The Pharma Innovation Journal 2022; SP-11(11): 2365-2371

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 2365-2371

www.thepharmajournal.com
Received: 01-08-2022
Accepted: 07-09-2022

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Identification of phytochemical profile of raw and cooked horse gram (*Macrotyloma uniflorum*) seeds by GC-MS/MS

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Abstract
Horse gram (*Macrotyloma uniflorum*) is an underutilized and unexplored food legume. The seeds of *Macrotyloma uniflorum* contain much more bioactive substances such as alkaloid, phenolic acid, tannin, flavonoids, fiber, essential fatty acid etc., which have significant metabolic and physiological effects. The horse gram is consumed mostly in the form of boiled and roasted. Even though, the horse gram is a richest source of phytochemical, the bioactive compounds are lost significantly during cooking process. The effects of four culinary treatments (pan boiling, pressure cooking, roasting) on the volatile component profile of horse gram were evaluated. The compounds identified were alcohols, aldehydes, ketones, esters, nitrogen compounds, alkenes, ethers, pyridines, phenols etc., All of the thermal treatments caused important changes in the volatile compound profile in particular an increase in carbonyl compounds. During cooking a significant (p<0.05) reduction in the content of several nitrogen compounds while an increase in aldehydes, alcohols, ketone, alkanes, esters was noted. During boiling significant (p<0.05) increases in the contents of several unsaturated aldehydes, alcohols, and benzene derivatives were observed, unlike roasting that mainly increased in the contents of pyrazines. Major differences observed in percentage of peak area showed that volatile flavor compounds of horse gram were significantly affected by type and processing conditions.

Keywords: Horse gram, pressure cooking, boiling, roasting, GC-MS/MS

Introduction
Horse gram (*Macrotyloma uniflorum*) is an underutilized and unexplored food legume. In India, Horse gram is grown on an area of 0.021 million hectares with a production of 0.012 million tonnes and national productivity of 469 kg per hectare (Vashishth *et al*., 2018) [1]. They are low in fat and excellent sources of protein, dietary fiber, a variety of micronutrients and macronutrient. The most important pharmacological activities of the horse gram are astringent, anthelmintic, antipyretic, anti-oxidant activity, urinary discharges and cardiovascular disease. The seeds of *Macrotyloma uniflorum* contain much more bioactive substances such as alkaloid, phenolic acid, tannin, flavonoids, fiber, essential fatty acid etc., which have significant metabolic and physiological effects. The horse gram is consumed mostly in the form of boiled and roasted (Suriyamoorthy *et al*., 2014) [2]. Thermal processes like cooking structurally modifies the legume seeds but also modifies functional properties which in turn increases digestibility of seeds. Several reports showed significant decrease in certain heat-labile nutrients during cooking of seeds. In a previous study observed that cooking decreased polyphenol content horse gram which could be attributed to either binding of polyphenols with other organic substances like protein or structural alterations of polyphenols. Even though, the horse gram is a richest source of phytochemical, the bioactive compounds are lost significantly during cooking process. Hence, the study was carried to evaluate the effect of different methods of cooking on phytochemical profile of horse gram.

Materials and Method
Sample preparation: The horse gram (Paiyur-2 variety) seeds were procured from Regional Research Station (TNAU), Paiyur. The horse gram seeds were cooked (pan boiling, pressure cooking, roasting). The raw and cooked horse gram seeds (5 g each) were taken in conical flask and methanol was added in the ratio of 1:10 and kept in incubating shaker for three days. The extract was collected and evaporated by using vacuum rotary flask evaporator.

