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Gas Chromatography/Mass Spectroscopy Analysis of Phytochemicals Present in Orange Peel Powder and in Bread Prepared Using It

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Orange peel is a rich source of phytochemicals and flavouring agent used in bread making. The phytochemicals present in the orange peel powder and bread prepared using orange peel powder was analyzed by gas chromatography/mass spectroscopy (GC/MS). The result shows that the methanolic and hexane extracts of orange peel powder contains phytocompounds with nature of fatty acids, ketones, cycloalkanes, sugar compounds, saturated hydrocarbons, amino acids, polyols, essential oil, carboxylic acids and antioxidants; out of which amino acids, aldohexose sugar and polyols shows higher peaks. But they were absent in the bread prepared with orange peel powder, which would have degraded due to high baking temperature. However, it shows the presence of boric acid, dehydrating agent, acylating reagent, alkane, saturated fatty acid and organic compounds. Out of which boric acid shows the highest peak. Considering, hexane extract both the orange peel powder and bread prepared using it shows the presence of fatty acids and hydrocarbon based phytocompounds.

Keywords: Phytochemicals, Bread, Orange peel powder, GC/MS.

INTRODUCTION

Bread was a stable food known for its taste, aroma and texture [1]. The most commonly used ingredients for bread making were flour, salt, sugar, fat, water and yeast where as egg, milk, nuts, fruits and vegetable were other ingredients add based on preference. In recent times, change in food habits and increase in population have influenced higher bread consumption [2,3].

Oranges were widely used in juice, marmalade and jam industries. At present, some industries have focused on utilizing byproducts of citrus fruits [4]. The oil obtained from orange peel was widely used as a flavouring agent in drinks and foods due to its fragrance property [5]. Orange peel was enriched with phyto-chemicals and fibers due to the presence of albedo and flavedo [6]. These phyto-chemicals help in preventing cancer, neurodegenerative and cardiovascular diseases [7]. Fiber content present in orange peel helps in controlling cholesterol, blood sugar level and heart disease. It also promotes weight loss and assists digestion [5]. Ascorbic acid, phenols, flavonones, carotenoids, nutraceuticals and other secondary metabolites which were important for human nutrients was predominatly found in an orange. Hydroxylated polymethoxyflavones and polymethoxyflavones were found in the orange peel [8]. Carotenoids such as β-carotone, zeaxanthin, criptoxanthin, lutein and flavanoids mainly glycosylated flavanones were present in the orange fruit [9-11]. When compared to orange juice, nutraceuticals was more predominately present in its peel which was responsible for functional food development [10-12].

This is the first study to show what types of phyto-chemical compounds present in bread supplemented with orange peel powder. Thus, present research is focused on analyzing the phyto-chemicals present in orange peel powder and bread prepared using orange peel powder by gas chromatography/mass spectroscopy (GC/MS). Phytochemicals in orange peel have different polarities. Thus, to extract phyto-chemicals effectively both the polar (methanol) and non-polar (hexane) solvents were used.

EXPERIMENTAL

Fully ripened orange (*Citrus sinensis*), flour, sugar, salt, dry yeast, fat were purchased from local markets. Other chemicals were purchased from Sigma.

Preparation of orange peel powder and bread: Oranges were washed thoroughly with distilled water and the peels were removed into small pieces and air dried for 10 to 14 days. Later, dried samples were powered and sieved to obtain the fine product. The orange peel powder was stored in airtight container at room temperature for further uses. Orange peel powder (3 %) was used in bread making above which might induce sour taste [5]. Thus, the dough was prepared by replacing flour with 3 % of orange peel powder (*i.e.*, 97 g of flour and 3 g of orange peel powder) whereas other ingredients such as 5 g of sugar, 2 g of salt, 2 g of yeast, 3 g of fat and 55-60 mL of water were mixed and kneaded. In ambient conditions, the dough was proofed for 2 h and baked at 200 ºC for 25 min. Later, bread crumbs were removed and dried in hot air oven at 40 ºC for 12 h and grained to powder form for further analysis.

Extraction and GC-MS analysis: Both methanol and hexane extracts of orange peel powder and bread were prepared by ultrasonic enhanced extraction method [13]. The sample (0.5 g) was mixed with 25 mL of solvent and sonicated for 10 min at 25 ºC. Later, it was filtered and subjected to further analysis. The sample (1 µL) was injected into GC-MS (GC trace ultra version 5, MS-thermo DSQ II, run time: 31 min, carrier gas: helium, flow rate: 1.0 mL/min, column: db35 ms capillary standard non-polar, oven temperature rose to 270 ºC from 40 ºC at 8 ºC min^{-1}).

