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GC-MS olfactometric characterization of odor active compounds in cooked red kidney beans (*Phaseolus vulgaris*)

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ABSTRACT

Red kidney beans are a staple pulse crop well known for its unique flavor characterized by kidney bean like, smoky, sulfury and earthy aroma notes. However, nature of compounds responsible for the unique beany odor of the cooked pulse has not been established. Steam distillation extracts of red kidney beans were subjected to Gas-chromatography olfactometry (GC-O) techniques namely detection frequency and aroma extract dilution analysis (AEDA). GC-O results suggest that methional with flavor dilution (FD) factor 21 is responsible for imparting the characteristic odor of the cooked red kidney beans. Apart from this p-vinyl guaiacol (FD 13) was identified as most important contributor towards smoky odor note. Sulfury note was mainly contributed by diethyl sulfide (FD 10) while 2-ethyl-3-methyl pyrazine (FD 13) was identified to be responsible for earthy note in cooked red kidney beans. Contribution of these compounds in characteristic aroma of cooked red kidney beans is reported here for the first time.

1. Introduction

Red kidney beans are an important pulse grown in the Indian subcontinent valued as a source of dietary protein. Among the different varieties grown, Kashmiri red variety is the most popular due to its unique flavor. Studies on red kidney bean aroma have been quite limited and only few of the major volatiles have been identified in various cultivars. Hexanal, (E,E)-3,5-octadiene-2-one, 1-penten-3-ol and benzaldehyde have been reported to be the prominent odor -active compounds of raw beans (Oomah et al., 2007). Although the aroma of the beans develops during cooking, the aroma impact compounds responsible for cooked bean odor have not been identified. We recently analyzed the major aroma compounds in raw and cooked beans of Kashmiri red, Chitra and Sharmili varieties (Mishra et al., 2017). Reduction in the content of several alcohols, aldehydes and terpene hydrocarbons with corresponding increase in terpene alcohols, sulfurous compounds, ketones and pyrazines during cooking was noted. Results revealed a significantly higher content of total volatile constituents in both the raw and cooked samples in Kashmiri red compared to the other two varieties. Kashmiri red variety also had the highest sensory scores for cooked kidney bean odor. Correlation of volatile profile data of cooked Kashmiri red variety obtained from GC/MS with quantitative descriptive sensory analysis (QDA) and odor activity values (OAV) using principal component analysis (PCA) suggested the possible role of methional, diethyl sulphide, dimethyl disulphide and dimethyl trisulfide with high OAV in contributing to the characteristic cooked kidney beans-like aroma, however, the results were not experimentally validated. Although compounds could be correlated with sensory quality but character impact compounds could not be identified. To further identify compounds responsible for imparting characteristic aroma, the present study aimed at evaluating the odor of cooked beans employing GC-Olfactometry (GC-O) techniques.

GC-olfactometry techniques are widely employed for determining odor of individual compounds and to identify aroma active compounds present in a food product (Acree et al., 1984; Grosch, 1993; Jordán et al., 2003). In GC-O human assessors are employed to detect and evaluate volatile compounds eluting from a GC column via a specifically designed odor port in place of conventional detectors such as mass spectrometer (MS) or a flame ionisation detector (FID) (Delahunty et al., 2006). GC-O techniques can be broadly classified into three types: detection frequency, dilution to threshold and direct intensity. To the best of our knowledge there are no reports on odor evaluation of red kidney beans by GC-O techniques. In present study volatile aroma isolate of Kashmiri

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red obtained by simultaneous distillation extraction (SDE) was therefore subjected to GC-O using detection frequency (DF) method and flavor dilution (FD) factor obtained by AEDA (aroma extract dilution analysis). This aided in understanding the contribution of predominant odorants based on their odor intensity and their impact on the final odor impression of the kidney bean extract.

2. Materials and methods

2.1. Materials

Kashmiri red variety of kidney beans was procured from local market, Chembur, Mumbai, India.