GC-MS/MS Analysis

GC-MS/MS was carried out using a TSQ 8000 Evo system (Thermo Fisher Scientific, Palo Alto, CA, USA). Separation was done on a nonpolar TG-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Code 26098-1640, Thermo Fisher Scientific, USA) with helium carrier gas at 1.2 mL/min constant flow. The injector was operated in split mode and kept at 250 °C. The oven temperature was programmed at 40 °C for 3 min, increased to 80 °C at 3 °C/min and kept at 80 °C for 1 min. Then the temperature was raised to 150 °C at 5 °C/min, held at 150 °C for 1 min, and finally raised at 10 °C/min to 280 °C, and held for 2 min. Mass spectra were obtained over a range of 33–800 m/z applying an electron energy of 70 eV. Ion source and transfer line temperatures were set to 280 and 300 °C, respectively. Volatile compounds were identified based on their mass spectra compared with those in the NIST mass spectral libraries (National Institute of Standards and Technology, Gaithersburg, MD, USA) with matching probability > 80% (Table 1) using the Trace Finder software (Thermo Fisher Scientific, Palo Alto, CA, USA) (Sudhagar *et al*., 2022) [12].

Statistical analysis

Each sample was measured three times (n = 3), which were presented as mean average. The data analysis (CRD) was done by XLSTAT software (Addinsoft, New York, USA).

Result and Discussion

Table 1 lists the phytochemicals identified in raw and cooked and horse gram seeds. These compounds can be clustered in the following chemical families: alcohols, aldehydes, alkanes, ketones, esters, ethers, nitrogen compounds, pyridines, phenols and other compounds.

Aldehydes

The amounts of aldehyde were increased by cooking; this can be explained by the fact that although enzymes are destroyed during thermal processing, the high temperatures accelerate the autoxidation processes, which are not enzymatically catalyzed. This effect was lower in pressure cooking because of the lower cooking duration. The formation of C_6 aldehydes in the plant is related to cell destruction (Das *et al*., 2014) [6]. The presence of C_6 aldehydes is largely responsible for the characteristic odour of the cooked legumes. The major formation pathway of the branched-chain aldehydes seems to be the oxidative deamination-decarboxylation, via Strecker degradation (Bharathi and Anand., 2016) [7]. The aldehydes are not detected in the raw samples and only detected in cooked samples.

Alcohols

Alcohols constitute the most important family of volatile compounds. The presence of alcohols having the same chain length as the carbonyl found among the plant volatiles provides good evidence for oxidoreductase activity in tissues. Their odour threshold value was higher than for aldehydes, so their influence in the aroma must be lower. The alcohol present in the samples were 1-Deoxy-d-arabitol, 1-(2-Methoxyethoxy)-2-methyl-2-propanol, Piperonyl alcohol, Mercaptoethanol, 2-Phenylisopropanol, Isoproterenol, Ethanol, Galactitol. The cooking process accelerate the formation of alcohol formation. The samples cooked by pan boiling method consist of highest amount of alcohol compared to other cooking method. The raw horse gram seeds do not contain any alcohol content.

Ketones

Some of the ketones may derive from unsaturated fatty acids such as linolenic acid (Ingle *et al*., 2020) [8]. It could also be the result of the oxidative cleavage of carotenoids such as lycopene and phytoene. Ketones contents increased with all the heat treatments pan boiling cooking. the raw sample contain lower amount of ketone compared to cooked samples.

Esters

Esters are formed by esterification of carboxylic acids and alcohols (Goswami., 2017) [9]. The esters were thermally degraded, the roasted and pressure-cooked sample contains lower and higher ester content, respectively.

Table 1: Identified phytochemicals and their average percentage area (*n*=3) of raw and cooked horse gram

S. No	Volatile compounds	Raw	Pan boiling	Pressure cooking	Roasting
Alcohols					
1.	1-Deoxy-d-arabitol	-	0.06	-	-
2.	1-(2-Methoxyethoxy)-2-methyl-2-propanol	-	-	0.05	-
3.	Piperonyl alcohol	-	0.24	-	-
4.	Mercaptoethanol	-	0.04	-	0.14
5.	2-Phenylisopropanol	-	-	0.1	-
6.	Isoproterenol	-	0.22	-	-
7.	Ethanol	-	-	-	0.24
8.	Galactitol	-	0.3	-	-
Total Alcohols		-	0.86	0.15	0.38
Aldehydes					
9.	Acetamidoacetaldehyde	-	-	-	0.04
10.	Phloroglucinaldehyde	-	0.2	0.3	0.41
11.	Acetaldehyde ethyl trans-2-hexenyl acetal	-	0.05	-	-
12.	3,4-Dihydroxybenzaldehyde	-	0.25	0.04	-
13.	2-Ethylhexanal ethylene glycol acetal	-	0.01	-	-
Total aldehydes		-	0.51	0.34	0.45
Ketones					
14.	5H-Isoindolo[1,2-b][3]benzazepin-5-one	-	-	0.01	-
15.	4-Methyl-thiazolidine-2-thione	-	-	-	0.02
16.	2'-Hydroxy-5'-methylacetophenone	-	0.02	-	-

