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Production of Flavors from Agro waste of Ocimumbasilicum L. by Different Microorganisms Using Solid-State Fermentation

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Abstract

Background: Nowadays, food and agricultural industries annually produce millions of tons of waste, resulting from food production and consumption. Treatments of agro-wastes have been focused on biotechnological flavor production, utilizing microbial fermentation or biotransformation. **Aim**: This research's major goal was to makenatural flavor compounds from *Ocimum basilicum* L. agro-wastes utilizing ten different microorganism strains (fungi, yeasts, and bacteria). **Materials and methods**: Flavors were produced from *O.basilicum* L. waste via microbial fermentation employing four fungal strains, five Bacilli bacterial strains, and *Saccharomyces cerevisiae* F-307 via solid-state fermentation techniques, then the volatiles were extracted by headspace and identified utilizing GC/MS. **Results:** The major component and its percentage differ between fermented and unfermented *O. basilicum* L. waste. Gas chromatography-mass spectrometry "GC/MS" analysis showed that fermentation with *T. viride*F-216 yielded (100% isoprenyl cinnamate), *B. subtilis* NRCM-62, *B. subtilis* NRCZ-144 yielded (47.87% and 79.45% isovaleric acid), *A. fumigatus* F-225, *B. subtilis* NRCR-22, *B. subtilis* NRCH-123 and *S. cerevisiae* F-307 yielded (91.13%,86.93%, 54.65%, and 41.12% bisabolene), *B. subtilis* NRCF-510 yielded (67.81% diacetyl), *A. niger* F-258 yielded (27.16% methyl pentanoate), *A. oryzae F-923* yielded (32.24% ethyl isovalerate), compared to control sample which yielded (79.17% chavibetol acetate). **Conclusion**: Using solid-state fermentation of *Ocimumbasilicum* L. agro-wastes, utilizing the ten microorganisms examined, can be applied to produce valuable and interesting natural flavors such as (Butter, Cheese, and Fruity aromas)

Keywords: Agro-waste, *Ocimumbasilicum* L., Solid-state fermentation, Biotechnology, Natural flavors

1. Introduction

The food and agricultural industries produce annually million tons of waste, resulting from the production and consumption of food, peels, pulps, aqueous residues, and others. Many of which raise serious disposal issues and, consequently, considerable costs to various industries. Therefore, using agro-wastes is the most popular aspect in biotechnological processes for producing high value-added products in terms of reducing production cost [1-2]. Recently, agro-wastes have focused on biotechnological flavor production by using microbial fermentation or biotransformation due to their high amount of reusable components for microorganisms [3-6]. Several studies were conducted on the production of natural flavor compounds from agro-wastes by microbial fermentation using various by-products such as cassava bagasse, sugar beet, beet molasses, coffee husk …….etc. [7-12].

The concept of waste as a material "which has no use" is changing to seeing waste as a resource by converting it into secondary material with modification. Wastes can thus be converted into ,valuable resources used at home or even sold for wealth. Waste recycling involves collecting discarded materials such as husks, peels, poultry droppings,

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cow dung, biomass, etc.…. and processing these materials and turning them into new products [13].

There has been an increasing trend towards more efficient utilization of agro-industrial residues, which are generated in large amounts during the processing of raw materials [14]. Most of these residues recently do not find any potential application, and their disposal in the environment causes serious pollution concerns. One possible application of these residues can be their utilization as a carbon source in bioprocesses. Several processes have been reported that utilize these residues as raw material to reduce bulk chemicals and value-added products such as ethanol, single-cell protein, edible mushrooms, enzymes, organic acids, amino acids ……. etc. The application of agro-industrial residues in bioprocesses not only provides alternative substrates, but it also helps solving their disposal problem. Many new avenues have opened for their utilization with the advent of biotechnological inventions, mainly in enzyme and fermentation technology.

Nowadays, the flavour and fragrance compounds synthesis biotechnological process plays an increasing role in the food, cosmetic, chemical, and pharmaceutical industries due to an increas growing reference of the consumer for natural food additives and other compounds of biological origin. Several reports and reviews have been published on the production of volatile compounds (aroma compounds) by microorganisms [15,16], including the production of these compounds by fermentation of simple nutrients such as sugars and amino acids. Although several bacteria, yeasts, and fungi have been reported to produce aroma compounds, a few species of yeasts and fungi have generally been referred, and only a few of these find industrial application due to their generally regarded as safe (GRAS) status.

Solid-State Fermentation (SSF)is a process carried out in a solid matrix with sufficient moisture content for microbial growth and metabolism requirements but almost no free water in the system [17]. Due to the limited amount of water, capital and operating costs are reduced because of lower working volumes per product yield and process wastewater and lower energy costs for sterilization and stirring [18-21]. Moreover, SSF of agro-industrial wastes simulates the natural environment of many microorganisms offering high productivity rates, higher product stability, and lower extent of catabolite repression [18]. Even though SSF remains a sustainable approach to producing natural aroma compounds using various agro-industrial residues. Solid-state fermentation (SSF) is considered a potential tool for utilizing agro-industrial residues for cultivating microorganisms and is deemed to be advantageous in many respects in comparison to liquid fermentation, mainly when yeasts or filamentous fungi are used in bioprocesses; the SSF would be preferred to several advantages such as higher productivity and lower pretreatment, downstream processing, and waste disposal costs. Since eliminating autoclave sterilization is expected to reduce the overall cost of the final products, Gas chromatography (GC-MS) analysis was employed to monitor changes in the composition of flavor-active compounds during the fermentation process.

