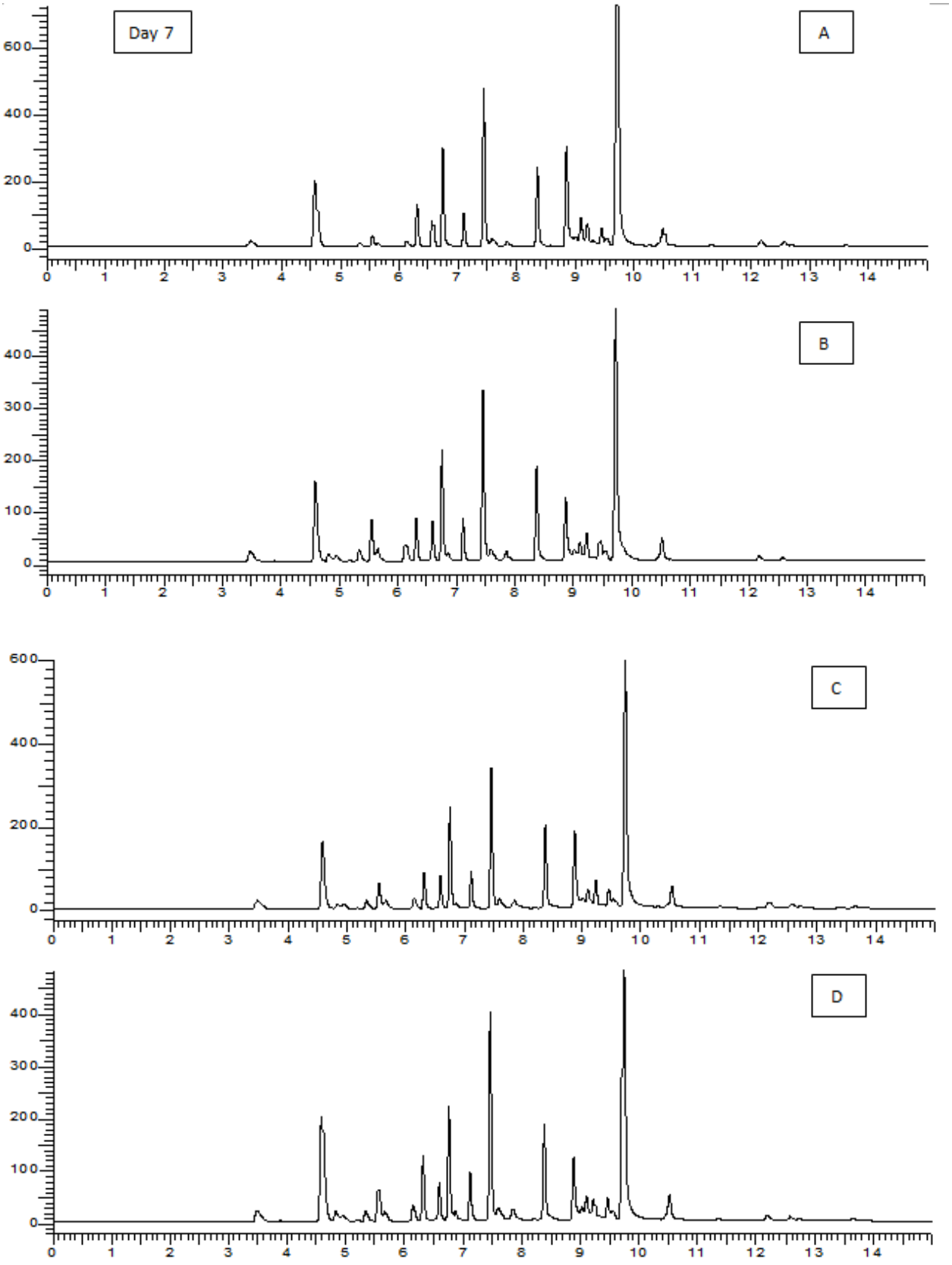


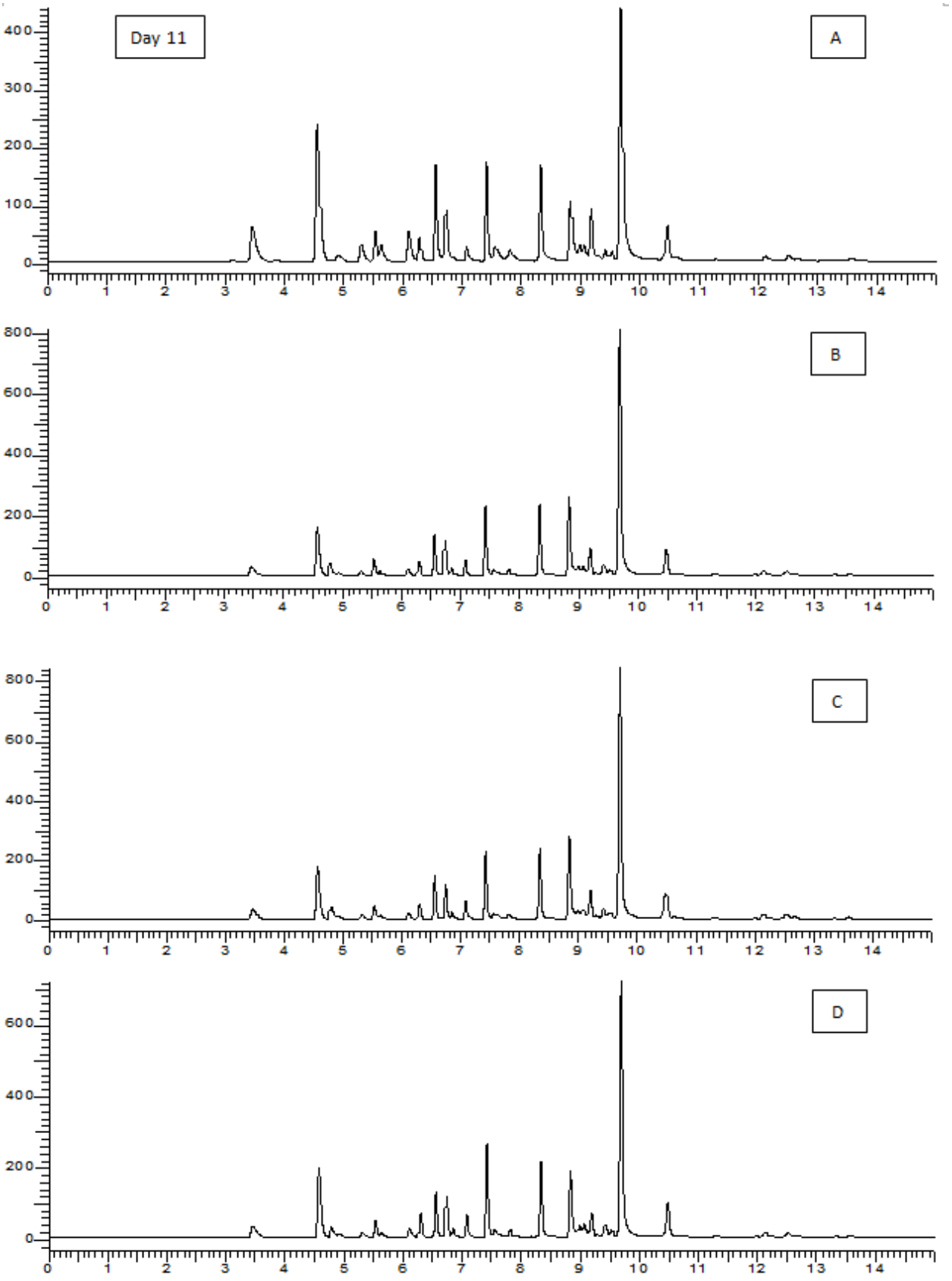
Figure 42 Banana test of ethylene and propylene-induced GCoU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GCoU plate; C. GCoU plate with 100 ppm ethylene; D. GCoU plate with 100 ppm propylene.

In Figure 42, the cells exposed to propylene did not delay banana ripening while the ones exposed to air or ethylene show obvious delayed banana ripening effects. However, due to the difference of ripening extent among the bananas from one bunch on Day 0, the results here are inconsistent with those from Figure 41.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D


Day 11
A
B
C
D

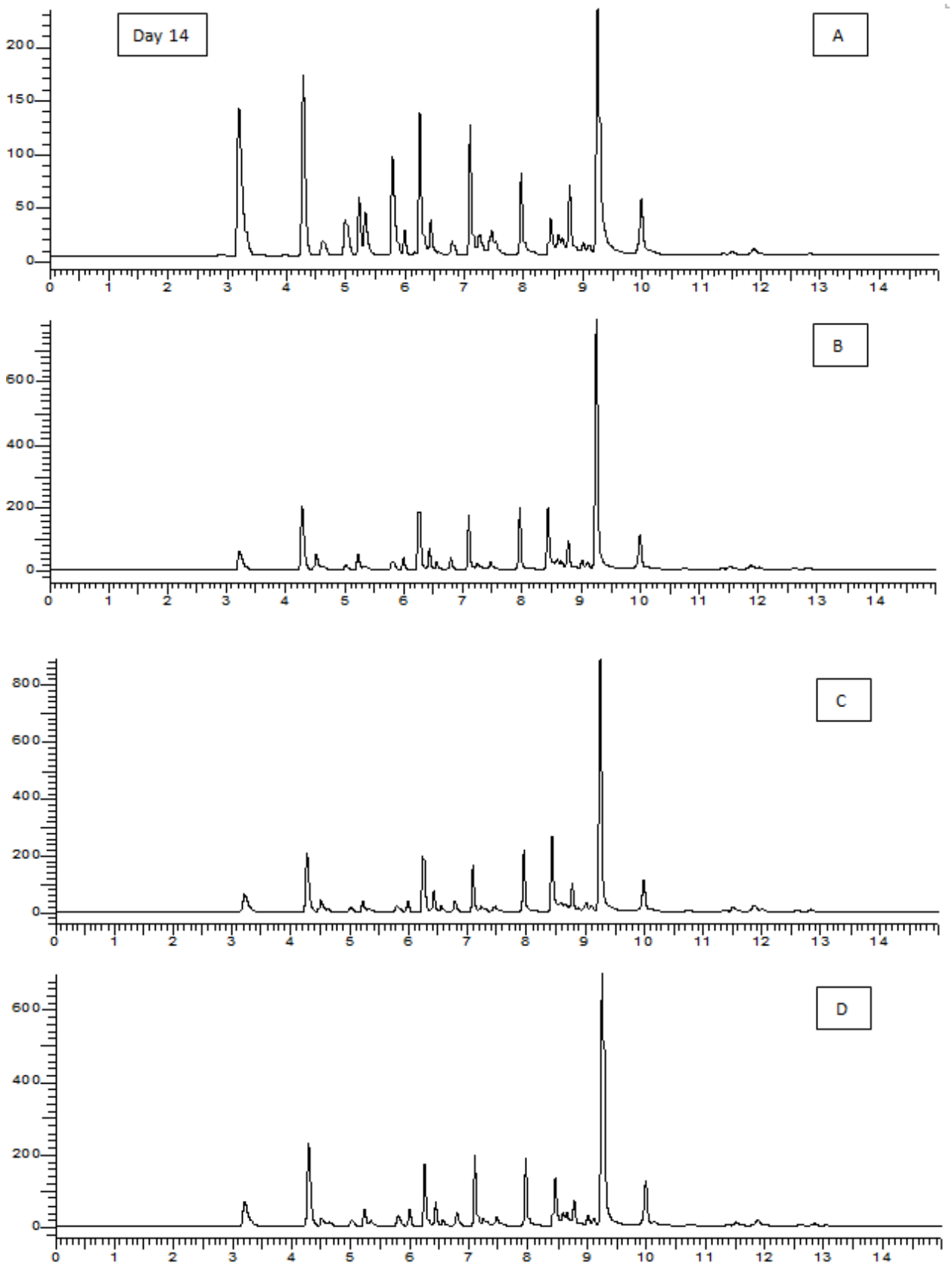

Day 14
A
B
C
D

Figure 43 HS-SPME-GC-FID of banana test of ethylene and propylene-induced *R. rhodochrous* DAP 96253 (GCoU plate). A. control; B. GCoU plate; C. GCoU plate with 100 ppm ethylene; D. GCoU plate with 100 ppm propylene.

From Day 11, the fungi growing on control inhibit the banana VOCs, so the results here are inconsistent with the banana photos (Figure 43).

d. Effects of ethyl acetate-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

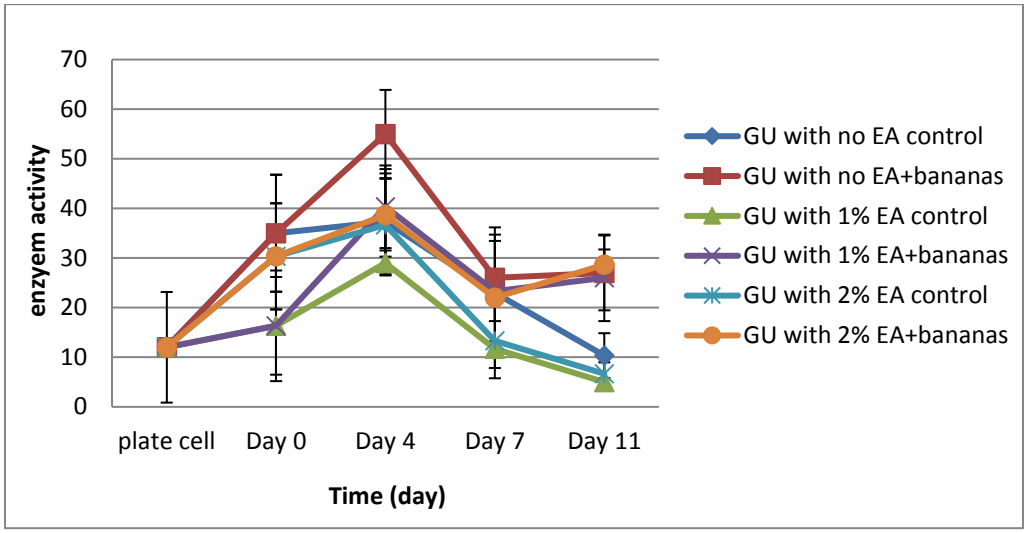
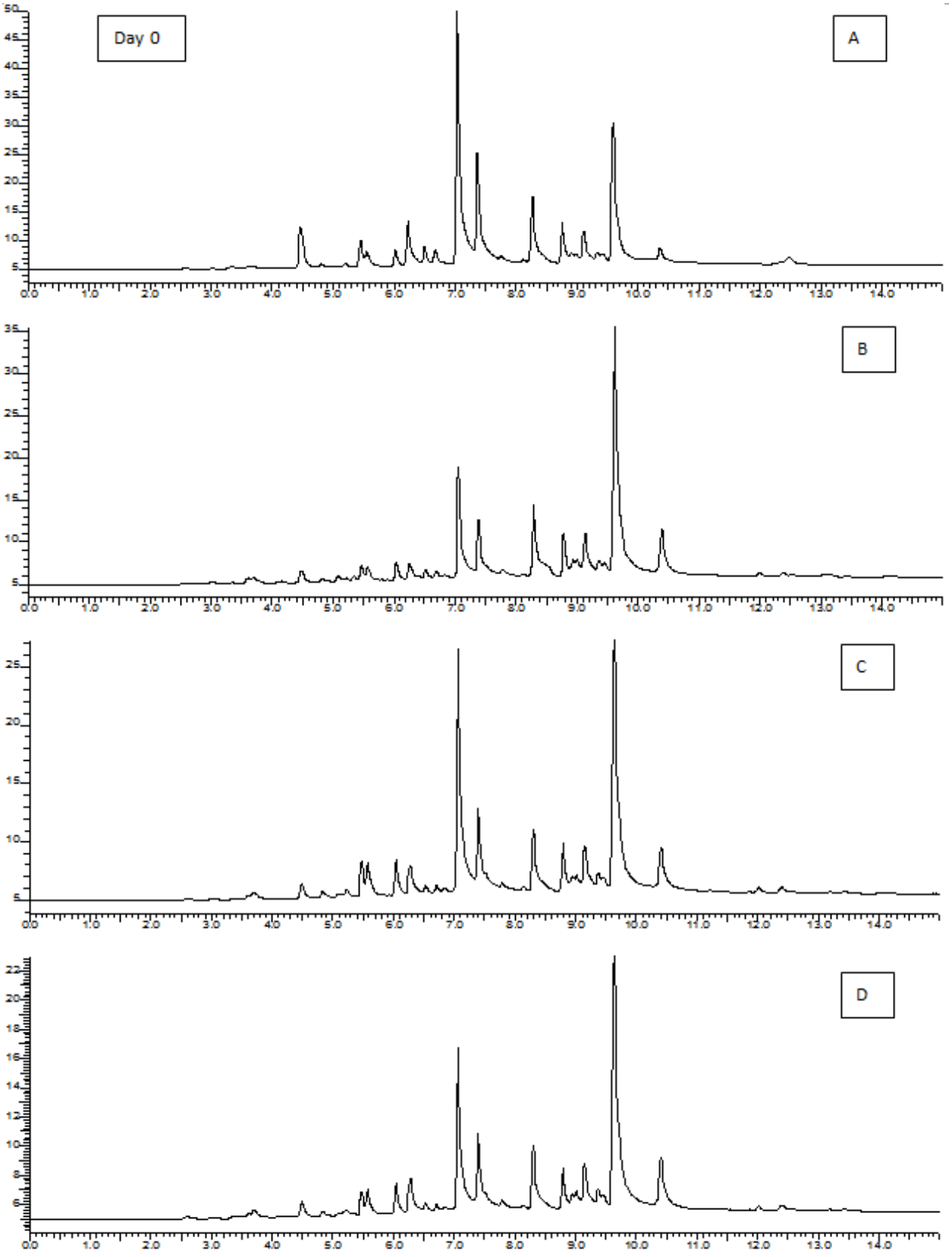


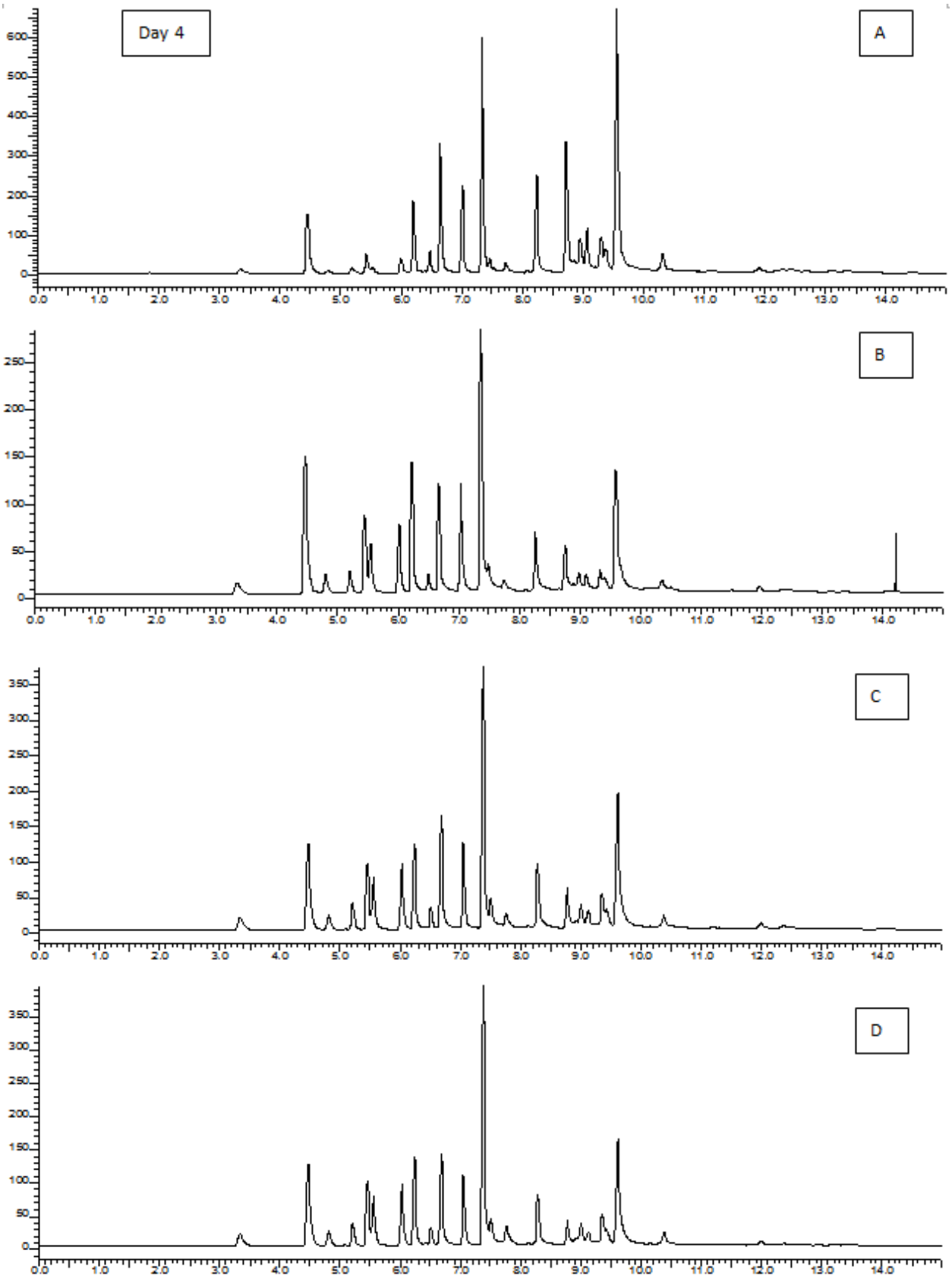
Figure 44 1-HMO activity of *R. rhodochrous* DAP 96253 (GU plate) with 1% and 2% ethyl acetate induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

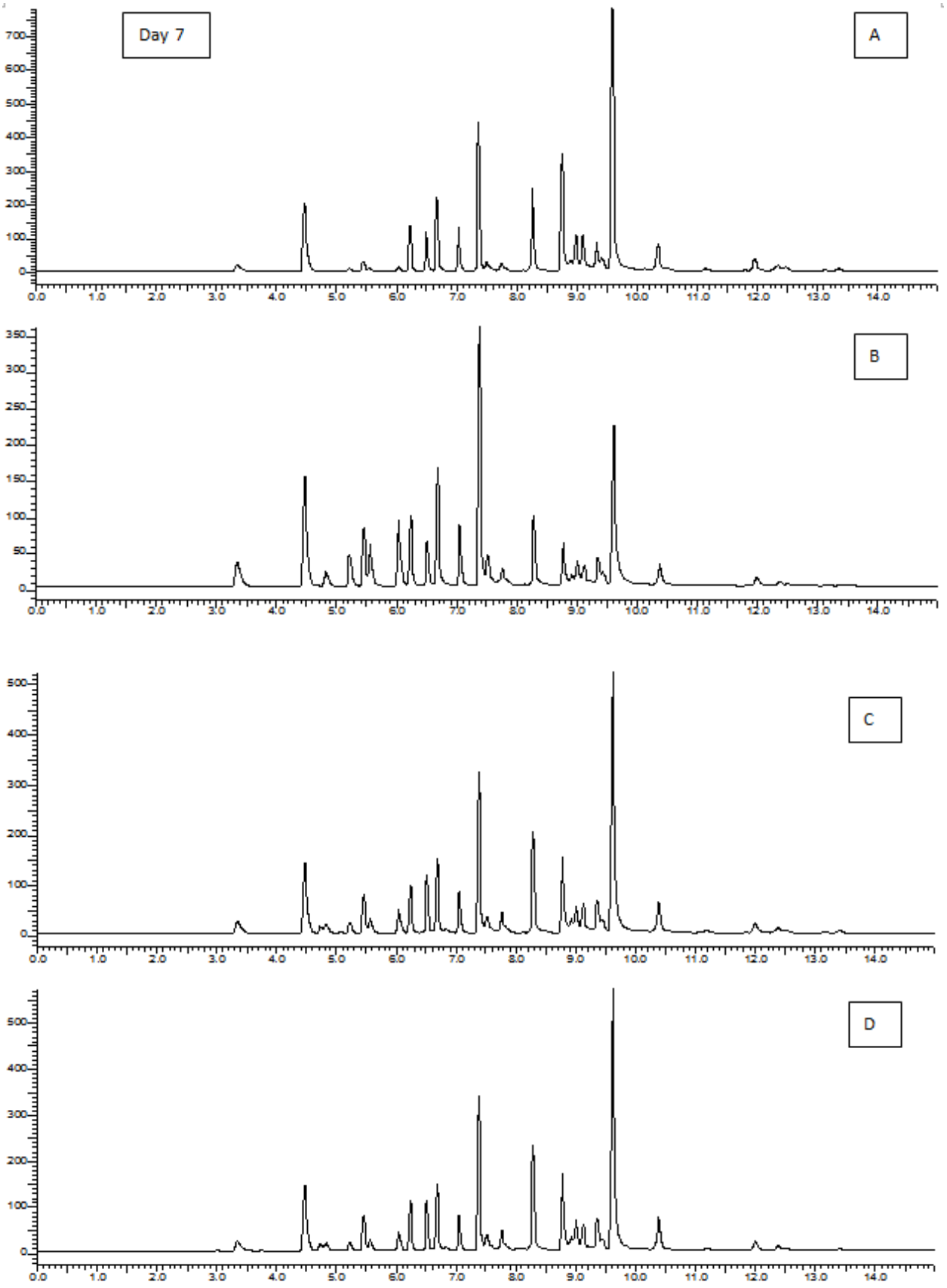
In Figure 44, 1-HMO activities of all the samples reach the highest value on Day 4. For the cells which are exposed to bananas, the ones with no ethyl acetate treatment contains the highest 1-HMO activity when they are exposed to bananas, while those with 1% ethyl acetate treatment show the lowest 1-HMO activity. For the cells with no banana exposure, the trend of 1-HMO activity is the same. Besides, bananas can induce 1-HMO activity obviously through the whole time-course fruit experiment.