17.	1-[2,4-Bis(trimethylsiloxy)phenyl]-2-[(4-trimethylsiloxy)phenyl]propan-1-one	0.15	0.11	0.04	0.09
18.	3-Buten-2-one	-	0.03	-	-
19.	4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one	-	-	0.01	-
20.	2,5-Dihydroxyacetophenone	-	0.17	0.12	-
21.	3,6'-Dimethoxy-2'-(tert.-butyldimethylsilyl)oxychalcone	-	-	0.08	-
22.	Androstane-11,17-dione	-	0.06	-	-
23.	4'-Hydroxyvalerophenone	-	-	0.1	0.67
24.	1H-isoindole-1,3(2H)-dione	-	-	-	0.09
25.	2,4,6-Cycloheptatrien-1-one	-	-	0.41	-
26.	5-Nitro-2-(4-phenylazo-phenyl)-isoindole-1,3-dione	-	-	0.40	-
27.	Acetovanillone	-	-	0.39	-
28.	2,6-Dihydroxyacetophenone	0.19	0.34	0.15	0.13
29.	1,3-Isobenzofurandione	-	0.19	-	-
30.	2,4,6-Cycloheptatrien-1-one	-	-	0.41	-
31.	4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one	-	-	0.1	-
32.	3-Amino-4-piperonyl-5(4H)-isoxazolone	0.07	-	-	-
33.	Ethanone	-	-	-	0.29
	Total ketones	0.41	0.92	2.22	1.29
	Alkanes				
34.	Propane, 2-ethoxy-	0.01	0.05	0.03	-
35.	Propane, 2-methoxy-2-methyl-	-	0.05	-	-
36.	Trifluoromethyltrimethylsilane	-	0.01	-	-
37.	1,3-Dioxolane	-	0.03	0.03	0.11
38.	Trisiloxane	0.51	0.12	0.33	0.07
39.	Pentasiloxane, dodecamethyl-	0.31	0.27	0.25	0.42
40.	1-Triethylsilyloxyheptadecane	-	0.1	0.09	-
41.	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	-	0.29	0.10	0.07
42.	p-Trimethylsilyloxyphenyl-bis(trimethylsilyloxy)ethane	-	-	0.41	0.29
43.	1-Pentamethyldisilyloxycyclopentane	-	0.06	-	-
44.	2,5,7,10-Tetraoxa-6-silaundecane	-	-	0.19	-
45.	Tris(tert-butyldimethylsilyloxy)arsane	-	-	-	0.22
46.	Cyclotetrasiloxane	0.41	0.42	0.52	0.36
47.	Bis[di(trimethylsiloxy)phenylsiloxy]trimethylsiloxyphenylsiloxane	-	-	0.79	-
48.	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	-	-	-	0.15
49.	1,7-Di(3-ethylphenyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane	-	-	-	0.32
50.	Silane	0.16	-	-	0.10
51.	Ethane	0.04	-	-	0.03
52.	Ethylmethylsilane	-	-	-	0.05
53.	3,6-Dioxa-2,4,5,7-tetrasilaoctane	-	-	-	0.16
54.	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene	0.07	0.07	0.08	0.05
55.	1-Heptene	0.08	0.06	0.14	0.08
56.	1,4-Cyclohexadiene	0.09	0.01	0.04	-
57.	Tricyclo[4.2.1.0(2,5)]non-7-ene	0.51	-	0.66	0.39
58.	1,2,3-Triphenyl-3-methyl-cyclopropene	-	-	0.33	-
59.	3,4-Dibromo-1-pentene	-	-	0.06	-
60.	1,3-Butadiene	-	-	-	0.18
61.	1-Pentene	0.08	-	0.08	0.11
	Total Alkanes	2.27	1.54	4.13	2.95
	Nitrogen compounds				
62.	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	0.06	0.03	0.04	-
63.	N,N-Dimethyl-1,2,3-trithian-5-amine	-	-	-	0.13
64.	Benzeneethanamine	-	0.32	0.46	0.59
65.	2'-Methoxy-N-methyl-2-oxo-2-phenylethylamine	-	-	0.09	-
66.	benzenamine	-	-	-	0.15
67.	9-Ethyl-N-(trimethylsilyl)-9H-carbazol-3-amine	-	-	-	0.2
68.	2,5-Dimethyl-1-(4-chlorophenyl)pyrrole	0.01	0.02	-	-
	Total Nitrogen compounds	0.7	0.37	0.59	1.07
	Esters				
69.	Methyl 3,4-dihydroxybenzoate	-	-	-	0.08
70.	Dibutyl phthalate	-	0.09	-	-
71.	[4-(1,1-dimethylethyl)phenoxy]-, acetate	-	0.03	-	-
72.	2-Methoxybenzoic acid, 2,3-dichlorophenyl ester	0.03	0.04	-	0.08
73.	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	-	0.31	-	-
74.	2-(3-Benzoylphenyl)propionic acid trimethylsilyl ester	-	0.09	-	-
75.	para-Isopropyl benzoic acid trimethylsilyl ester	-	-	0.16	-
76.	Silicic acid, diethyl bis(trimethylsilyl) ester	-	0.25	0.62	0.49
77.	3-Methyl-2-(trimethylsilyl)oxybenzoic acid trimethylsilyl ester	-	0.33	0.32	0.48