Sweet basil (*Ocimumbasilicum L*.) is one of the medicinal plants most widely cultivated

in Egypt and used in flavors, fragrances, pharmaceuticals, and cosmetics. Its leaves and flowers

have been used in traditional medicine to treat kidney malfunctions, diarrhea, headaches, coughs,

and many other symptoms [22]. Based on its bioactive constituents and the cultivation environment, sweet basil is classified into four main chemotypes: methyl chavicol, linalool, methyl eugenol, and methyl cinnamate. Meanwhile, other researchers have shown the application of basil wastes in feed, composting mixtures, and biogas production [23,24,25]. According to Adel et al. [26,27], the Egyptian *Ocimumbasilicum* L. represents the methyl chavicol-linalool chemotype with higher antioxidant activity. basil wastes in a dried/ oil form as an aromatic ingredient rich in bioactive compounds and offering functional properties, that could be successfully incorporated into food products. The composition of the bioactive compounds (phenolic acids and flavonoids) and antioxidant activity were significantly higher in basil wastes (*O. basilicum* L.) [28]. Up down knowledge, there are no studies about using basil-waste as a source for SSF to produce natural flavours. Therefore, the current study aimed to prospect the protentional of basil waste as substrate for microbial flavour production by applying different strains of bacteria, fungi, and yeasts to obtain natural flavours from the waste of basil followed by Gas Chromatography-Mass spectrometry.

2. Materials and methods

2.1. Plant waste and chemicals

Basil waste *(Ocimumbasilicum* L.) leaf and stem waste was collected from El-Qalag, Qalyubiyya, Egypt. A taxonomist identified the waste at the Department of Medicinal and Aromatic Plants Research, National Research Center (Cairo, Egypt). Diethyl ether and the mixture of *n*-alkanes C_6–C_{20}, purchased from Sigma Aldrich Chemical Co. All other chemicals are analytical grade.

2.2. Microorganisms and Inoculum preparation.

Four strains of fungi, namely ***Aspergillus niger* F258**, ***Aspergillus. Fumigatus* 225. *AspergillusoryzaeF*-923** and ***Trichoderma viride* F-216** and five Bacilli bacterial strains ballooning to Bacillus subtilis, namely ***Bacillus subtilis*NRCH-123, *Bacillus subtilis* NRCR22, *Bacillus subtilis* NRCZ 144, *Bacillus subtilis* NRCF 510 and *Bacillus subtilis* NRCM 62,** as well as one yeast called ***saccharomyces cerevisiae* F-307 as listed in Table (1)** were obtained from Microbial Chemistry Lab. National Research Center, Dokki, Cairo. Egypt. The inoculum was made from 5 days old potato dextrose agar (PDA) cultures. The inoculum (containing 106-107 spores) was suspended in Yeast extract, peptone, Malte (YPM) broth. The suspension, when necessary, was diluted with sterile water to give a spore count within the predetermined range. One ml of inoculums was added to each cooled sterilized (autoclaved at 121ºC for 20 minutes and 15 psi.) in 250 capacity conical flasks.

Table (1) Symbol of various strains used in flavour production by SSF

No	Microorganisms
Control	-
T1	Aspergillus.fumigatusF-225
T2	Trichoderma viride F-216
T3	Aspergillus.oryzaeF-923
T4	Aspergillus niger f-258
T5	Bacillus subtilis NRCR-22
T6	Bacillus subtilis NRCH-123
T7	Bacillus subtilis NRCF -510
T8	Bacillus subtilis NRCM-62
T9	Bacillus subtilis NRCZ-144
T10	Saccharomyces cerevisiae F-307

2.3. Fermentation of Basil waste

The fermentation was carried out under solid-state fermentation in 250 mL Erlenmeyer flasks containing 5 g of basil waste moistened to 50% with distilled water and incubated with 1 mL spore suspension (106 spores). The cultures were incubated at 30 °C for three days for solid-state fermentation.

2.4. Flavour compounds extraction.

2.4.1. Isolation of Headspace Volatiles:

The volatiles in the headspace of each sample under investigation were isolated by using a dynamic headspace system. The samples were purged for 3h with nitrogen gas (grade of N2<99.99) at 100 ml/min flow rate. The headspace volatiles were swept into cold traps containing diethyl ether and held at -10°C. The solvents containing the volatiles were dried over anhydrous sodium sulfate for 1 h. The volatiles were obtained by evaporating the solvents and concentrated with rotary under 40°C to a final volume of 10 µl under reduced pressure [29].

2.5. Gas chromatography-mass spectrometry (GC – MS) analysis

A gas chromatography (Hewlett–Packard model 5890) coupled to a mass spectrometer (Hewlett–Packard-MS (5970) was used for analysis. Volatiles were separated using a fused silica capillary column DB-5 (60 m × 0.32 mm i.d. × 0.25 µm film thickness). The oven temperature was maintained initially at 50ºC for 5min, and then programmed from 50 to 250ºC at a rate of 4º C /min. Helium was used as the carrier gas, at flowrate of 1.1 ml/min. The sample size was 2 µl, split ration 1:10, the injector temperature was 220ºC. Mass spectra in the electron impact mode (EI) were obtained at 70 eV, and scan m/z range from 39 to 400 amu. The retention indices (Kovats index) of the separated volatile components were calculated with reference to the retention time of a series of alkanes (C6–C20) as external standard run at the same conditions. The isolated peaks were identified by matching with data from the mass spectra library (National Institute of Standard and Technology, NIST) and comparison with those of authentic compounds and published data [30].

3. Results and Discussion

3.1. Effect of solid-state fermentation using fungi, bacteria, and yeast on volatile components of postharvest waste of *Ocimumbasilicum* L.