Figure 45 Banana test of ethyl acetate-induced GU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GU plate; C. GU plate with 1% ethyl acetate; D. GU plate with 2% ethyl acetate.

In Figure 45, GU plate cells with no ethyl acetate treatment can delay banana ripening the best while the ones with 1% ethyl acetate treatment show the worst effect.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D

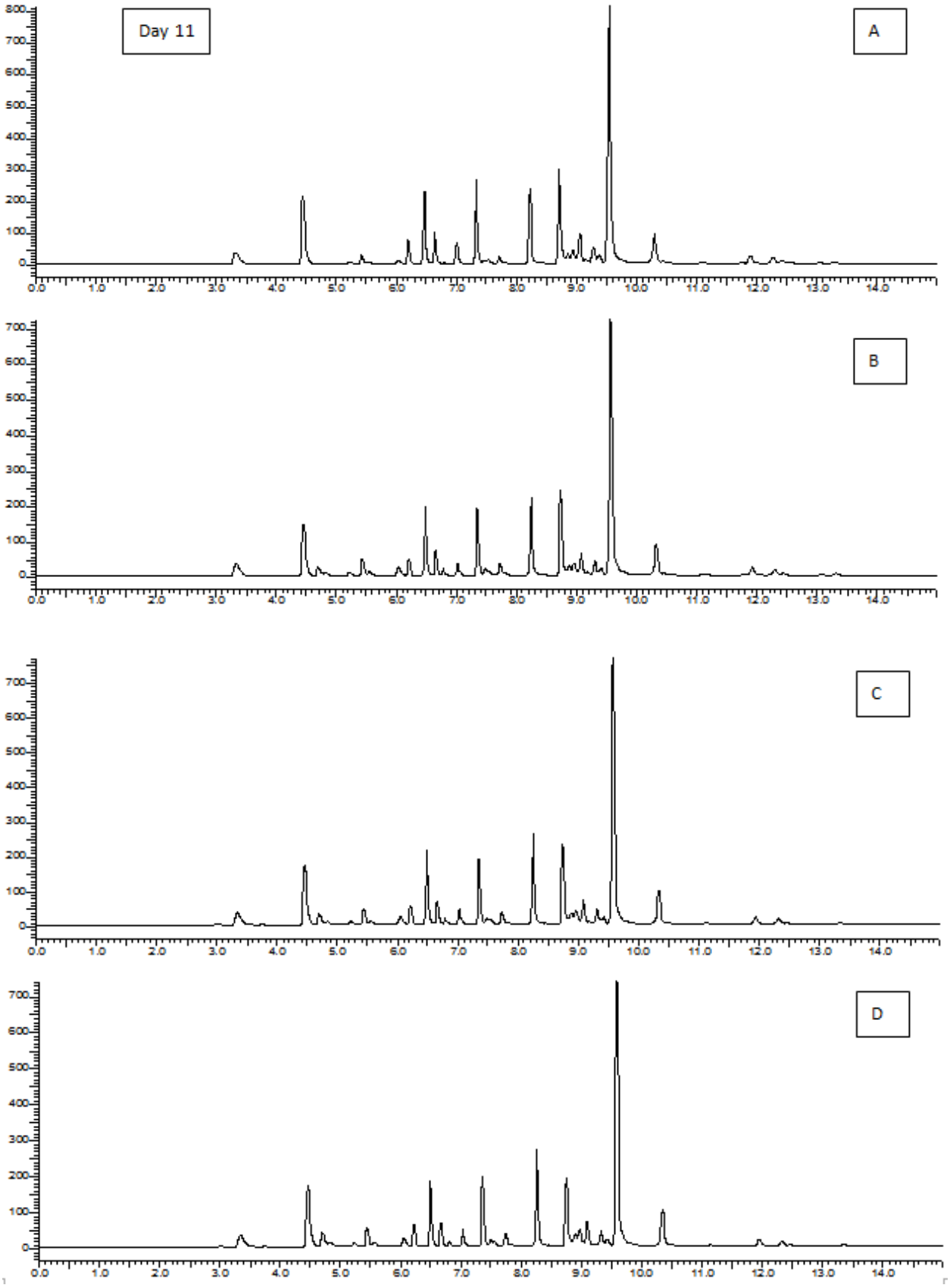

Day 11
A
B
C
D

Figure 46 HS-SPME-GC-FID of banana test of ethyl acetate-induced *R. rhodochrous* DAP 96253 (GU plate). A. control; B. GU plate; C. 1% ethyl acetate-induced GU plate; D. 2% ethyl acetate-induced GU plate.

From Day 7, banana VOCs from the containers with GU cells are much fewer than those from the control, which is consistent with the banana photos (Figure 46).

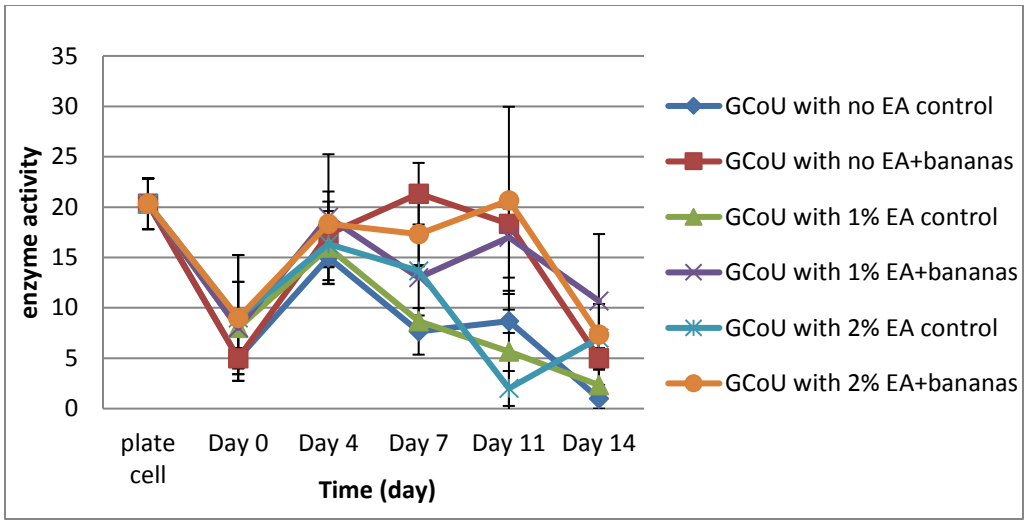
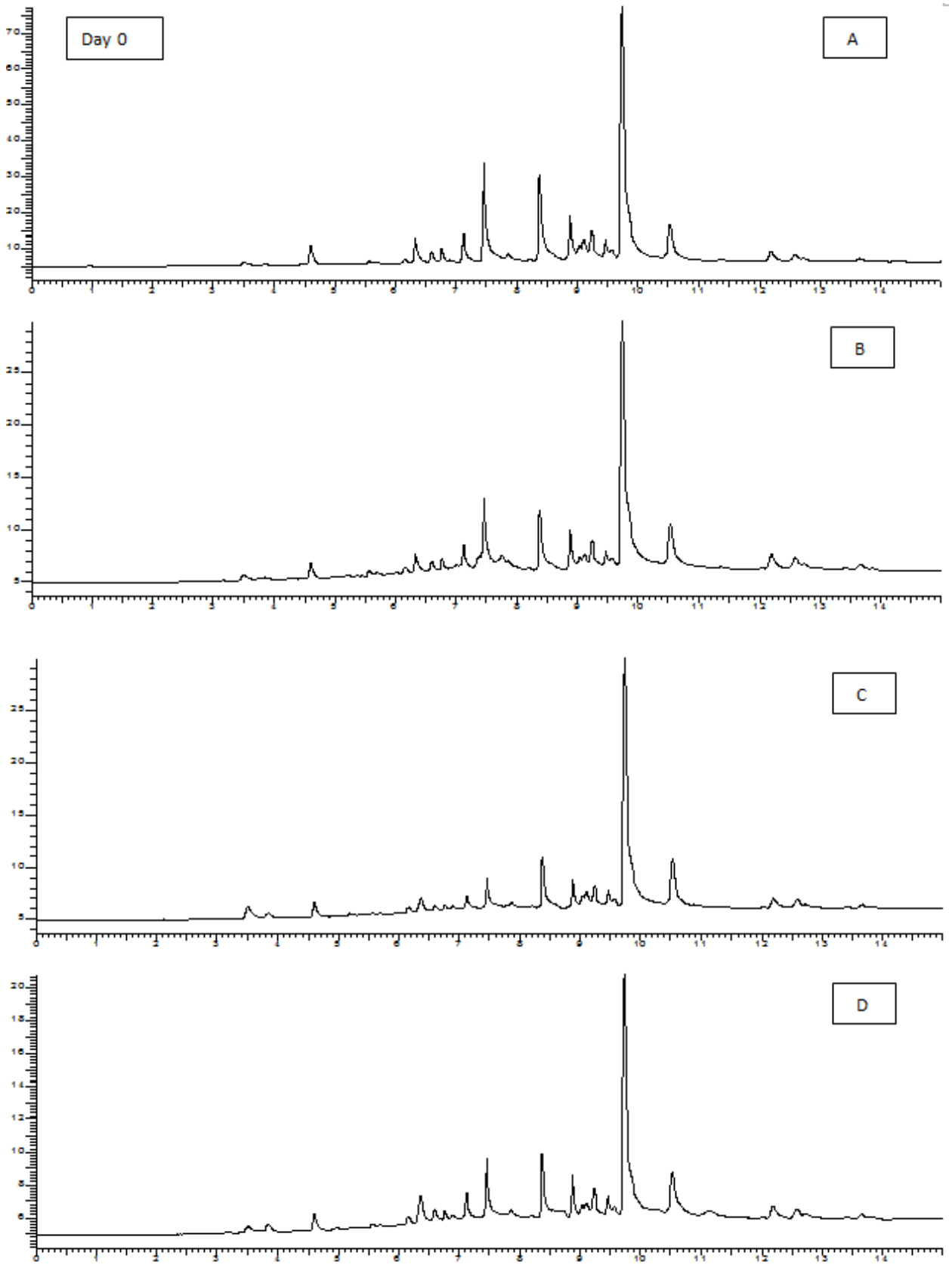


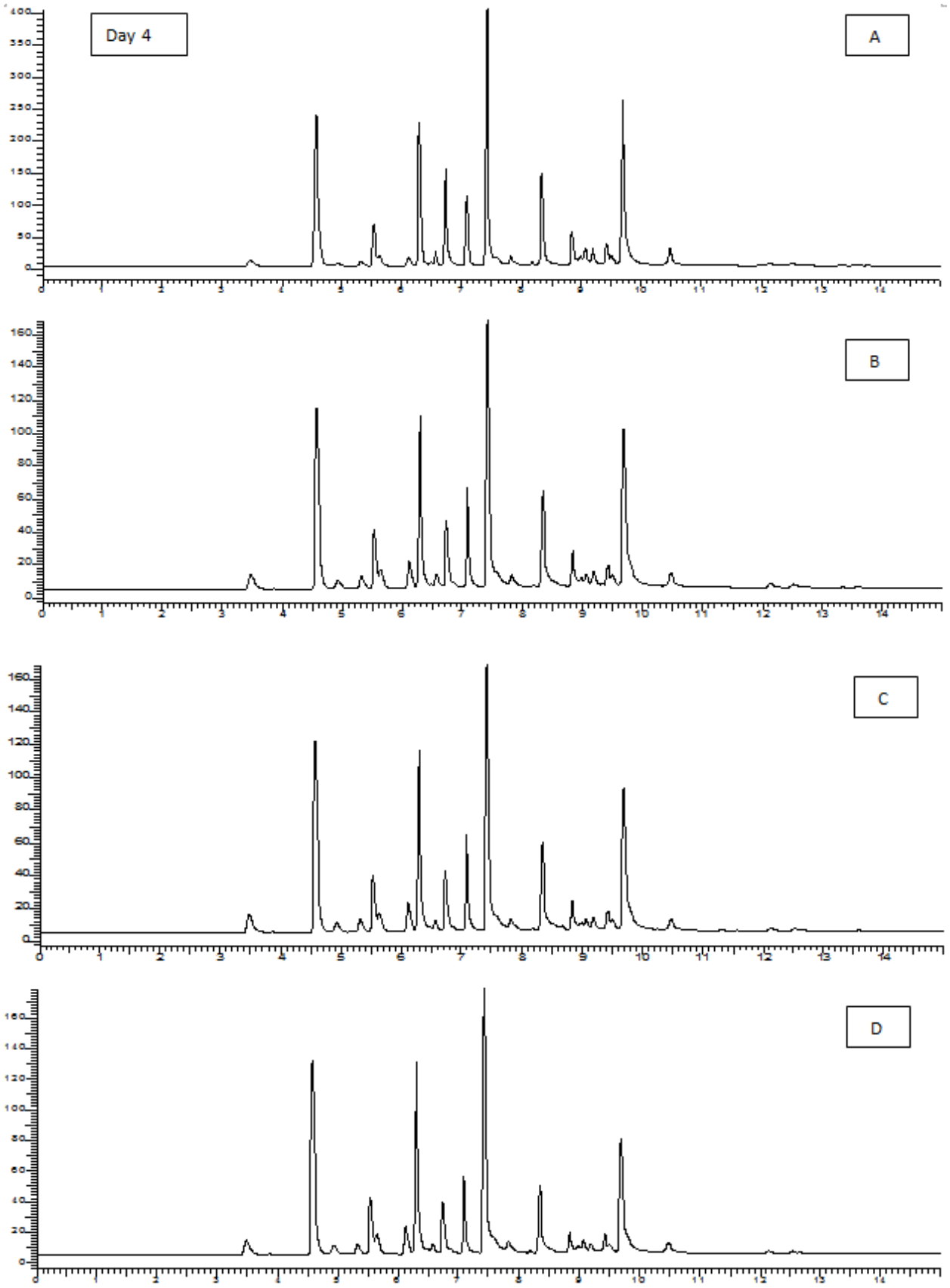
Figure 47 1-HMO activity of *R. rhodochrous* DAP 96253 (GCoU plate) with 1% and 2% ethyl acetate induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

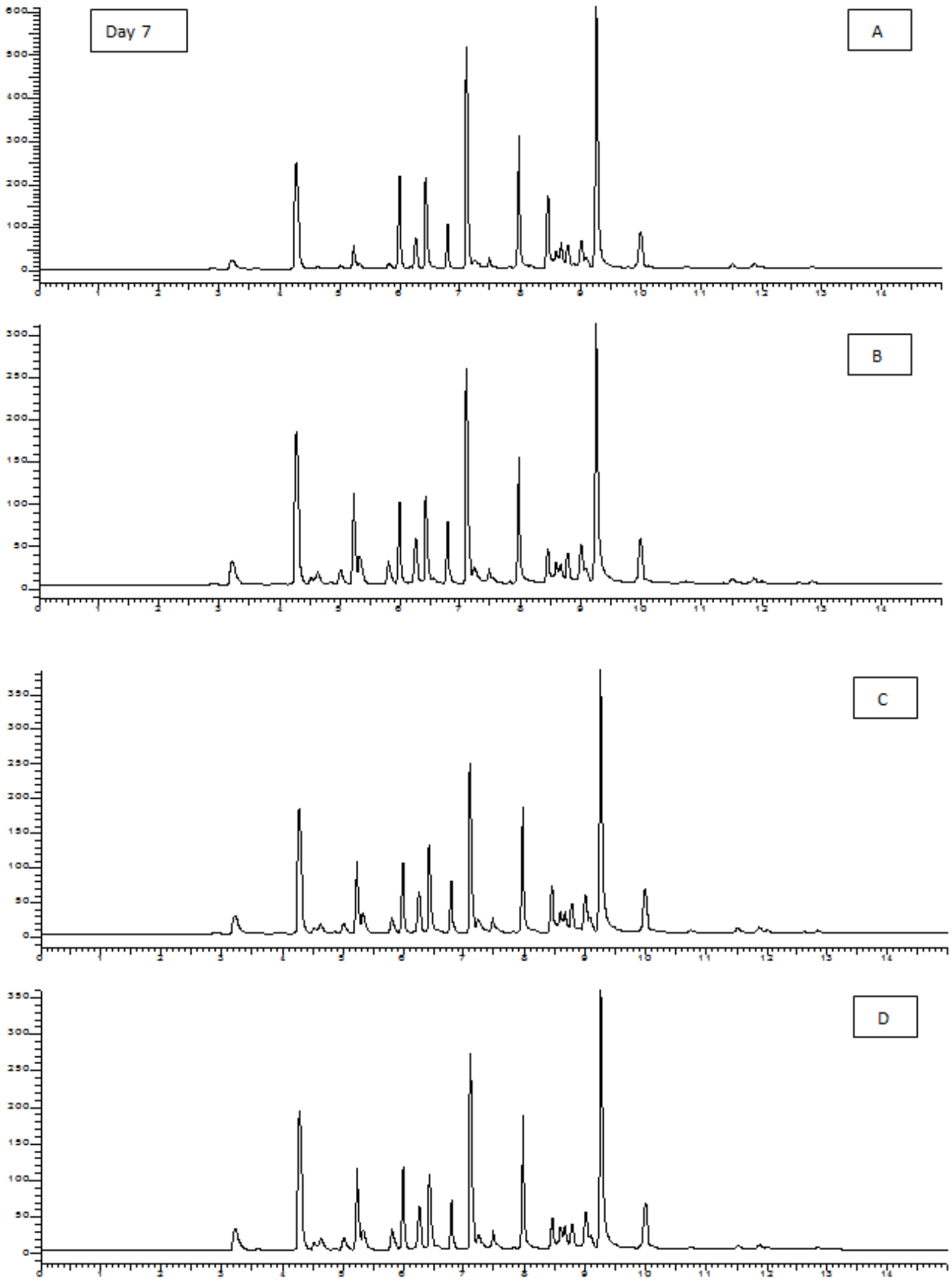
In Figure 47, bananas can enhance 1-HMO activity of GCoU plate cells. On Day 14, the ones exposed to 1% ethyl acetate maintain the highest 1-HMO activity while the ones with no ethyl acetate exposure exhibit the lowest 1-HMO activity, which is consistent with the photo.

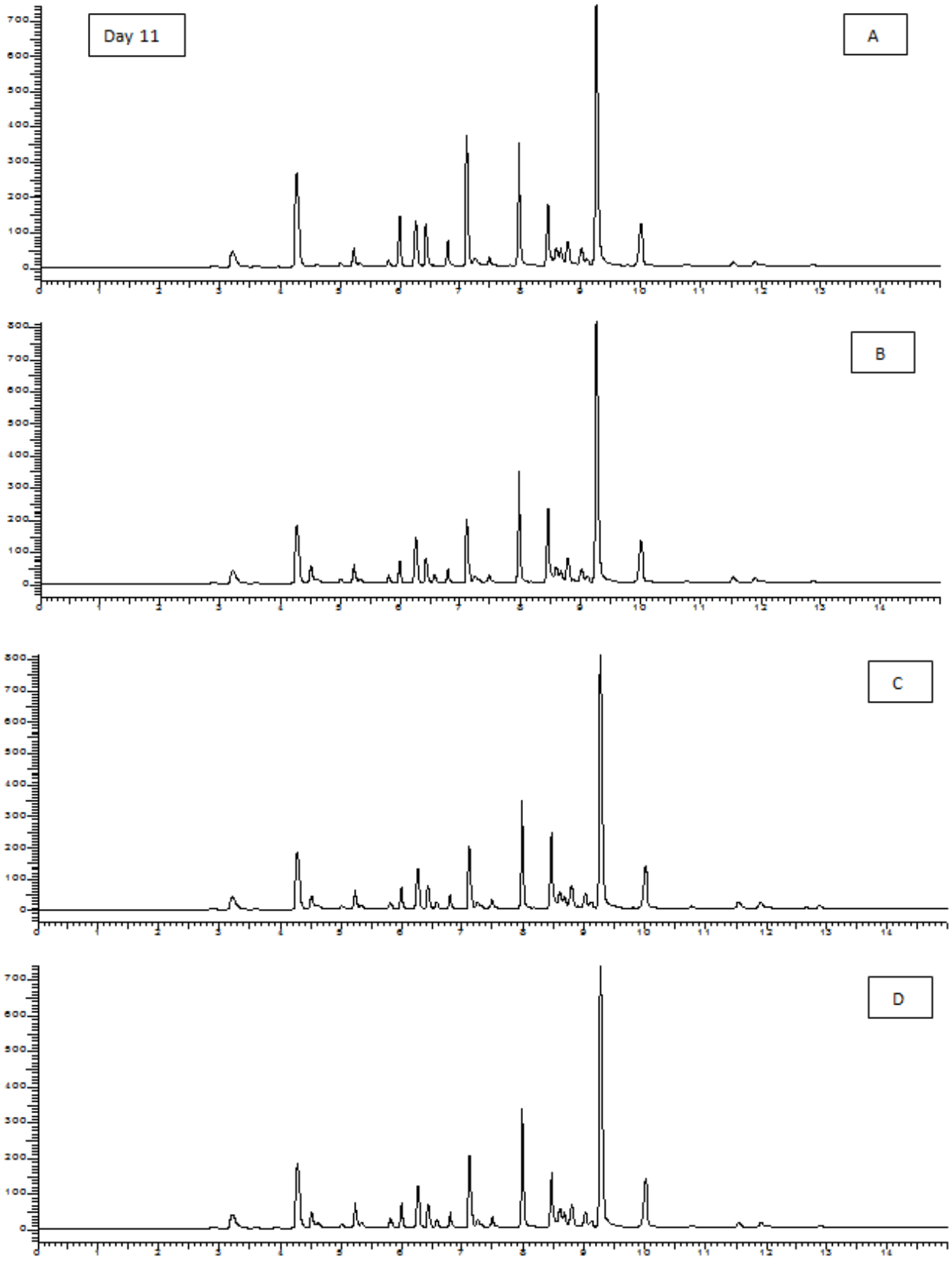
Figure 48 Banana test of ethyl acetate-induced GCoU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GCoU plate; C. GCoU plate with 1% ethyl acetate; D. GCoU plate with 2% ethyl acetate.