2.2. Chemical standards

Diethyl ether (analytical grade reagent) was obtained from S.D. Fine Chem. Ltd., Mumbai, India and was double distilled before use. Sodium sulphate (Chemco fine chemicals, India), methional, hexanal, benzothiazole, decanal, p-vinyl guaiacol, dimethyl trisulfide and 2-ethyl-3,5-dimethyl pyrazine, 2,6-dimethyl pyrazine and benzaldehyde were purchased from Sigma-Aldrich, USA. Benzene acetaldehyde and 1-octen-3-ol were purchased from Fluka, USA. 3-methyl butanol was purchased from Reachim, Russia.

2.3. Simultaneous distillation extraction (SDE)

SDE involves use of steam for extracting volatile compounds. Since samples were subjected to high temperature (100 °C) during SDE for 2 h, conditions were considered similar to that of cooking. SDE was carried out as per procedure reported earlier for cooked kidney beans (Mishra et al., 2017). Briefly, 250 g of kidney beans were subjected to SDE using peroxide free double distilled diethyl ether as solvent for extraction. The obtained SDE isolate was concentrated to a volume of 5 mL using Kuderna Danish apparatus and then reduced to a volume of 100 μl using gentle stream of nitrogen gas. 1 μl of the above concentrate was then used for GC/MS and GC-O analysis.

2.4. Gas chromatography-mass spectrometry (GC/MS) and gas-chromatography olfactometry (GC-O)

SDE extracts were analyzed using Shimadzu GC–MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with an olfactory detection port (ODP-2, Gerstel, Germany). The column used was DB-5 (J&W Scientific, California, USA) capillary column (length, 30 m; i. d., 0.25 mm and film thickness, 0.25 μm). A splitter was used at the end of column to split column effluent (1:1), one part being directed to MS, while the other part to sniffing port (ODP). Temperature programming for analysis were: column temperature was increased from 40 to 200 °C at a rate of 4 °C/min, maintained at initial temperature and 200 °C for 5 min, and then increased to 280 °C at the rate of 10 °C/min, held at final temperature for 20 min. Injector and interface temperatures were maintained at 210 and 280 °C, respectively. Helium was used as carrier gas with flow rate of 0.9 mL/min. Peaks were identified using mass spectral data of authentic standards (section 2.2) and mass spectral libraries (Wiley/NIST) provided with the instrument as well as by comparing retention index (RI) values of the compounds from available literature reports.

2.5. Detection frequency (df)

The GC-O analysis was carried out by a trained panel of six analysts and detection frequency of aroma notes was recorded. The number of times a note was perceived by sensory panel was defined as its detection frequency. The sniffing was divided in two sessions of 20 min each and each panelist participated in each session separately to avoid tiredness.

2.6. Aroma extract dilution analysis (AEDA)

To confirm the contribution of various volatiles towards characteristic aroma of kidney beans, aroma extract dilution analysis (AEDA) was performed by three most sensitive panelists in detection frequency experiment and repeated twice by each panelist. SDE isolate was injected (1.5 μl) in different split ratios: splitless, 2, 4, 6, 8, 10, 13, 17, 21 and 25. The maximum split ratio in which aroma was perceived was defined as the flavor dilution (FD) factor of that particular compound.