A total of 90 volatile compounds were identified in the headspace of *Ocimumbasilicum* L. in all samples under investigation. All these compounds are listed with their area percentage in **Table (2),** and the typical gas chromatograms are given in (**Figures. 1-4**)

Table (2) Volatile components isolated in headspace of control and fermented samples of basil waste by Different Microorganisms Using Solid-State Fermentation

Volatile Compounds	KI[a]	Control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Identification Method [b]
Not Identified	≥570	-	-	-	12.44	-	-		-	-	-	-	MS&KI
Diacetyl	572	1.6	-	-	2.48	-	-	-	67.81	2.08	-	-	MS&KI
ethyl ether	589	2.44	-	-	-	-	-	0.29	15.44	24.55	1.34	-	MS&KI
ethyl acetate	591	-	-	-	23.16	-	-	-	-	6.11	-	-	MS&KI
2-Methylfuran	628	-	-	-	-	-	-	-	-	0.97	-	-	MS&KI
Methyl propanoate	634	-	-	-	-	0.91	-	-	4.06	-	-	0.81	MS&KI
Isobutanol	661	-	-	-	-	-	-	0.81	-	-	-	-	MS&KI
Not Identified	662	-	-	-	-	-	-	-	1.02	-	-	-	MS&KI
3-methylButen-1-ol	724	-	-	-	0.56		0.22	-	-	0.69	1.54	-	MS&KI
4-Methyl-2-pentanone	726	0.87	-	-	-	-	-	-	-	0.4	-	-	MS&KI
Norbornene	729	-	-	-	-	-	-	-	-	-	-	0.74	MS&KI
Dimethyldisulfide	753	-	-	-	0.2	-	-	-	-	0.52	0.61	-	MS&KI
Butanoic acid	772	-	-	-	-		-	0.42	-		-	-	MS&KI
Butyl acetate	809	0.71	-	-	-	19.67	-		-	8.57	5.91	-	MS&KI
MethylPyrazine	818	1.01	-	-	-	22.26	-	5.78	-		-	-	MS&KI
Methyl pentanoate	٨٢١		-	-	-	27.16	-	-	-		-	-	MS&KI
Isovaleric acid	826	-	-	-	-	-	-	-	-	47.87	79.45	-	MS&KI
Furfural	829	-	-	-	-	-	-	-	-	2.27	3.74	-	MS&KI
2-Methylthiazol	838	-	-	-	2.58	-	-	0.48	-	-	-	-	MS&KI
Ethyl isovalerate	848	-	-	-	32.24	-	-	-	-	-	-	0.37	MS&KI
(3Z)-Hexenol	851	-	-	-	2.05	-	-	-	-	-	-		MS&KI
2-Furanethanol	866	-	-	-	-	-	-	1.33	-	-	-	-	MS&KI
2-Methylbutyl acetate	875	-	-	-	-	-	-	0.59	-	-	-	-	MS&KI
2,5-Dimethylpyrazine	902	-	-	-	-	-	-	2.38	-	-	-	-	MS&KI
Ethyl2-methyl-4-pentenoate	926	-	-	-	-	-	-	0.56	-	-	-	3.25	MS&KI
Menthene	985	-	-	-	-	-	-	0.63	-	-	-	-	MS&KI
2-ethyl-3-methyl Pyrazine	1002	-	-	-	-	-	-	2.04	-	-	-	-	MS&KI
Furan2-acetyl-5-methyl-,	1031	-	-	-	-	-	-	0.55	-	-	-	-	MS&KI
Ocimene	1033	0.76	-	-	-	-	-	-	-	-	-	-	MS&KI

2-Hydroxy-3-methyl-2-cyclopentenone	1035	-	0.36	-	-	-	1.13	1.31	-	-	-	1.33	MS&KI
Methyl-cyclohexenone	1046	-	-	-	-	-	-	1.75	-	-	-	0.16	MS&KI
2-butyl-Thiophene	1058	-	-	-	-	-	-	0.5	-	-	-	-	MS&KI
Octenol	1065	-	-	-	-	-	-	0.65	-	-	-	0.05	MS&KI
Vertocitral	1076	-	-	-			0.37		-	-	-	-	MS&KI
Nonanal	1102	-	-	-	-	-	-	2.83	-	-	-	-	MS&KI
trans-Vertocitral C	1105	-	-	-	-	-	-	0.61	-	-	-	-	MS&KI
Terpineol	1130	-	-	-	-	-	-	0.43	-	-	-	-	MS&KI
Lavandulol	1166	-	-	-	-	-	-	0.34	-	-	-	-	MS&KI
Dihydro carvone	1191	0.58	-	-	-	-	-		-	-	-	-	MS&KI
Decanol	1198	-	-	-	-	-	-	0.86	-	-	-	-	MS&KI
Hexadienolisobutanoate	1203	-	-	-	-	-	-	3.96	-	-	-	-	MS&KI
Carveol	1216	-	-	-	-	-	-	0.98	-	-	-	-	MS&KI
Carvacrol	1245	-	-	-	-	-	-	0.48	-	-	-	-	MS&KI
Hexenyl angelate	1275	-	-	-	-	-	-	1.02	-		-	-	MS&KI
Nonan	1277	0.69	-	-	-	-	-	-	-		-	-	MS&KI
Vanillin	1302	-	-	-	-	-	-	2.21	-		-	-	MS&KI
Isoamyl benzyl ether	1310	-	-	-	-	-	-	0.64	-		-	-	MS&KI
Decadienal (2E,4E)	1315	-	-	-	-	-	-		-	0.92	1.51	-	MS&KI
Jasmonol	1328	-	0.33	-	-	-	0.32	0.92	-	-	-	18.67	MS&KI
Eugenol	1350	-	-	-	-	-	-	-	-	-	-	27.55	MS&KI
Anisaldehyde	1364	-	-	-	-	-	-	-	-	0.62	-	-	MS&KI
Carvacrol acetate	1370	-	-	-	-	-	-	0.57	-	-	-	0.13	MS&KI
Ethyl cinnamate	1377	-	0.32	-	-	-	-	-	-	-	-		MS&KI
γ-Terpinen	1391	1.32	-	-	-	-	-	-	-	-	-	-	MS&KI
Dodecanal	1402	-	-	-	-	-	-	0.63	-	-	-	-	MS&KI
Curcumene	1480	-	-	-	-	-	2.05	1.75	-	-	-	-	MS&KI
Citronellol isobutanoate	1482	-	1.94	-	-	-	-	-	-	-	-	-	MS&KI
Germacrene D	1484	-		-	-	-	-	-	-	-	-	1.14	MS&KI
Nerylisobutanoate	1490	-	0.94	-	-	-	0.83	0.62	-	-	-		MS&KI
Farnesene	1506	-	-	-	-	-	-	0.45	-	-	-	0.22	MS&KI
Myristicin	1518	-	-	-	-	-	-	-	-	-	-	0.1	MS&KI

