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Research Paper

Microbial Production from Fruit and Floral Waste: A Review

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ABSTRACT:
The citrus peels contain cyclic monoterpene limonene. Limonene is the major component in the oil of citruspeel. In orange oil greater than 90% D-limonene are present. D-limonene extract by the distillation methodfromorangepeel(AmanPandayetal.,2015).AccordingtoRivasetal.,theorangepeelisinfactconstitutedbysolublesugars-16.9%,starch-3.75%,fiber(cellulose-9.21%,hemicelluloses-10.5%,lignin-0.84%andpectin-42.5%), ashes-3.50%,fats-1.95 % andproteins-6.50 % (Rivas et al., 2008).Citric acid is an intermediate of thetricarboxylic acid cycle (TCA). The first patent for citric acid production by Aspergillus niger utilising sugarsolutions was reported in 1913 (Tornado et al., 2011).Orange peels contains soluble sugars and pectin as themain components. According to Rivas et al. (2006).The large amount of this waste is still dumped every year,which causes both economic and environmental problems such as high transportation cost, lack of dumpingsite,andaccumulationofhighorganiccontentmaterial.Therefore,moreeffectiveandsustainablealternativesforusingorangepeelwastes(Wikandarietal.,2015;GrohmannandBaldwin,1992; Marınetal.,2007).
Rose is well known plant for aroma production and has number of industrial uses.2-phenylalcohols are one ofthemostimportantaromaticalcoholstogiveroselikeAromainfoodproducts.Thehigheralcoholsareproducedby microbes during fermentation from α-keto acids, by degrading amino acid via "Ehrlich pathway" (Styger etal., 2011).Natural flavours are important quality molecules in a wide range of products like food, cosmetics,beveragesandpharmaceuticals.Nowadays,mostofthemoriginatefromchemicalsynthesisorextractionfrom plants (Carlquist et al., 2014). The 2-phenylethanol (2-PE) aromatic alcohol, with a global production equalingabout 10000 tons per year (Hua and Xu, 2011), is one of the most significant volatile substances.Naturalflavours are important quality molecules in a wide range of products like food, cosmetics, beverages andpharmaceuticals.Nowadays,mostofthemoriginatefromchemicalsynthesisorextractionfromplants(Carlquist et al., 2014). The 2-phenylethanol (2-PE) aromatic alcohol, with a global production equaling about10000tons peryear (Huaand Xu,2011),is oneofthemostsignificantvolatilesubstances.
Key Words: *Citrus,Orangepeels,Limonene,2-phenylethanol,Microbes,Aspergillusniger, Fermentation,CitricAcid,Aroma,Alcohol.*

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I. INTRODUCTION:

Orange fruit contain 70% to 90% water concentration. Orange like citrus fruits are excellent source of vitaminC, A and B, fiber (pectin, cellulose, hemicelluloses& lignin.), minerals (likepotassium and calcium) anti-oxidantcompoundslikephenolicandcarotenoids(Arnarson,2019).Citrusprocessingindustrygeneratestoneofwasteannually (salma and Ibrahim, 2018). India Produce 25 lakh tonnes of orange every year. Orange peel is aprimary waste in production of orange juice so the waste is accumulated in bulk and make environmentalproblems. (Gotmare and Gade, 2018) The concentrations of total aldehydes increasewith fruit maturity butdeclines with over-maturity.Citric acid is one of the most important organic acids. **T**he citrus peels containcyclicmonoterpenelimonene.Limoneneis themajorcomponentin theoilofcitruspeel.Inorangeoilgreater than 90% D-limonene are present. The D-limonene, occurring common in nature as the fragrance of orange,useasaflavoring agentinfoodmanufacturing,incosmetic,andinpharmaceuticaltheycanhidetheunpleasant tastesofdrug.Mantzouridouetal.(2015)usedSaccharomycescerevisiaeforsolidstatefermentation of orange peel waste to produce yeast flavour and other valuable chemicals. They obtainedmaximum volatile aroma esters and total polyphenols, respectively, from fermented orange peel. The largeamount of this waste is still dumped every year, which causes both economic and environmental problemssuch as high transportation cost, lack of dumping site, and accumulation of high organic content material.Therefore, more effective and sustainable alternatives for using orange peel wastes(Wikandariet al., 2015;Grohmann and Baldwin, 1992; Marınet al., 2007).Rose is well known plant for aroma production and hasnumberofindustrialuses.Roseshavemanyindustrialusesalongwithitsantimicrobialproperties.Mostlyrosesare used in cosmetic products. Rose has sweet, pleasurable smell, which is favourable by a lot of people mostof the people prefer the smell of rose has most recognised and popular aroma in the world. Essential oil ofrose is quite expensive, but has major uses in pharmaceutical and medical. It is also used to cure chronicinsomnia (Jane, 2015)Quality of sleep is important during pregnancy and can be improved by changing sleepenvironment both internally and externally. Rose petals, stem, route, leaves all of them have their ownproperties and are useful in many ways. Many microorganisms are found from rose plant; they are known asendophytic organisms.Rose plant has been studied withrespect to its endophyticfungi characteristics.(Catalina et al., 2007). There are many other products that can be produced by rose, such as dye, cosmetics,essentialoils,perfumes,jam,aromaticproducts,andmedicines.Inmanyfermentedfoodproducts,2phenyl ethanol and 2-phenyl acetate are responsible for rose like smell. 2-phenylethanol (2-PE) has highestindustrialvalueforitsroselikesmellwhichismostlyusedincosmeticindustries.Somemicrobialtransformationprocessesarealsoabletoproduce2-PEbutitsyieldislimitedduetostressoforganicsolventson microorganisms. 2- PE can naturally obtain from flowers like rose, but the extraction method is costly.Market price of naturally produced 2-PE is 1000$/kg whereas, chemically production of 2-PE cost 5$/kg,therefor2-PEbio-productionhasgetmoreattention(Xinyaoetal.,2016).Therearebundleofmicroorganismsare present which can produce 2-PE, such as bacteria, Fungi, and yeast (Masuo et al., 2015 and Gathins et al.,2015).