In Figure 48, GCoU cells exposed to 1% ethyl acetate can delay the upper bunch of bananas better than those exposed to 2% ethyl acetate/no ethyl acetate.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D


Day 11
A
B
C
D

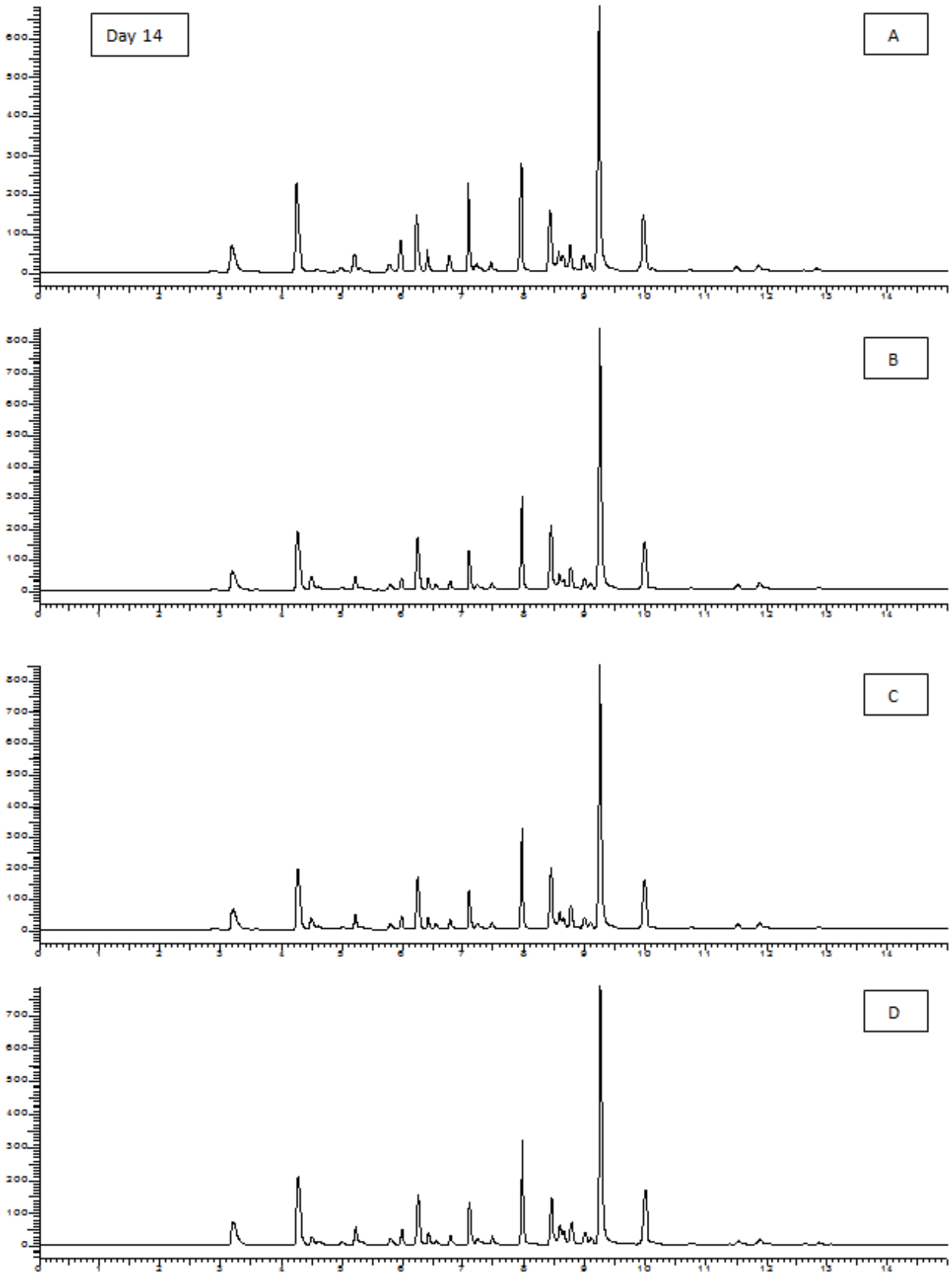

Day 14
A
B
C
D

Figure 49 HS-SPME-GC-FID of banana test of ethyl acetate-induced *R. rhodochrous* DAP 96253 (GCoU plate). A. control; B. GCoU plate; C. 1% ethyl acetate-induced GCoU plate; D. 2% ethyl acetate-induced GCoU plate.

From Day 11, the amounts of banana VOCs from all the four containers are similar, which is consistent with the banana photos (Figure 49).

e. The coordinative effects of organic red delicious apples on delayed fruit ripening by GU and GCoU plate cells of *R. rhodochrous* DAP 96253

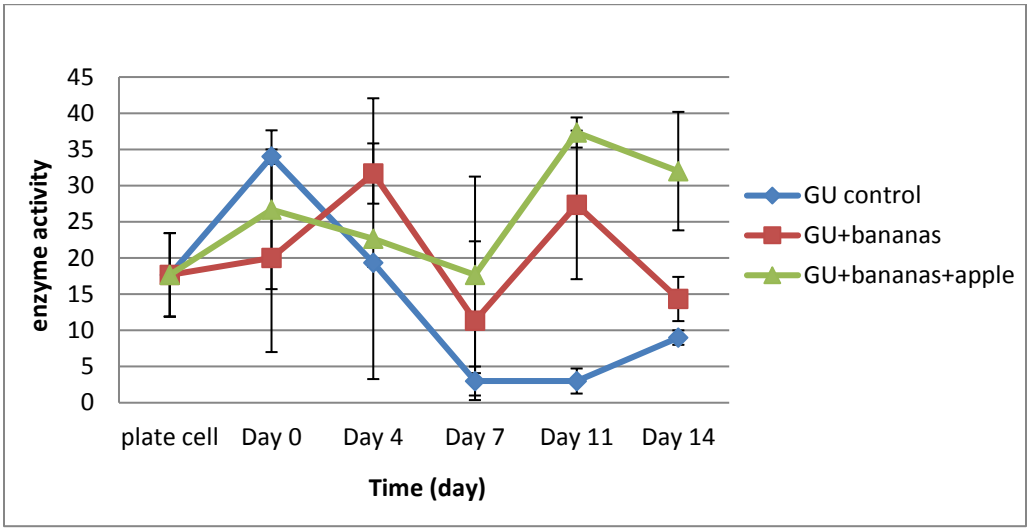
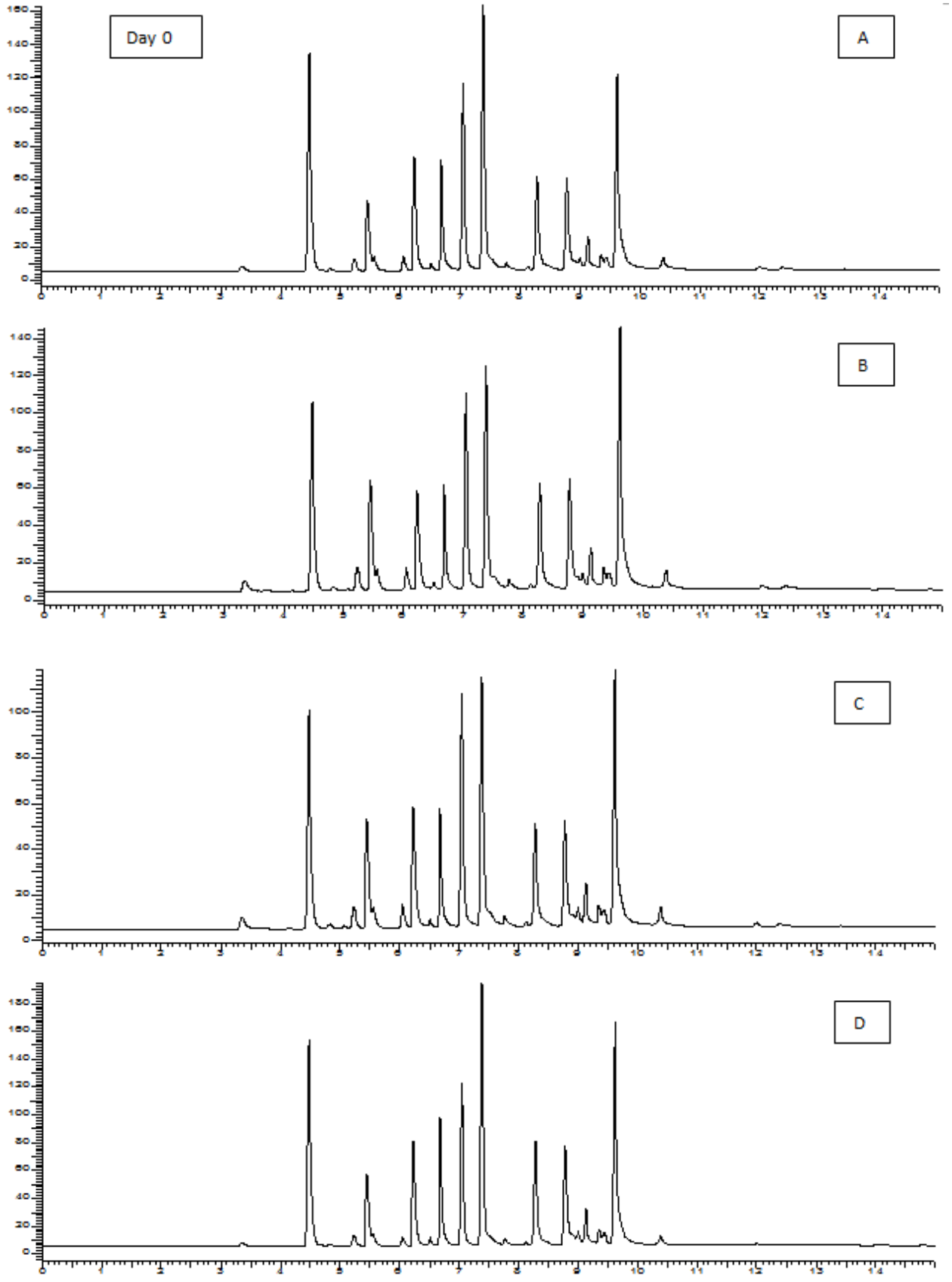


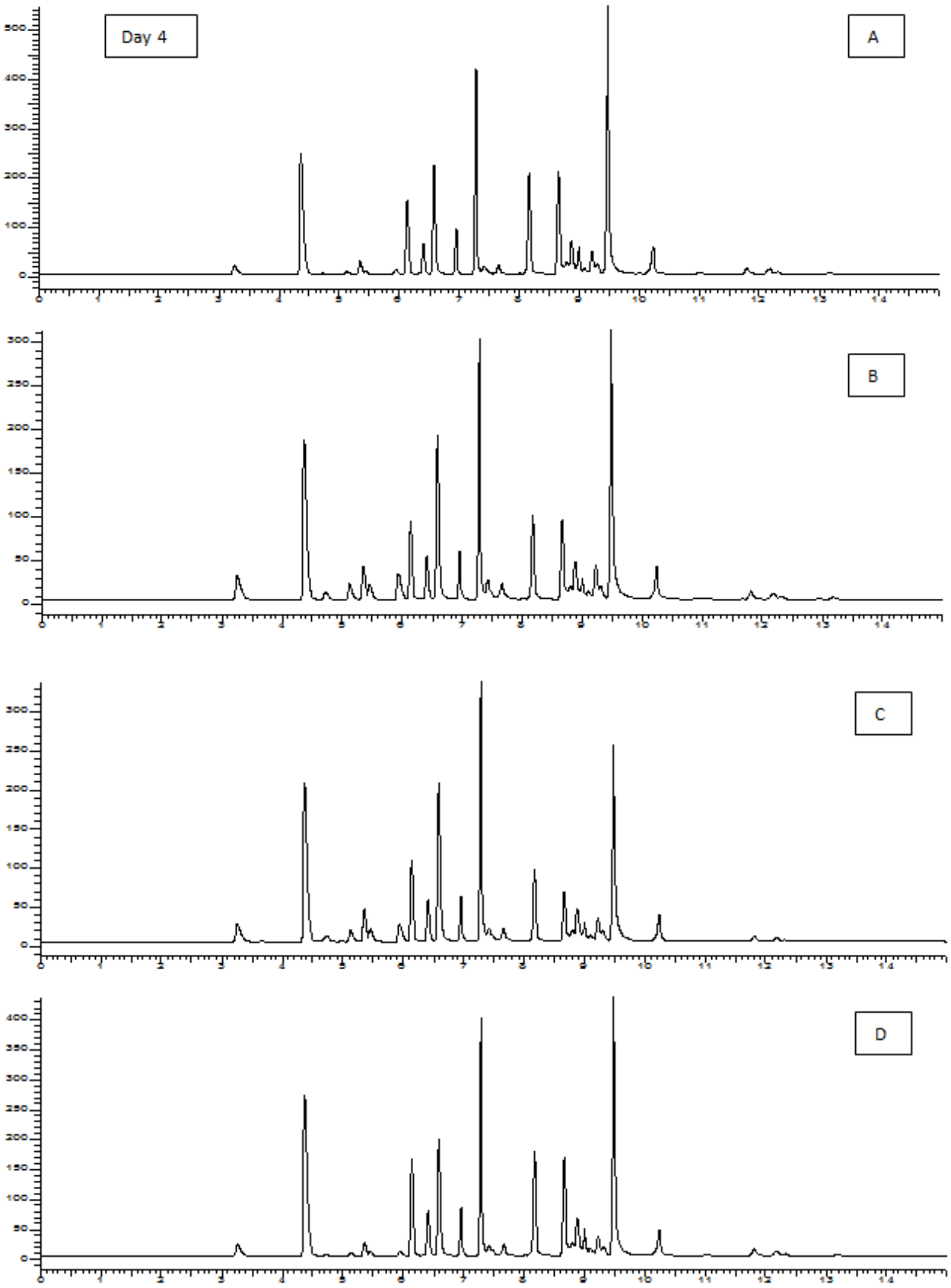
Figure 50 1-HMO activity of *R. rhodochrous* DAP 96253 (GU plate) with organic apple coordination. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

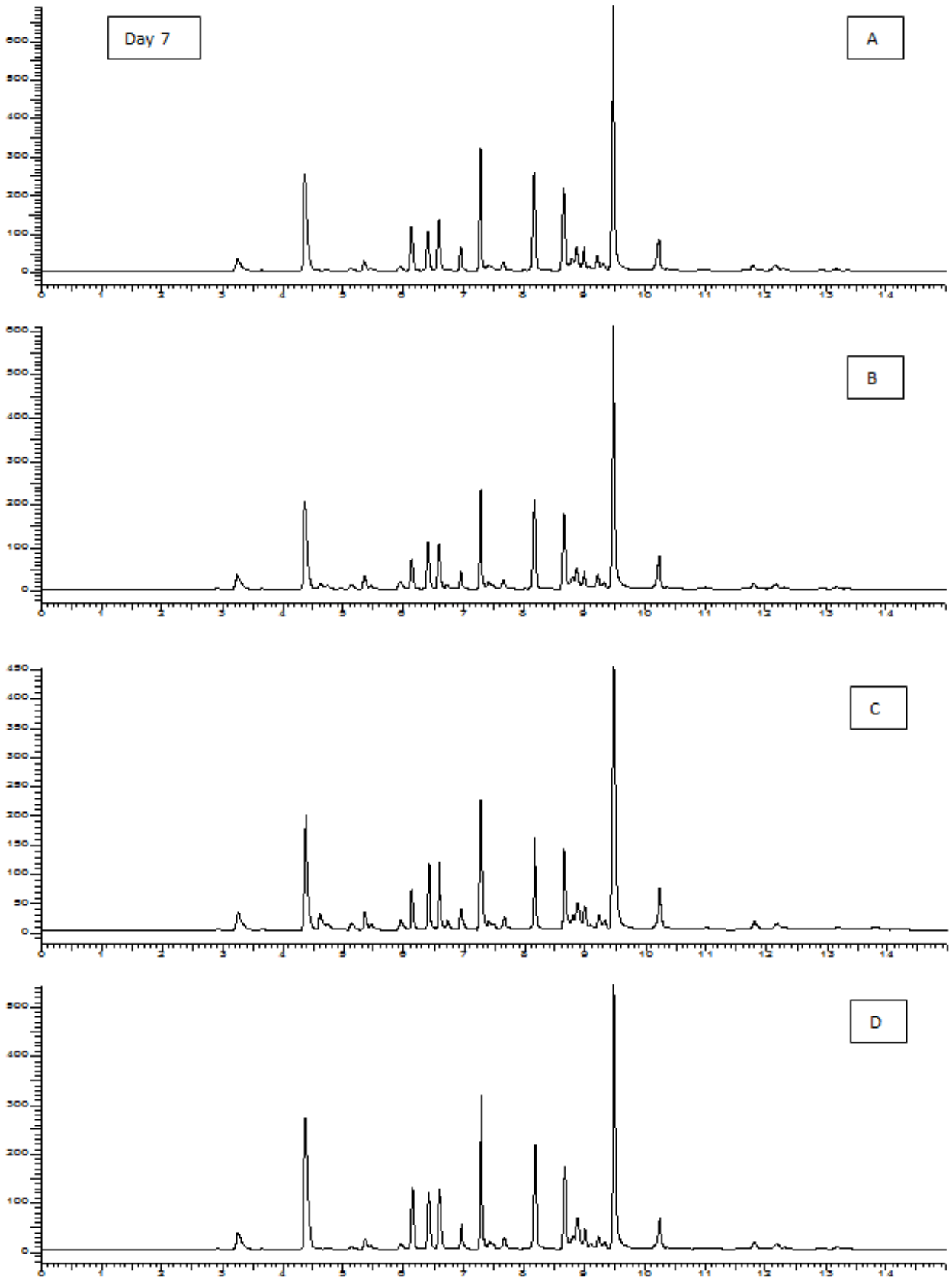
In Figure 50, the cells exposed to both bananas and an organic apple maintain the highest 1-HMO activity and the ones exposed to no fruit show the lowest 1-HMO activity from Day 7.

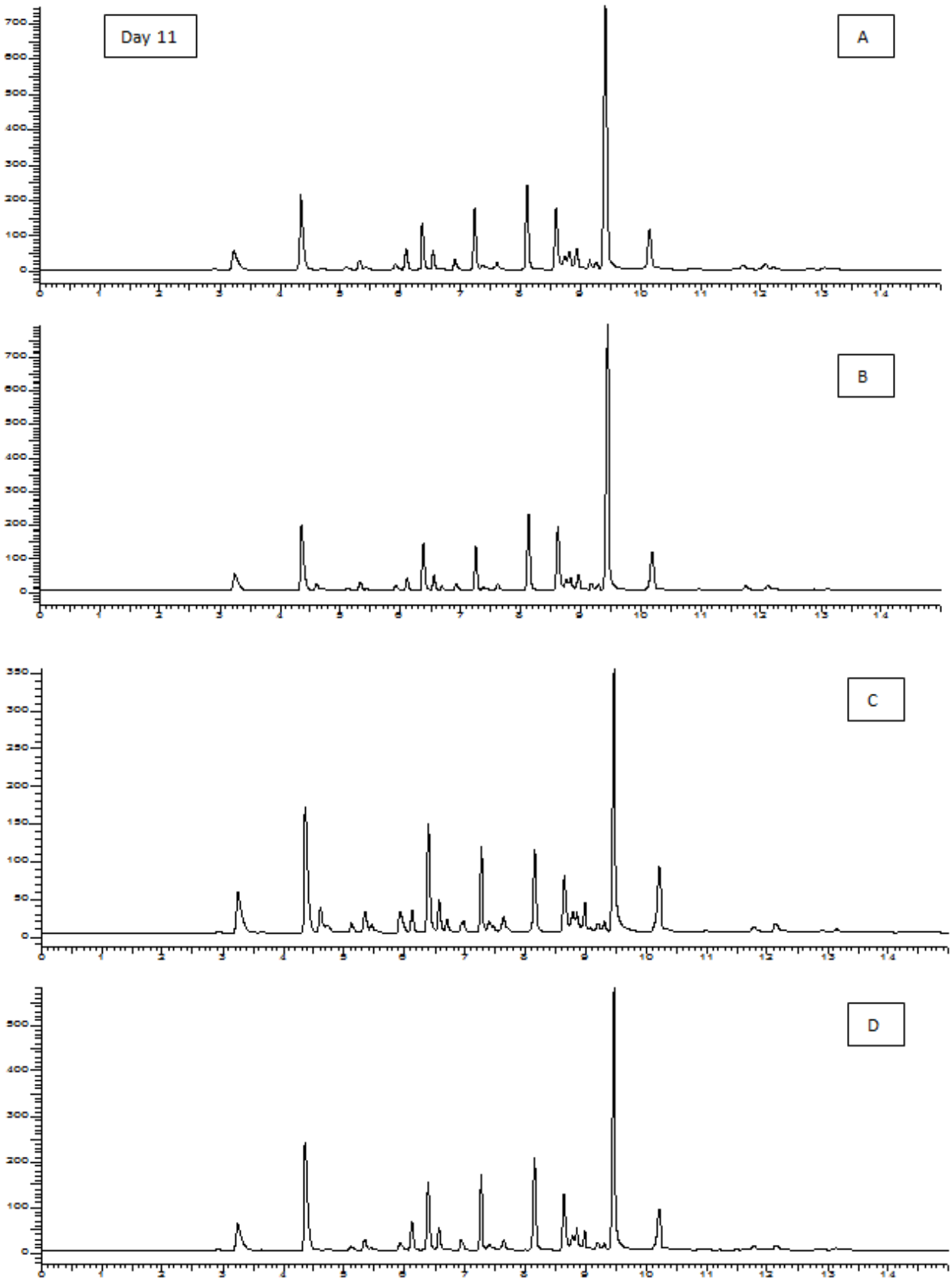
Figure 51 Banana test of organic apple-coordinated GU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GU plate; C. GU plate with an organic apple; D. an organic apple.

In Figure 51, the cells exposed to an organic apple perform similar effects on delaying banana ripening compared with the ones with no organic apple exposure. Compared with control, neither of these cells can delay banana ripening effectively. However, the cells show some effective delayed banana ripening results on Day 11.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D


Day 11
A
B
C
D

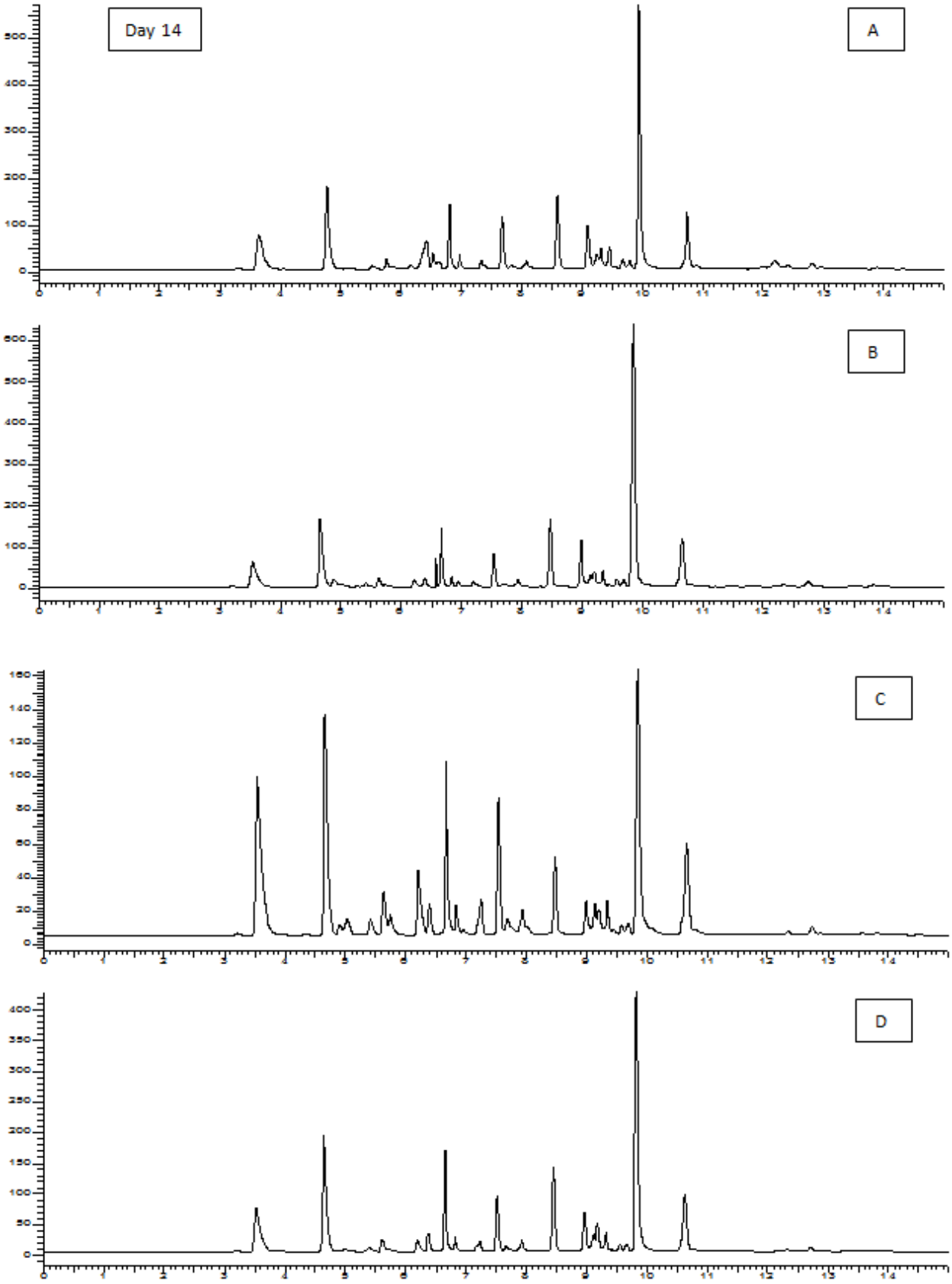

Day 14
A
B
C
D

Figure 52 HS-SPME-GC-FID of banana test of organic apple-coordinated *R. rhodochrous* DAP 96253 (GU plate). A. control; B. GU plate; C. organic apple+GU plate; D. organic apple.