Chavibetol acetate	1525	79.17	-	-	-	-	-	-	-	-	-	-	MS&KI
ϒ-Bisabolene,	1529	-	91.13	-	-	-	86.93	54.65	-	-	-	41.12	MS&KI
α-Cadinene	1537	-		-	-	-		0.89	-	-	-		MS&KI
Furopelargone A	1538	-	0.41	-	-	-			-	-	-		MS&KI
Copaen-11-ol	1540	-	-	-	-	-			-	-	-	0.58	MS&KI
Santalenone	1576	-	0.5	-	-	-	-	-	-	-	-	0.15	MS&KI
Apofarnesol	1591	0.81	-	-	-	-	-		-	-	-	-	MS&KI
α-Amylcinnamyl alcohol	1683	-	-	-	-	16.87	-	-	-	-	-	-	MS&KI
(2Z,6Z)- Farnesal	1684	-	-	-	-	-	2.37	-	-	-	-	-	MS&KI
α-trans-Bergamotol	1691	-	-	-	-	-	0.55	-	-	-	-	-	MS&KI
Apodophylene	1707	-	-	-	-	3.13		-	-	-	-	-	MS&KI
Niranin	1716	-	-	-	-		0.48	-	-	-	-	-	MS&KI
Elemodiol	1747	-	-	-	-	-	-	1.27			-	-	MS&KI
Cinnamaldehyde	1774	0.66	-	-	-	-	-	-	-	-	-		MS&KI
α-Eudesmol acetate	1794	3.34	-	-	-	-	-	-	-	-	-		MS&KI
Cadinene	1803	-	-	-			0.39	0.34	-	-	-		MS&KI
Cembrene	1935	-	0.26	-	0.25	1.08	0.67	0.21		0.37	0.62	0.21	MS&KI
Cembrene A	1966	2.7	-	-		-	-	-		-	-	-	MS&KI
Isoprenyl cinnamate	1970	-	-	100	1.35	4.15	3.42	-	7.61	-	-	-	MS&KI
Dolabradiene	1973	-	1.88	-	-		-	-	-	-	-	-	MS&KI
Ferula lactone I	1974	-	-	-	-	2.85	-	2.3	-	3.51	5.27	-	MS&KI
Occidol acetate	1975	-	-	-	-		-	-	-	-	-	2.86	MS&KI
Pseudo phytol	1989	-	-	-	-	1.92	-	-	-	-	-	-	MS&KI
4-hydroxy-Stilbene	2043	2.28	-	-	-	-	-	-	-	-	-	-	MS&KI
Benzyl cinnamat	2091	0.52	-	-	-	-	-	-	-	-	-	-	MS&KI
Heneicosane	2106	-	-	-	4.83	-	-	-	-	-	-	-	MS&KI
Isoprenyloxycoumarin	2118	-	-	-	16.4	-	-	-	-	-	-	-	MS&KI
Decyl anthranilate	2145	-	-	-	-	-	-	-	3.51	-	-	-	MS&KI
Incensole	2162	-	-	-	0.41	-	-	-	-	-	-	-	MS&KI
Total		99.739	98.07	100	98.95	100	99.73	99.03	99.45	99.729	99.99	99.44	MS&KI

:**KI**: Linear Kovat indices: Compounds listed according to their elution on DB-5 column.

ь: compounds identified by GC-Ms (MS) by comparison of MS and KI of standard compounds run under similar GCMS conditions, (-)-: not detected

The analysis of the control sample (unfermented) basil consisted mainly of 16 volatile compounds**.** The main volatile compounds wereChavibetol acetate (79.17%) followed by α-Eudesmol acetate (3.34%), CembreneA (2.7%), and 4-hydroxy Stilbene (2.28%)and ϒ-terpinene(1.32%). The major compounds in the HD oil were estragole (64.47%), β-linalool (16.88%), anethole (8.67%), and methyl cinnamate (3.48%), Adel et al. (2019), [26]. These findings contrast with our reports studied on headspace extraction. Due to the known drawbacks of the hydrodistillation technique, like losses and degradation of some volatile compounds, due to long extraction times under thermal or hydrolytic effects.