II. PROPOSEDMETHODOLOGY

CHEMISTRYOFORANGEPEELS:

Orange Peels are receiving great attention for their high concentration of limonene. This compound is a cyclicmonoterpene(C10H16),presentingtwodifferentopticalisomers:Limonene&L-limonene.Anin-situproduction of enzymes for recovery of oil from the orange peels is very attractive.The methods employ eithersubmerged fermentation or solid-state fermentation (SSF), the latter being more common. In this study, solidstate fermentation of fresh orange peels by *Aspergillus* sp. to extract extra-cellular enzymes and orange peeloilarenotedandtheeffectofmethodsoforangepeelsterilization,importanceofselectionofthefermentationstrain,externalnitrogensource,andparticlesizeofthepeelsarealsostudied(Labrath&Gaikar,2020).

CHEMISTRYOFROSEPETALS:

In1913,itwasobtainedthefirstpatentintheUnitedStatesforamethodofproducingcitricacidbyAspergillusniger in sugar solutions (Soccol et al., 2006).2-Phenylethanol(2-PE) is an important flavor and fragrancecompoundwitharose-likeodour.Thenatural2-phenylethanolismainlyextractedfromrosepetalsthatinvolveahigh-cost process.Specificstrainsofyeastlike*Saccharomycescerevisiae,Hansenulaanomala*arealsocapableofproducingaromacompoundsbybioconversionof2-phenylalanineinto2-phenylethanol(Starketal.,2002).

SUBSTRATE:

Sweet oranges and red roses were used as a sample for the isolation of microorganisms, which was collectedfromthedifferentregions.Yeastwasisolatedfromslightlyrottensweetorangepeelswhichwasleftintheairtight plasticbagfortwodaysandthenweresoakedinsterilediStilledwaterfor3hoursbeforeusedasprimarysample.Fungiwasisolatedfrom rosepetalswhichwasleftintheopenfortwodays and then soaked in sterile distilled water for another 3 hours. orange presswasobtained from a citrusprocessingfruitusingFMCjuiceextractors(FMCCorporation, Floridadivision,LakeladFL,USA).Theliquorwascentrifugedat2100gmfor10minandthesupernatantwas usedasthefermentationmedium.

CULTURES:

Orangepeelandrosepetalswerecutintosmallpiecesusingsterilescalpelandplacedintodistilledwaterseparatelyforthreehours.Afterserialdilutionofsample,it wasthenspreadontodifferentagarplatessuchasGYEagarplateforyeast,sabouraud'sagarplateforfungi.Orangepeelsa mpleandrosepetalssamplewerespread on GYE agar plate and sabouraud's agar plate respectively. All the plates were incubated for 24-Hours.Thecitricacid-producingstrainsofAspergillusnigerNRRL567andNRRL599wereobtainedfromtheCABInternationalMycological Institute (Surrey,Uk)andthestrainNRRL567fromtheAmericanTypeCultureCollection(Rockville,MD,USA).Eachculturewasgrownonpotatodextroseagarslants(Merck)for5days.ASpore inoculum was prepared by adding 7 ml of sterilized distilled water to each slant and shaking vigorouslyfor1min.