78.	Isophthalic acid, di(2-methoxyethyl) ester	-	0.04	-	-
79.	2-Fluoro-5-trifluoromethylbenzoic acid, butyl ester	-	-	-	0.18
80.	Hydrocinnamic acid, p-methoxy-.beta.-methyl-, methyl ester	-	-	-	0.17
81.	Isophthalic acid, monoamide, N-(3-methylphenyl)-, ethyl ester	-	0.53	-	-
82.	1,3,5-Benzenetricarboxylic acid, trimethyl ester	-	0.17	0.39	-
83.	Terephthalic acid, diisopropyl ester	-	0.31	-	-
84.	Benzenepropanoic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	-	0.20	-	0.36
85.	Acetic acid, bis[(trimethylsilyl)oxyl]-, trimethylsilyl ester	-	0.26	0.28	0.14
86.	N-[4-[(trimethylsilyl)oxy]benzoyl]-, methyl ester	-	0.15	-	-
87.	Arsenous acid, tris(trimethylsilyl) ester	-	0.66	-	-
	Total esters	0.03	3.46	1.77	1.98
	Ethers				
88.	methyl ether	-	-	0.05	-
89.	trimethylsilyl ether	-	0.24	0.23	-
90.	Acridine-2,9-diol, bis(trimethylsilyl) ether	-	-	0.07	-
91.	mono(tert-butyldimethylsilyl) ether	-	-	0.13	-
92.	Phloroglucinaldehyde, tris(trimethylsilyl) ether	0.15	0.20	0.30	0.41
93.	3,4-Dihydroxybenzaldehyde, bis(trimethylsilyl) ether	-	0.25	0.04	-
	Total Ethers	0.15	0.69	0.82	0.41
	Pyridine				
94.	Thieno[2,3-b]pyridine, 5-ethyl-3-nitro-	-	0.36	-	-
95.	3-Methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridine	-	-	0.31	-
	Total Pyridine	-	0.36	0.31	-
	Phenols				
96.	4-(Diethylaminomethyl)-2,5-dimethylphenol	0.05	-	0.16	-
97.	4-tert-Amylphenol	-	-	0.14	-
	Total phenols	0.05	-	0.30	-
	Others				
98.	Pyrogallol	0.6	0.21	0.09	-
99.	Glycolic acid	0.04	-	-	-
100.	Protocatechoic acid	-	-	0.25	-
101.	Butanoic acid, 2-oxo-	-	0.02	-	-
102.	Pipecolic acid	0.03	0.04	-	-
103.	2-Hydroxyskatole	-	0.04	-	-
104.	Oxalic acid	0.02	-	-	0.04
105.	Benzenepropanoic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-	-	0.32	-	0.36
	Total other compounds	0.69	0.63	0.34	0.4