In Figure 52, from Day 7, the banana VOCs from the container with GU cell and an organic apple and the one with only an organic apple are fewer than those from the control. However, the banana photos show that the bananas with GU cell and an organic apple are more ripened than control. So this should be from the effects of fungi growing on the bananas. The chilling injury can also inhibit the formation of banana VOCs (the container with only an organic apple).

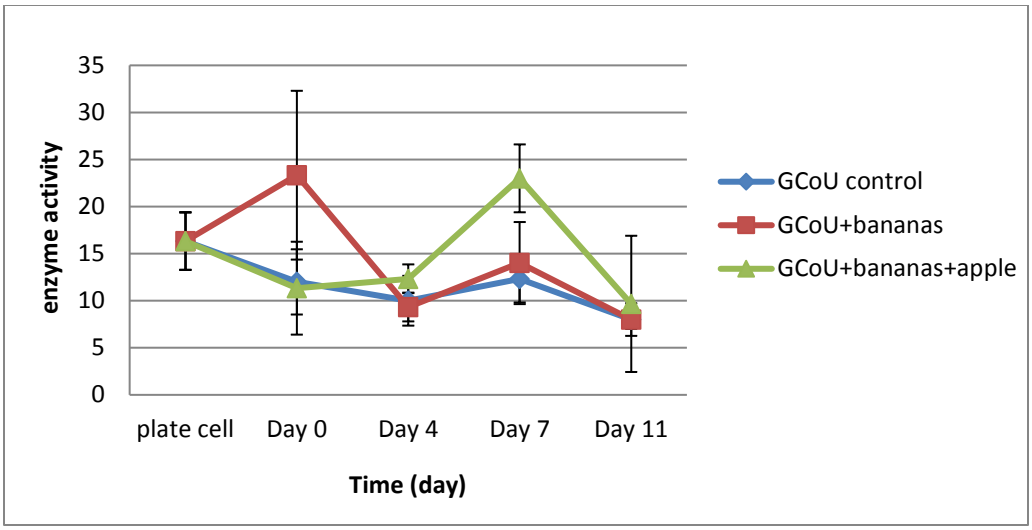
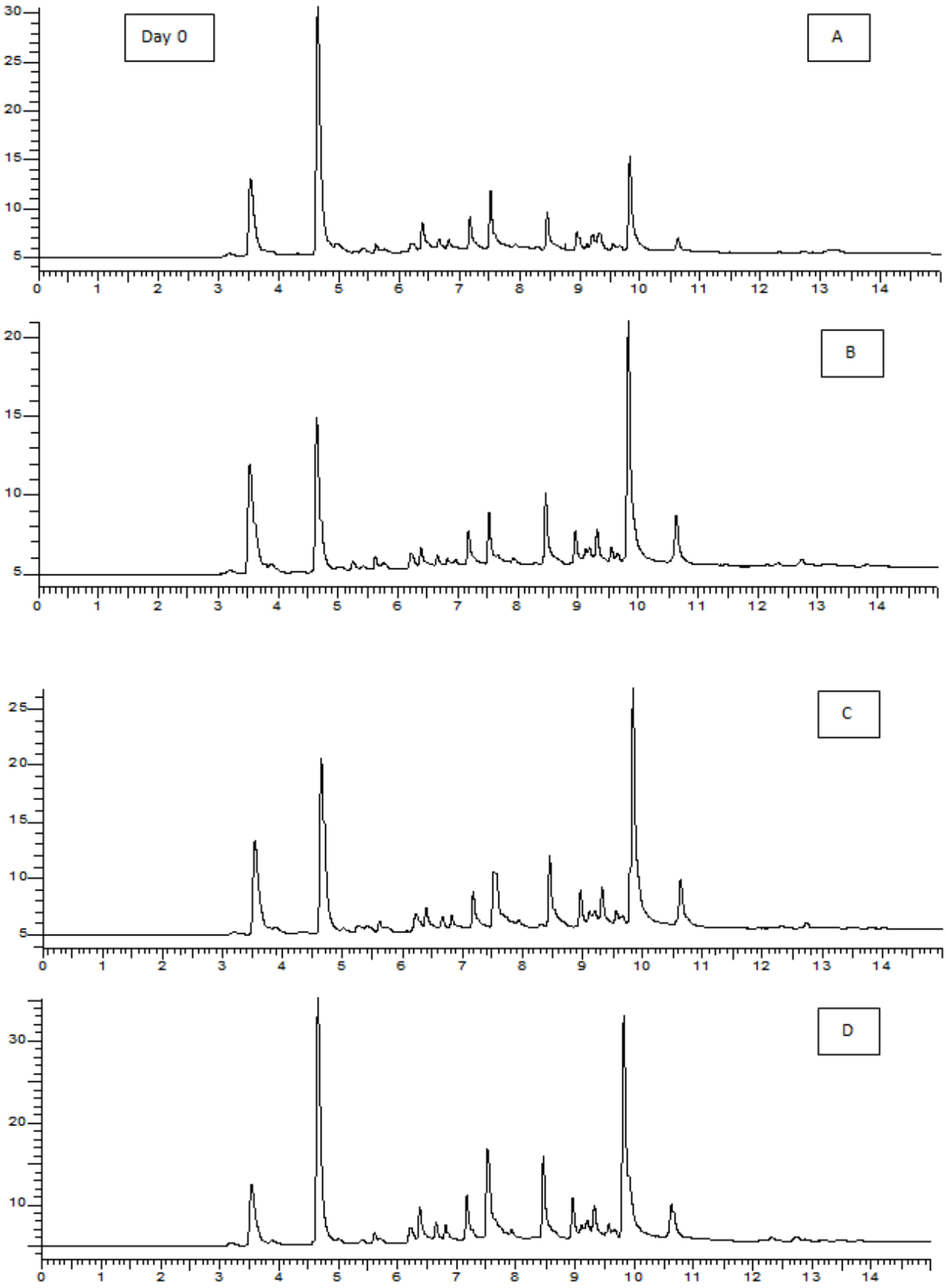


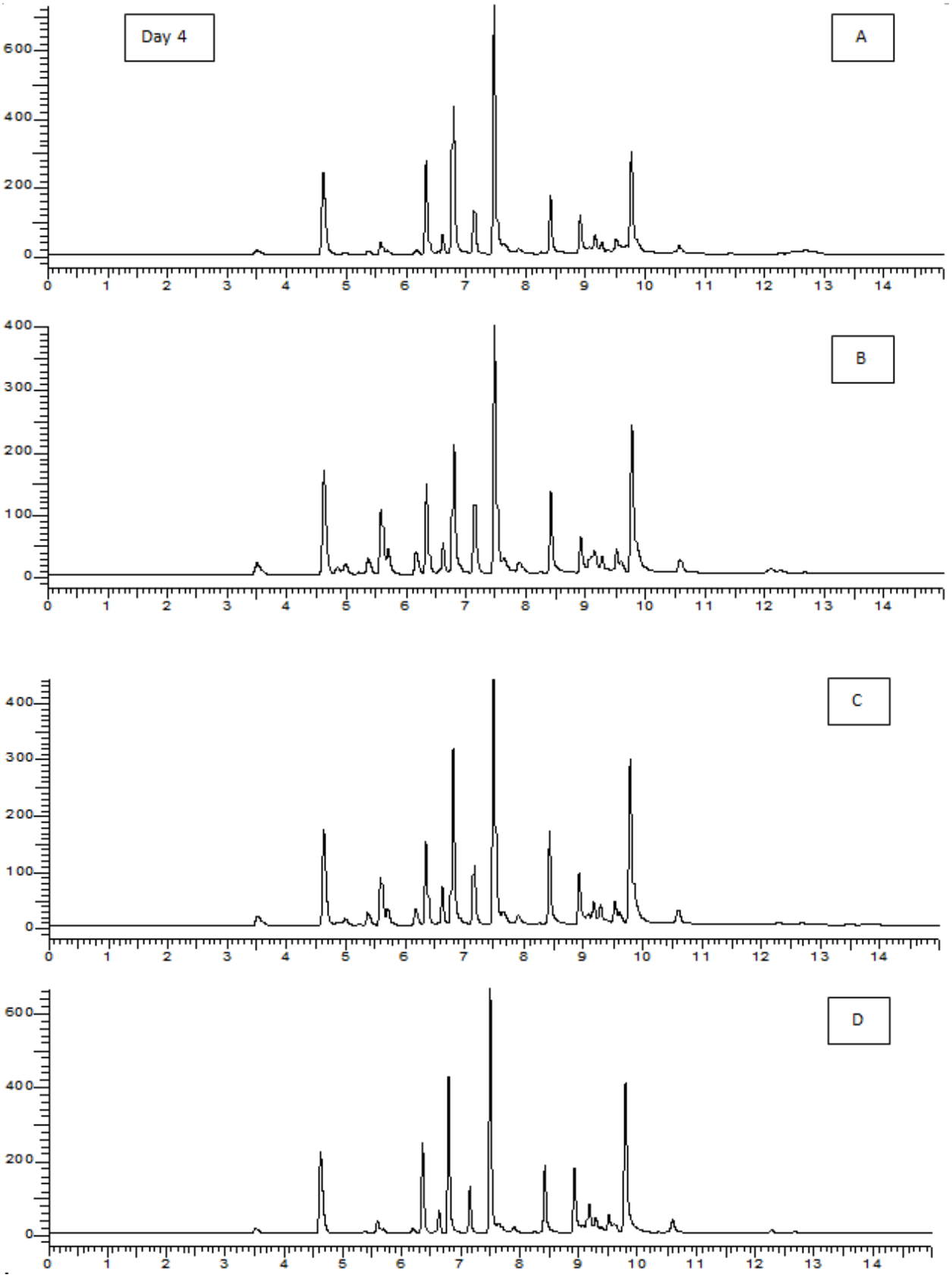
Figure 53 1-HMO activity of *R. rhodochrous* DAP 96253 (GCoU plate) with organic apple induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

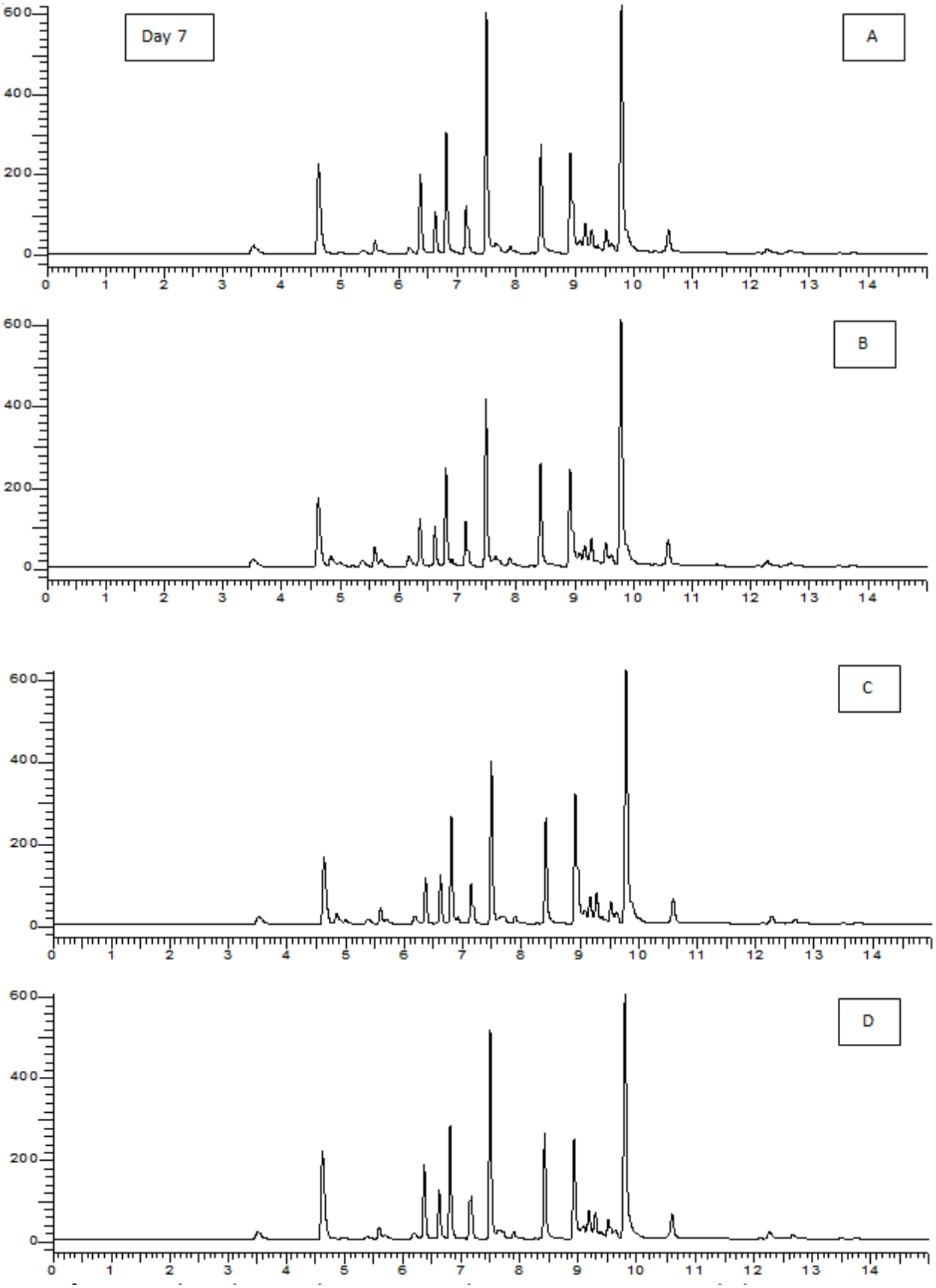
In Figure 53, from Day 7, 1-HMO activity of the cells exposed to bananas and an organic apple is the highest and the one of the cells with no fruit exposure is the lowest.

Figure 54 Banana test of organic apple-coordinated GCoU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GCoU plate; C. GCoU plate with an organic apple; D. an organic apple.

In Figure 54, the cells exposed to an organic apple show worse delayed banana ripening effect than the ones with no organic apple exposure. Compared with control, neither of these cells shows the ability of delaying banana ripening.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

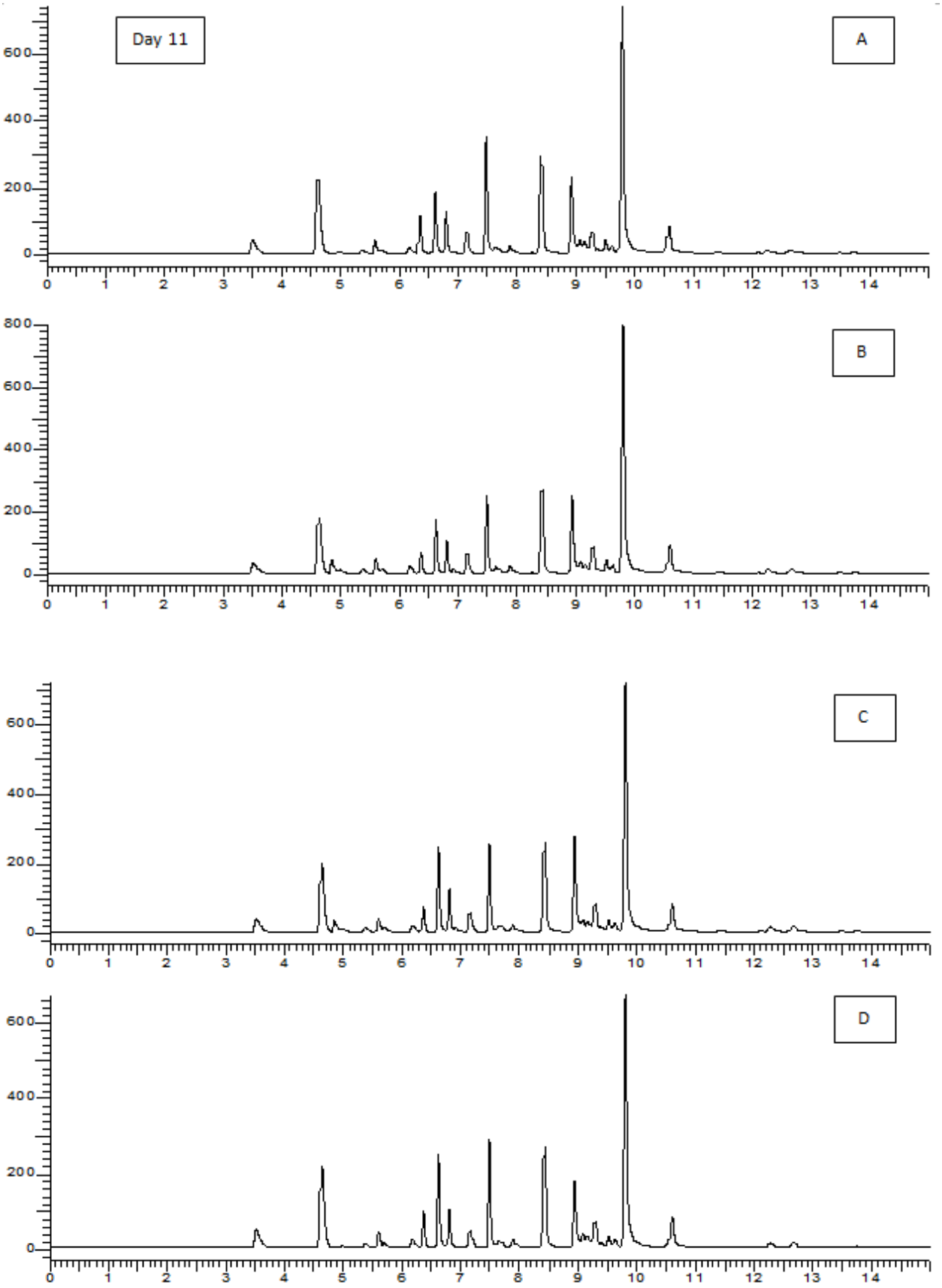

Day 11
A
B
C
D

Figure 55 HS-SPME-GC-FID of banana test of organic apple-coordinated *R. rhodochrous* DAP 96253 (GCoU plate). A. control; B. GCoU plate; C. organic apple+GCoU plate; D. organic apple.

In Figure 55, from Day 7, the banana VOCs from the container with an organic apple and the one with GCoU cell and an organic apple are similar with those from control.

f. Effects of peach juice-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

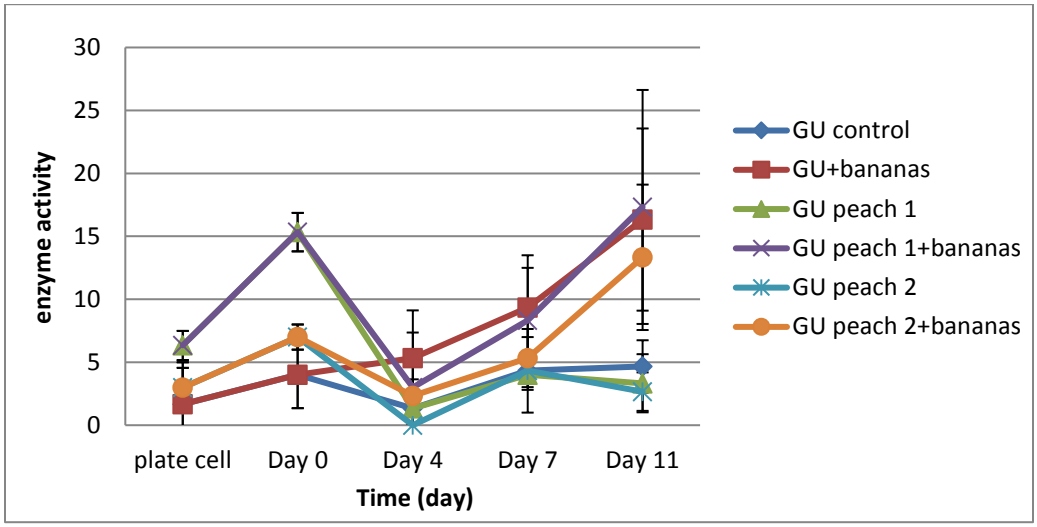
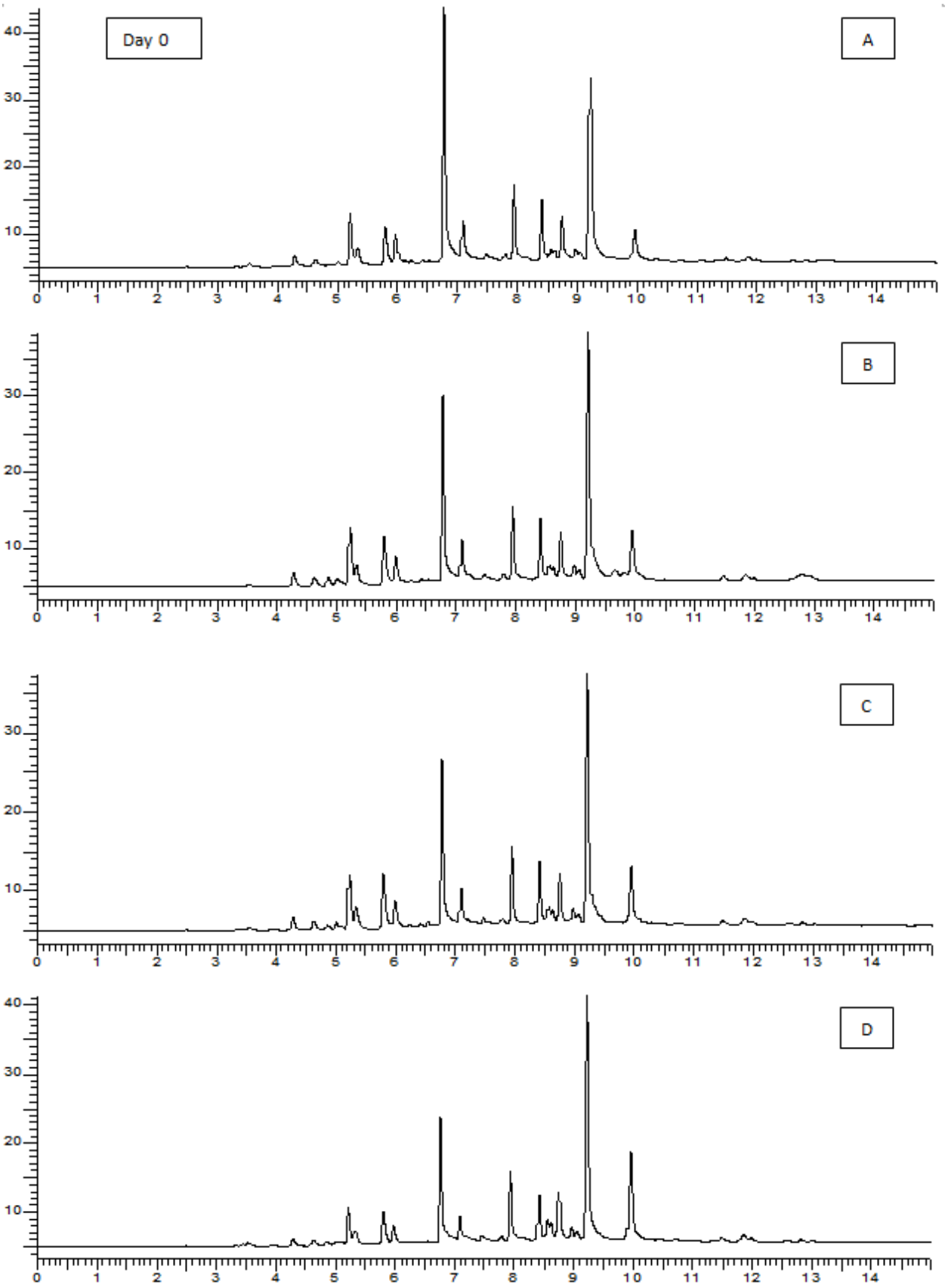