III. FERMENTATIONMEDIUM

The PH of the OPL medium was adjusted to 3.0-6.2 with concentrated hydrochloric acid prior to sterilization.Themedium100gwasdispensedinto250mlErlenmeyerflasks.Theflaskswereautoclavedat20min.Eachflaskwasinoculatedwith0.4mloftheinoculum.Methanol(0,20,40,60ml)wasaddedandflaskswereincubated200rpm.Allsampleswere preparedintriplicate.

ANALYTICALMETHODS:

GC MS assay method for 2-phenylethanol headspace was analyzed using a gas chromatography with Agilenttechnologies technology with a capillary column HP-5. Citric acid and phenyl ethanol levels in the experimentsweredeterminedbystudyingthekineticprofileofcitricacidproductionusingaHPLCandThinlayerchromatography. Sweet oranges and red roses were used as a sample for the isolation of microorganisms.Orange peel sample and rose petals sample were spread on GYEagar plate and sabouraud's agar platerespectively. All the plates were incubated for 24-hour at 37°C. After incubation for 24 hours at 37°C all theplates were observed carefully for their colony characteristics. After identification of microorganisms twodifferent fermentation broths were prepared.Fruit waste fermentation broth with yeast extract in 100 mlwaterwithorangepeelpowderasrawmaterialwaspreparedforthefermentationofcitricacidandlimonene.Floral waste fermentation broth with yeast extract in 100 ml water with fresh rose petals as raw material waspreparedforthefermentationofβ-phenyl ethanol.Isolatedyeast,fungiandcyanobacteriawereinoculatedinboth kind of fermentation broths separately and were incubated at 37°C for five days. Determination ofdifferent product was performed after every 24 hours and result were noted. Titration method is used todeterminetheamountofalcoholinfermentationbroth.Thisiswidelyusedmethod.Citricacidestimationwascarriedoutusingacid-basedtitrationmethod.Thereasonweusedtitrimetricmethodwasbecauseitissimple,accurate, rapid and cost-effective method and can be carried out in any simple laboratory. Oil recovery andestimationisquitedifficultmethodandlimoneneisaterpenewhichmakesitmoredifficulttoanalyses.Bromatetitration method is very easy to handle and give accurate results. β-phenyl ethanol can easily vaporize in theairanddifficulttokeepwithoutalittlecoldtemperature.Weusedice-bathtokeepitcoolduringtheestimationprocess.Forstandard,extractofrosepetalswasused.estimationandextractionofproductsinalcohol,phenol,citric acid, limonene. Phytochemical analysis in Carbohydrate test in blue precipitate, Tannins test in greenishblue, Saponins test foam production, Glycoside test red and blue separate layers, Flavonoid test yellowishorange,Alkaloidtestinred-browncolor.

IV. DETERMINATION

ALCOHOL:

Alcohol is one of the main contents produces during fermentation. Titration method is used to determine theamount of alcohol in fermentation broth. This is widely used method.Alcohol is calculated using alcoholstandardcurve.Theprocessstartswith1mlaliquottakingintesttube.4mLdistilledwaterwasaddedwith10ml of 0.2N $K_2Cr_2O_7$. Tubes were incubated in boiling water-bath for 30 min. After incubation and cooling itdown to the room temperature 20% KI (4 ml) was added and mixed thoroughly. Solution was titrated against0.1N$Na_2S_2O_3$tillthecolourchangetowinered,thenfewdropsof1%starchwasaddedandcolourturnedtobluish-green which was again titrated against 0.1N $Na_2S_2O_3$ till the colour change to light blue. Value of B-Ewas calculated andplottedingraph(ShindeandPatil,2016).

PHENOL:

The Folin-Ciocalteu method is described in various pharmacopeia for determination of phenolic compounds.Blue colour is formed during the reaction because of phosphotungstic-phosphomolibdenum complex, wherethe maximum absorption depends on the concentration of phenolic compound. TheFolin-Ciocalteu was usedtoperformphenolestimationtest.Forthereferencechemicalstandard,stocksolutionofgallicacid(20μg/ml)prepared in distilled water was used, and for the test sample, incubated fermentation broths were used withundiluted and 1:10 diluted sample.1 mL of each solution was transferred to separate tubes containing 1 mLFolin-Ciocalteureagent.Allthetubeswerekeptatroomtemperaturefor3minandthen10%Na_2CO_3(10mL)was added. Tubes

were incubated for 30 min at room temperature. After incubation samples were analyse inUV-Vis spectrophotometer forabsorbance at740nm,waterwasusedas blank.
(Melloetal.,2013)