Alkanes

The alkane compounds present in pulses originate mainly from the oxidative decomposition of lipids. They generally have weak odours and do not contribute much to flavors in foods; however, they may modify the volatility and flavor-imparting properties of other volatile compounds (Abdullah *et al*., 2022) [10]. The alkanes content was highest in the pressure cooked and roasted samples. the higher cooking temperature may contribute to the generation of alkanes compounds. 1-Pentene, 1,3-Butadiene, 1-Heptene, Propane, 2-ethoxy-, Propane, 2-methoxy-2-methyl- are the some of the alkanes present in the raw and cooked samples.

Nitrogen compounds

The nitrogenous flavor compounds (pyrrole) were detected very trace amount (0.01 per cent) in the raw samples. The compounds were generally formed or increased after heating (cooking and roasting) most likely due to the Maillard reaction (Vandarkuzhali *et al*., 2017) [11]. The roasted samples had highest amount of nitrogen compounds; it may be due to degradation of proteins. The cooking processes led to either significantly increased values for nitrogenous compounds.

Ethers

The ether content of raw, pan boiled, pressure cooked and roasted samples were 0.15, 0.69, 0.8, 0.41 percentage of area, respectively. The cooking process increases formation of ethers to five to six-fold. The ether compounds present the samples are methyl ether, trimethylsilyl ether, Acridine-2,9-diol, bis(trimethylsilyl) ether, mono(tert-butyldimethylsilyl) ether, Phloroglucinaldehyde, tris(trimethylsilyl) ether, 3,4-Dihydroxybenzaldehyde, bis(trimethylsilyl) ether.

Pyridine and Phenols

The roasted sample do not contain both the pyridine and phenol compounds. The very trace amount pyridine content was present in raw samples. Thieno[2,3-b]pyridine, 5-ethyl-3-nitro-, 3-Methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridine are the pyridine compounds present in the samples. The phenol compound present in the samples are 4-(Diethylaminomethyl)-2,5-dimethylphenol and 4-tert-Amylphenol.

Other Compounds

The raw and cooked samples contains some miscellaneous compounds such as Pyrogallol, Glycolic acid, Protocatechoic acid, Butanoic acid, 2-oxo-, Pipecolic acid, 2-Hydroxyskatole, Oxalic acid, Benzenepropanoic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy].

