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Effect of Lactic Acid Bacteria Concentrations on the Composition of Bioactive Compounds in a Fermented Food Formulation

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Original Research Article

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Abstract: The volatile compound composition of food provides an indication of the benefits that this food can provide to the consumer. Thus the present study aimed at identifying the bioactive compounds present in formulated fermented diet made from 20% red kidney bean (*Phaseolus vulgaris* L.), 60% mung bean (*Vigna radiate*), 10% irish potato (*Solanum tuberosum*), and 10% ripe fresh papaya (*Carica papaya*) fruits inoculated with different concentrations of *Lactococcus lactis* sp. (*Lc. lactis* sp.) strain using the Gas Chromatography- Mass Spectrophotometer (GC-MS). The formulated diet consisted the control diet containing no lactic bacteria, diet 1 (1×10^6 CFU/ml), and diet 2 (2×10^6 CFU/ml). The results of the GC-MS revealed the presence of twenty volatile compounds in each diet. Five compounds namely Benzyl Alcohol; 2-propyl-1-pentanol; 1,3-diethyl benzene; 1-Tridecyn-4-ol; Phthalic acid, and cyclobutyl isobutyl ester were identified in all diets. Three volatile compounds namely Benzyl alcohol, 2-propyl-1-pentanol, and 1,3-diethyl benzene were identified as the dominant compounds in control; whereas two (Phthalic acid, cyclobutyl isobutyl ester, (E)-9-Dodecenoic acid, methyl ester) and three more (9.12-Octadecadienoic acid, methyl ester; 9-Octadecenoic acid (Z) -, methyl ester, and Tetradecanoic acid, 10,13-dimethyl-, methyl ester) bioactive compounds were detected in diet 1 and diet 2 respectively. Benzyl alcohol was the principal dominant bioactive compound in all the diets. The present study indicates that inoculation and increasing in the concentration of lactic bacteria in diet led to the identification of 2 and 3 other major volatile compounds with biology activities which could be highly important in pharmacology and nutraceuticals.
Keywords: Fermented diet, *Lactococcus lactis* sp., GC-MS, volatile compounds, bioactive compounds.

INTRODUCTION

Bioactive compounds are defined as components of food that influence physiological or cellular activities in the animals or humans when consumed [1]. They exhibit beneficial effects such as antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression [2]. The knowledge of the composition of volatile compounds in food has greatly increased during the past decades; some fermented foods are very rich source of bioactive compounds. Fermented foods and beverages are one of the indispensable components of the dietary culture of every community in the world [3]. Fermented foods with probiotics having functional properties may provide beneficial effects on health [4]. Microorganisms transform the chemical constituents of raw materials during fermentation and enhance the nutritive value of the products, enrich bland diets with improved flavor and texture, preserve perishable foods, fortify products with essential amino acids, health-promoting bioactive compounds, vitamins, and minerals; degrade undesirable compounds and anti-nutritive factors, impact antioxidant and antimicrobial properties, improve digestibility, and stimulate probiotic functions [3]. It is known that LAB added as starter cultures are capable of transforming lactic acid, citrate, lactate, protein and fats into volatile compounds that together with amino acids and other products play a critical role in the development of flavors [5, 6]. Many bacterial species coexist in dynamic communities and produce a wide diversity of secondary metabolites as cues potentially involved in competition and cooperation; enabling them to adapt to biotic and abiotic stresses [7, 8]. Some research works have been done in this domain: The production of volatile compounds by the probiotic strain, *Lactobacillus plantarum* NCIMB 8826, in cereal-based media (oat, wheat, barley and malt) was investigated [9]; the effect of *Pichia kudriavzevii* OG32 on volatile compounds produced in fermented cereal-based functional food using cereal-mix substrate were also investigated [10]. Thus this study aimed at evaluating the effect of different concentrations of *Latococcus lactis* sp. on bioactive compounds present in formulated diet after fermentation. Specifically available local raw materials

such as Irish potato, red kidney bean, mungbean, and papaya were used to formulate, prepare, inoculate, ferment and identify volatile compound by GC-MS.