3. Results and discussion

Fig. 1 depicts the GC/MS profile of cooked kidney beans along with the detection frequencies of odor notes detected during GC-O analysis. A total of 14 aroma notes were detected during df experiments and the corresponding compounds were identified using MS (Fig. 1). Major odor notes identified were green (hexanal), cooked red kidney beans (methional), peanut (benzaldehyde), smoky or beany (p-vinylguiacol) with a detection frequency of 100 percent (Fig. 1). Apart from these odour notes, flowery (benzene acetaldehyde), and earthy (2-ethyl-3-methyl pyrazine) notes were detected with a frequency >80 %. To further ascertain the role of above compounds in imparting characteristic aroma to cooked kidney beans, the SDE extract was subjected to aroma extract dilution analysis (AEDA). Results obtained are demonstrated in Table 1. The highest FD of 21 was obtained for methional which was described by the panelists to possess cooked kidney beans odour, indicating its important role in imparting characteristic odour to cooked beans. Low odor threshold for methional (0.2 ppb) as reported in literature (Landaud et al., 2008) further corroborates its high FD observed in the present study. Other significant odorants observed in AEDA having high FD (≥10) were diethyl sulfide (pungent, sulfury), hexanal (green), benzaldehyde (peanut), 2-ethyl-3-methyl pyrazine (earthy) and p-vinyl guiacol (smoky, beany). Apart from these, dimethyl trisulfide (cauliflower, sulfury) and 2-ethyl-3,5-dimethyl pyrazine (roasted seeds/peanut) also exhibited relatively higher FD. Interestingly, the compounds such as diethyl sulfide, dimethyl trisulfide and 2-ethyl-3,5-dimethyl pyrazine which exhibited comparatively lower detection frequencies, were observed to possess significantly higher dilution factors in AEDA method (Fig. 1 and Table 1). On the other hand, benzene acetaldehyde having high df (>80) demonstrated correspondingly lower flavor dilution factor in AEDA (Table 1). Reason for low FD factors observed for benezene acetaldehyde, 1-octen-3-ol, benzothiazole and decanal could be their low concentration of 6.24 μg kg^{-1}, 0.95 μg kg^{-1}, 1.8 μg kg^{-1} and 20.5 μg kg^{-1} respectively in the cooked beans as reported earlier (Mishra et al., 2017). Therefore, aroma of these compounds could be detected by assessors in concentrated extracts, but not in diluted extracts during AEDA. In a previous study on the effect of various thermal treatments on the volatile profile of kidney beans, hexanal (1.95 %), benzaldehyde (1.77 %) and p-vinylguaiacol (1.66 %) were also reported as major volatile compounds in cooked red kidney beans (Ma et al., 2016). On the other hand, the compounds such as methional, and 2-ethyl-3-methyl pyrazine which exhibited high flavor dilution factors in present study, were not reported by these researchers. No sensory evaluation was performed by them and role of identified volatile compounds in imparting characteristic aroma of cooked kidney beans was also not inferred.

In our earlier studies, aroma of cooked red kidney beans has been shown to be characterized by presence of sulfury, smoky, boiled potato, earthy/raw potato and red kidney beans like odor notes (Mishra et al., 2017). Compounds such as methanethiol, methional, diethyl sulfide, dimethyl disulfide, and dimethyl trisulfide (Mishra et al., 2017) were postulated to have a possible role in imparting cooked kidney beans aroma. However, based on the results of both the GC-O techniques, it could be clearly established that methional is associated with characteristic red kidney beans like aroma and also has a high FD factor (Fig. 1 and Table 1). Aroma of this compound is reported to be musty, tomato, earthy, vegetable and cheesy like (Escudero et al., 2000; Burseg and De