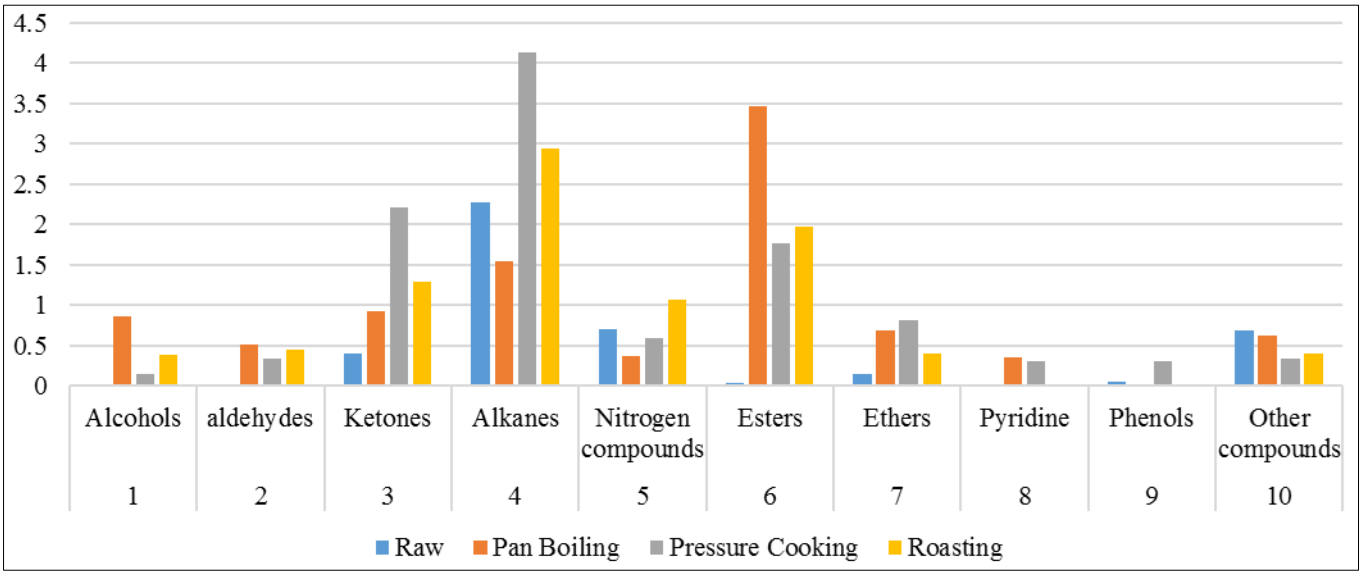
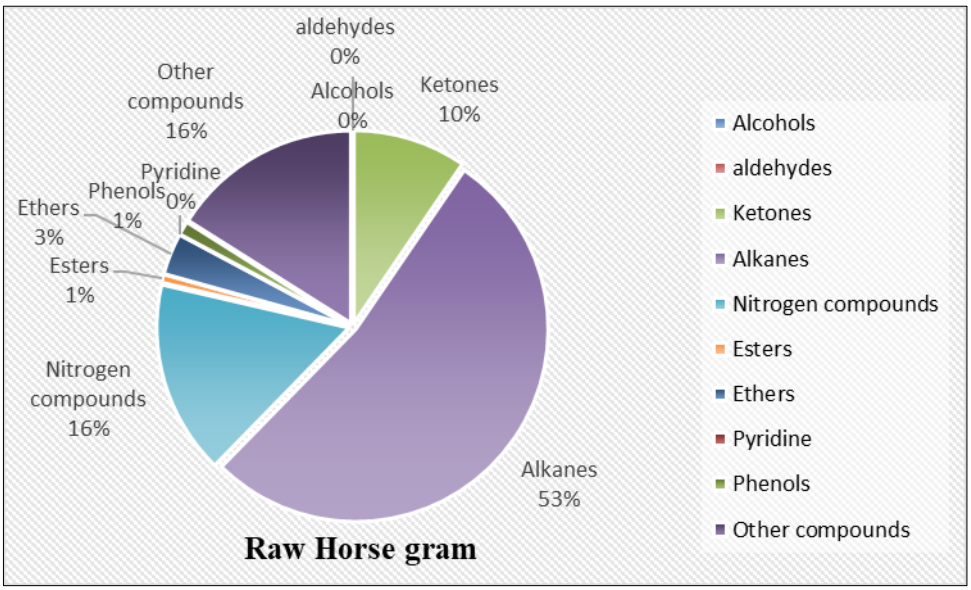
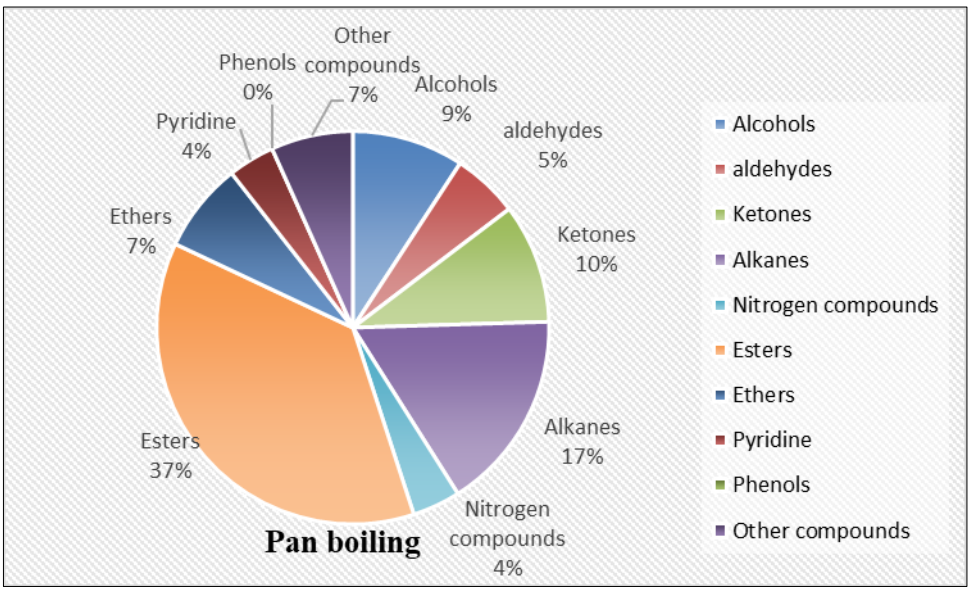