MATERIALS AND METHODS

Materials

Dry red kidney beans (*Phaseolus vulgaris*) were purchased from a local market of Dschang, Cameroon. Dry dehulled mungbeans (*Vigna radiate*), potatoes (*Solanum tuberosum*), and papaya (*Carica papaya*) were purchased from a local supermarket in Mysore, India.

Lc. lactis sp strain was obtained from the Laboratory of Biochemistry, Medicinal Plant, Nutrition and Food science (LABPMAN) of the University of Dschang-Cameroon. *Lc. lactis* sp. culture was propagated (1%, v/v) twice in M17 broth, and incubated at 37 °C for 18 h without agitation before experimental use. 5 ml was collected and the centrifuged (8,000 g, 10 min, 4 °C) cells were washed twice with sterile physiological solution and re-suspended in 2.5 ml of the same solution.

Formulation, preparation and fermentation

The complementary food was formulated using 20% red kidney bean, 60% mung bean, 10% irish potato, and 10% ripe fresh papaya fruits. Red kidney beans were soaked in distilled water for 18 h, removed and dehulled manually; dehulled raw mungbean was soaked in distilled water for 2 h; Irish potato was cleaned, washed, peeled and diced. Papaya fruit was washed, peeled and diced. Raw materials were mixed with 50 ml of distilled water and ground using Meenũmix model M-86 (Meen Equipment, India) during 2 min. 200 ml of distilled water was added progressively to the paste, homogenized and precooked for 15 min on hotplate. 0.5 g of salt was also added to the paste. Into three 250 ml Erlenmeyer each, 100 ml of paste was introduced, cooked for 1 h to destroy anti nutritional factors and sterilized by autoclaving at 121 °C for 15 min. When the pastes reached room temperature, they were inoculated with different concentrations of culture 1×10^6 CFU/ml named as diet 1 and 2×10^6 CFU/ml named as diet 2. Pastes were incubated at selected temperature for 18 h in adequate conditions. Un-inoculated paste treated in the same way was used as a control.

Volatile Compound Analysis

Extraction of volatile compounds

5 ml of each 18 h fermented diet was taken, mixed with 5 ml dichloromethane (DCM) kept in a freezer for 24 h, and then macerated with a mortar. The homogenized culture in DCM solvent was transferred to a separating funnel. The bottom dichloromethane layer was taken in the dry tubes. The solution was dried by adding a pinch of anhydrous sodium sulphate ($Na_2SO_4.10H_2O$). The dried solution was transferred in a graduated test tube. It was sealed with cellophane tape. The cells were stored in a freezer (-20 °C). The sample was concentrated to 1 ml using Nitrogen concentrator, and 1µl was injected into a Gas Chromatograph (GC 8000 Series, Fisons Instruments).

Analysis of volatile compounds

The concentrated extract was analyzed for volatile compounds using a GC-MS. The gas chromatograph (Perkin Elmer Auto system XL) was interfaced to a mass spectrometer (Perkin Elmer Turbo Mass Gold). The separation of volatiles was carried out in ELITE 1 non-polar capillary column (30 m X 0.25 mm (ID); 0.25 µm film thickness). One microliter of the extract was injected (split ratio 1:10) and the carrier gas was helium at a flow rate of 1 ml/ min. The oven temperature was held at 100 °C for 6 min, heated at 4 °C/min to 150 °C, then at 8 °C/ min to 220 °C and held at 220 °C until an approximate run time of 40 min. The mass spectrophotometer was operated in the electron impact mode, and mass spectra were taken using an ionization voltage of 70 eV. The mass scan range was 40-400 AMU, with a scanning speed of 2.0 sec. Data acquisition and generation of chromatograms and spectra were done with Turbo Mass software [11].