CITRICACID:
Citric acid estimation was carried out using acid-based titration method.The reason we used titrimetricmethodwasbecauseitissimple,accurate,rapidandcost-effectivemethodandcanbecarriedoutinanysimplelaboratory. Sample was collected from fermentation broth by directly filter it and then centrifuge the sampleat 1000 rpm for 5min. For standard, citric acid was directly extracted out from orange peel, and differentconcentrationswerepreparedandanalyzed.First20mLsamplewastakeninflaskandfewdropsofphenolphthalein indicator was added. Solution was titrated using 0.1N NaOH solution. Final observation wascolour change from colorless to pink indicates the presence of citric acid. Final volume of NaOH was noteddown. (Eidetal., 2014)

LIMONENE:
Oilrecoveryandestimationisquitedifficultmethodandlimoneneisaterpenewhichmakesitmoredifficulttoanalyse.Bromatetitrationmethodisveryeasytohandleandgiveaccurateresults.Titrationmethod:
Anemptyglassbottlewasweighedonweighingmachine.5mLfermentationbrothwastakeninbottleandwasweighagain,then15mL2-propanol wasaddedandweighagain,andthesolutiontransferredintoflask.Then10mLmethylorangeindicatorpreparedinHClwasaddedandtitratedagainst0.1Npotassiumbromide-bromate solution till the colour changed to yellowish- orange. Standard was performed using extract fromorangepeel.Amountoflimonenewascalculatedusing simpleformulaas follows:
Amountoflimonene=(0.0034)(Bn)(100)(An+En)/(W)(En)Where,
0.0034=0.1NKBrO3/KBr
Bn=Voloftitrantused(B–Evalue)

En=Weightofemulsion(Eg-Ag)
Eg=Weightofbottle+alcohol+sampleAg=Weightofbottle +alcohol
An= Weight of alcohol (Ag - T)T=weightofbottle
W= Weight of alcohol emulsion (Eg - T)(Scottetal.,1966)

β-PHENYLETHANOL:
β-phenyl ethanolcaneasilyvaporizeintheairanddifficulttokeepwithoutalittlecoldtemperature.Weusedice-bath to keep it cool during the estimation process. For standard, extract of rose petals was used.In thiscolorimetricmethod5mLfermentationbrothwastakenintotheflaskwith10%solutionofKNO3inH2SO4(2mL).
Flask was then placed into ice-bath for 30 min and then transferred into water-bath for another 30 min.Letitcooland4mLNH4OHand9mLH2Owasadded,mixedwell.Absorbancewasmeasuredat540nm(McFarlaneandThompson.,1964)

V. APPLICATION

Traditionally, (+)-limonene is used as a flavoring compound in citrus-flavored products such as soft drinksandcandyandasafragranceingredientin householdcleaningproductsandperfumes(Duetz etal.,2003).
Asaflavourandfragranceingredient,limonenehasarelativelyhighpricebecauseofthe quality requirementsin this field. For this application, chirality is important. (+) Limonene (also called R- or d-limonene) has apleasant, orange-like odor whereas the (−) form (also called S- or l-limonene) has a harsher turpentine-likeodor (Friedman and Miller, 1971). Limonene has minor applications in other products. For example, it is usedasinsecticide(Ciriminnaetal.,2014)andisbeinginvestigatedformedicalapplicationsduetoitsantimicrobialandanti-cancer properties(Inouyeetal.,2001;Milleretal., 2010)

Citricacidmainlyusedinfoodindustry,pharmaceuticals,chemicalindustry,cosmetics,printings,foodpreservative, electro pickling, copper plating, beverage & others. Some specific applications are given below(Soccol,2003; Pandeyetal.,2001; Vandenberghe,1999;Grewal,1995).

1. Citricacidmonohydrateiswidelyusedasorganicacid&pHcontrolagent,flavouringandpreservativeinfoodproductionlikeascandy,cookies,biscuits,jams,jellies,snacks,instantfoodsandsauces.
2. It is used as acidity regulator and antioxidant in beverage such as alcoholic beverage, carbonated soft drink,syrups,juice drinks, tea&coffee, ice-cream,sports&energydrink.
3. Itcanbeusedinthrombininhibitorandfungicideinpharmaceutical.

Anti-Infective Agents, 2-phenylethanol on humans and other animals that destroy harmful microorganisms orinhibit their activity. They are distinguished from disinfectants, which are used on inanimate objects. Use asPreservatives,inpharmaceuticalpreparationstoprotectthem fromchemicalchangeormicrobialaction.Theyincludeanti-bacterialagents andantioxidants(Corre,1990).