Gas-Chromatography–mass spectrometry analysis of basil fermented with (***Aspergillus.fumigatusF-225)*** consists of ten volatile compounds. The volatile profile consisted mainly ofϒ-Bisabolene (91.13%) followed by Citronellol isobutanoat (1.94%) and Dolabradiene (1.88%). GC-MS identified one Compound as volatiles of *Ocimumbasilicum* L., waste fermented with (***Trichoderma viride***), it was Isoprenyl cinnamate (100%). Fermentation with (***Aspergillus.oryza*e F-923**) yielded 13 volatile compounds. The volatile profile consisted mainly of Ethyl isovalerate (32.24%)followed by ethyl acetate(23.16%) and Isoprenyloxycoumarin (16.41%).A10 volatile compounds were identified in fermentation with (***Aspergillus niger f-258***). The volatile profile consisted mainly of Methyl pentanoate(27.16%)followed by methyl-Pyrazine (22.26%), Butyl acetate (19.67%), and Amyl cinnamyl alcohol(16.87%). A thirteen volatile compounds were identified in fermentation with ***(Bacillus subtilis* NRCR-22**) consisted mainly ofϒ-Bisabolene (86.93%)followed by isoprenyl cinnamate (3.42%), Farnesal (2.37%) and Curcumene (2.05%), whereas fermentation with (***Bacillus subtilis* NRCH-123**) yielded 39 volatile compounds The volatile profile consisted mainly ofϒ-Bisabolene (54.65 %) followed by Methyl pentanoate (5.78%) and 2,5-Dimethylpyrazine (2.38%). In the case of fermentation with (***Bacillus subtilis* NRCF-510**), sex volatile compounds were identified. The volatile profile consisted mainly of diacetyl (67.81%) followed by Isoprenylcinnamate (7.61 %) and Methyl propanoate (4.06%), whereas in the case of (***Bacillus subtilis* NRCM-62**) 14 volatile compounds were identified; mainly of isovaleric acid (47.87%) followed by ethyl acetate (6.11%) and Butyl acetate (8.57%)**,** but fermentation with (***Bacillus subtilis* NRCZ-144)** consists of nine volatile compounds mainly of isovaleric acid (79.45%) followed by butyl acetate (5.91%) and ferula lactone I (5.27%)**.** Then finally, Gas chromatography-mass spectrometry analysis of basil fermented with (saccharomyces cerevisiae F-307) consists of eighteen volatile compounds. The volatile profile consisted mainly ofϒ-Bisabolene (41.12%)followed by Eugenol (27.55%) and Jasmonol (18.67%).

From the above results, we found that a high percentage for the production of bisabolene by fermentation with ***A. fumigatus*** F-225(91.13%), while fermentation with ***T. viride***F-216 yielded (100% isoprenyl cinnamate), but in the case of fermentation with ***B. subtilis*** NRCZ-144 yielded (79.45% isovaleric acid), ***B. subtilis*** NRCF-510 yielded (67.81% diacetyl), ***A. niger*** F-258 yielded (27.16% methyl pentanoate), ***A. oryzae F-923*** yielded (32.24% ethyl isovalerate), compared to control sample which yielded (79.17% chavibetol acetate).s

The natural plant product Bisabolene serves as a precursor to producing a wide range of industrially relevant chemicals. Bisabolene (C15H24) is the simplest monocyclic sesquiterpene and a bioactive compound in natural plant essential oils. It has three structural isomers, namely α-bisabolene, β-bisabolene, and γ- bisabolene, and each isomer has distinctly different properties and applications. The plant terpenoid bisabolene has a wide range of applications in cosmetic, chemical, pharmaceutical, and nutraceutical industries [31, 32]. Traditionally, bisabolene is extensively used as a high-value fragrance and flavour compound in many industries because bisabolene has delightful fruity and balsamic aroma [33], thus can be used as a food flavoring.

Furthermore, bisabolene is being investigated as anti-inflammatory and anti-cancer agents and thus would greatly benefit the medical community [34]. In addition, bisabolene could also serve as an essential starting material for synthesizing various commercially valuable products [31, 32]. At present, the industrial production of bisabolene is mainly achieved by direct extraction from plant tissues. However, this method has many disadvantages, such as limited raw material source, low yield of the product, and complicated separation steps [35]. Likewise, chemical syntheses of bisabolene suffer from the complexity of the production equipment and the low conversion rate of

raw materials [36]. These processes are also energy-intensive and can cause environmental issues. As a result, there is an ever-increasing demand to develop an alternative and renewable route to bisabolene. Among the alternative approaches, biosynthesizing bisabolene in the microbial cell. Several studies have successfully engineered S. cerevisiae for sesquiterpene production [37], which is following our research.

Diacetyl is mainly related to butter flavour. Therefore, is extensively used in the imitation of butter and other dairy flavors and whenever butter notes are desirable in food. Lactic acid bacteria and other microorganisms produce this compound in several foods (e.g., *Lactococcus lactis, Lactobacillus sp., Streptococcus thermophilus, Leuconostocmesenteroides*). The production of dairy flavour compounds, such as butyric acid, lactic acid, and diacetyl in mixed cultures of lactic acid bacteria growing in starch-based media, has been reported [38]. But also, we found that fermentation of *Ocimumbasilicum* L. using ***B. subtilis*** NRCF-510 yielded diacetyl.

Zhuhoujiang is a popular fermented soybean paste in China. The aroma profiles of Zhuhoujiang were systemically analyzed by gas chromatography–olfactometry/mass spectrometry (GC /MS). A total of 100 aroma compounds were detected, and 24 compounds were identified as the important aroma compounds by odor intensities and odor-activity values[39]. Ethyl isovalerate, ethyl isobutyrate, linolenic acid, and eucalyptol were demonstrated to be the key aroma compounds of Zhuhoujiang by omission tests. have been detected in samples with significant higher aroma intensities than the other ethyl esters. Ethyl isobutyrate and ethyl isovalerate were also reported as the major aroma compounds in Chinese horse bean-chili-paste [40].

3-Methylbutyric acid (actual isovaleric acid) and 2-methyl butyric acid are volatile branched-chain acids involved in Swiss cheese flavour [41]. The presence of isovaleric acid is related to the intensity and aromatic richness of several kinds of cheese. The concentration of isovaleric acid in Swiss cheeses varies over a large range. In many of the available reports, the term ‘‘isovaleric acid’’ referred to the mixture of 3-methyl-butyric acid and 2-methyl-butyric acid, which were not separated by the chromatographic method used to analyze volatile acids. The term ‘‘methyl butyric acids’’ was preferred to refer to the mixture of both acids. 3-Methylbutyric acid and 2-methyl butyric acid originate from leucine and isoleucine catabolism, respectively, via the first step of transamination followed by oxidation step. The microflora that produces methyl butyric acids in Swiss cheese has never been completely clarified. Most Swiss cheese ecosystem species can produce methylbutyric acids in vitro, as recently shown using resting cells of different species incubated in the presence of leucine and a-ketoglutarate: Lactobacillus helveticus, Lactobacillusdelbrueckii subsp. lactis [42].