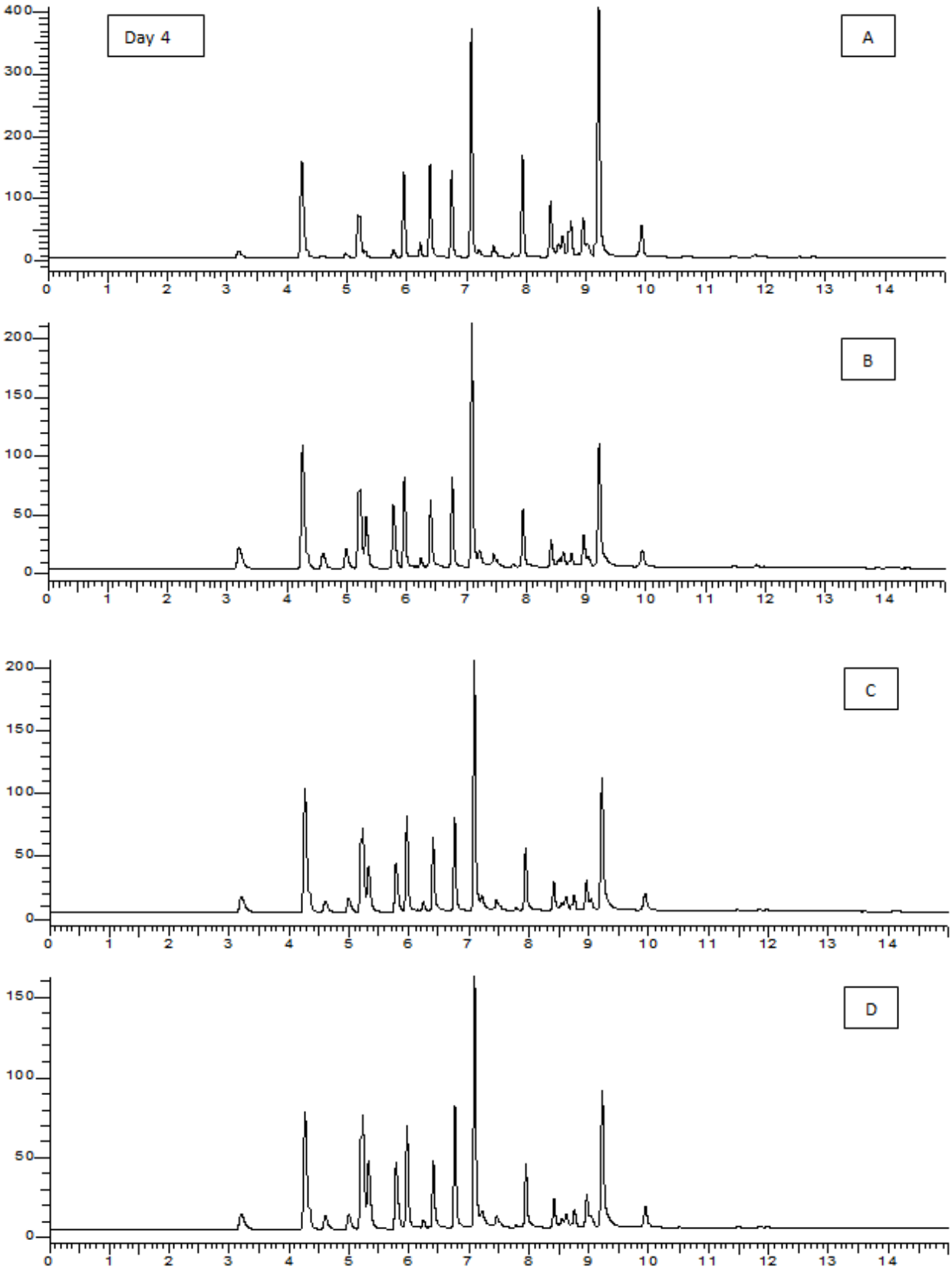
Figure 56 1-HMO activity of *R. rhodochrous* DAP 96253 (GU plate) with peach juice induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

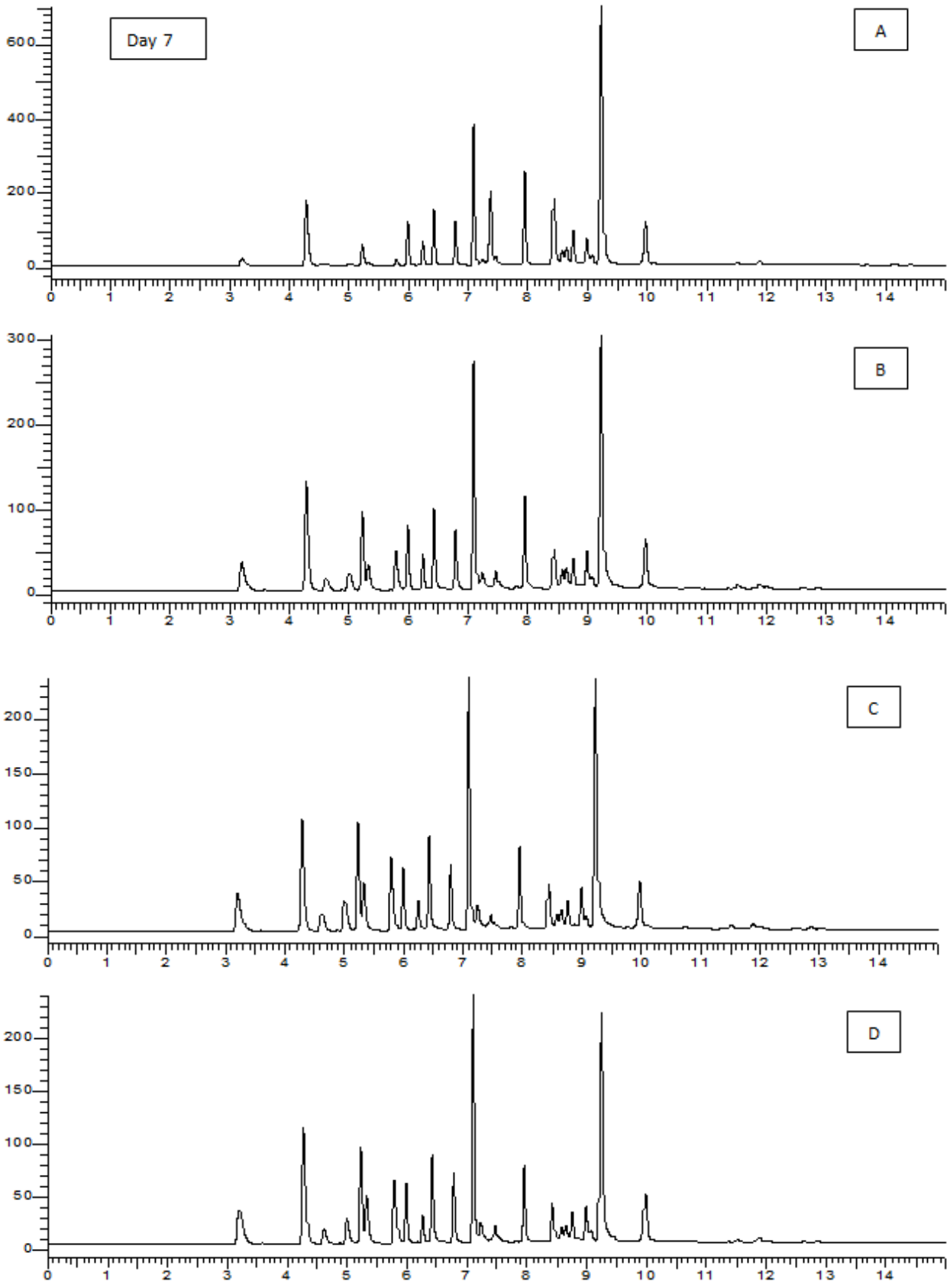
In Figure 56, GU plate cells grown on peach juice 1 maintain higher 1-HMO activity on Day 0, when 1-HMO activity of GU plate cells grown on peach juice 2 ranks the second high and the ones grown on no peach juice have the lowest 1-HMO activity. From Day 4, 1-HMO activity of GU plate cells grown on no peach juice is the highest when they are exposed to bananas.

Figure 57 Banana test of peach juice-induced GU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GU plate; C. GU plate with peach juice 1; D. GU plate with peach juice 2.

Banana test in Figure 57 shows very consistent results with 1-HMO in Figure 56. The higher the 1-HMO activity is, the better the cells can delay fruit ripening, although the difference of delayed fruit ripening results of these three samples is not obvious.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D

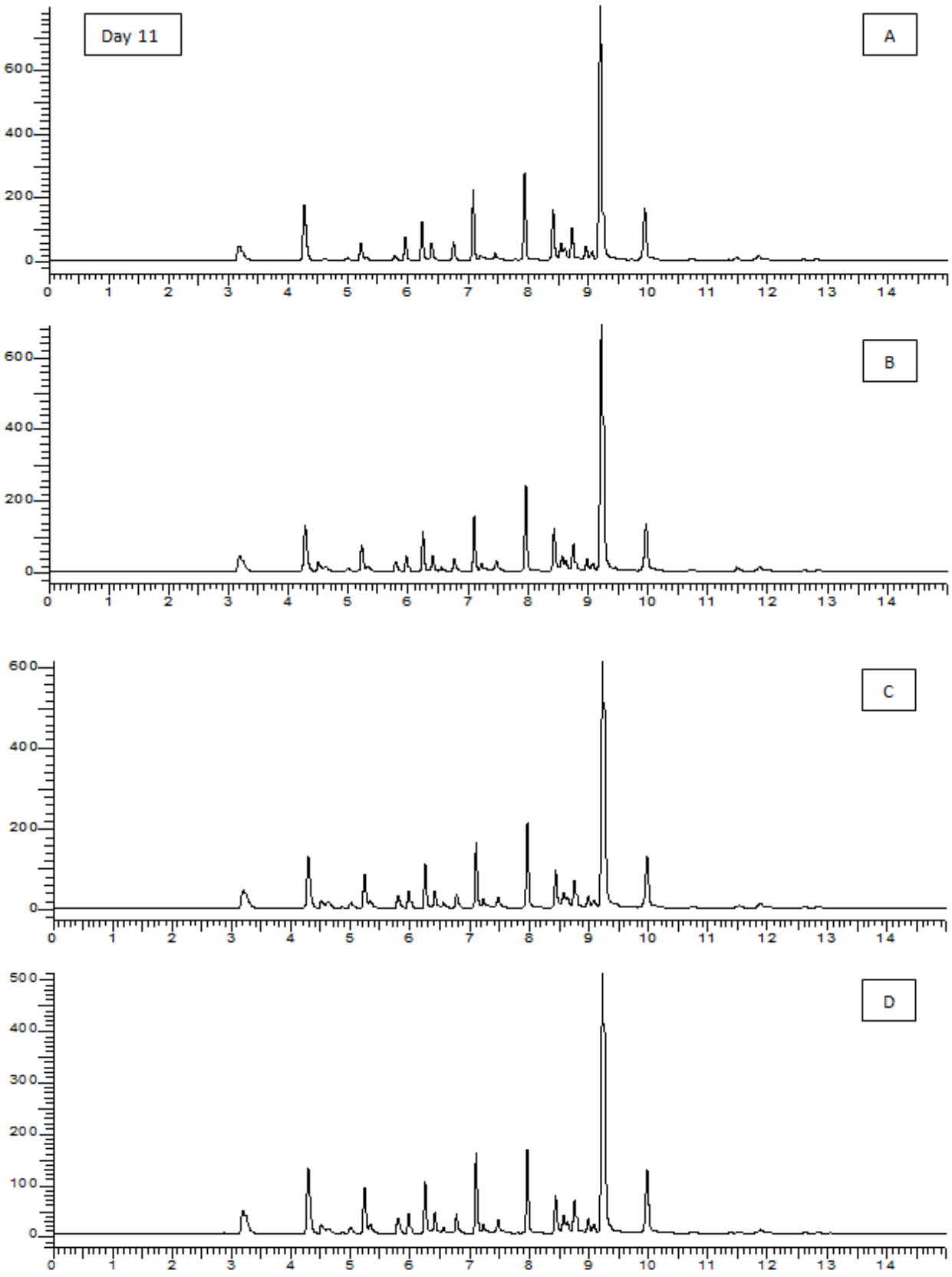

Day 11
A
B
C
D

Figure 58 HS-SPME-GC-FID of banana test of peach juice-induced *R. rhodochrous* DAP 96253 (GU plate). A. control; B. GU plate; C. peach juice 1-induced GU plate; D. peach juice 2-induced GU plate.

On Day 4 and 7, the banana VOCs from control are much higher than those from the other 3 samples and this is consistent with the banana photos in Figure 57 (Figure 58).

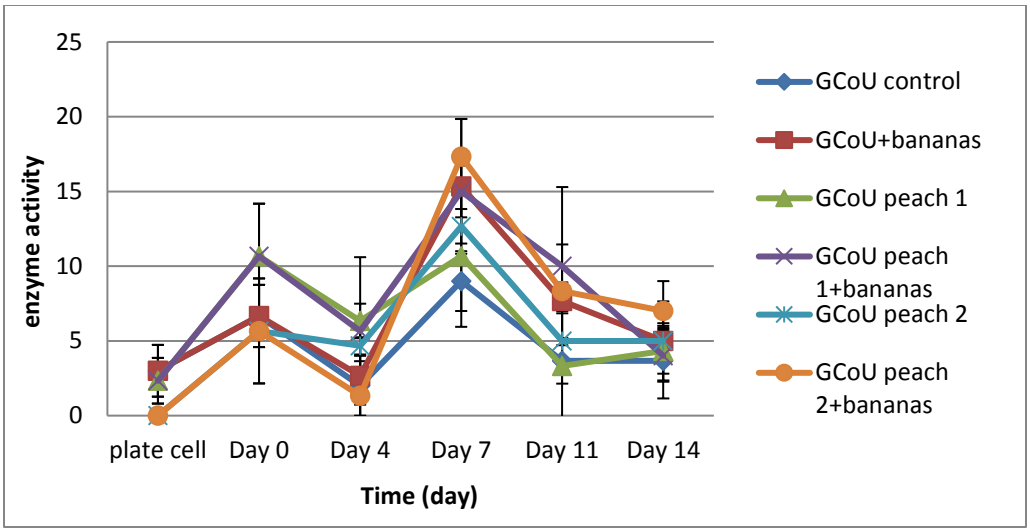
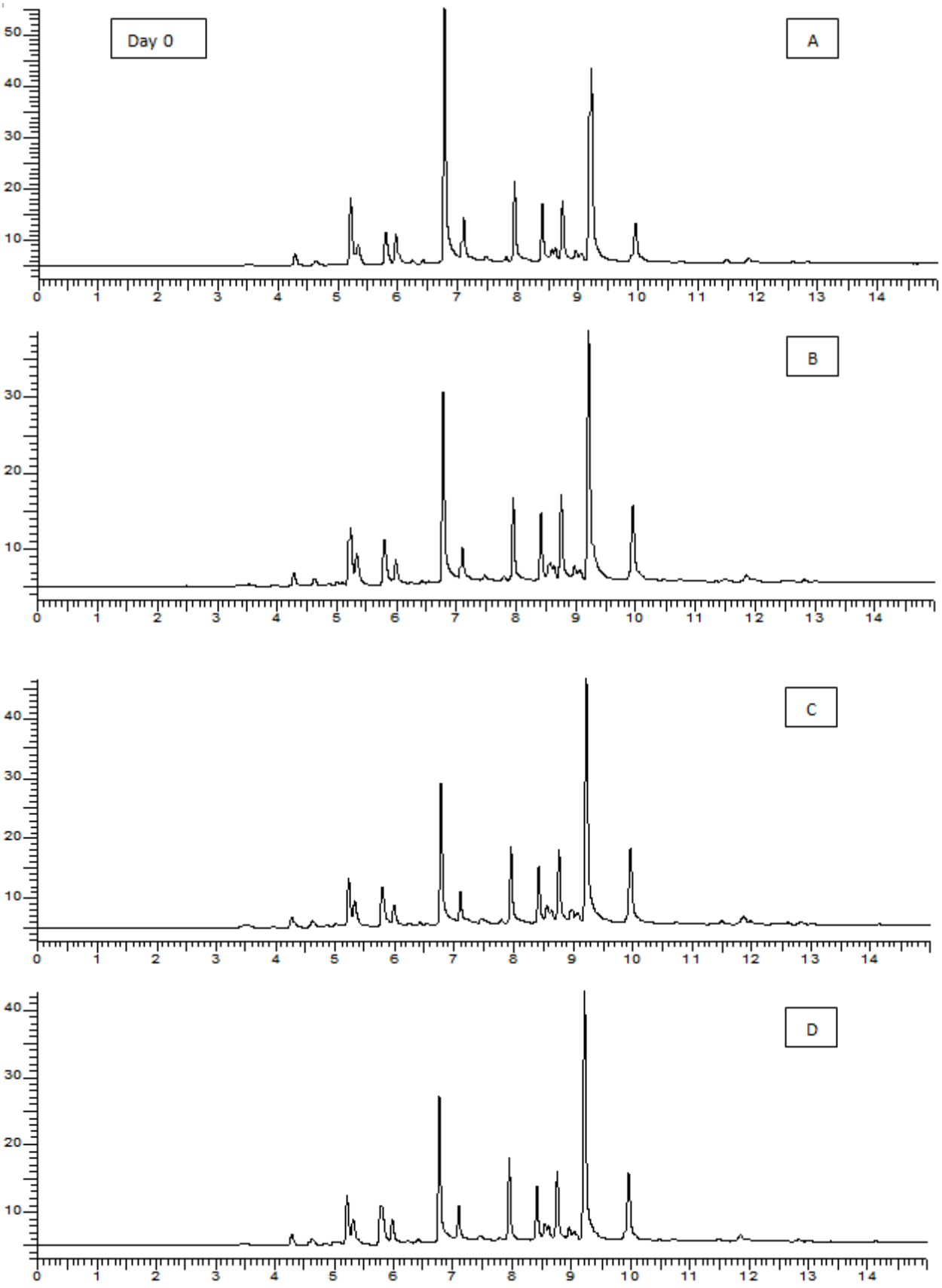


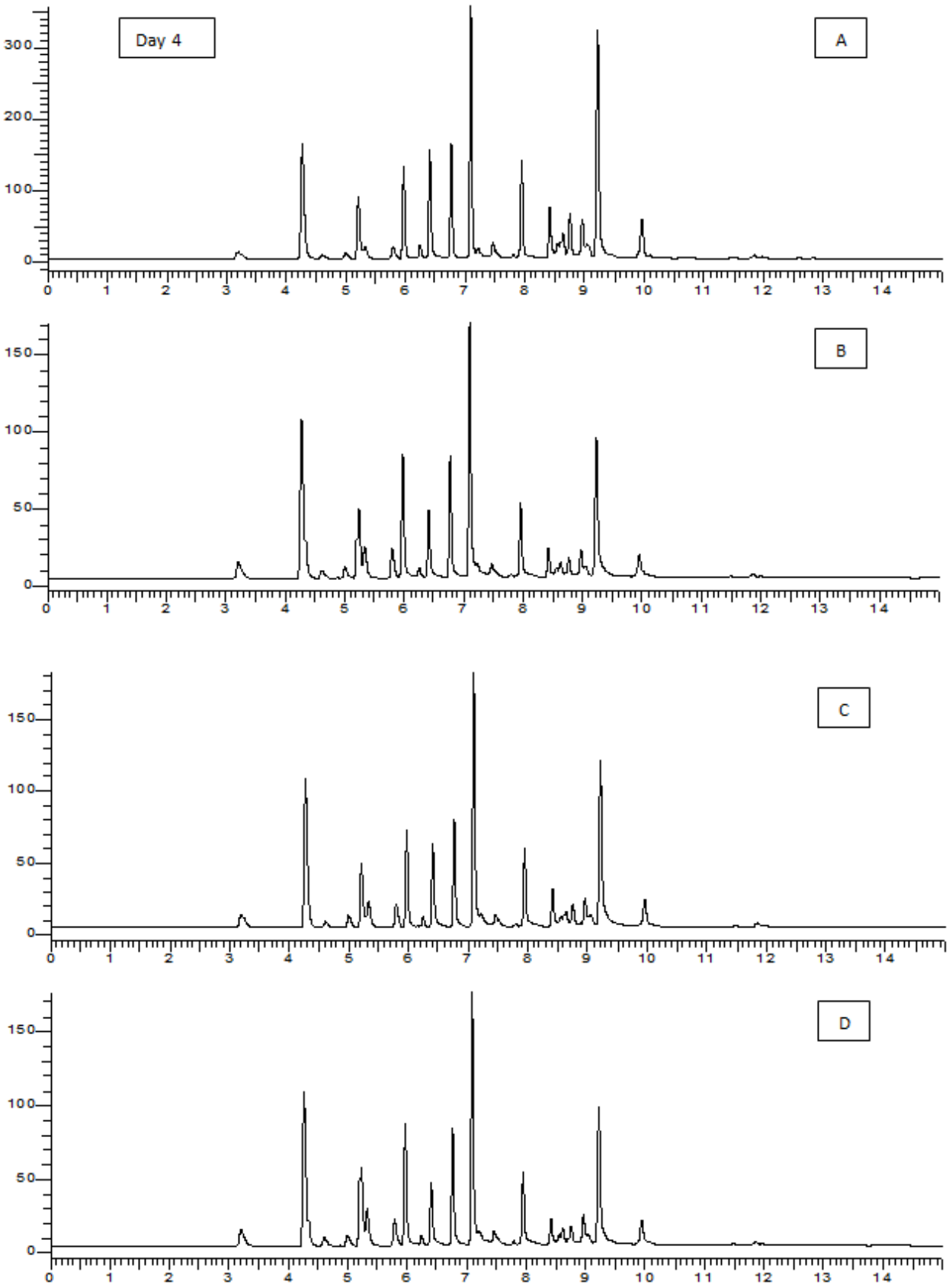
Figure 59 1-HMO activity of *R. rhodochrous* DAP 96253 (GCoU plate) with peach juice induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

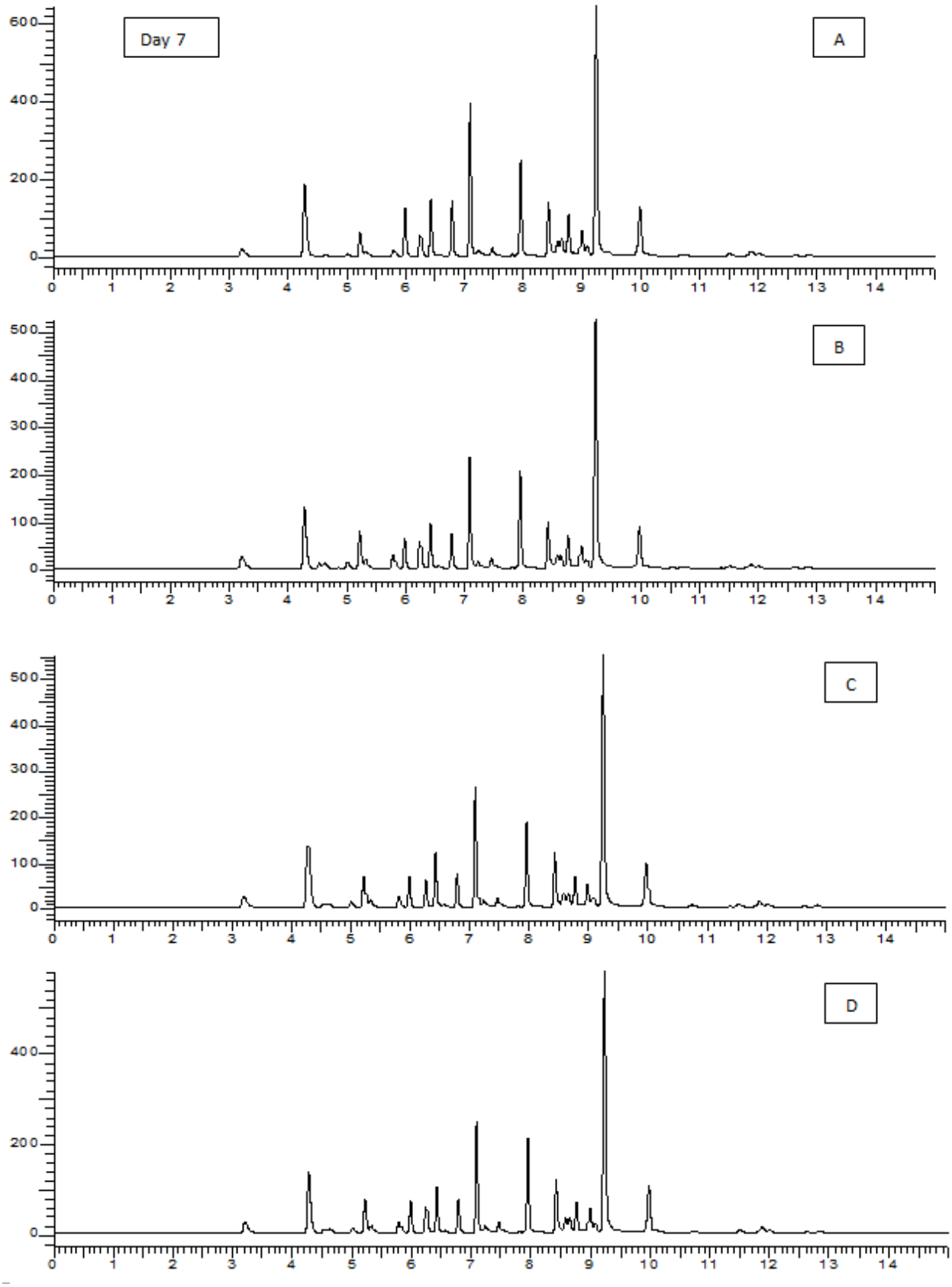
In Figure 59, GCoU cells perform similar 1-HMO activity trend as GU cells on Day 0. From Day 4, the cells grown on peach juice show higher 1-HMO activity than the ones grown on no peach juice, although peach juice concentration is not the main factor in 1-HMO activity.