Identification and quantification of volatile compounds

The identification of volatile compounds was performed by comparing the mass spectra with standard mass spectra database from the NIST Ver. 2.1 2009 Mass Spectra Library, Gaithersburg, Maryland, USA. The relative percentage of each volatile compound was calculated by comparing the peak area with the total area as follows:

$$\text{Relative percentage of each volatile compound} = \frac{\text{Compound peak area}}{\text{Total compounds peak area}} \times 100$$

RESULTS AND DISCUSSION

The GC-MS chromatograms (Fig 1, 2, and 3) and volatile compounds identified in each diet due to the effects of cell concentrations after fermentation are shown in table 1, 2, and 3. The biological activities of major compounds are also given in the respective tables. The GC MS analysis of each diet indicated the presence of 20 volatiles compounds. The three dominant identified compounds in control diet were Benzyl Alcohol (88.90%), 2-propyl-1- pentanol (5.97%), and 1,3-diethyl benzene (2.82%). Benzyl Alcohol (86.56%), 2-propyl-1- pentanol (6.59%), 1,3-

diethyl benzene (2.70%), Phthalic acid, cyclobutyl isobutyl ester (0.92%), and (E) -9-Dodecenoic acid, methyl ester (0.73%) were the five dominant compounds found in diet 1. Six principals compounds were detected in diet 2 namely Benzyl Alcohol (79.83%); 9,12-Octadecadienoic acid, methyl ester (6.50%); 2-propyl-1- pentanol (5.94%); 1,3-diethyl benzene (2.65%); 9-Octadecenoic acid (Z)-, methyl ester (2.05%), and Tetradecanoic acid, 10,13-dimethyl-, methyl ester (1.18%). It has to be noted that three major volatile compounds namely Benzyl Alcohol, 2-propyl-1- pentanol, and 1,3-diethyl benzene, and some other minor compound such as 1-Tridecyn-4-ol, and Phthalic acid, cyclobutyl isobutyl ester were present in all the three diets, this may be resulting in the fact that they have the same matrix. Also, Benzoic acid, 3-methyl-, 2-oxo-2-phenylethyl ester; 2,4,6,8-Tetramethyl-1-undecene; (E)-9-Dodecenoic acid, methyl ester, and 2-Methylheptanoic acid were detected only in inoculated diets with *Lactococcus lactis* sp. strain. This could be explained by the fact that these volatile compounds are product resulting from the growth of the bacteria in these two diets. Benzyl Alcohol was the principal bioactive compound identified in large amount in all diets. It's odor qualities are flowery, boiled cherries, moss, roasted bread, rose [12]. It is known to possess an antioxidant activity [13], this property would be useful for food preservation and could be benefit for health of the consumer. It was also identified in cereal-mix fermented with probiotic *Pichia kudriavzevii* OG32 [10]. The GC-MS results revealed several bioactive compounds in the different diets and most of them possessed biological activities known to have therapeutic value in various ailments via their antimicrobial, antioxidant, antifungal, antibacterial and anticancer activity. Among the various volatile compounds identified, most of them exhibited antimicrobial activity; this property could be benefit to the preservation of diets by inhibiting the proliferation of pathogenic bacteria responsible for the degradation of food.