VI. CONCLUSION

In1913,itwasobtainedthefirstpatentintheUnitedStatesforamethodofproducingcitricacidby*Aspergillusniger* in sugar solutions (Soccol et al., 2006). Orange peel was employed in this work as raw material for theproductionofcitricacid(CA)bysolid-statefermentation(SSF)ofAspergillusnigerCECT-2090(ATCC9142,NRRL599)inErlenmeyerflasks(Tornadoetal.,2011).Microbialproductionofcitric acidandlimoneneusingorangepeelpowderandβ-phenyl ethanolusingrosepetalshaveshowngreatamountofproductionbyyeastSaccharomyces sp., fungi Aspergillus sp. Microbial production of citric acid, limonene, 2-phenylethanolhasbeen carried out successfully using various method, still confirmanalysis using GC-MS or FTIR or TLC isnecessary. Even though, it is quite difficult to workthe microorganisms we used gave quite the satisfactoryresults. Microbial production of citric acid, limonene,2-phenylethanol has been carried out successfully usingvariousmethod.Infutureaspectsgeneticanalysisofmicroorganisms,anti-fungal&anti-bacterial.Themediumusedthroughoutthisstudycontainedtotalsugarsasglucose: fructose: sucroseratioof1.0;0.8;0.7,respectively.

REFERENCES:

[1]. Rivas, B., Torrado, A., Torre, P., Converti, A., & Domínguez, J. M. (2008). Submerged citric acidfermentation on orange peel autohydrolysate. Journal of Agricultural and Food Chemistry, 56(7),2380-2387.

[2]. Torrado, A. M., Cortés, S., Salgado, J. M., Max, B., Rodríguez, N., Bibbins, B. P., ... & Domínguez, J. M.(2011). Citric acid production from orange peel wastes by solid-state fermentation. Brazilian JournalofMicrobiology,42(1),394-409.

[3]. Chreptowicz,K.,Wielechowska,M.,Główczyk-Zubek,J.,Rybak,E.,&Mierzejewska,J.(2016).Productionofnatural2-phenylethanol:Frombiotransformationtopurifiedproduct. FoodandBioproducts Processing,100,275-281.

[4]. Etschmann,M.M.W.,Sell,D.,&Schrader,J.(2003).Screeningofyeastsfortheproductionofthearomacompound2-phenylethanolinamolasses-basedmedium.BiotechnologyLetters,25(7),531-536.

[5]. Etschmann, M., Bluemke, W., Sell,D., &Schrader,J. (2002). Biotechnological production of 2-phenylethanol.Appliedmicrobiologyandbiotechnology,59(1),1-8.

[6]. Soccol, C. R., de Souza Vandenberghe, L. P., Medeiros, A. B. P., Karp, S. G., Buckeridge, M., Ramos, L.P.,...&Torres,F.A.G.(2010).Bioethanolfromlignocelluloses:statusandperspectivesinBrazil.Bioresourcetechnology,101(13),4820-4825.

[7]. Torrado,A.M.,Cortés,S.,Salgado,J.M.,Max,B.,Rodríguez,N.,Bibbins,B.P.,...&Domínguez,J.M.(2011). Citric acid production from orange peel wastes by solid-state fermentation. Brazilian JournalofMicrobiology,42(1),394-409.

[8]. Etschmann, M. M. W., & Schrader, J. (2006). An aqueous–organic two-phase bioprocess for efficientproductionofthenaturalaromachemicals2-phenylethanoland2-phenylethylacetatewithyeast.AppliedMicrobiologyand Biotechnology, 71(4),440-443.

[9]. Labrath, Y. P., &Gaikar, V. G. (2020). Solid State Fermentation of Orange Peels for Production ofCellulase,PectinaseandRecoveryofOrangeOilusingAspergillusSpeciesNCIM1432.

[10]. Correa,C.R.,&Kruse,A.(2018).Supercriticalwatergasificationofbiomassforhydrogenproduction–Review.TheJournalofSupercriticalFluids,133, 573-590.

[11]. SaerensSMG,VerstrepenKJ,VanLaere SDM,Voet ARD,VanDijck P,Delvaux FR,TheveleinJM(2006)The80Saccharomyces cerevisiae EHT1 andEEB1 genes encode novel enzymeswith medium-chainfattyacidethylestersynthesis andhydrolysiscapacity.TheJournalofBiologicalChemistry

[12]. Styger G, Prior B, Bauer FF (2011b) Wine flavor and aroma. Journal of Industrial Microbiology andBiotechnology.Vol38, PP: 1145–1159.ISSN:1367-5435.

[13]. Eden A, Van Nedervelde L, Drukker M, Benvenisty N, Debourg A (2001) Involvement of branched-chain amino acid aminotransferases in the production of fusel alcohols during fermentation in yeast.AppliedMicrobiologyandBiotechnology(55),PP: 296–300.ISSN: 1432-0614

[14]. StygerG,JacobsonD,BauerFF(2011a)IdentifyinggenesthatimpactonaromaprofilesproducedbySaccharomycescerevisiaeandtheproductionofhigheralcohols.AppliedMicrobiologyandBiotechnology(91),PP:713–730.ISSN:1432-0614.