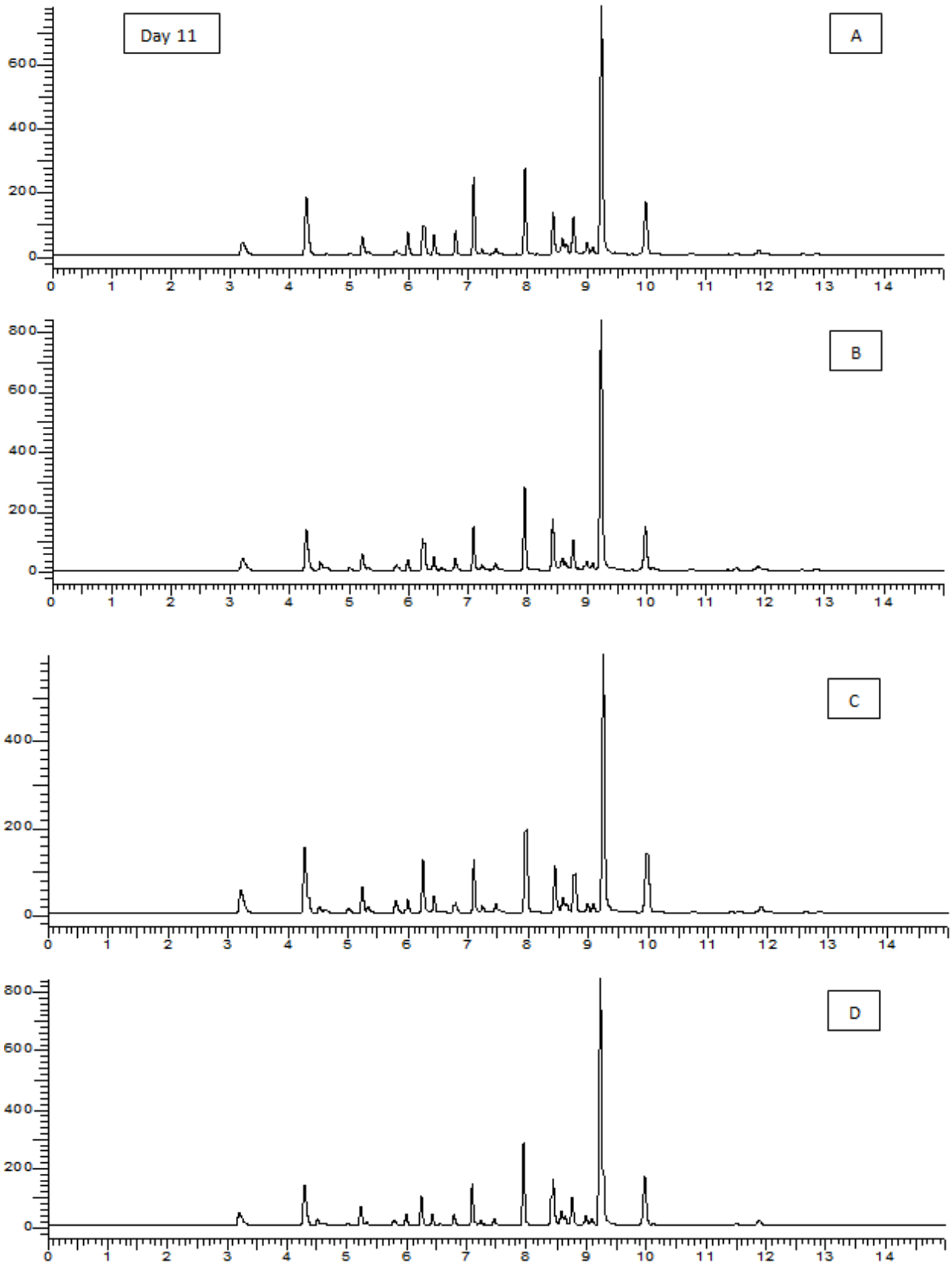
Figure 60 Banana test of peach juice-induced GCoU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GCoU plate; C. GCoU plate with peach juice 1; D. GCoU plate with peach juice 2.

On Day 14, banana test is consistent with 1-HMO activity: GU cells grown on peach juice 2 show the best delayed fruit ripening ability while the ones grown on peach juice 1 show the worst delayed fruit ripening ability although the difference is not very obvious (Figure 60).


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D


Day 11
A
B
C
D

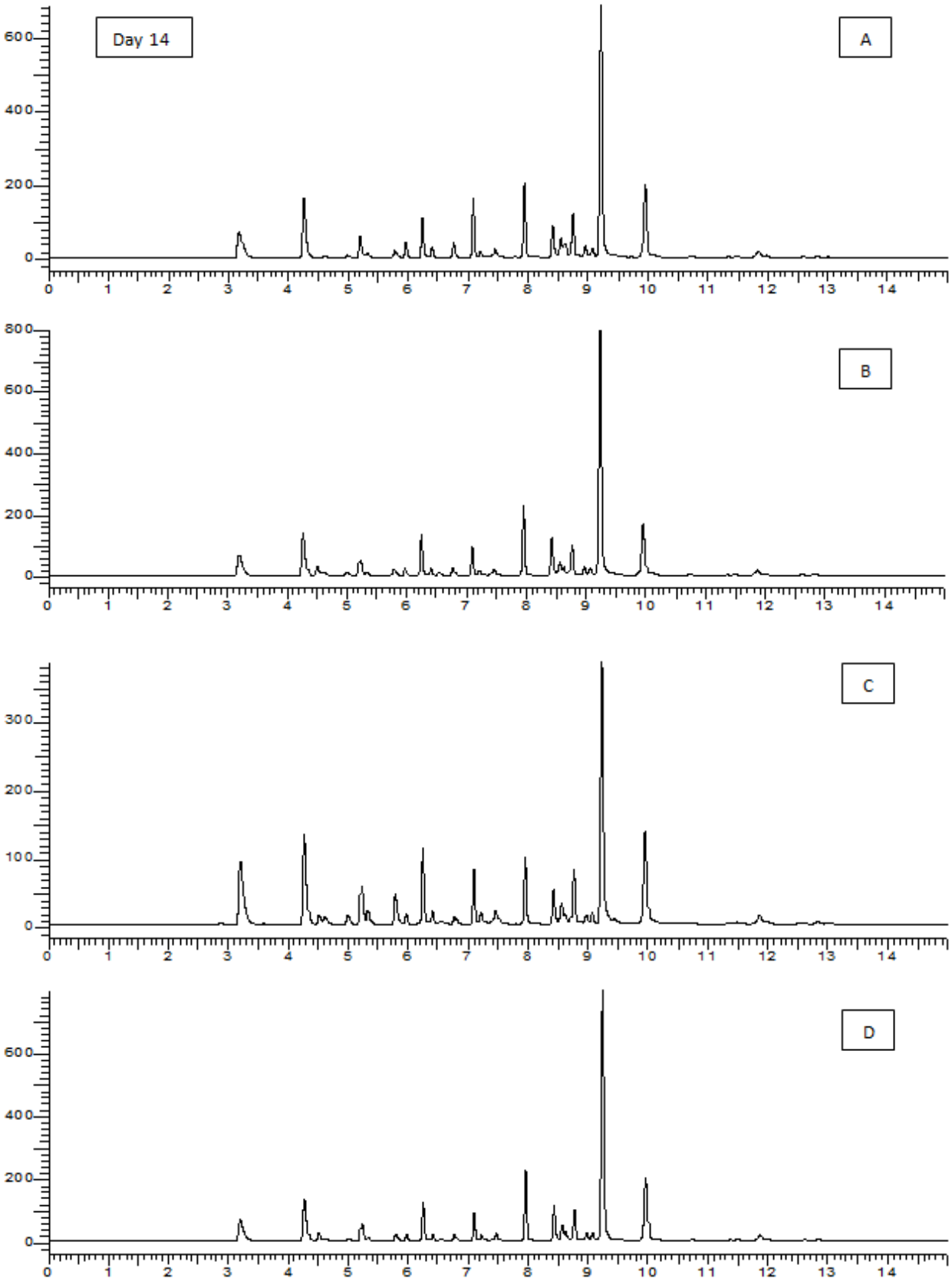

Day 14
A
B
C
D

Figure 61 HS-SPME-GC-FID of banana test of peach juice-induced *R. rhodochrous* DAP 96253 (GCoU plate). A. control; B. GCoU plate; C. peach juice 1-induced GCoU plate; D. peach juice 2-induced GCoU plate.

In Figure 61, the banana VOCs from all the samples are similar except of the ones from GCoU plate cells with peach juice 1 induction and this result is consistent with the banana photos in Figure 60. Fungi could be a factor for decreased banana VOCs from GCoU plate cells with peach juice 1 induction, however, the exact reason why fungi can decrease banana VOCs is still unclear.

g. Effects of whole banana and banana pulp-induced GU and GCoU plate cells of *R. rhodochrous* DAP 96253 on delayed fruit ripening

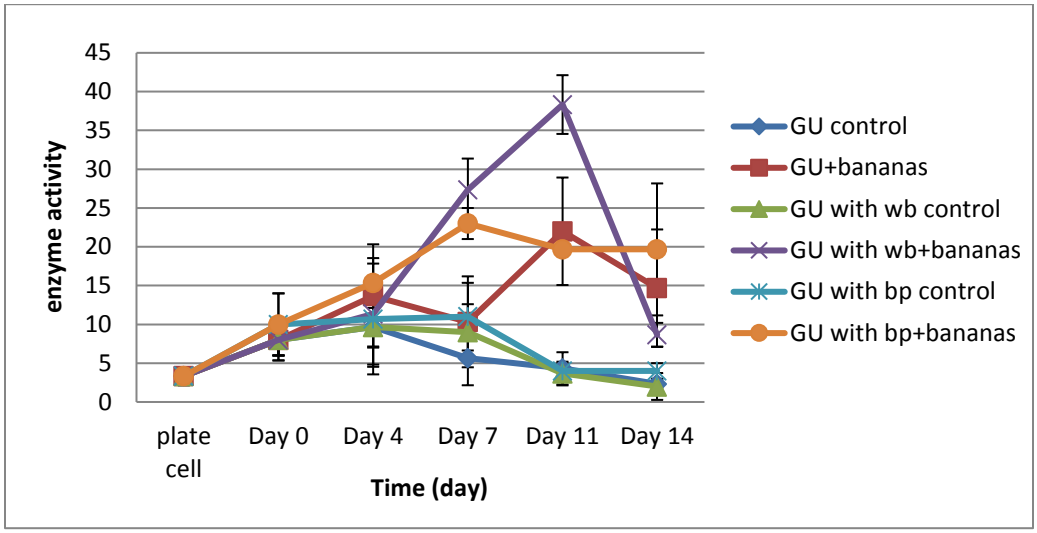
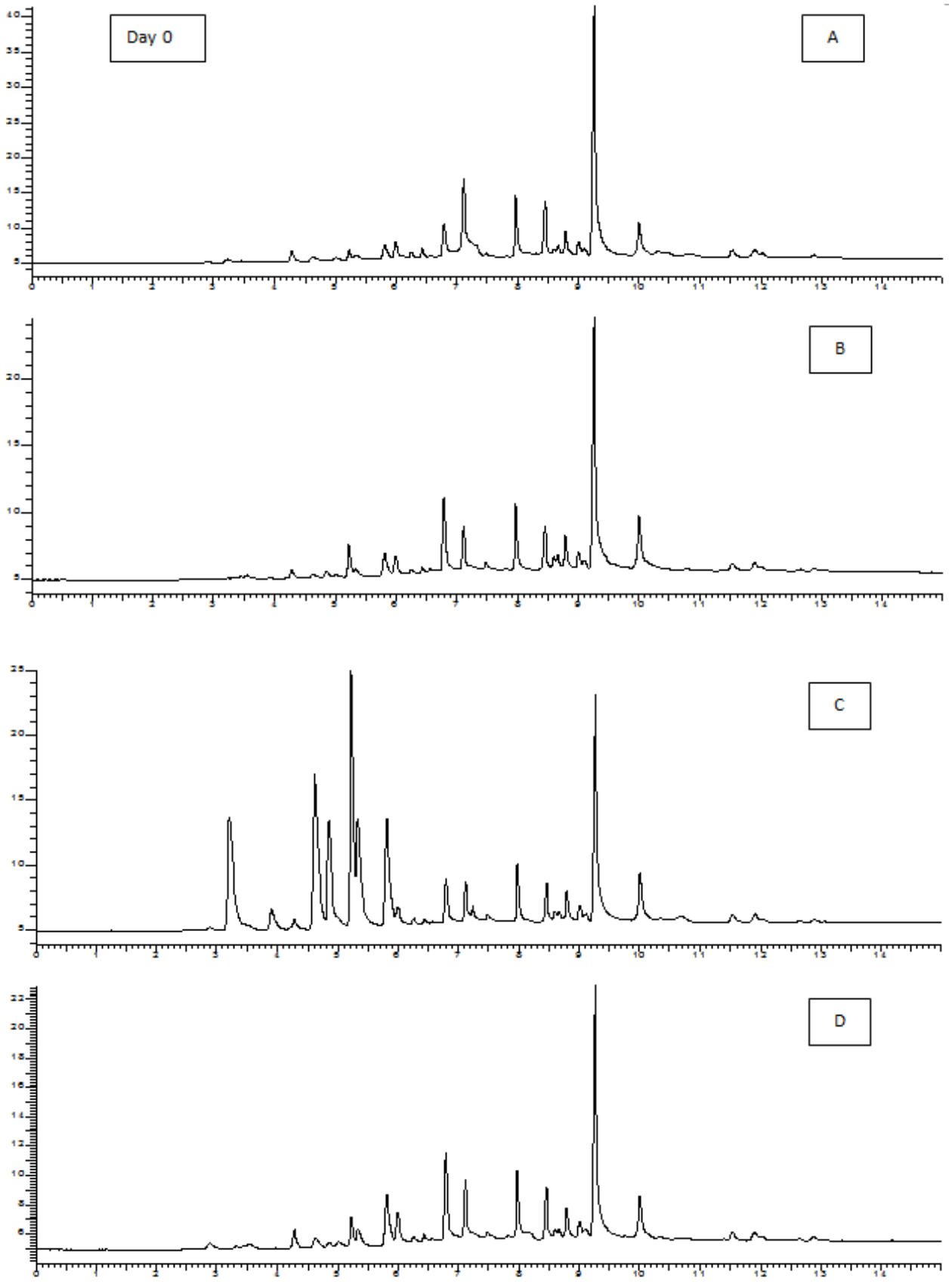


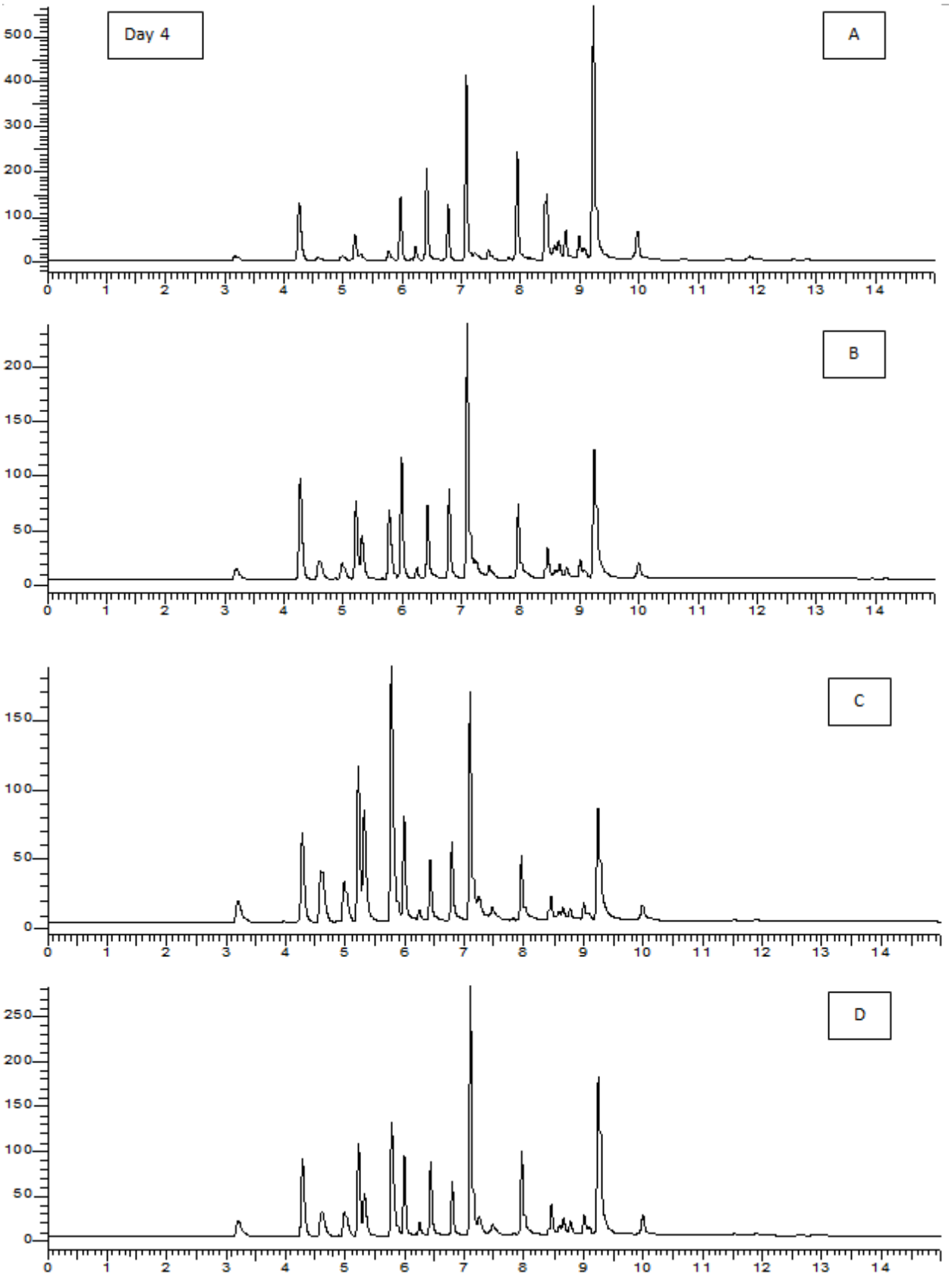
Figure 62 1-HMO activity of *R. rhodochrous* DAP 96253 (GU plate) with whole banana and banana pulp induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

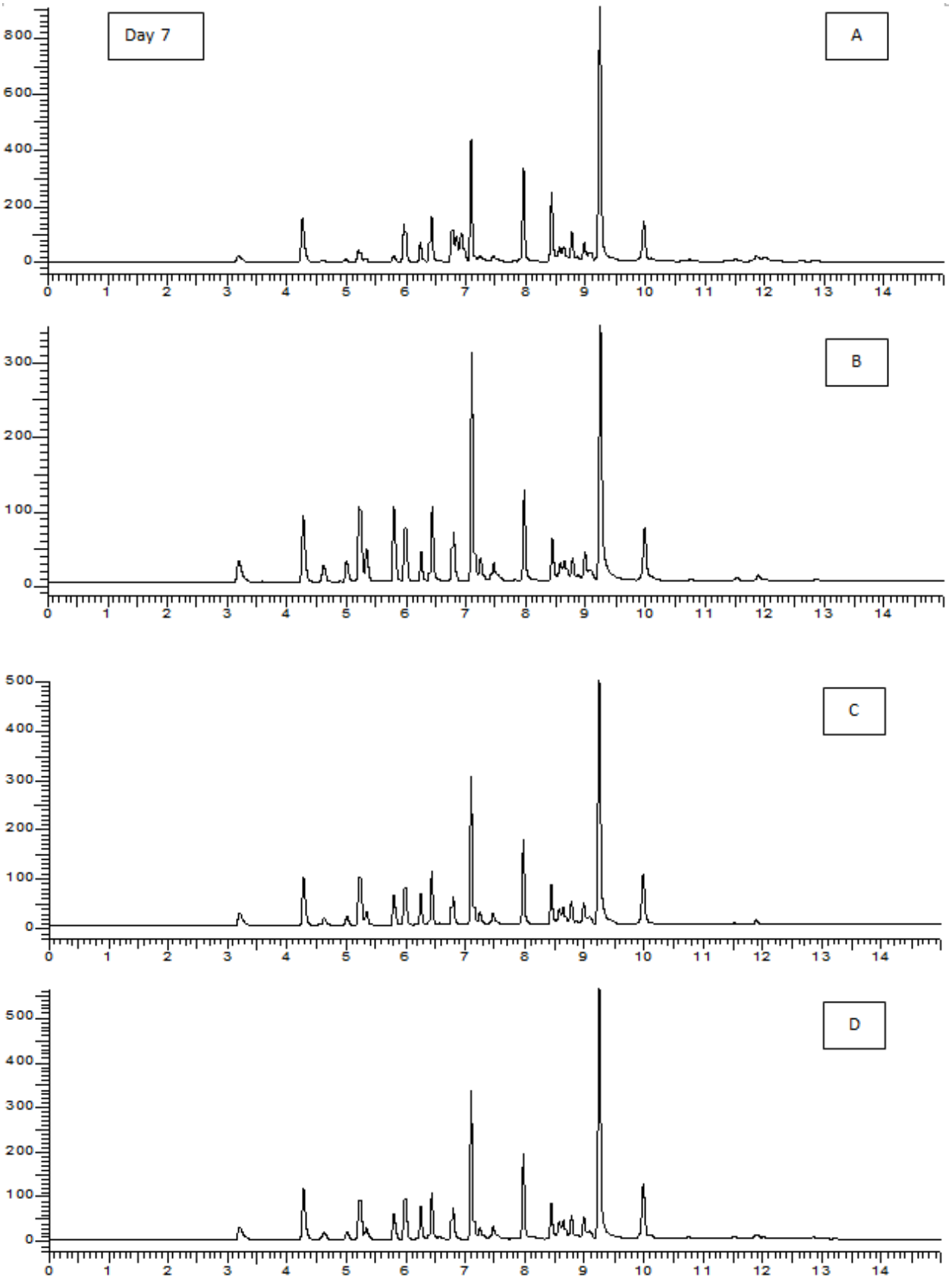
The differences of 1-HMO activity of different cells are not very obvious till Day 7, when the ones exposed to whole bananas maintain the highest 1-HMO activity while the ones with no pre-banana exposure have the lowest 1-HMO activity. On Day 11, 1-HMO activity of the ones exposed to banana pulp drops to the lowest (Figure 62).