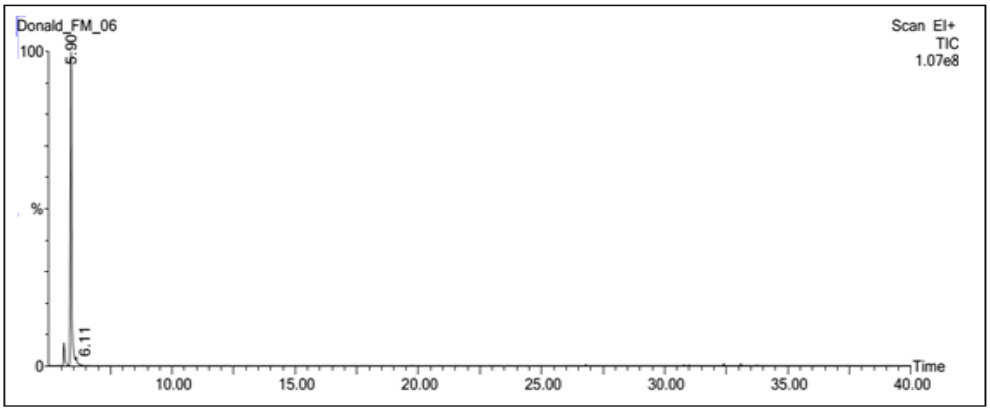

Fig-1: GC-MS chromatogram of volatile compounds of control diet

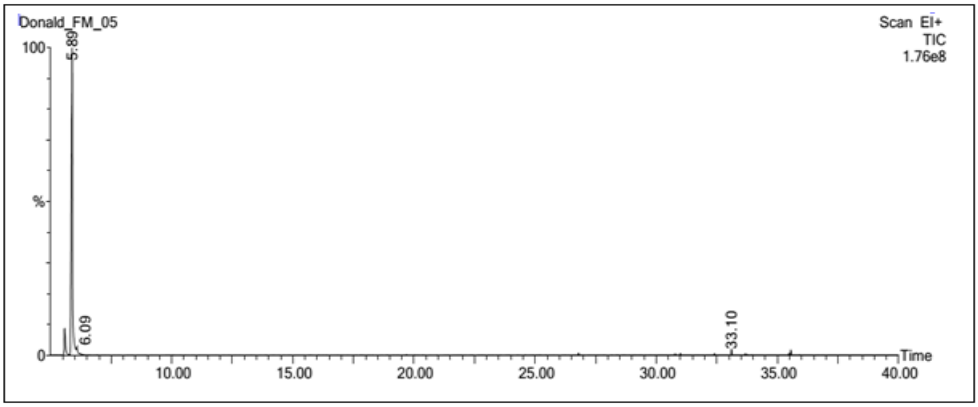

Fig-2: GC-MS chromatogram of volatile compounds of diet 1

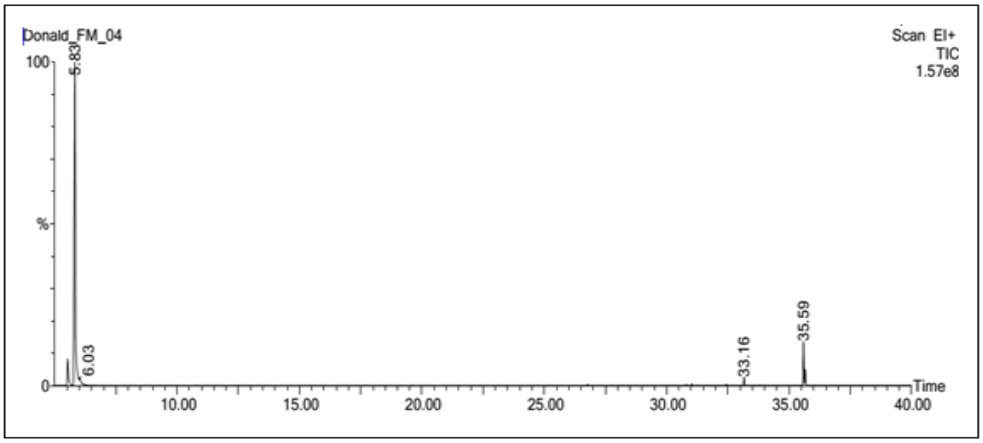

Fig-3: GC-MS chromatogram of volatile compounds of diet 2

Table-1: Volatile compounds identified by GC-MS in control diet

N°	RT	compounds	Molecular formula	MW	Yield (%)	Activity
1	5.61	2-propyl-1- pentanol	$C_8H_{18}O$	130.23	5.97	Anti-microbial, anti-fungal [14].
2	5.90	Benzyl Alcohol	C_7H_8O	108.14	88.90	Antioxidant [13].
3	6.12	1,3-diethyl benzene	$C_{10}H_{14}$	134.22	2.82	-
4	6.24	Isopropoxycarbamic acid, ethyl ester	C_6H_{13}	147.17	0.57	-
5	6.96	2-(1-decylundecyl)-1,4-dimethyl benzene	$C_{29}H_{52}$	400.72	0.04	-
6	19.72	1-Dodecyn-4-ol	$C_{12}H_{22}$	182.30	0.06	Oligosaccharide Provider [15].
7	20.02	Butanedial	$C_4H_6O_2$	86.09	0.03	Antimicrobial [16].
8	26.80	1,1,3-trimethyl cyclopentane	C_8H_{16}	112.22	0.19	-
9	30.78	Benzenecarbothioic acid, S-propyl ester	$C_{10}H_{12}OS$	180.26	0.17	Anticancer, antidiabetic [15].
10	30.99	1-Tridecyn-4-ol	C13H24O	196.33	0.13	Oligosaccharide Provider [15].
11	32.05	Octanoic acid, tripropylsilyl ester	$C_{17}H_{36}O_2Si$	300.55	0.03	Antimicrobial [15].
12	32.39	Phthalic acid, heptyl tridec-2-ny-1-yl ester	$C_{28}H_{42}O_4$	442.63	0.35	Antimicrobial [15].
13	33.00	(S)-I-Alanine ethylamide	$C_5H_{12}N_2O$	116.16	0.03	Anticancer [15].
14	33.09	Phthalic acid, 6-ethyl-3-octyl butyl ester	$C_{22}H_{34}O_4$	362.51	0.37	Antimicrobial [15].
15	33.33	I-Alanine, N-octanoyl-, propyl ester	$C_{14}H_{27}NO_3$	257.37	0.03	Anticancer [15].
16	33.67	2-(aminooxy)- pentanoic acid	$C_5H_{11}NO_3$	133.15	0.05	Antimicrobial [15].
17	33.76	Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	276.33	0.14	Antimicrobial [15].
18	33.95	Oxalic acid, cyclobutyl heptyl ester	$C_{12}H_{20}O_4$	228.29	0.06	Antimicrobial [15].