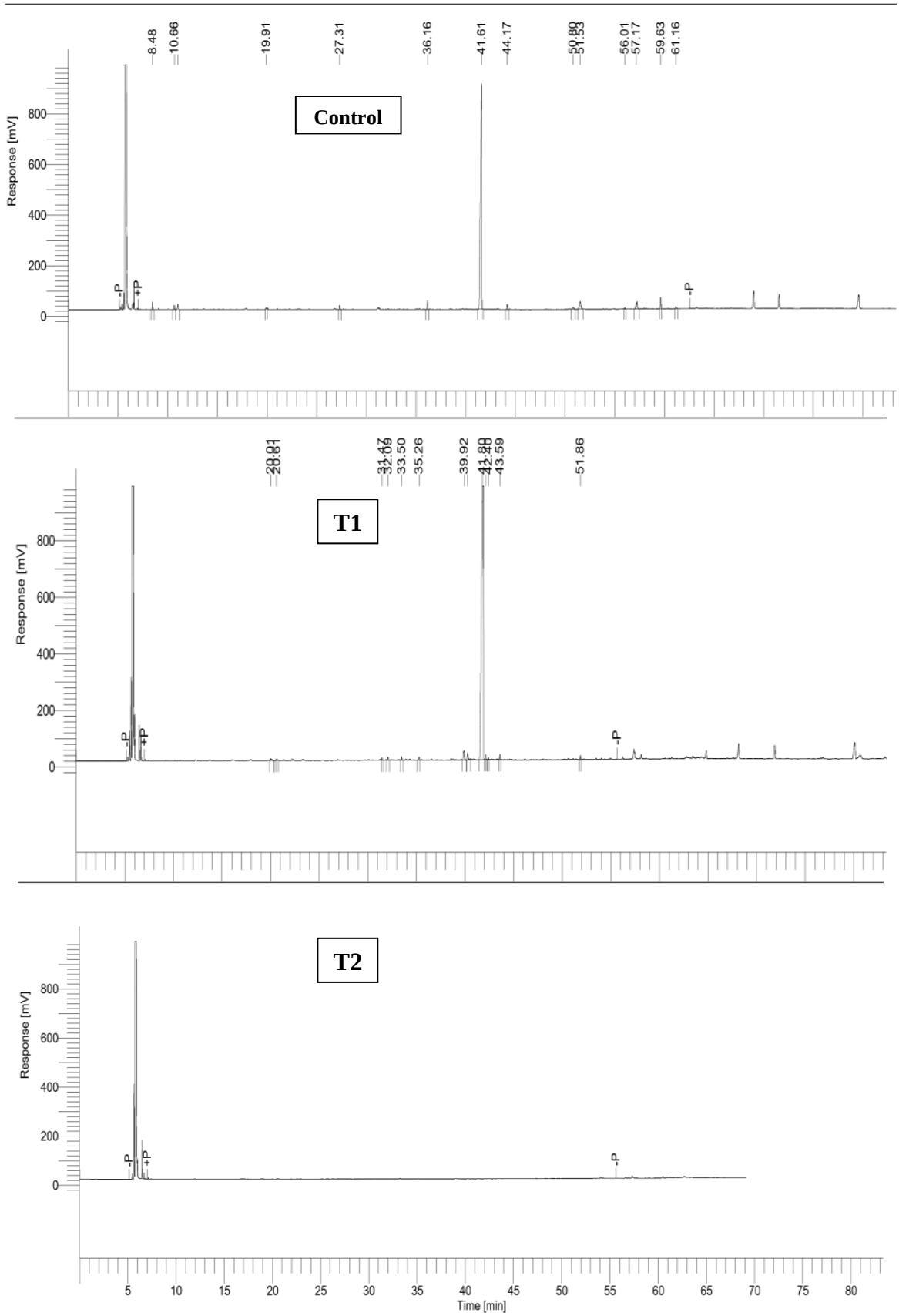
Fermentation is one of the most developed biotechnology. In general, it is done on plant-based food as a substrate [43]. However, in the last decade, the attention to fermentation technology has led to improved quality of herbal medicines through the production and improvement of bioactive metabolites essential for the medication [44]. For example, fermentation of Chinese herbal medicines using *Aspergillus oryzae* [45] and ginseng using *Bacillus, Lactobacillus, dan Pediococcus* [46] can increase total phenolic content and antioxidant activity.

A. niger is one of the most prominent enzyme-producing microorganisms produced from its metabolic processes [47]. Many studies have used A. niger as a strain of microorganisms used to increase the capacity of bioactive compounds [48-50].