RESULTS AND DISCUSSION

Fig. 1 shows the GC-MS chromatogram of methanol and hexane extracts of orange peel powder and bread sample prepared using orange peel powder. Retention time, compounds identified, molecular formula, molecular weight, nature and applications are presented in Table-1.

Total 16 compounds are identified in the methanolic extract of orange peel powder. The nature of the phyto-chemical compounds identified are fatty acids, ketones, cycloalkanes, sugar compounds, saturated hydrocarbons, amino acids, polyols, essential oil, carboxylic acid and antioxidants. Even though many phyto-compounds are identified only 3 namely pentanamide, 5-hydroxy-, D-allose and 1,3-cyclopentanedimethanol show high peaks at 14.86, 18.84 and 20.25 retention times.

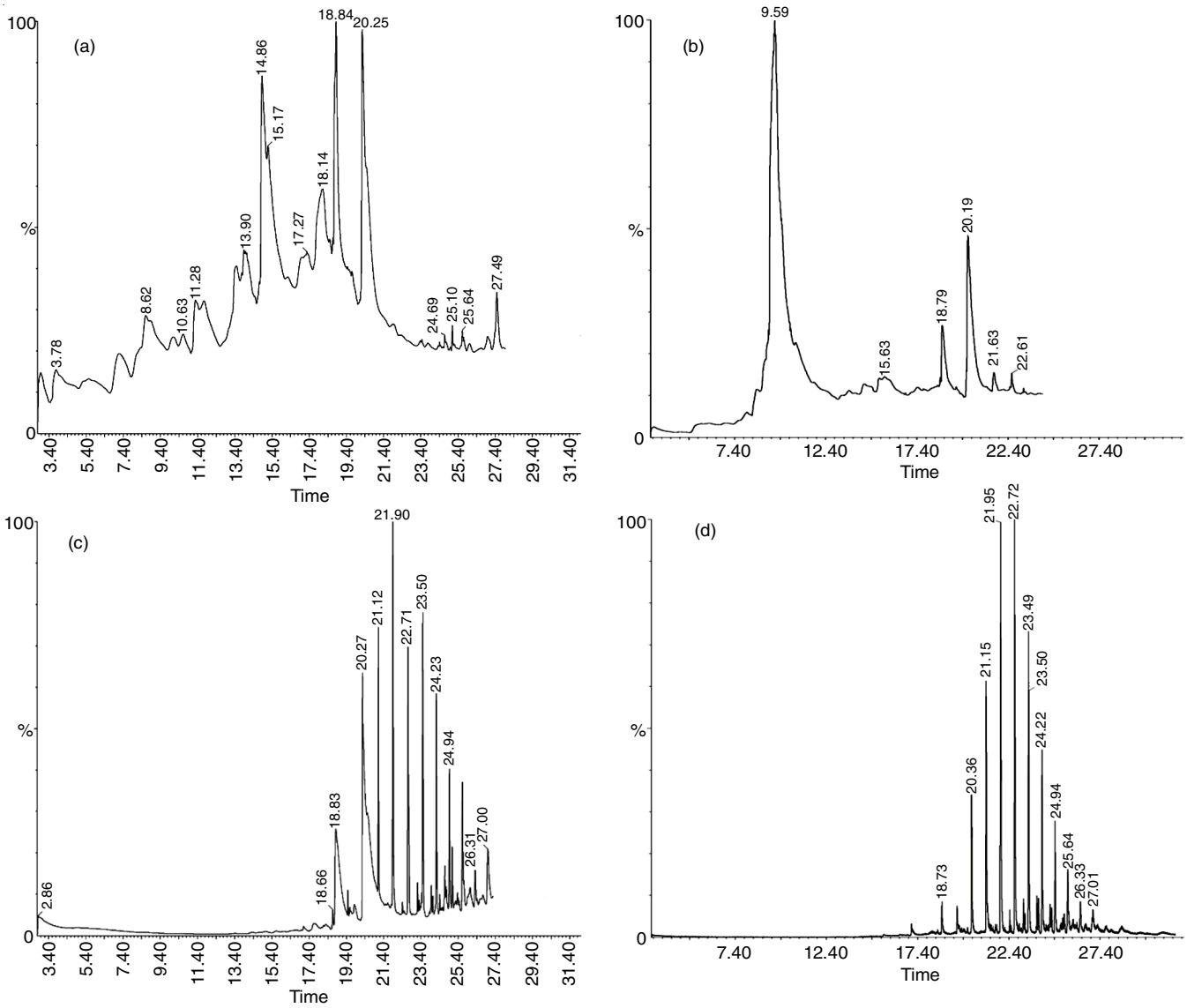

Fig. 1. Chromatogram of GC/MS analysis (a) methanolic extract of orange peel powder, (b) methanolic extract of bread with orange peel powder, (c) hexane extract of orange peel powder, (d) hexane extract of bread with orange peel powder