[15]. JaneBucklePhD,RN,inClinicalAromatherapy(ThirdEdition),2015.ISBN:9780702064869.

[16]. Xinyao Lu, Yuqin Wang, Hong Zong, Hao Ji, Bin Zhuge, and Zhuoli Dong (2016). Bioconversion ofphenylalanineto2-phenylethanolbythenovelstresstolerantyeastCandidaglycerinogenesWL2002-5.Bioengineered.Vol7(6): 418–423.ISSN:2165-5987.

[17]. Masuo S, Osada L, Zhou S, Fujita T, Takaya N. (2015). Aspergillus oryzae pathways that convertphenylalanineintotheflavourvolatile2-phenylethanol.FungalGeneticsandBiology.Vol77,PP:22-30.ISSN:1087-1845.http://dx.doi.org/10.1016/j.fgb.2015.03.002

[18]. Gethins L, Guneser O, Demirkol A, Rea MC, Stanton C, Ross R P, Influence of carbon and nitrogensource on production of volatile fragrance and flavour metabolites by the yeast Kluyveromycesmarxianus,Yeast(2015);Vol32:PP: 67-76;ISSN:1097-0061.

[19]. Wikandari R., Nguyen H., Millati R., Niklasson C., &Taherzadeh M. J. (2015). Improvement of biogasproduction from orange peel waste by leaching of limonene. BioMed research international, Vol2015.ISSN:2314-6141https://doi.org/10.1155/2015/494182

[20]. K. Grohmann and E. A. Baldwin (1992). Hydrolysis of orange peel with pectinase and cellulaseenzymes.BiotechnologyLetters,vol.14,no.12,PP:1169–1174,ISSN:1573-6776.https://doi.org/10.1007/BF01027023

[21]. F. R. Marın, C. Soler-Rivas, O. Benavente-Garc´ıa, J. Castillo, and J. A. Perez-Alvarez (2007). By-productsfromdifferentcitrusprocessesasasourceofcustomizedfunctionalfibres.FoodChemistry,vol.100, no.2, PP:736–741,

ISSN:0308-8146.

[22]. Battista F., Remelli G., Zanzoni S., &Bolzonella D. (2020). Valorization of residual orange peels:Limonenerecovery,volatilefattyacids,andbiogasproduction.ACSSustainableChemistry&Engineering,8(17),PP:6834-6843.ISSN:2168-0485https://doi.org/10.1021/acssuschemeng.0c01735

[23]. Labrath Y. P., &Gaikar V. G. (2020). Solid State Fermentation of Orange Peels for Production ofCellulase,PectinaseandRecoveryofOrangeOilusingAspergillusSpeciesNCIM1432.doi:10.21203/rs.3.rs-123470/v1

[24]. RivasB.;TorradoA.;TorreP.;ConvertiA.;DomínguezJ.M.(2008).Submergedcitricacidfermentationon orange peel autohydrolysate. Journal of Agricultur and food Chemistry. 56, PP:2380–2387. ISSN:1520-5118.

[25]. Soccol C.R.; Vandenberghe L.P.S.; Rodrigues C.; Pandey A. (2006). New Perspectives for Citric AcidProductionandApplicationofFoodTechnologyandBiotechnology.44,PP:141–149.ISSN1330-9862.

[26]. KapoorK.K.;ChaudharyK.;TauroP. (1982).PrescottandDunn'sIndustrialMicrobiology,4thedn.G.Reed(Ed),AVIPublishingCo,Wesrport,CT.https://doi.org/10.1023/A:1008821413239

[27]. Torrado A. M., Cortés S., Salgado J. M., Max B., Rodríguez N., Bibbins B. P., ... & Domínguez J. M.(2011). Citric acid production from orange peel wastes by solid-state fermentation. Brazilian JournalofMicrobiology,Vol42,PP:394-409.ISSN:1517-8382.

[28]. Roccia A., Hibrand-Saint Oyant L., Cavel E., Caissard J. C., Machenaud J., Thouroude T. &Baudino S.(2019). Biosynthesis of 2-phenylethanol in rose petals is linked to the expression of one allele ofRhPAAS.Plantphysiology, Vol179(3),PP:1064-1079.ISSN: 0032-0889

[29]. Carlquist M., Gibson B., Yuceer Y.K., Paraskevopoulou A., Sandell M., Angelov A.I. (2014). Processengineeringforbioflavourproductionwithmetabolicallyactiveyeastsminireview.Yeast.32,PP:123-143.ISSN:1097-0061. https://doi.org/10.1002/yea.3058

[30]. KovachevaN.,RusanovK.,AtanassovI.(2010).IndustrialCultivationofOilBearingRoseandRoseOilProductioninBulgariaduring21STCentury,DirectionsandChallenges.BiotechnologyandBiotechnologicalEquipment.Vol24, PP: 1793-1798.ISSN: 1314-3530.