Fig 1: Phytochemical profile of raw and cooked Horse gram seeds (Area %)





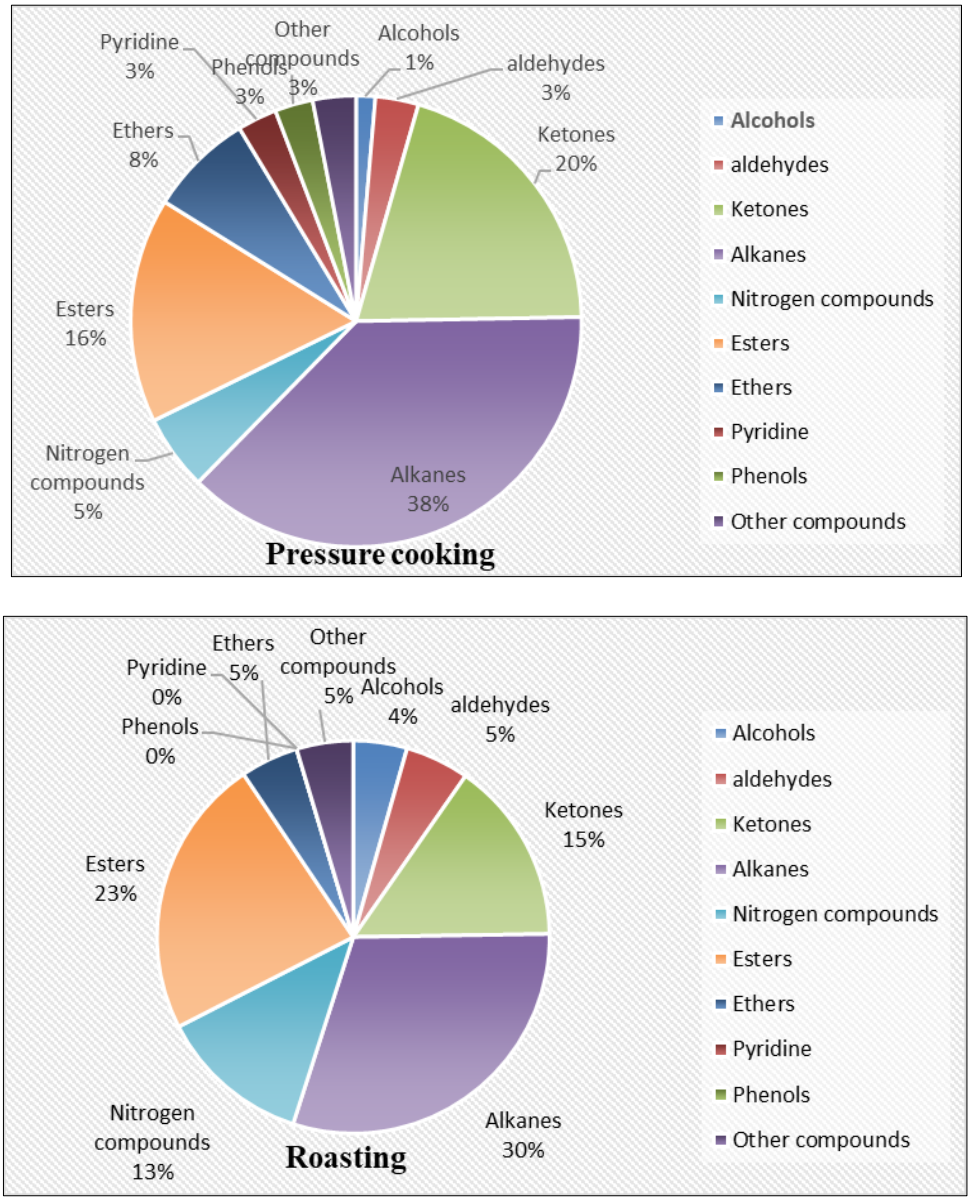


Fig 2: Distribution of volatile compounds in raw and cooked horse gram (relative percentage)

Conclusion

The cooked seeds of horse gram contain highest amount of phytochemical such as aldehyde, ketone, organic acid, amino acids than the raw seeds. the cooking process leads to the formation aromatic compounds. The retention of phytochemicals was highest in the pressure-cooked samples. The pan boiling and roasting leads to the significant loss of phytochemical which are sensitive to heat. The compounds responsible for the aroma of the samples were formed during roasting.

References

1. Vashishth R, Semwal AD, Pal Murugan M, Sharma GK. Post-harvest processing, cooking and textural properties of horse gram (*Macrotyloma uniflorum*) varieties. Journal of Food Measurement and Characterization. 2018;12(1):57-268.
2. Suriyamoorthy P, Subrhamanian H, Kanagasapabathy D. Comparative phytochemical investigation of leaf, stem, flower and seed extracts of *Macrotyloma uniflorum* L. Indo Am J Pharm Res. 2014;4(11):5415-5420.
3. Morris JB, Wang ML, Grusak MA, Tonnis B. Fatty acid, flavonol, and mineral composition variability among seven *Macrotyloma uniflorum* (Lam.) Verdc. accessions. Agriculture. 2013; 3(1):157-169.
4. Kawsar SMA, Mostafa G, Nahar N, Huq E. Chemical Constitutions and Hemolytic Activity of *Macrotyloma uniflorum* Linn. International Journal of Natural and Engineering Sciences. 2009;3(1):69-72.