TABLE-1
PHYTOCHEMICAL COMPONENTS IDENTIFIED BY GC-MS

Retention time (min)	Compound identified	m.f.	m.w.	Nature	Application
(a) Methanolic extract of orange peel powder					
3.78	2,6-Heptadien-1-ol, 2,4-dimethyl-	$C_9H_{16}O$	140	Fatty Alcohols	Flavouring agent
8.62	2-Butanone, 4-(acetyloxy)-	$C_6H_{10}O_3$	130	Methyl ethyl ketone	Sweet odor similar to butterscotch
10.63	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	$C_7H_{10}O_6$	190	Cycloalkanes	Flavouring agent
11.28	Fructofuranose, 2,6-anhydro-1,3,4-tri-O-methyl-, beta.-D-	$C_9H_{16}O_5$	204	Fructose in furanose form	Sugar compound
13.90	Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-	$C_{10}H_{14}O$	150	Saturated hydrocarbon	Fragrance agent
14.86	Pentanamide, 5-hydroxy-	$C_5H_{11}O_2N$	117	Amino acid	Anti microbial drug
15.17	1,3,5-Triazine, hexahydro-1,3,5-tris(1-methylethyl)-	$C_{12}H_{27}N_3$	213	Nitrogen-containing heterocycle compound	Herbicides
17.27	1,6;3,4-Dianhydro-2-O-acetyl-.beta.-D-talopyranose	$C_8H_{10}O_5$	186	Sugar compound	Food additive
18.14	Methyl(methyl 4-O-methyl-.alpha.-D-mannopyranoside)uronate	$C_9H_{16}O_7$	236	Tautomeric form of mannose	Processing aids and additives
18.84	D-Allose	$C_6H_{12}O_6$	180	Aldohexose sugar	Sweetening agents
20.25	1,3-Cyclopentanedimethanol	$C_7H_{14}O_2$	130	Polyols	Fragrance agent
24.69	5-Acetoxymethyl-2,6,10-trimethyl-2,9-undecadien-6-ol	$C_{17}H_{30}O_3$	282	Essential oil	Food texturizing agent
25.10	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (All-E)-	$C_{30}H_{50}$	410	Squalene organic compound	Oil preparation
25.64	Bicyclo[3.1.1]heptan-3-one, 2-(but-3-enyl)-6,6-dimethyl-	$C_{13}H_{20}O$	192	Saturated hydrocarbon	Flavouring agent
27.18	Propanedioic acid, propyl-	$C_6H_{10}O_4$	146	Dicarboxylic acid	Acidity controller, natural preservative, fragrance and flavour enhancer
27.49	2h-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimet	$C_{31}H_{52}O_3$	472	Antioxidant	Nutritional supplement
(b) Methanolic extract of bread containing orange peel powder					
9.59	Boronic acid, ethyl-, bis(2-mercaptoethyl ester)	$C_6H_{15}O_2S_2B$	194	Compound related to boric acid	Preservative
15.63	Cyclohexanol, 2-methyl-	$C_7H_{14}O$	114	Organic syrup colourless compound	Flavouring agent
18.79	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Saturated fatty acid	Flavouring agents
20.19	Bicyclo[4.1.0]heptane, 7-pentyl-	$C_{12}H_{22}$	166	Alkane	Application not known
21.63	10-Undecenoyl chloride	$C_{11}H_{19}OCl$	202	Acylating reagent	Defoamer
22.61	Orthoformic acid, tri-2-butenyl ester	$C_{13}H_{22}O_3$	226	Hypothetical compound	Mild dehydrating agent
(c) Hexane extract of orange peel powder					
18.66	8-Heptadecene	$C_{17}H_{34}$	238	Double-bond stereos	Flavouring compound
18.83	Nonanoic acid	$C_9H_{18}O_2$	158	Saturated fatty acid	Flavouring agent
20.27	Nonane, 4,5-dimethyl-	$C_{11}H_{24}$	156	Alkane hydrocarbon	Fuel additive
21.12	Triacontane	$C_{30}H_{62}$	422	Aliphatic hydrocarbon	Food and medical application
21.90	Sulfurous acid, 2-ethylhexyl tridecyl ester	$C_{21}H_{44}O_3S$	376	Colorless liquid with a pungent sulfur odor	Reducing agents and disinfectants
22.71	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380	Alkane hydrocarbon	Adsorbents and absorbents
23.50	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	Fatty acid derivatives	Flavouring agent
24.94	Hexadecane, 1-iodo-	$C_{16}H_{33}I$	352	Fatty acid derivatives	Flavouring agent
26.31	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	Fatty acid derivatives	Flavouring agent
27.00	2,2,4,4,5,5,7,7-Octamethyloctane	$C_{16}H_{34}$	226	Alkane hydrocarbon	Perfuming agent
(d)Hexane extract of bread containing orange peel powder					
18.73	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}O_2F_5$	696	Ester	Preservative
20.36	2-Undecene, 5-methyl-	$C_{12}H_{24}$	168	Organic compound	Fragrance agent
21.15	Nonane, 2,5-dimethyl-	$C_{11}H_{24}$	156	Alkane hydrocarbon	Fuel additive
21.95	Hentriacontane	$C_{31}H_{64}$	436	Alkane hydrocarbon	Clouding agent; glazing agent; texturizer; thickener
22.72	Hentriacontane	$C_{31}H_{64}$	436		
23.49	Hentriacontane	$C_{31}H_{64}$	436		
23.50	4-Pentenoic acid, 2-acetyl-, ethyl ester	$C_9H_{14}O_3$	170	Unsaturated fatty acid	Flavouring agent
24.22	Hexane, 3-ethyl-3-methyl-	C_9H_{20}	128	Hydrocarbon	Flavouring agent
24.94	Hentriacontane	$C_{31}H_{64}$	436	Alkane hydrocarbon	Clouding agent; glazing agent; texturizer; thickener
25.64	17,21-Dimethylheptatriacontane	$C_{39}H_{80}$	548	Alkane hydrocarbon	Food and medical application
26.33	Decane, 3,8-dimethyl-	$C_{12}H_{26}$	170	Alkane hydrocarbon	Component of kerosene and petrol
27.01	Isoheptadecanol	$C_{17}H_{36}O$	256	Fatty alcohol	Flavouring agent