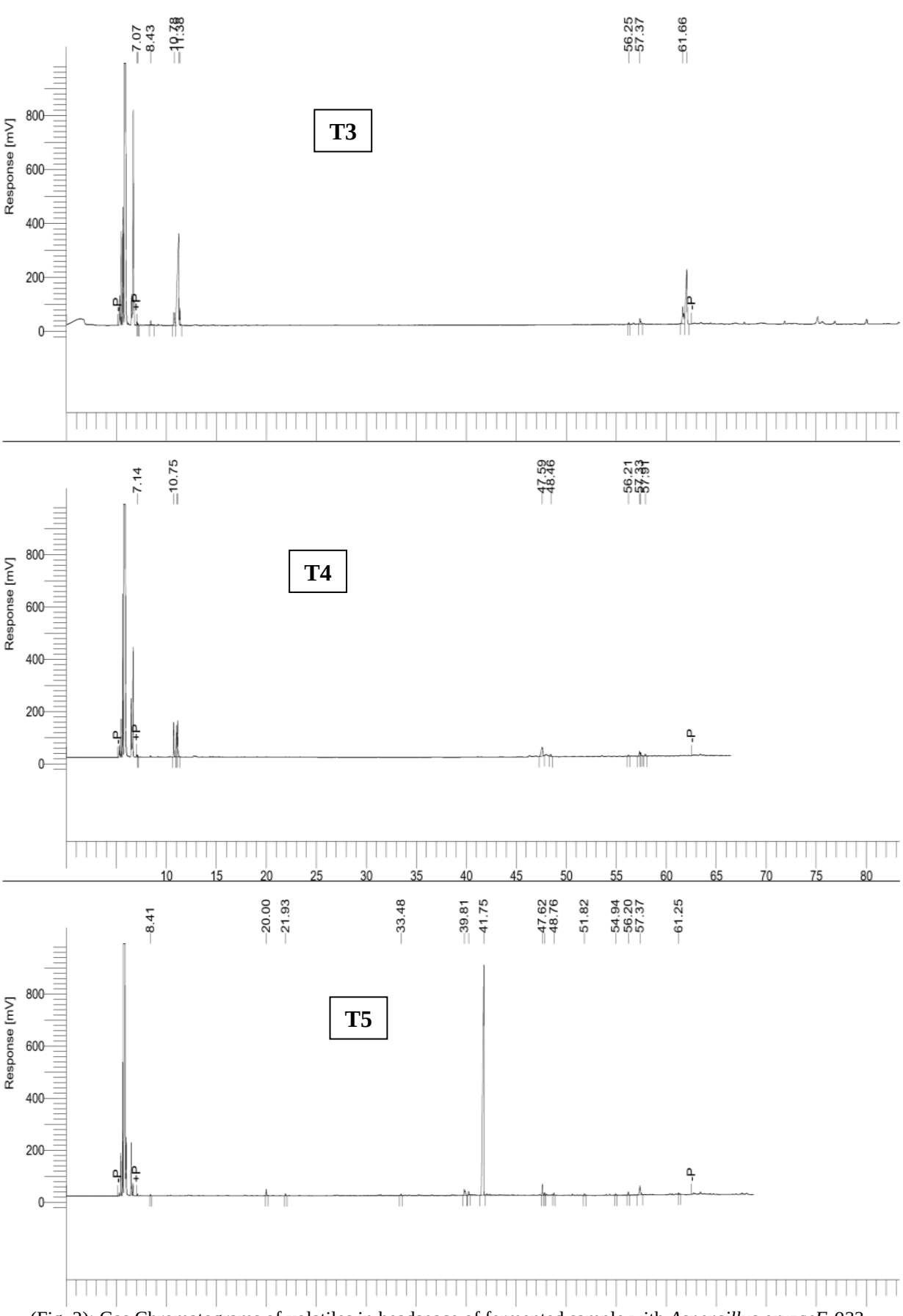
SSF was found to be very suitable for the production of pyrazines. [51-52] studied the biosynthesis of 2,5-dimethyl pyrazine (2,5-DMP) and tetramethylpyrazine (TMP) using SSF cultures of Bacillus subtilis on soybeans.

4. Conclusion Tables and figures

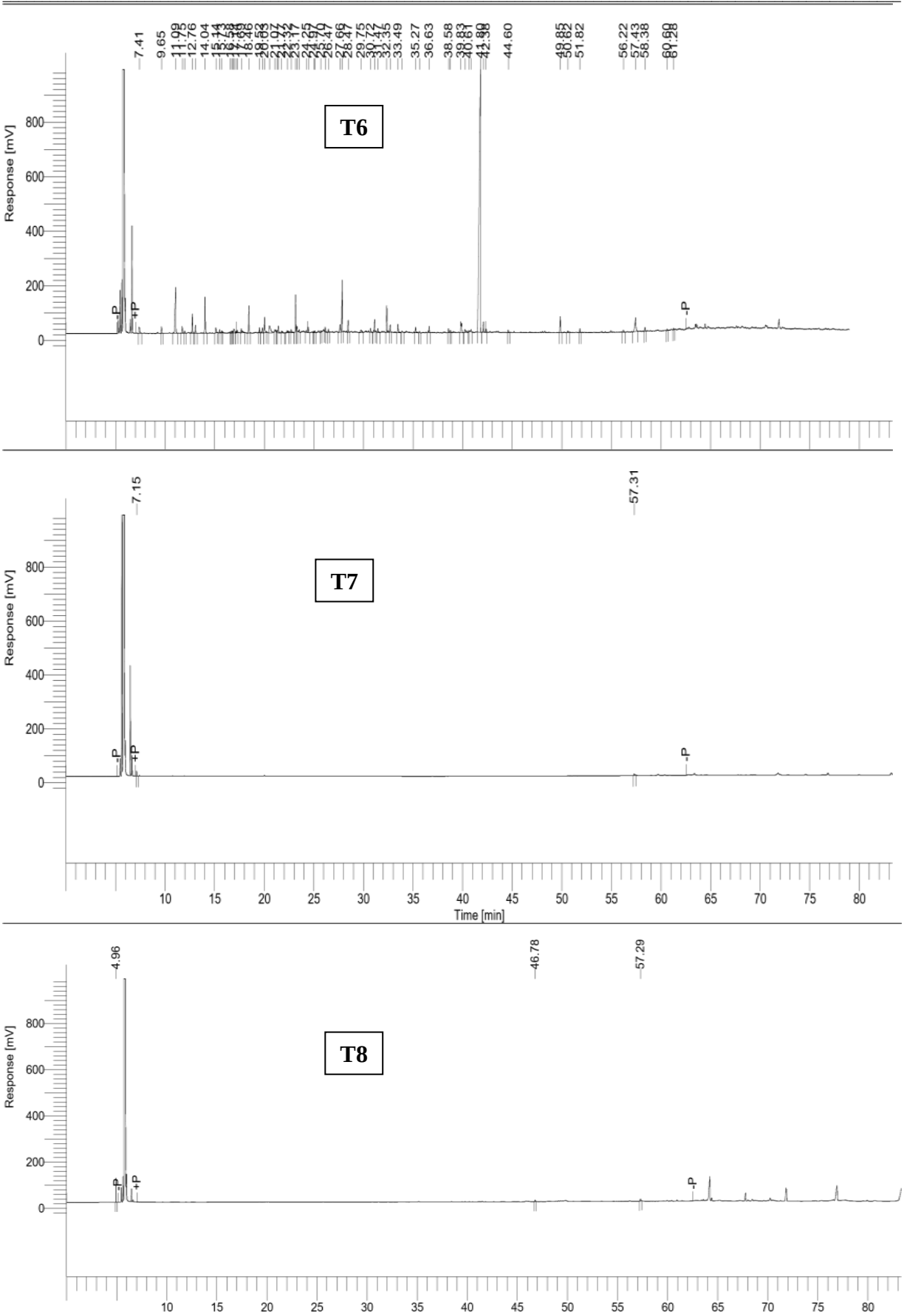
The results of this study point out for the first-time using Solid State Fermentation (SSF) as an effective biotechnology method of *Ocimumbasilicum* L. waste to produce natural flavour (butter, cheese, and fruity aroma) by using ten microorganisms including Four strains of fungi (T1-T4) and five Bacilli bacterial strains,(T5-T9) as well as *saccharomyces cerevisiae* F-307,(T10). and its high industrial importance