[31]. Cui Z., Yang X., Shen Q., Wang K., Zhu T., (2011) Optimization of biotransformation conditions forproduction of 2-phenylethanol by a Saccharomyces cerevisiae CWY132 mutant. Nat. Prod. Res. 25,PP: 754-759.ISSN:2673-1665.

[32]. HuaD.,XuP.(2011).Recentadvancesinbiotechnologicalproductionof2-phenylethanol.BiotechnologyAdvances.Vol29,PP: 654-660.https://doi.org/10.1159/000455169

[33]. Garavaglia J., Flores S., Pizzolato T., Peralba M., Ayub M. (2007). Bioconversion of Lphenylalanineinto2phenylethanolbyKluyveromycesmarxianusingrapemustcultures.WorldJournalofMicrobiologyandBiotechnology.Vol23,PP: 1273–1279.ISSN: 1573-0972.

[34]. Huang C.J., Lee S.L., Chou C.C. (2000). Production and molar yield of 2-Phenylethanol by Pichiafermenter L-5 as affected by some medium components. Journal of Bioscience and Bioengineering,Vol90:2, PP:142-147, ISSN: 1389-1723,

[35]. Yin S., Zhou H., Xiao X., Lang T., Liang J., Wang C., (2015.) Improving 2-phenylethanol production viaEhrlich Pathway using genetic engineered Saccharomyces cerevisiae strains. Current Microbiology.70,PP: 762-767.ISSN:1432-0991.https://doi.org/10.1159/000455169

[36]. Kim B., Cho B.R., Hahn J.S. (2014a). Metabolic engineering of Saccharomyces cerevisiae for theproduction of 2 phenylethanol via Ehrlich pathway. Biotechnology and Bioengineering. Vol 111,PP:115-124.ISSN:0006-3592.

[37]. StarkD,MunchT,SonnleitnerB,et al.Extractivebioconversionof 2-phenylethanol fromL-phenylalanine by Saccharomyces cerevisiae. Biotechnol Prog. (2002); Vol 18(3) PP: 514–523. ISSN:8756-7938.https://doi.org/10.1021/bp020006n

[38]. Zanetti M, Ternus ZR, Dalcanton F, de Mello MMJ, de Oliveira D, et al. (2015) MicrobiologicalCharacterization of Pure Geraniol and Comparison with Bactericidal Activity of the Cinnamic Acid inGram-Positive and Gram- Negative Bacteria. Journal of Microbiology, Biochemistry Technology. 7:4PP: 186-193https://doi.org/10.3390/ijms22041717

[39]. LiR.,WangK.,WangD.,XuL.,ShiY.,DaiZ.,&ZhangX.(2021).Productionofplantvolatileterpenoids(roseoil)byyeastcellfactories.Green Chemistry.https://doi.org/10.3390/molecules1912 19292

[40]. NationalCenterforBiotechnologyInformation(2021).PubChemCompoundSummaryforCID22311,Limonene.RetrievedJuly26,2021fromhttps://pubchem.ncbi.nlm.nih.gov/compound/Limonene.

[41]. Koolaji N., Shammugasamy B., Schindeler A., Dong Q., Dehghani F. &Valtchev P. (2020). Citrus peelflavonoids as potential cancer prevention agents. Current developments in nutrition, Vol 4(5), PP:25.ISSN:2475-2991.https://doi.org/10.1093/cdn/nzaa025

[42]. NationalCenterforBiotechnologyInformation(2021).PubChemCompoundSummaryforCID311,Citricacid.RetrievedJuly26,2021from https://pubchem.ncbi.nlm.nih.gov/compound/Citric-acid.

[43]. NationalCenterforBiotechnologyInformation(2021).PubChemCompoundSummaryforCID6054,2- Phenylethanol. Retrieved July 26, 2021 fromhttps://pubchem.ncbi.nlm.nih.gov/compound/2-Phenylethanol.

[44]. Boluda-AguilarM.;Lopez-GomezA.(2013).Productionofbioethanolbyfermentationoflemon(CitruslimonL.peelwastespretreatedwithsteamexplosion.IndustrialCropsandProducts.Vol41,PP:188–197.ISSN:0926.