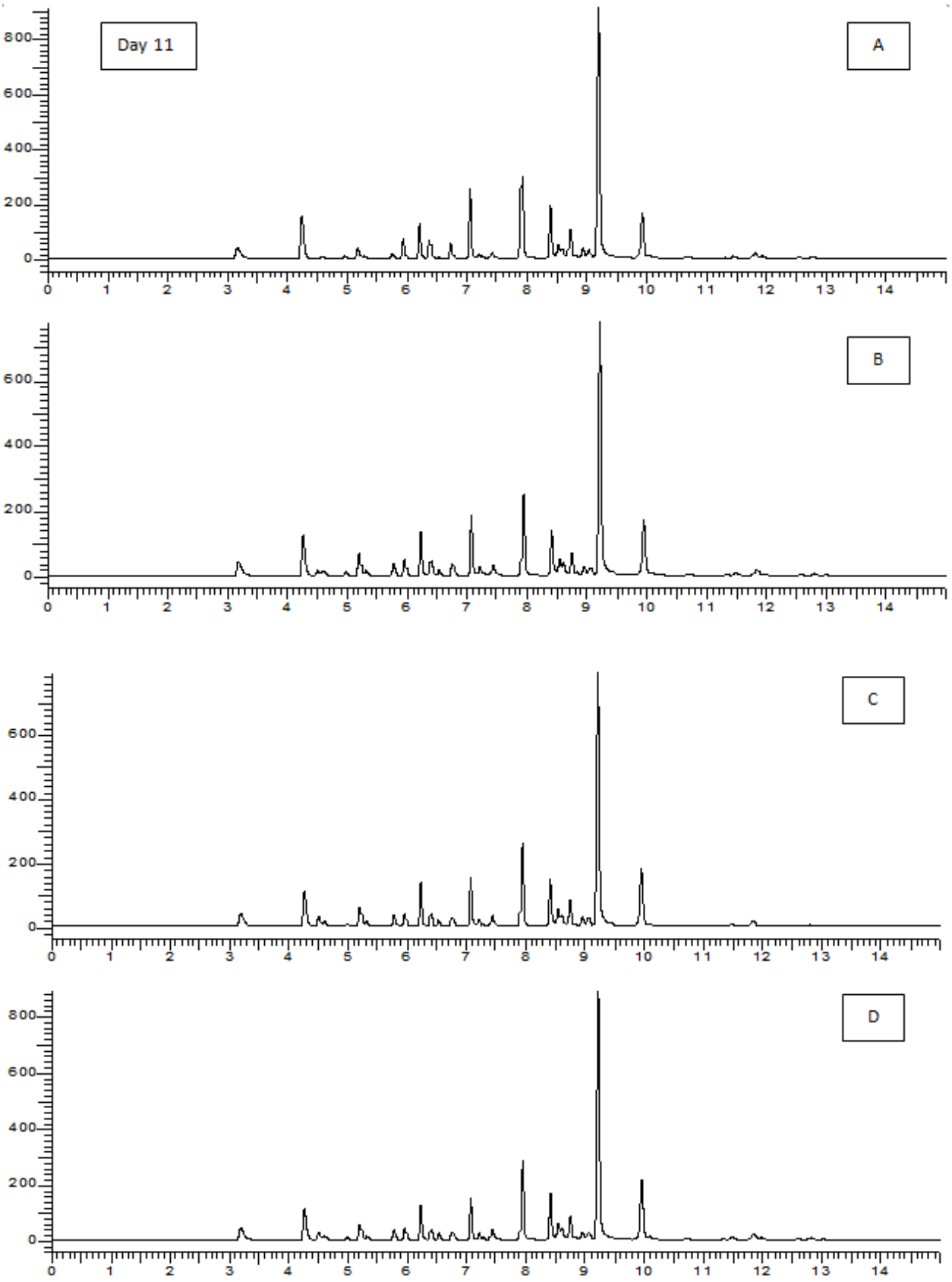
Figure 63 Banana test of banana-induced GU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GU plate; C. GU plate with whole bananas; D. GU plate with banana pulp.

In Figure 63, the upper bunch of bananas on Day 11 shows consistence with 1-HMO activity in Figure 62. However, the lower bunch of bananas with GU plate cells is even more ripened than control, showing inconsistence with 1-HMO activity.


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D


Day 11
A
B
C
D
0
200
400
600
800
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

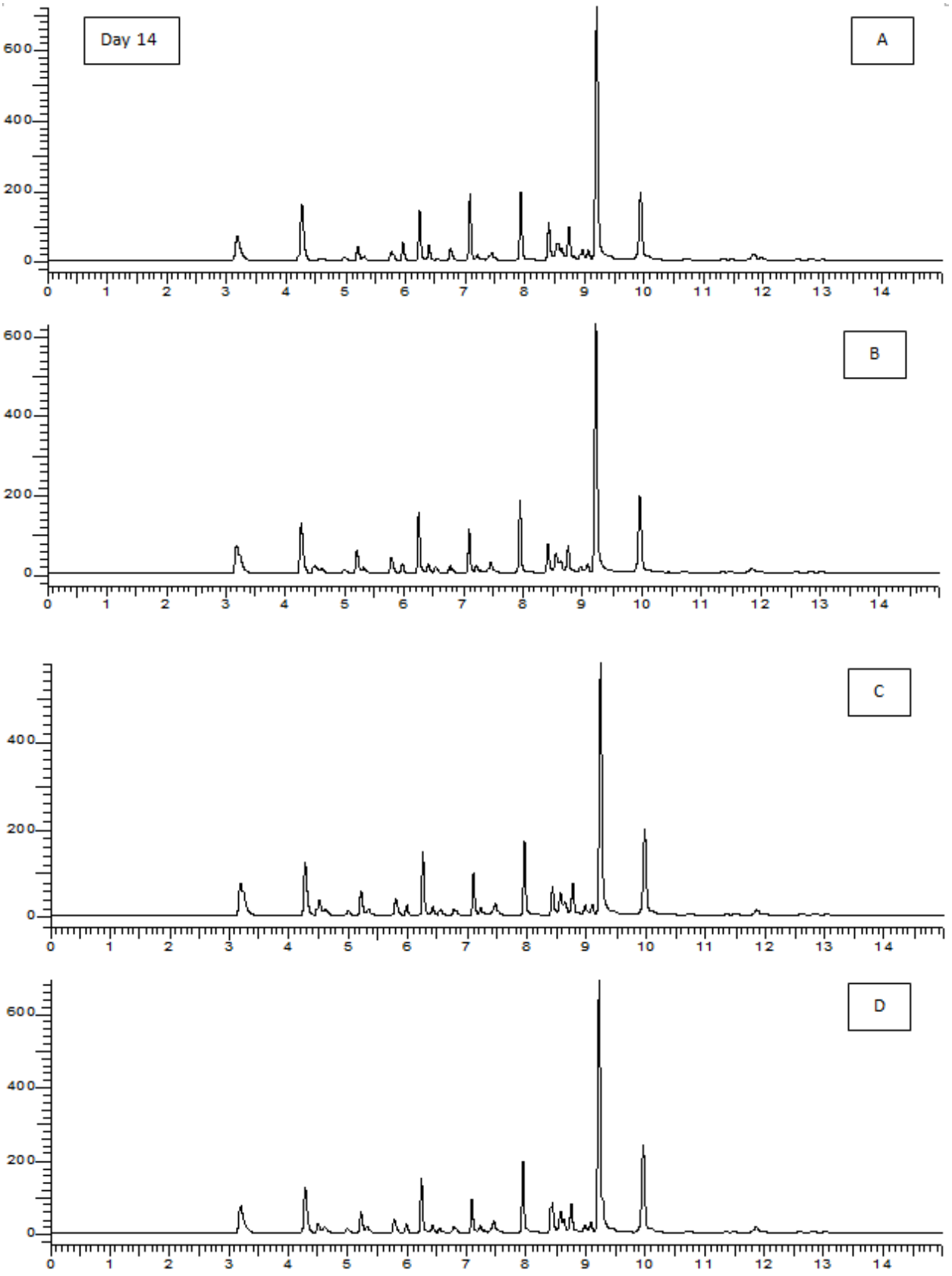

Day 14
A
B
C
D
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 64 HS-SPME-GC-FID of banana test of banana-induced *R. rhodochrous* DAP 96253 (GU plate). A. control; B. GU plate; C. whole banana-induced GU plate; D. banana pulp-induced GU plate.

From GC results in Figure 64, it is clear that the banana VOCs from all the samples are consistent with the upper bunch of banana photos in Figure 63.

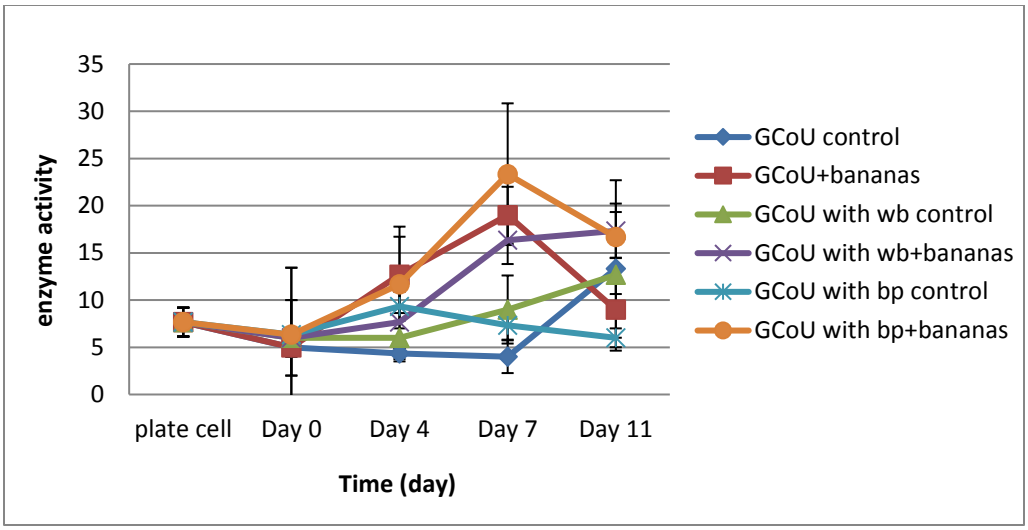
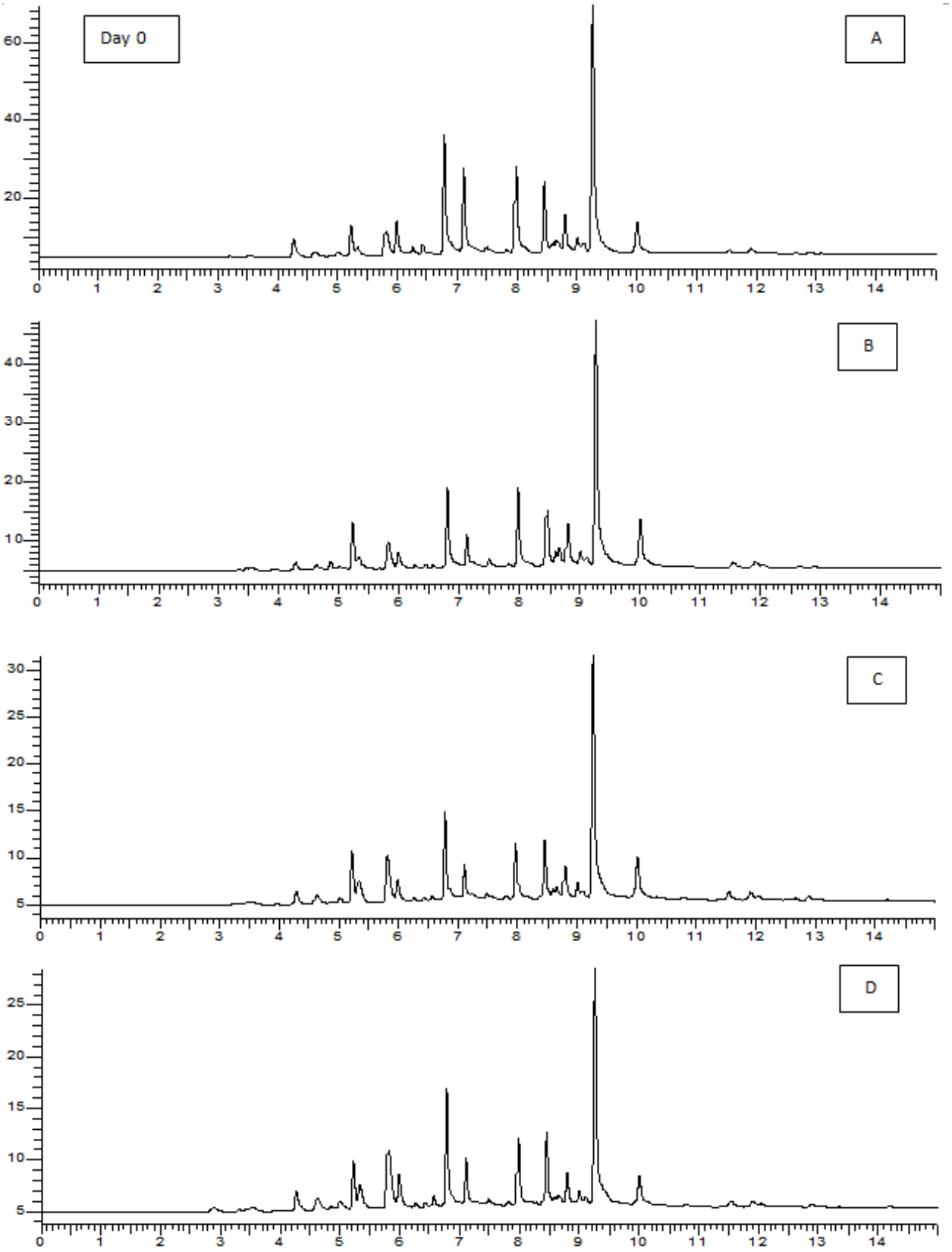


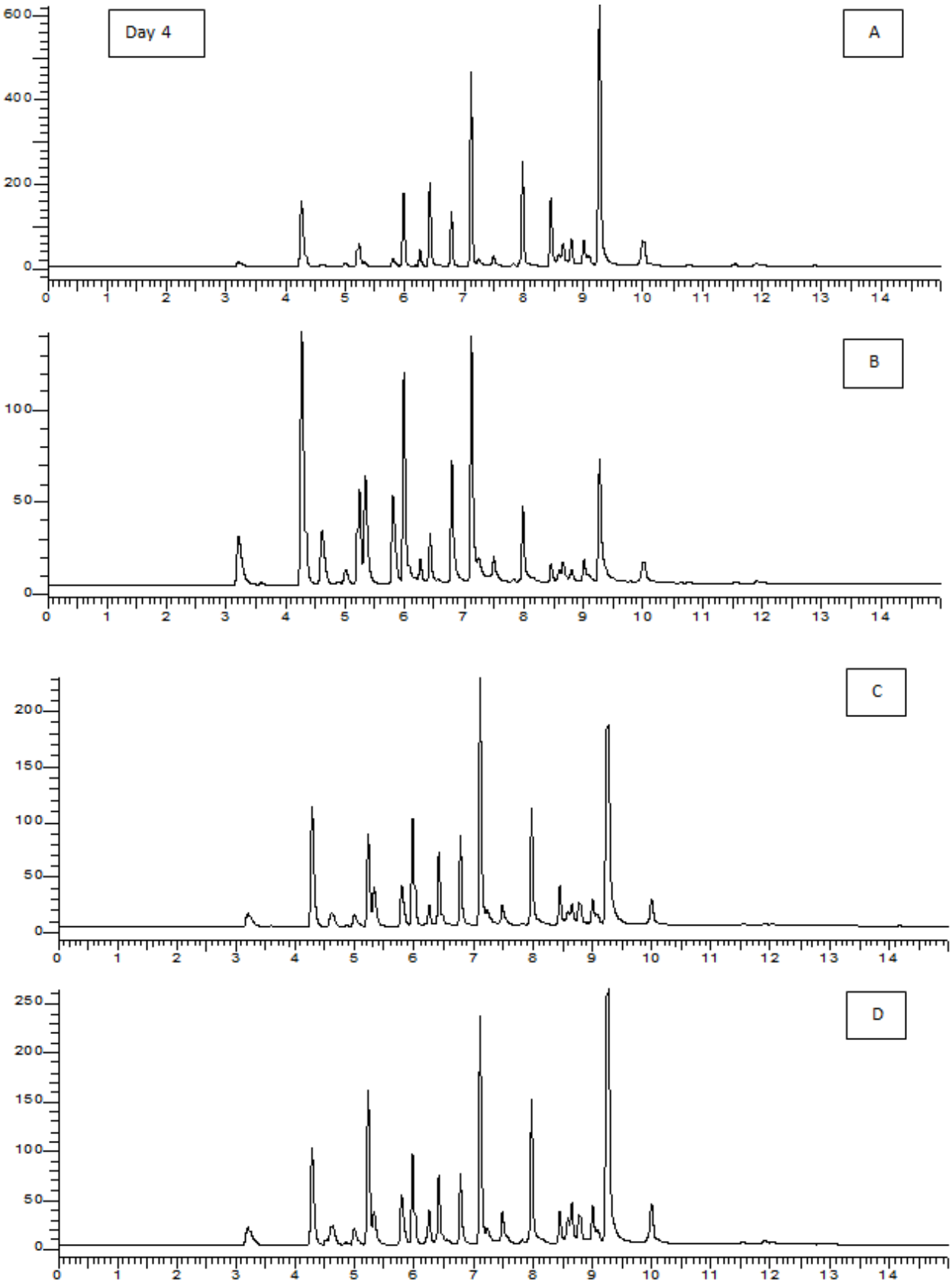
Figure 65 1-HMO activity of *R. rhodochrous* DAP 96253 (GCoU plate) with whole banana and banana pulp induction. *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

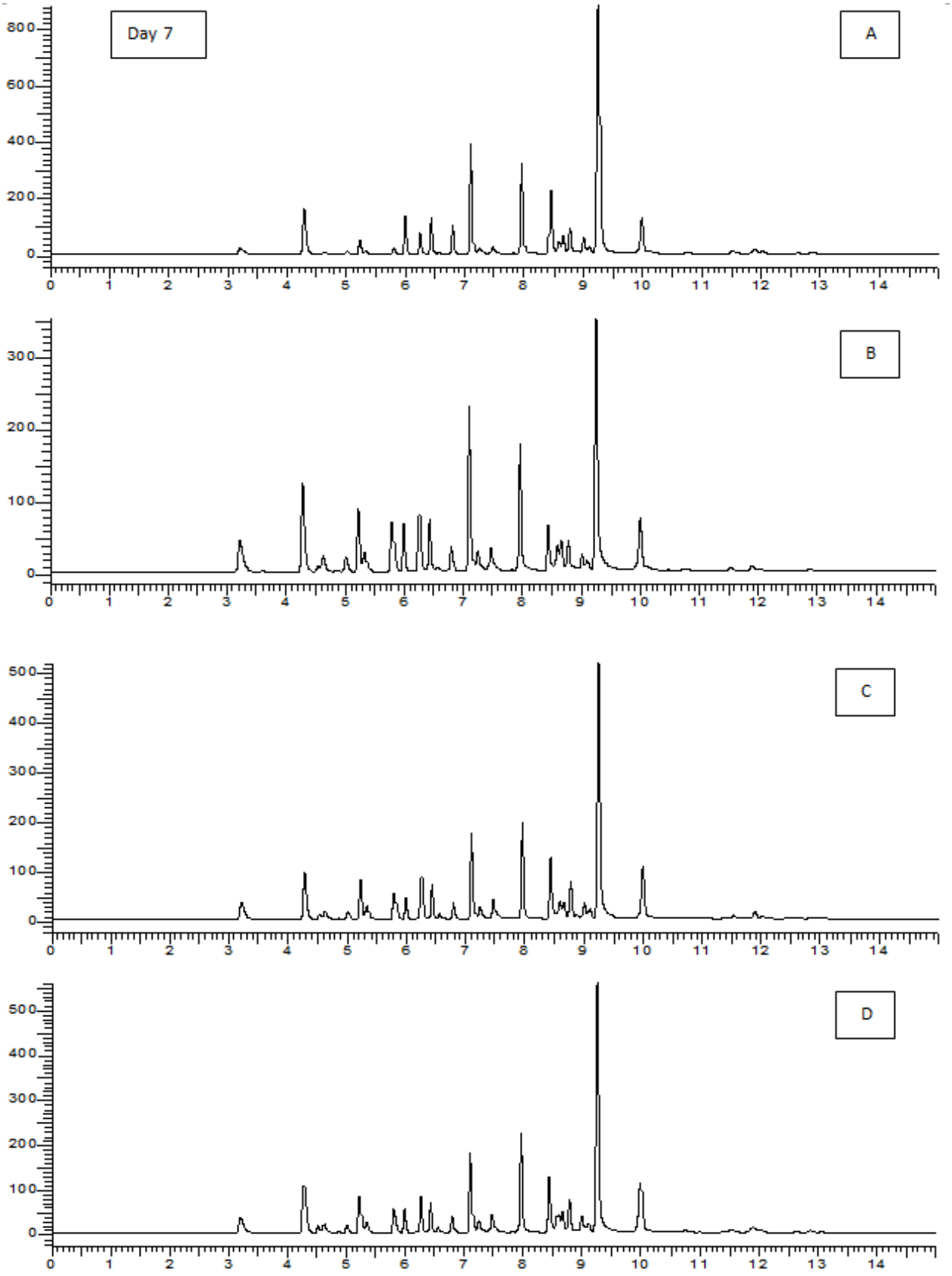
In Figure 65, pre-banana exposure cannot enhance 1-HMO activity of GCoU cells. On Day 7, 1-HMO activity of *R. rhodochrous* DAP 96253 pre-exposed to banana pulp is the highest.

Figure 66 Banana test of banana-induced GCoU plate cells of *R. rhodochrous* DAP 96253. A. control; B. GCoU plate; C. GCoU plate with whole bananas; D. GCoU plate with banana pulp.

On Day 11, GCoU plate cells pre-exposed to whole bananas show the best delayed fruit ripening result which can be seen from the upper bunch of bananas, however, fungi grown on the lower bunch of bananas affects the results at the same time (Figure 66).