This shows that the orange peel is rich in amino acid, aldohexose sugar and polyols. Methanolic extract of bread containing orange peel powder is identified to contain only six phytochemical compounds. Dehydrating agent, acylating reagent, alkane, saturated fatty acid, organic colourless compound and boric acid are the nature of the identified phytocompounds. Out of these, boric acid shows the highest peak at retention time 9.59. This shows that the bread prepared with orange peel powder is rich in boric acid. On comparing both the methanolic extracts, it is clear that phytocompounds with the nature of amino acid, polyols and antioxidants present in orange peel powder are absent in the bread prepared using orange peel powder. The compounds of this nature would be degraded due to high baking tempera-ture.

The hexane extract of orange peel powder contains 10 phytochemical compounds. All the 10 compounds are identified most of them are fatty acid and hydrocarbon in nature. The same nature is observed in the hexane extract of bread prepared using orange peel powder. The nature of the compounds identified (*i.e*. fatty acid and hydrocarbon) are same in both methanolic and hexane extracts the type of phytochemical compound was different. In hexane extract of orange peel powder the phyto-chemicals responsible for the fatty acid nature are nonanoic acid, 2,6,10,15-tetramethyl heptadecane, 1-iodo hexadecane, and for hydrocarbons nature are 4,5-dimethyl nonane, triacontane, 1-iodo octadecane, 2,2,4,4,5,5,7,7-octamethyl octane. Similarly in hexane extract of bread prepared with orange peel powder, the phytochemicals responsible for fatty acid nature are isoheptadecanol and 4-pentenoic acid, 2-acetyl ethyl ester and for hydrocarbon nature are 2,5-dimethyl nonane, hentriacontone, 3-ethyl-3-methyl hexane, hentriacontane, 17,21-dimethylheptatriacontane and 3,8 dimethyl decane.

Conclusion

GC/MS analysis of orange peel powder shows the presence of many important phyto-chemical compounds. It is the first study to analyze the phytochemicals present in the bread supplemented with orange peel powder using GC/MS. Though amino acids, polyols and antioxidants based phytocompounds are present in orange peel powder, however, found to be absent in the bread prepared using it. These compounds may be degraded due to high baking temperature while other types of phytocompounds are present in both the orange peel powder and in bread prepared using orange peel powder.

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