[45]. L.P.S. Vandenberghe, C.R. Soccol, A.Pandey, J.M. Lebeault (1999). Review: Microbial production ofcitricacid.BrazilianArchiveofBiologyandTechnology.Vol42,PP:263–276.https://doi.org/10.1590/S1516-89131999000300001.

[46]. W.A. Duetz, A.H. Fjällman, S. Ren, C.Jourdat, B. Witholt. (2001).Biotransformation of d-Limonene to(+)trans-CarveolbyToluene-GrownRhodococcusopacusPWD4Cells.Appliedandenvironmentalmicrobiology.67(6): PP:2829-2832.ISSN: 1098-5336.

[47]. Nautical O.H., Tiwari K.K. (2012).Supercritical Carbon DioxideExtraction of Indian Orange Peel OilandHydroDistillationComparisononTheirCompositions.ScienceandTechnology.2(3)PP:27-31.DOI:10.5923/j.scit.20120203.01.

[48]. ScallietG,JournotN,JullienFetal.(2002).Biosynthesisofthemajorscentcomponents3,5-dimethoxytolueneand1,3,5-trimethoxybenzenebynovelroseO-methyltransferases.FEBSLetters. Vol523,PP:113–118.ISSN:0014-5793.

[49]. RayneS,ForestK.(2016).Carboxylicacidesterhydrolysisrate constantsforfoodandbeveragearomacompounds.FlavourandFragranceJournal.Vol 31,PP:https://doi.org/10.1002/ffj.3327.

[50]. Jakobsen HB, Olsen CE. Influence of climatic factors on emission offlower volatiles in situ. Planta.(1994);192:365–371https://doi.org/10.1007/BF00198572.

[51]. V.A.ShindeandR.B.Patil(2016).ProductionofEthanolbySaccharomyces cerevisiae Using OrangePeelsandBananaPeels,InternationalJournalofCurrentMicrobiologyandAppliedSciences,Vol:5PP:280-284, ISSN:2319-7706107.

[52]. Scott,W.CliffordandM.K.Veldhuis(1966),Rapidestimationofrecoverableoilbybromatetitration,JournalofAssociationofOfficialAnalyticalChemists,ISSN:0004-5756.

[53]. Mohammed ShaibuAuwal, Sanni Saka, Ismail Alhaji Mairiga, KyariAbbaSanda, Abdullahi ShuaibuandAminaIbrahim(2014),Preliminaryphytochemicalandelementalanalysisofaqueousandfractionated podextractsof Acacia

nilotica (Thornmimosa), Veterinary research forum,5(2): 95–100,PMID: 25568701.
[54]. W.D.McFarlaneandK.D.Thompson(1964),Colorimetricmethodsfordeterminationofaromaticalcohols inbeer. JournaloftheInstituteofBrewing, 70: 497-502.
[55]. Joseph K. Adu, Cedric D. K.Amengor, Naomi Kabiri, EmmanuelOrman, Stella AblaGameliPatamia,andBerniceKorkorOkrah(2019),ValidationofaSimpleandRobustLiebermann–BurchardColorimetricMethodfortheAssayofCholesterolinSelectedMilkProductsinGhana,InternationalJournalofFoodScience,Volume 2019,Article ID: 9045938.
[56]. ChristyGnanaTheebaP.andSasiKumarR.(2015),Phytochemicalexamination,antioxidantpotentialandinvitroantibacterialstudiesofcrudeextractsofPartheniumhysterophorusLinn.Leaves,JournalofChemicalandPharmaceuticalResearch,7(4):219-225, ISSN:0975-7384.
[57]. XinyaoLu,YuqinWang,HongZong,HaoJi,BinZhuge,andZhuoliDong,Bioconversionof–phenylalanine to 2-phenylethanol by the novel stresstolerantyeast Candida glycerinogenesWL2002-5Bioengineered.2016;7(6):418–423.
[58]. Joseph K. Adu, Cedric D. K.Amengor, Naomi Kabiri, EmmanuelOrman, Stella AblaGameliPatamia,andBerniceKorkorOkrah(2019).ValidationofaSimpleandRobustLiebermann–BurchardColorimetric Method for the Assay of Cholesterol inSelected Milk Products in Ghana,InternationalJournalofFoodScience,Volume 2019,Article ID: 9045938.
[59]. BraddockRJ,CadwalladerKR1992Citrusby-productsmanufactureforfooduse.FoodTechnol46(2)105-110.
[60]. Battista F., Remelli G., Zanzoni S., &Bolzonella D. (2020). Valorization of residual orange peels:Recovery, volatile fatty acids, and biogas production. ACS Sustainable Chemistry & Engineering,8(17),PP:6834-6843.ISSN:2168-0485https://doi.org/10.1021/acssuschemeng.0c01735.