5. Shivakumara KN. Review on friedel-crafts acylation of benzene derivatives using various catalytic systems. Int. J Adv. Chem. Res. 2021;3(1):25-31. DOI: 10.33545/26646781.2021.v3.i1a.32
6. Das S, Vasudeva N, Sharma S. Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy. Organic and medicinal chemistry letters. 2014;4(1):1-4.
7. Bharathi V, Anand AV. Chemical characterization from GC-MS studies of ethanolic extract of Macrotyloma uniflorum. Research Journal of Pharmacy and Technology. 2016;9(3):238-240.
8. Ingle KP, Al-Khayri JM, Chakraborty P, Narkhede GW, Suprasanna P. Bioactive Compounds of Horse Gram (*Macrotyloma uniflorum* Lam. [Verdc.]). Bioactive Compounds in Underutilized Vegetables and Legumes; c2020. p. 1-39.

9. Goswami RP. Modulation of Bioactive Compounds in Underutilized Legume Horse Gram (*Macrotyloma uniflorum*) by Germination and Fermentation (Doctoral dissertation); c2017.
10. Abdullah BM, Jaleel AA, Fatema SI, Pathan JM. Identification of bioactive compounds present in kulthi (*Macrotyloma uniflorum*) seed extract by gas chromatography-mass spectrometry. Research Journal of Pharmacy and Technology. 2022;15(2):814-818.
11. Vandarkuzhali P. Effect of processing on *Macrotyloma uniflorum* (horse gram) for development and quality evaluation of convenience food (Doctoral dissertation, Department of Food Science and Technology); c2017.
12. Sudhagar R, Priyanka S, Chockalingam V, Sendhilvel V, Souframanien J, Raja K, *et al*. Development and Metabolic Characterization of Horse Gram (*Macrotyloma uniflorum* Lam.(Verdc.)) Mutants for Powdery Mildew Resistance. Agronomy. 2022;12(4):800.