19	36.58	N-(aminocarbonyl)-propanamide	$C_4H_8N_2O_2$	116.12	0.03	Anticancer [15].
20	39.31	Dimethyl 1-(1,2,4-triazol-3-ylamino)butylphosphonate	$C_8H_{17}N_4O_3P$	248.22	0.03	-

Table-2: Volatile compounds identified by GC-MS in diet 1

N°	RT	compounds	Molecular formula	MW	Yield (%)	Activity
1	5.59	2-propyl-1- pentanol	$C_8H_{18}O$	130.23	6.59	Anti-microbial-anti-fungal [14].
2	5.89	Benzyl Alcohol	C_7H_8O	108.14	86.56	Antioxidant [13].
3	6.09	1,3-diethyl benzene	$C_{10}H_{14}$	134.22	2.70	-
4	6.23	Benzoic acid, 3-methyl-, 2-oxo-2-phenylethyl ester	$C_{16}H_{14}O_3$	254.28	0.41	Antimicrobial [15].
5	6.30	o-toluic acid, tridec-2-ynyl ester	$C_{21}H_{30}O_2$	314.46	0.16	Antimicrobial, anticancer [15].
6	6.39	3-ethyl benzaldehyde	$C_9H_{10}O$	134.17	0.03	-
7	6.94	2-ethyl-6-phenyl-1,3,4-thiadiazolo (3,2-a) (1,3,5)-triazine-5,7-dione	$C_{12}H_{10}N_4O_2S$	274.30	0.05	-
8	19.69	2,4,6,8-Tretramethyl-1-undecene	$C_{15}H_{30}$	210.40	0.12	Antibiotic [17].
9	19.99	2,2,5-trimethyl-3,4-hexanedione	$C_9H_{16}O_2$	156.22	0.04	-
10	26.79	6-methyl-1-octene	C_9H_{18}	126.24	0.31	-
11	30.79	Benzyl benzoate	$C_{14}H_{12}O_2$	212.25	0.21	Antiparasitic, antimicrobial [18].
12	31.00	1-Tridecyn-4-ol	C13H24O	196.329	0.20	Oligosaccharide Provider [15].
13	32.40	Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	276.33	0.27	Antimicrobial [15].
14	33.10	Phthalic acid, cyclobutyl tridecyl ester	$C_{25}H_{38}O_4$	402.57	0.92	Antimicrobial [15].
15	33.68	n-Decanoic acid	$C_{10}H_{20}O_2$	172.27	0.22	Anticancer, antimicrobial [15].
16	33.76	Phthalic acid, butyl hexyl ester	$C_{18}H_{26}O_4$	306.40	0.09	Antimicrobial [15].
17	33.96	1-Hexene, 2,4,4-triethyl-	$C_{12}H_{24}$	168.32	0.07	-
18	35.48	Bicyclo[7.1.0]decane	$C_{10}H_{18}$	138.25	0.24	Antimicrobial [15].
19	35.55	(E)-9-Dodecenoic acid, methyl ester	$C_{13}H_{24}O_2$	212.33	0.73	Anticancer, antimicrobial [15].
20	35.93	2-Methyl heptanoic acid	$C_8H_{16}O_2$	144.21	0.07	Antimicrobial [15].