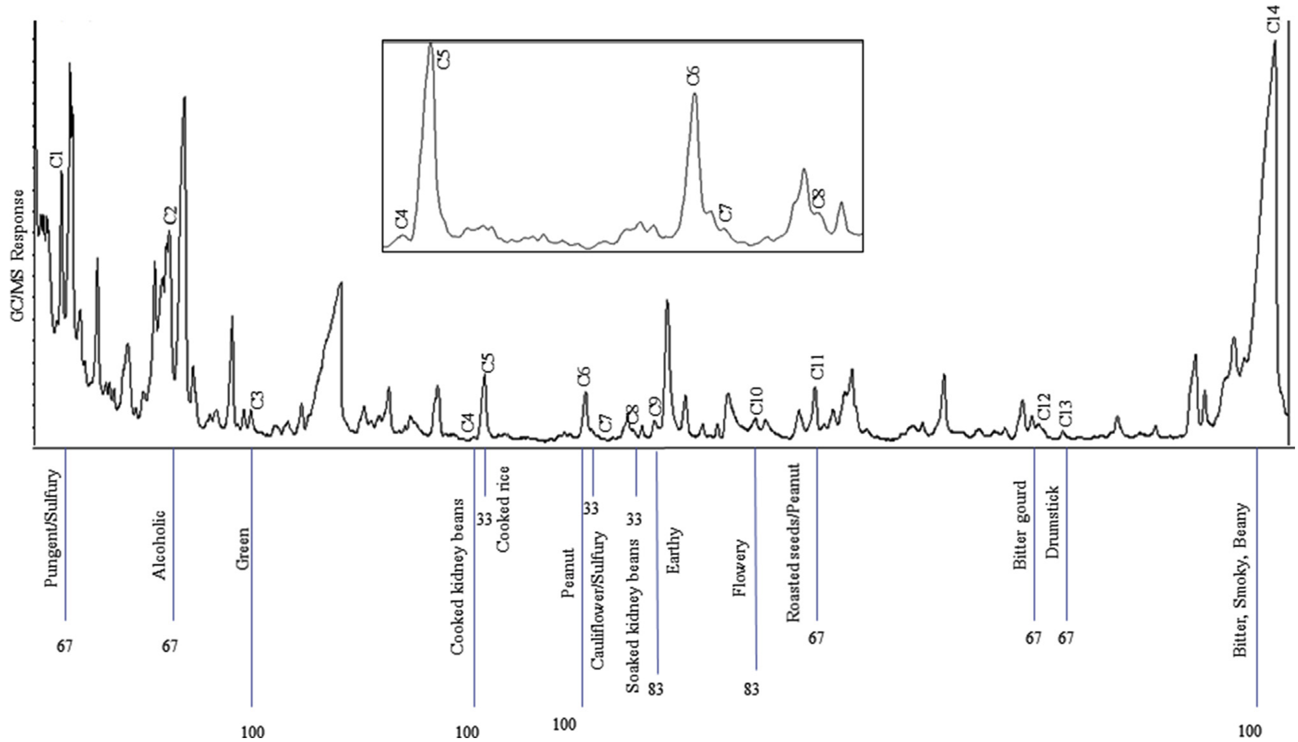

Fig. 1. GC-MS chromatogram of cooked kidney beans (SDE extract) with corresponding aroma active compounds as identified in GC-O (detection frequency method).

Jong, 2009). We have earlier reported that methional is not present in raw kidney beans but is formed only during cooking (Mishra et al., 2017) via Strecker degradation of amino acid methionine. To the best of our knowledge, this is the first report wherein the role of methional in imparting characteristic aroma to red kidney beans has been demonstrated. No odor notes could be perceived corresponding to methanethiol and dimethyldisulfide in GC-O experiments. Diethyl sulfide and dimethyl trisulfide having pungent/sulfury and cauliflower/sulfury odor notes, respectively were identified to be responsible for another important sulfury aroma note in cooked kidney beans during GC-O studies (Table 1). GC-O studies also suggested greater contribution of diethyl sulfide in sulfury note of kidney beans with higher df and FD of 67 and 10, respectively as compared to dimethyl trisulfide having values of 33 and 8, respectively (Fig. 1 and Table 1). In previous work no compounds could be correlated with sulfury odor note. All these compounds are formed only during cooking and are not present in raw kidney beans (Mishra et al., 2017). Sulfur compounds are generally formed from methionine and cysteine amino acids. Strecker degradation of methionine results in formation of methional which on further oxidation forms methanethiol. Dimethyl disulfide and dimethyl trisulfide are then produced by oxidation of methanethiol (Chin and Lindsay, 1994). These compounds are widely distributed and play an important role in aroma quality of various foodstuffs such as meat, fermented products (cheese, beer and wine), fruits (strawberry, yellow passionfruit) and vegetables

Table 1
Odor notes identified using Aroma extraction dilution analysis (AEDA) of cooked kidney beans.