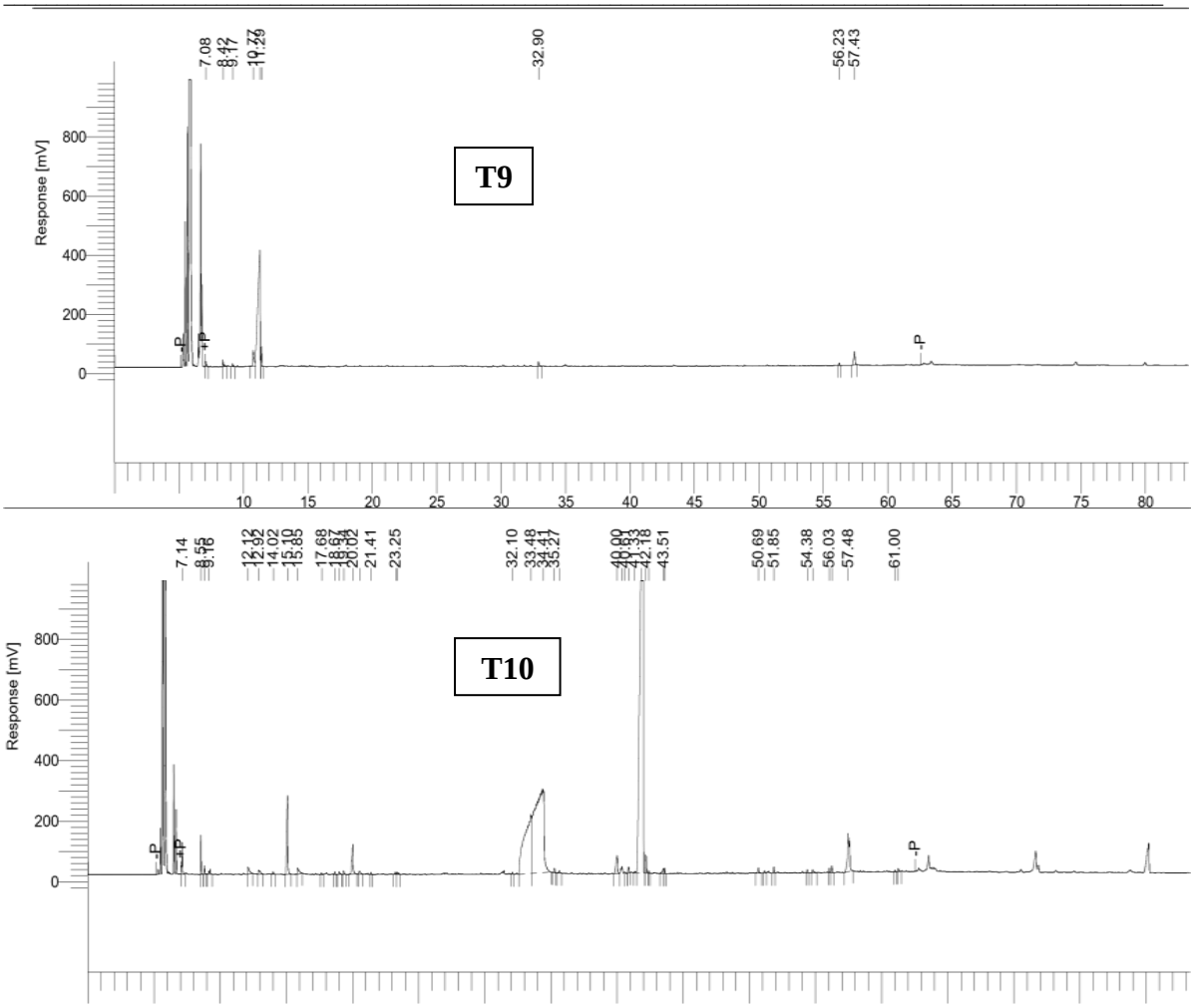
(F. 1 (Fig.1):Gas Chromatograms of volatiles in headspace of control, fermented sample with *Aspergillus.fumigatus*F-225 and *Trichoderma viride* F-216



(Fig. 2): Gas Chromatograms of volatiles in headspace of fermented sample with *Aspergillus.oryzaeF-923, Aspergillus niger f-258* and *Bacillus subtilis NRCR-22*



(Fig. 3): Gas Chromatograms of volatiles in Headspace of fermented sample with *Bacillus subtilis* NRCH-123 , *Bacillus subtilis* NRCF -510 and *Bacillus subtilis* NRCM-62



(Fig. 4): Gas Chromatograms of volatiles in headspace of fermented sample with *Bacillussubtilis*NRCZ-144 and *saccharomyces cerevisiae* F-307

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