Table-3: Volatile compounds identified by GC-MS in diet 2

N°	RT	compounds	Molecular formular	MW	Yield (%)	Activity
1	5.53	2-propyl-1- pentanol	$C_8H_{18}O$	130.23	5.94	Anti-microbial-anti-fungal [14].
2	5.83	Benzyl Alcohol	C_7H_8O	108.14	79.83	Antioxidant [13].
3	6.03	1,3-diethyl benzene	$C_{10}H_{14}$	134.22	2.65	-
4	6.17	Benzoic acid, 3-methyl-, 2-oxo-2-phenylethyl ester	$C_{16}H_{14}O_3$	254.28	0.66	Antimicrobial [15].
5	6.34	2-(1-methylethyl)-1,4,4-triphenyl-1,2-diazetidin-3-one	$C_{23}H_{17}ON_2$	337	0.03	-
6	6.37	1-Phenyl-1-decanol	$C_{16}H_{26}O$	234.38	0.03	-
7	6.89	1H-1,2,4-Triazole, 3-chloro-5-methyl	$C_3H_4ClN_3$	117.536	0.03	Anti-HIV-Integrase, antidote (Heavy metal, Hydrazine) [15].
8	19.63	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210.40	0.06	Antibiotic [17].
9	26.77	4-Octene, 2,6-dimethyl-, [S-(E)]-	$C_{10}H_{20}$	140.27	0.02	-
10	30.80	2,5-Cyclohexadiene-1,4-dione, 5-bromo-2,3-dimethyl-, 1-oxime, o-benzoyl-	-	-	0.17	Anticancer [15].
11	31.02	1-Tridecyn-4-ol	$C_{13}H_{24}O$	196.33	0.20	Oligosaccharide Provider [15].
12	32.44	Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	276.33	0.19	Antimicrobial [15].
13	33.16	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	$C_{17}H_{34}O_2$	270.46	1.18	Antimicrobial [15].
14	33.74	Di-n-propyl ether	$C_6H_{14}O$	102.18	0.05	Anticancer [15].
15	33.82	Phthalic acid, butyl tridec-2-yn-1-yl ester	$C_{25}H_{36}O_4$	400.55	0.07	Antimicrobial [15].
16	34.04	Oxalic acid, cyclobutyl heptyl ester	$C_{13}H_{24}O_4$	244.33	0.05	Antimicrobial [15].
17	35.59	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.48	6.50	Antimicrobial [15].
18	35.66	(E)-9-Dodecenoic acid, methyl ester	$C_{13}H_{24}O_2$	212.33	2.05	Anticancer, antimicrobial [15].
19	36.05	2-Methylheptanoic acid	$C_8H_{16}O_2$	144.21	0.06	Antimicrobial [15].
20	36.74	6H-[1,3]Dioxolo[5,6] benzofuro[3,2-c] [1]benzopyran, 6a, 12a-dihydro-2,3-dimethoxy-	$C_{18}H_{16}O_6$	328.32	0.02	-

CONCLUSION

At the end of the present study which was aimed at evaluating the impact of bacterial cell concentration (*Lactococcus lactis* sp.) on the volatile compounds produced in fermented diets, the findings revealed that the inoculation of diets with *Lactococcus lactis* sp. strain increased the formation of more major volatile compounds with known biological activity. The presence of multiple bioactive compounds identified in the inoculated diets may be of significant benefit with an immense health effects on humans and can prove to be good functional food.

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