Odor[a]	Compound identified (Notation)[b]	LRI[c]	split ratio (FD)[d]	Odor threshold (ppb) in water	Identification[h]
Pungent, sulfury	Diethyl sulphide (C1)	699	10	0.9 (in wine)[f]	LRI, MS
Alcoholic	3-Methylbutanol (C2)	727	6	250[e]	LRI, MS, Std
Green	Hexanal (C3)	800	10	5[e]	LRI, MS, Std
Cooked kidney beans	Methional (C4)	910	21	0.2[e]	LRI, MS, Std
Cooked rice	2,6-Dimethyl pyrazine (C5)	915	6	200[e]	LRI, MS, Std
Peanut	Benzaldehyde (C6)	965	10	350[e]	LRI, MS, Std
Cauliflower, sulfury	Dimethyl trisulfide (C7)	972	8	0.005[a]	LRI, MS, Std
Soaked kidney beans	1-Octen-3-ol (C8)	988	4	1[e]	LRI, MS, Std
Earthy	2-Ethyl-3-methyl pyrazine (C9)	999	13	0.4[g]	LRI, MS
Flowery	Benzeneacetaldehyde (C10)	1050	2	4[e]	LRI, MS, Std
Roasted seeds/peanut	2-Ethyl-3,5-dimethyl pyrazine (C11)	1080	8	1[g]	LRI, MS, Std
Bitter-gourd	Decanal (C12)	1202	2	0.1[e]	LRI, MS, Std
Drumstick	Benzothiazole (C13)	1238	4	80[e]	LRI, MS, Std
Bitter, smoky, beany	p-Vinyl guaiacol (C14)	1335	13	3[e]	LRI, MS, Std

[a] Odor as perceived by panellists during GC-O (AEDA).
[b] Compounds identified corresponding to perceived odor notes with notation representing compounds in Fig. 1.
[c] Kovat's index on RXi-5 column during GC-O.
[d] Flavor dilution factor in terms of split ratio, expressed for each note as average FD of the three panelists.
[e] http://www.leffingwell.com/odorthre.htm.
[f] Landaud et al.,(2008).
[g] http://www.leffingwell.com/pyrazine.htm.
[h] Identification: methods of identification: LRI (Linear retention index), MS (Mass spectrum), Std (standard).

(onion, cabbage and cooked potato) (Landaud et al., 2008).

2-Ethyl-3, 5-dimethyl pyrazine and p-vinylguaiacol were correlated with smoky odor note present in red kidney beans (Mishra et al., 2017). In present study role of these compounds for smoky odor is further established. During GC-O studies aroma of 2-ethyl-3, 5-dimethyl pyrazine was characterized as roasted seed/peanut while p-vinyl guaiacol had bitter/beany/smoky aroma (Table 1). Results obtained also clearly established more prominent role of p-vinyl guaiacol in providing smoky odor to kidney beans due to its higher df and FD values of 100 and 13, respectively (Fig. 1 and Table 1). Corresponding values of these attributes for 2-ethyl-3, 5-dimethyl pyrazine was 67 and 8, respectively. p-Vinylguaiacol was shown to be formed only during cooking, while content of 2-ethyl-3, 5-dimethyl pyrazine increased eight times during cooking of red kidney beans (Mishra et al., 2017). Lignin pyrolysis during thermal processing is known to result in the formation of p-vinylguaiacol (Azadfar et al., 2015).

Another important aroma note identified by QDA analysis of red kidney beans was the earthy note which was found to be associated with 2-ethyl-3-methyl pyrazine, trimethyl pyrazine and thymol (Mishra et al., 2017). Interestingly, out of these three compounds, aroma of only 2-ethyl-3-methyl pyrazine could be observed during GC-O studies. Aroma of this compound was identified as earthy (Table 1). This compound also demonstrated high FD of 13 with df of 83 which further ascertained its role for providing earthy note in kidney beans odor. Pyrazines are generally formed by Maillard reaction between amino acids and carbohydrates (Shibamoto and Bernhard, 1977) and pyrolysis of serine and threonine (Baltes and Bochmann, 1987).

In the present study apart from the major aroma notes identified in the previous report, we could also identify the compounds responsible for additional aroma notes perceived by the panelists. These include alcoholic (3-methyl butanol), green (hexanal), cooked rice (2,6-dimethyl pyrazine), flowery (benzene acetaldehyde), bittergourd (decanal) and drumstick like (benzothiazole) (Table 1 and Fig. 1). The results obtained in the present study emphasize the crucial role of GC-O methods for determining the nature of volatile compounds responsible for characteristic aroma of a product. So although, we could obtain an idea about aroma contributing compounds by correlating the aroma notes and sensory analysis results in the previous study, role of more precise GC-O techniques in identifying aroma compounds responsible for characteristic aroma of cooked kidney beans is established in the present study for the first time.