Day 0
A
B
C
D


Day 4
A
B
C
D


Day 7
A
B
C
D

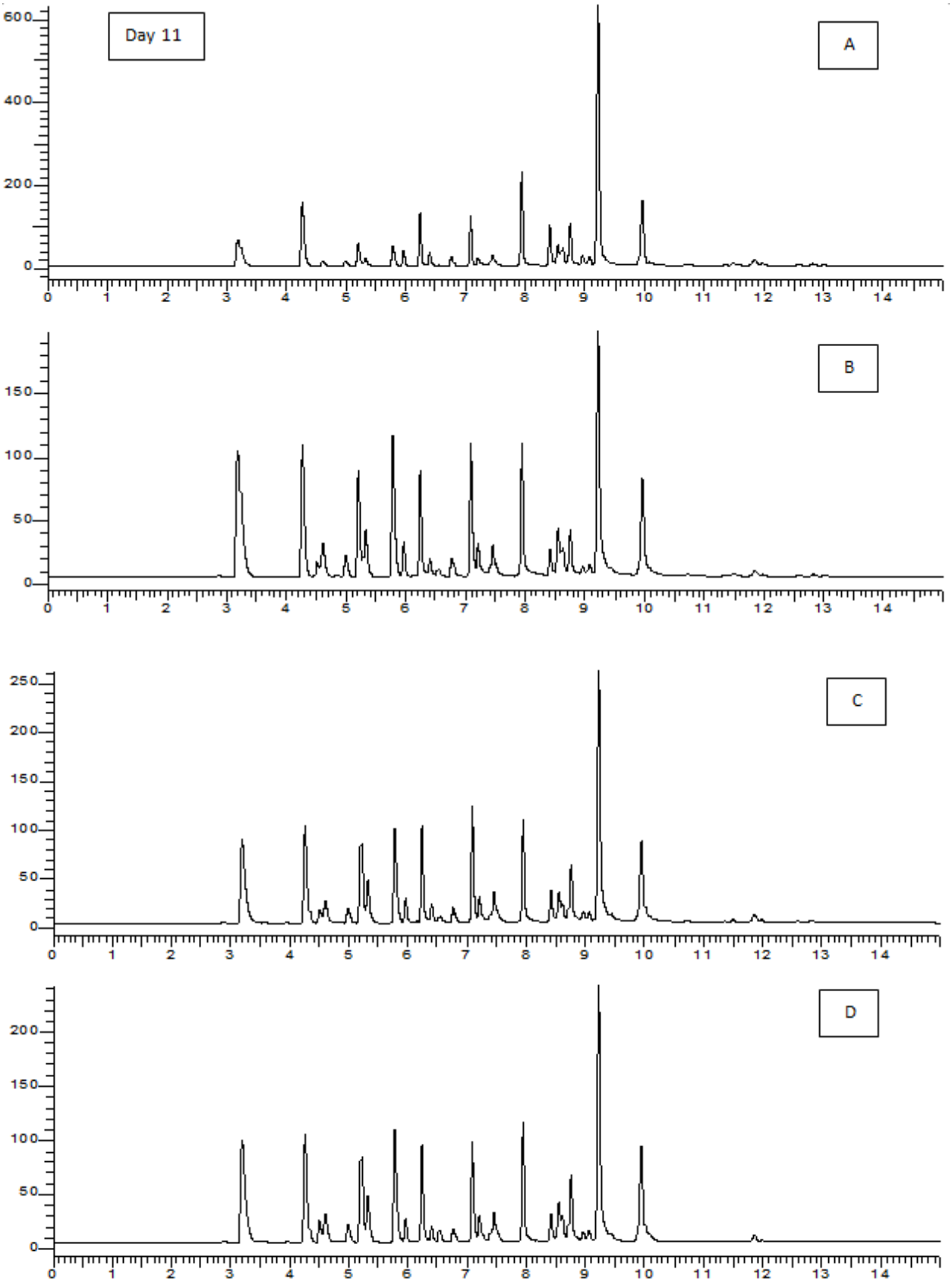

Day 11
A
B
C
D

Figure 67 HS-SPME-GC-FID of banana test of banana-induced *R. rhodochrous* DAP 96253 (GCoU plate). A. control; B. GCoU plate; C. whole banana-induced GCoU plate; D. banana pulp-induced GCoU plate.

Since there are more fungi growing on the lower bunch of bananas from the samples with GCoU plate cells, the banana VOCs are decreased dramatically and it is impossible to conclude any correlation with banana photos in Figure 66 (Figure 67).

3.6 Mycelial Growth of *R. rhodochrous* DAP 96253

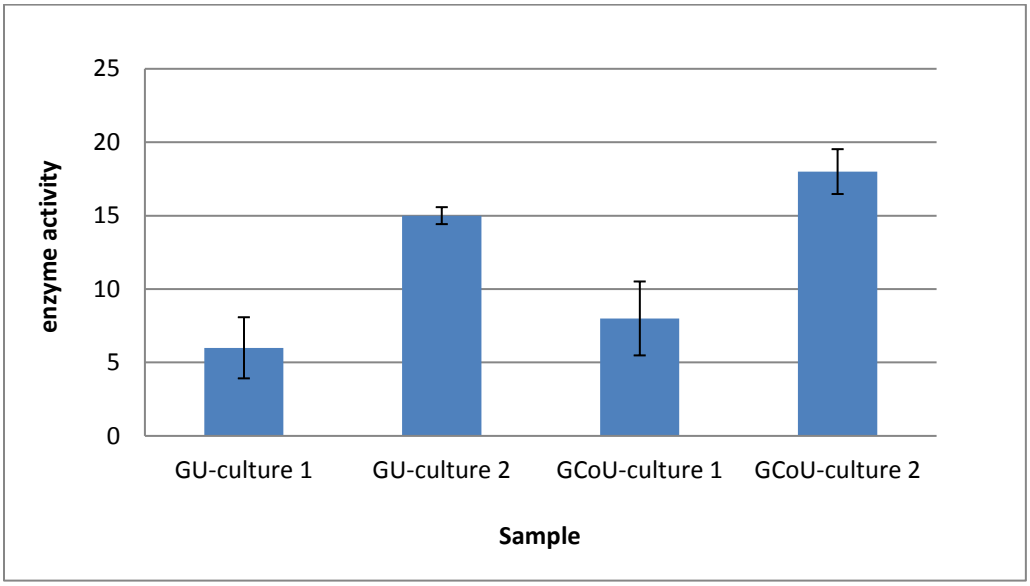


Figure 68 1-HMO activity of *R. rhodochrous* DAP 96253 (GU and GCoU plate cells from two cultures). *units (nmol of 1,2-epoxyhexane · hour)/g (cdw).

In Figure 68, 1-HMO activities of *R. rhodochrous* DAP 96253 grown on both media from culture 1 are lower than the ones from culture 2. For each culture, 1-HMO activity of *R. rhodochrous* DAP 96253 grown on GU is lower than that of GCoU.


A
10 µm


B
10 µm

Figure 69 Photomicrographs of *R. rhodochrous* DAP 96253 plate cells (GU and GCoU plate cells from two cultures) (40x10). A. GU-culture 1, B. GU-culture 2, C. GCoU-culture 1, D. GCoU-culture 2.

Figure 69 clearly shows classic *R. rhodochrous* surface growth. In Figure 69A, there are some rudimentary filaments of 10-20 µm in length. Other filaments have fragmented into small rods, which give the appearance of false branching. In Figure 69B, most of the filaments have

fragmented into small rods. A few filaments of 10 μm in length remain. The GCoU plate cells in Figures 69C and D show much longer filaments of 20-40 μm (Figure 69C) or 10-15 μm (Figure 69D) but again fragmenting into small rods. While differences are noted in the filament length for GU and GCoU plate cells, these difference may be due to the different media or differences in surface tension. Regardless, the photomicrographs show the rudimentary filaments that break down into small rods that are classic for *R. rhodochrous* growth.

4 DISCUSSION

Glucose, fructose and galactose are monosaccharides; sucrose, trehalose, maltose and lactose are disaccharides; maltodextrin is a polysaccharide, and all contain a glucose moiety. Excluding lactose, all of the other carbohydrates can be considered in terms of glucose units (maltose was added in the past as a "slow-release" form of glucose because it reduced the growth of contaminants during fermentation). All of these supplements are degraded via a glycolytic pathway that typically feeds the TCA cycle. As such, the amount of reduced pyridine nucleotide produced will be essentially the same. Reduced pyridine nucleotides are necessary for the activity of 1-HMO. However, and this is an important point regarding *R. rhodochrous* DAP 96253: the induced cells contain an oxidoreductase which provides catalytic reduced pyridine nucleotide regenerated in the enzyme complex in situ, thus freeing the 1-HMO from the requirement of metabolically supplied NADH . Reductase was discovered in the analysis of the *R. rhodochrous* DAP 96253 genome (John Neville, 2016 unpublished) and purified from *R. rhodochrous* DAP 96253 (Shirley Belshazzar, 2016 unpublished).

What is most interesting, is that 1-HMO (along with NHase, amidase and cyanidase) is induced in media containing carbohydrates. Furthermore, in the carbohydrate supplemented media, Isocitrate lyase (ICL), the key enzyme of the glyoxylate bypass was significantly induced. In sum, under induction conditions where no hydrocarbons or lipids/fats are used, 1-HMO, and ICL typically associated with hydrocarbon metabolism, are significantly enhanced.

It is clear that 1-HMO activity of *R. rhodochrous* DAP 96253 is unstable when cells are stored at 37°C. From earlier work (Tucker, 2007; Wang, 2013), it was quite clear that the carbohydrate supplements had a significant impact on NHase stability. With respect to 1-HMO stability, when cells are stored at either 4°C or 25°C, the carbohydrate supplements did not have

a significant impact on stability. Our current knowledge suggests that NHase/amidase may play a critical role in delayed ripening, as does 1-HMO. That being the case, and in the effort to develop a very rugged catalyst, we now know that methods involving medium supplementation which provided significant stability for NHase show little or no effect on 1-HMO stability. Furthermore, in parallel work (Barlament, 2016), the use of glucose supplementation during fermentation has resulted in improving induced cell yield by 2-3x.

Sokolovska *et al.* (2003) found that different carbon sources (sodium alkanoates, glucose and alkanes) could affect the permeability of *R. erythropolis* E3. Tucker (2007) found that using selected carbohydrate supplements has a significant impact on the outer cell wall composition. Additional research should explore the relationship between cell permeability of *R. rhodochrous* DAP 96253 cells when grown in carbon supplemented media during fermentation at scale. Here we have compared the differences of 1-HMO activity and stability of *R. rhodochrous* DAP 96253 affected by eight kinds of carbohydrates under different storage temperatures and time, which aims at unitizing *R. rhodochrous* DAP 96253 effectively and efficiently in degrading harmful alkenes from pollutants.

Placing the induced cells of *R. rhodochrous* DAP 96253 in the presence of whole bananas improved 1-HMO activity. HS-SPME-GC/MS analysis of ripening bananas clearly shows a number of VOCs are present. When induced cells are placed with ripening bananas there is a significant decrease in the signature banana VOCs. Common theory has been and still is currently, that enzymes tend to be most stable when they are bound to their respective substrates (this theory has been effectively used in stabilizing detergent enzymes by formulating these enzymes with substrate analogs). The substrate stabilization is very important in the case of enzymes such as 1-HMO, NHase, amidase and cyanidase which are multi-component enzymes.

Keeping the sub-units and components together in an active form is a necessity for maintaining the activity of these enzymes. The fact that 1-HMO activities increased may be due to stabilization and reformation of the 1-HMO active complex. Exploring how the 1-HMO active enzyme complex may be stabilized is a significant topic for future research.

There are many biocatalyst immobilization methods and calcium-alginate bead belongs to entrapment (End and Schoning, 2004). Our results show that these immobilized beads still maintain 1-HMO activity even after storage at 4°C for 28 days. Wu and Wisecarver (1991) added 0.02% calcium alginate into PVA-boric acid immobilized beads of *Pseudomonas*, which prevented the beads from agglomeration. Doria-Serrano *et al.* (2001) evaluated hydrogels composed of different ratios of PVA and calcium alginate and found that the ratio 80/20 (wt%) showed the best characteristics compared with the other ratios. Here we added the same weight of PVA as calcium-alginate to form the calcium-alginate PVA beads and have found that they can maintain 1-HMO activity in *R. rhodochrous* DAP 96253 more stably than calcium-alginate beads. For further study, we may try adding different weights of PVA to calcium alginate and evaluate 1-HMO activity. In addition, other immobilization methods should be investigated. Understanding 1-HMO in *R. rhodochrous* DAP 96253 and further exploring immobilization techniques have the potential to expand the utility and practical applications of 1-HMO.

We know that induced cells of *R. rhodochrous* DAP 96253 are capable of delaying the ripening of a number of fruits (e.g., peaches, avocados, tomatoes, bananas, etc.). While we knew from previous research that the induced cells of *R. rhodochrous* DAP 96253 were capable of degrading ethylene and cyanide (two of the major plant volatile hormones produced in the Yang Cycle). We also knew from GC analysis that the induced cells of *R. rhodochrous* DAP 96253 altered the fruit volatile fingerprint. However, we did not know the nature of the

compounds that were involved. With the recent addition of HS-SPME-GC-MS capabilities, we are now able to look at the VOC profile of fruits in the presence or absence of induced cells of *R. rhodochrous* DAP 96253.

CAR/PDMS fiber was used to adsorb head-space VOCs of bananas in the absence and presence of induced cells. Desorbing the SPME fiber onto the GC-MS resulted in a total ion chromatogram (TIC) identified six unique compounds (Figure 30). We have found that during the process of banana ripening (control), these six VOCs are accumulating more from Day 0 to Day 7 while decreasing after Day 7 except butanoic acid, 3-methyl-, 3-methylbutyl ester. In Figure 29, the amounts of banana VOCs which come out between peak 1 and peak 2 shown in Figure 30 (e.g., 1-butanol, 2-pentanone, isobutyl acetate, etc.), are different in these 3 samples (the ones from GU and GCoU are less than those from control on Day 4 and Day 7). The changes of the amounts of VOCs can be from gene regulations of bananas themselves and transforming from or to other VOCs at the same time. We may also try using other types of fibers to compare the results if necessary.

When we used ethylene and propylene to attempt to induce 1-HMO activity in *R. rhodochrous* DAP 96253 before conducting fruit tests, the results showed that both of them could induce 1-HMO activities of GU and GCoU plate cells. We also used ethyl acetate (one of the banana VOCs identified in control ripening bananas) to induce 1-HMO activity in *R. rhodochrous* DAP 96253 and found out that it could induce 1-HMO activity of GCoU plate cells. Interestingly, peach juice induced 1-HMO activity of GU plate cells. Pre-banana exposure (banana pulp or whole bananas) induced the level of 1-HMO activity of GU plate cells. Apples, which are noted for high ethylene production, increased 1-HMO activities of both GU and GCoU plate cells of *R. rhodochrous* DAP 96253.

It is clear that the activity and stability of 1-HMO and related enzymes in induced growing cells, and most probably induced resting cells are influenced by plant volatile hormones. In general, where delayed ripening was noted, the concentration of plant volatile hormones was significantly reduced for a period of time, but ultimately reached the same levels as seen in control ripening fruit. It is noted, that the products of enzymatic reactions can be profoundly influenced by reaction conditions, and by the nature of and amount of substrates available (this is however, well beyond the scope of this research).

The metabolic pathway of glucose in *R. rhodochrous* DAP 96253 yields NADH, which is necessary for activating molecular oxygen. *R. rhodochrous* DAP 96253 is able to produce ammonia from urea by urease (Kelly Cannon, 2015 unpublished) and ammonia can react with ethylene epoxide to yield ethylenediamine (Gilson and Winters, 1983), so urea may further accelerate the reaction catalyzed by 1-HMO through degrading ethylene epoxide in delayed fruit ripening. Ethylenediamine would be quickly metabolized by NHase/amidase. Cobalt is a necessary element for living organisms but it will be harmful to them at a high concentration (Komeda *et al.,* 1997). Cobalt is a critical component of NHase in *R. rhodochrous* J1. NHase can catalyze nitriles to amides by hydration and amides will become acids and ammonia. There are two kinds of NHase which can be induced by cobalt. One of them is a high molecular-mass NHase and the other is a low molecular-mass NHase. Urea is used as a co-inducer for the former one to produce acrylamide from acrylonitrile. Cyclohexanecarboxamide is used as a co-inducer for the latter one to produce nicotinamide from 3-cyanopyridine (Kobayashi *et al.,* 1992; Komeda *et al.,* 1996 a; Komeda *et al.,* 1996 b).

Komeda *et al.* (1997) found that cobalt was uptaken into *R. rhodochrous* J1 cells by NhlF transporter which was encoded by *nhlF*. Cobalt can make *R. rhodochrous* DAP 96253

more sensitive to antibiotics due to the changes of cell permeability (Tucker, 2007). From the results that we have got, it is very obvious that GCoU plate cells of *R. rhodochrous* DAP 96253 have higher abilities of degrading styrene, 1-hexene, 100 ppm ethylene in air, 100 ppm propylene in air and VOCs from whole bananas. However, it is hard to conclude which plate cells (GU or GCoU) will yield higher 1-HMO activity and keep better 1-HMO stability. It is also difficult to tell which plate cells (GU or GCoU) could work better in delayed fruit ripening. Epoxides are very unstable, so it is one shortcoming of the 24-hour monooxygenase assays we are using. It will be necessary to check activity and stability of the enzymes that are associated with epoxide degradation. We could also try increasing cobalt ion concentration or adding other kinds of heavy metal ions to compare the results. Here we used M9 minimal medium (no glucose) as the buffer of cell suspension set up for fruit test. In order to maintain 1-HMO acitivity and stability better, we could try looking for some other buffers. We set up 2+2 bananas (from two bunches) in the containers as the target fruit, still, we can add more bananas to fruither improve the statistical confidence of our research. For many years, visual analysis has been used to judge the ripening stages of bananas, but some other new methods can still be explored (testing starch and sugar contents would be very helpful, however these are destructive tests). Even though these uncertain factors exist, we can still come to an agreement that *R. rhodochrous* DAP 96253 cells (no matter grown on GU or GCoU plates) can delay fruit ripening better when the cells show higher 1-HMO activity and maintain better 1-HMO stability. This research, and related research in the laboratory, confirm that GCoU plate cells are superior at delayed ripening than GU plate cells.

It is very interesting to see that *R. rhodochrous* DAP 96253 can delay fruit ripening and the fruits can give a feedback on 1-HMO activity and stability in *R. rhodochrous* DAP

96253. 1-HMO can be considered as an indicator of the ability of the cell catalyst on delayed fruit ripening. Since Rhodococcus spp. are common to plants and agricultural soils, together with the results we have found here, the hypothesis that *Rhodococcus* have co-evolved with plants could be strengthened. It is also interesting to note that 1-HMO activity in *R. rhodochrous* DAP 96253 decreased from Day 14 after increasing till Day 11, so it could be concluded that the VOCs of bananas at late ripening stage inhibit 1-HMO activity in *R. rhodochrous* DAP 96253.

During the whole growth phase, *Rhodococcus zopfii* developed rudimentary mycelia which fragmented into irregular rod- or coccus-shaped cell units (Stoecker *et al.*, 1994). Trichloroethene (TCE) degradation in *R. rhodochrous* B-276 is highly dependent upon the growth phase (Saeki *et al.*, 1999). Rhodoccoci start the growth cycle from cocci or rods, which gradually develop into filaments which then fragment into small cells giving the appearance of rudimentary mycelium (Goodfellow, 1989). Longer filaments of *R. rhodochrous* DAP 96253 suggest a longer period of filament extension prior to fragmentation, which may affect 1-hexene absorption ability and lead to disassociation of 1-HMO components. This observation should be followed with fermentation-based studies which examine the physiology of the *Rhodococcus* cells when grown to high density in conventional stirred tank reactors.

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APPENDICES

A Manufacturers of the chemicals used in the experiments included.

Chemicals	Catalog #	Manufacturers
Nutrient Broth	234000	Becton Dickinson, Sparks, MD
Agar	281210	Becton Dickinson, Sparks, MD
Yeast Extract	288620	Becton Dickinson, Sparks, MD
Malt Extract	218630	Becton Dickinson, Sparks, MD
Urea	U16-3	Fisher Scientific, Fair Lawn, NJ
$CoCl_2 \cdot 6H_2O$	255599	Sigma-Aldrich, St Louis, MO
Glucose	G8270	Sigma-Aldrich, St. Louis, MO
Trehalose	625625	EMD Millipore, San Diego, CA
Sucrose	21938	USB, Cleveland, OH
Maltose	M5885	Sigma-Aldrich, St. Louis, MO
Fructose	F-0127	Sigma-Aldrich, St. Louis, MO
Lactose	L-3625	Sigma-Aldrich, St. Louis, MO
Galactose	216310	Becton Dickinson, Sparks, MD
Maltodextrin	419672	Sigma-Aldrich, St. Louis, MO
NaH_2PO_4	S0751	Sigma-Aldrich, St. Louis, MO
Na_2HPO_4	S9763	Sigma-Aldrich, St. Louis, MO
Tris-HCl	111846	USB, Cleveland, OH
Sodium Alginate	A-0682	Sigma-Aldrich, St. Louis, MO
PVA	363146	Sigma-Aldrich, St. Louis, MO
NBP	N14204	Sigma-Aldrich, St. Louis, MO

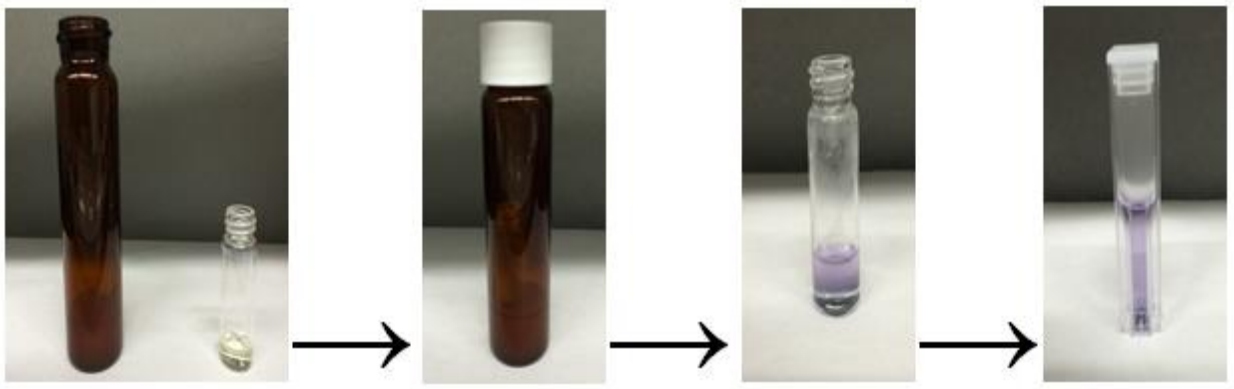
1-Hexene	320323	Sigma-Aldrich, St. Louis, MO
Styrene	S4972	Sigma-Aldrich, St. Louis, MO
1,2-Epoxyhexane	377171	Sigma-Aldrich, St. Louis, MO
Styrene Oxide	S5006	Sigma-Aldrich, St. Louis, MO
Acetone	179124	Sigma-Aldrich, St. Louis, MO
Triethylamine	T0886	Sigma-Aldrich, St. Louis, MO
Ethylene Glycol	102466	Sigma-Aldrich, St. Louis, MO
Ethyl Acetate	14133-13	Pharmco-AAPER, Shelbyville, KY
$Na_2HPO_4 \cdot 7H_2O$	S9390	Sigma-Aldrich, St. Louis, MO
KH_2PO_4	PX1562	EMD Chemicals, Gibbstown, NJ
NaCl	S271-3	Fisher Scientific, Fair Lawn, NJ
NH_4Cl	A4514	Sigma-Aldrich, St. Louis, MO
$MgSO_4$	2500-01	JT Baker, Center Valley, PA
$CaCl_2$	C1016	Sigma-Aldrich, St. Louis, MO

B M9 minimal medium (no glucose) recipe.

1. Make M9 salts: 64 g of $Na_2HPO_4 \cdot 7H_2O$, 15 g of KH_2PO_4, 2.5 g of NaCl and 5.0 g of NH_4Cl are dissolved into ddiH_2O to adjust to 1 L of solution (autoclaved to be sterile).

2. Make M9 minimal medium: 200 mL of M9 salts, 2 mL of 1M $MgSO_4$ (sterile), 100 l of 1M $CaCl_2$ are mixed with ddiH_2O (sterile) to adjust to 1 L of solution.

C Process flow of the monooxygenase assays.



D CAR/PDMS fiber in a 4.4-L polyester container with whole bananas and *R. rhodochrous* DAP 96253.

E Perkin Elmer Autosystem XL GC-FID.

F Agilent Technologies 7890A/5977A GC/MSD.