4. Conclusion

GC-O techniques aided in identification of several compounds such as benzaldehyde, p-vinyl guaiacol, benzene acetaldehyde, 2-ethyl-3-methyl pyrazine, 2-ethyl-3,5-dimethyl pyrazine, methanethiol, diethyl sulfide, methional and dimethyl trisulfide as responsible for red kidney beans aroma. Methional as the character impact compound for kidney beans aroma was further established. Sulfury notes were mainly contributed by diethyl sulfide and dimethyl trisulfide, while smoky note was predominantly due to presence of p-vinyl guaiacol and 2-ethyl-3,5-dimethyl pyrazine. 2-Ethyl-3-methyl pyrazine was the contributory compound towards earthy aroma. Results obtained from this study can help in formulation of red kidney beans aroma for potential applications in food industry.

Declarations

Author contribution statement

Prashant K. Mishra: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jyoti Tripathi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sumit Gupta: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Prasad S. Variyar: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

Acree, T.E., Barnard, J., Cunningham, D.G., 1984. A procedure for the sensory analysis of gas chromatographic effluents. Food Chem. 14, 273–286.
Azadfar, M., Gao, A.H., Chen, S., 2015. Structural characterization of lignin: a potential source of antioxidants guaiacol and 4-vinylguaiacol. Int. J. Biol. Macromol. 75, 58–66.
Baltes, W., Bochmann, G., 1987. Model reactions on roast aroma formation - V. Mass spectrometric identification of pyridines, oxazoles, and carbocyclic compounds from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. Z. Lebensm. Unters. Forsch. 185, 5–9.
Burseg, K., De Jong, C., 2009. Application of the olfactoscan method to study the ability of saturated aldehydes in masking the odor of methional. J. Agric. Food Chem. 57, 9086–9090.
Chin, H.W., Lindsay, R.C., 1994. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. Food Chem. 49, 387–392.
Delahunty, C.M., Eyres, G., Dufour, J.P., 2006. Gas chromatography-olfactometry. J. Sep. Sci. 29, 2107–2125.
Escudero, A., Hernández-Orte, P., Cacho, J., Ferreira, V., 2000. Clues about the role of methional as character impact odorant of some oxidized wines. J. Agric. Food Chem. 48, 4268–4272.
Grosch, W., 1993. Detection of potent odorants in foods by aroma extract dilution analysis. Trends Food Sci. Technol. 4, 68–73.
Jordán, M.J., Margaría, C.A., Shaw, P.E., Goodner, K.L., 2003. Volatile components and aroma active compounds in aqueous essence and fresh pink guava fruit puree (Psidium guajava L.) by GC-MS and multidimensional GC/GC-O. J. Agric. Food Chem. 51, 1421–1426.
Landaud, S., Helinck, S., Bonnarme, P., 2008. Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. Appl. Microbiol. Biotechnol. 77, 1191–1205.
Ma, Z., Boye, J.I., Azarnia, S., Simpson, B.K., 2016. Volatile flavor profile of Saskatchewan grown pulses as affected by different thermal processing treatments. Int. J. Food Prop. 19, 2251–2271.
Mishra, P.K., Tripathi, J., Gupta, S., Variyar, P.S., 2017. Effect of cooking on aroma profile of red kidney beans (Phaseolus vulgaris) and correlation with sensory quality. Food Chem. 215, 401–409.
Oomah, B.D., Liang, L.S.Y., Balasubramanian, P., 2007. Volatile compounds of dry beans (Phaseolus vulgaris L.). Plant Foods Hum. Nutr. 62, 177–183.
Shibamoto, T., Bernhard, R.A., 1977. Investigation of pyrazine formation pathways in glucose-ammonia model systems. Agric. Biol. Chem. 41, 143–153.