

Characterization of Volatiles Compounds Emitted from Freeze-dried *Lactobacillus plantarum* CWBI-B1419 During Storage

Ibourahema Coulibaly^{1*}, Elisée Kporou Kouassi², Clement Kouassi Kouassi³, Daouda Kone⁴

Abstract— The powder of freeze-dried *Lactobacillus plantarum* CWBI-B1419 was analyzed at three stages during storage. The water content, the rate of viable bacteria were monitored and the volatiles compounds were extracted from the powders by the technic of sampling of the dynamic headspace and analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The analysis revealed 109 volatiles compounds identified aldehydes, alcohols, alkenes, alkanes, esters, ketones and compounds derived from amino acids such as the predominates groups. Ten volatiles were selected according to their high percentage of peak areas. Seven of these volatils were analyzed by following their evolution during storage. Among these compounds we noticed a decreased of ethanoic acid and cyclotetrasiloxane octamethyl unlike acetic acid, propionic acid, 2-methyl-, butanoic acid and 3-methylbutanoic with increasing percentages of significantly ($p < 0.1$) during 90 days of storage. The contents of volatiles from *Lactobacillus plantarum* CWBI-B1419 is a function of the storage period and also the quatntité polyunsaturated fatty acids. Despite the high content of polyunsaturated fatty acids, catabolism of amino acids and synthesis of esters led to important changes in the composition of volatile products of lactic acid bacteria from oxidation reactions of lipids.

Keywords: Freeze-dried cells, *Lactobacillus*, (GC-MS), Volatils compound; Storage, Solid-phase microextraction (SPME).

I. INTRODUCTION

The composition of volatiles compounds gives characteristic aroma profiles that are specific for different types of dry-cured meat sausages [1]-[5]. Unsaturated fatty acids can be oxidised easily by diverse oxidation mechanisms. Light exposure and presence of photosensitisers such as riboflavin

Ibourahema Coulibaly Unit and Research in Agroforestry, Laboratory of Microbiology, Bio-Industry and Biotechnology (LMBIB), Department of Biochemistry and Microbiology, University Jean Lorougnon Guédé, BP. 150 Daloa, Ivory Coast

Elysée Kouassi Kporou Unit and Research in Agroforestry, Laboratory Interactions Host - microorganism, Environment and Evolution (LIHME), University Jean Lorougnon Guédé, BP. 150 Daloa, Ivory Coast –

Kouassi Kouassi Clément Unit and Research in Agroforestry Laboratory of Microbiology, Bio-Industry and Biotechnology (LMBIB), University Jean Lorougnon Guédé, BP. 150 Daloa, Ivory Coast

Daouda koné Faculty of Biosciences, Plant Physiology Laboratory (PPL), University Felix Houphouët Boigny of Cocody, 22 BP. 582 Abidjan 22, Ivory Coast,

and chlorophyll can accelerate lipid oxidation through type I pathway of excited photosensitisers and singlet oxygen oxidation or type II pathway [6]. Changes of volatile profiles can be used as indicators for determining the degree of oxidation in fats and oils [7]. The most important odor and flavour compounds in dry-cured meat sausages are formed by the decomposition of fatty acids and amino acids [8], [9]. Free unsaturated fatty acids (UFA) will easily oxidize to lipid hydroperoxides, which are unstable and rapidly decompose to secondary oxidation volatiles compounds such as aldehydes, alcohols, ketones, furans, alkanes, and alkenes [10]. Amino acid catabolism gives different volatiles compounds like sulphides, pyrazines, and branched-chained amino acids (BCAA) [8]. The aroma of fresh lactic acid bacteria can be attributed to secondary lipid oxidation compounds formed from hydroperoxides either by lipoxygenases or autooxidation of UFA and PUFA [11], [12]. During storage, increasing levels of these compounds can lead to the development of rancid off-flavors in bacteria fatty acid. The objectives of this study were to determine the effects of light irradiation and oxygen effect on the formation of volatiles compounds from polyunsaturated fatty acid model systems. In addition, the general changes in volatils during cold storage as well as the influence of 4 differents milk protein ingredients were studied. The volatile compounds were collected using dynamic headspace sampling and separated and identified by use of dynamic headspace gas chromatography-mass spectrometer (GC-MS). Previous studies [13], have shown that in order to obtain an adequate lipid to protein ratio, extra protein needs to be added to secure physical stabilization of the lactic acid bacteria oil throughout processing. However, intrinsic properties of commercial protein powders can influence on the oxidative stability.

II. MATERIALS AND METHODS

Microorganisms and inoculum

A selected *Lb. plantarum* CWBI-B1419 bacterial strain (CWBI, Wallon Center for Industrial Bio-Industry Unit, Gembloux Agricultural University, Belgium), isolated from poultry farm was used as inoculum in this study. MRS broths inoculated with strain were incubated at 30°C for 18 h. The bottles were incubated at 30°C to the exponential phase of growth (18h). The bacterial suspensions were then used to inoculate the fermenting media at 1%(v/v). In all cases, the initial microbial concentration was approximately 7.5log10 CFU/ml. The cell pellets obtained were resuspended in 5 ml (50% v/v) glycerol and frozen at -80 °C. The supernatants obtained after centrifugation (Sorvall RC2-B, Sorvall, USA) at 2,500 × g for 20 min, were decanted.

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Productions and storage conditions

The strains were grown in 100 L bioreactor containing MRS medium for 18 h and then concentrated 20 times by centrifugation. The supernatants obtained after centrifugation (Sorvall RC2-B, Sorvall, USA) at $2,500 \times g$ for 20 min, were decanted with 2%, w/w glycerol and 5%, w/w maltodextrine. Cells were freeze-dried in a low freeze-drier (Leybold, Belgium) with a standard program by increasing the temperature gradually from -45°C to 25°C at 0.9 mbar pressure (30h) followed by 15h at 0.15 mbar. All fermentations were done in duplicate and average values reported. Freeze-dried samples were stored during 90 days, in portion size (2 ± 0.5 g in each portion) at 20°C in aluminum foil packets which were vacuum-sealed. Samples were withdrawn for analysis each 30 days and kept at -20°C (48-72 h) before analysis

Dry cell weight, water Content and survival rate

The dry cell weight of 1 g of powder was determined at the end of freeze drying. The water content (100 g dry weight)-1 of the freeze-dried samples during storage at 20°C was determined each 30 days. The samples were dried in a convection oven until constant weight and results are mean of triplicate determinations. The water activity (a_w) of the freeze-dried samples was measured at 20°C using a water activity meter Novasina (Novasina, Pfäffikon, Switzerland). Standard salt solutions (Novasina) of known water activity were used for calibration of the sensor at the measuring temperature. Readings were taken until four sequential readings gave the same results. Results obtained were the average of three determinations. The viable counts were obtained by plate count method after 48h. Percentage survival after 90-day storage was calculated as $100 \times N/N_0$, where N is the CFU/g of the freeze-dried sample at a given time and N_0 is the CFU/g of the sample at the end of freeze-drying.

Data analysis of SPME

Two grams of freeze-dried cells were added to a 20-mL glass sample vial (Perkin Elmer, Mass., U.S.A.) and fitted with a self-sealing septum at its top, through which the solid-phase microextraction (SPME) syringe needle (bearing a fiber coated with $8 \mu\text{m}$ thick film of Carboxen-polydimethylsiloxane, Supelco, Bellefonte, Pa., U.S.A.) was introduced and maintain in the headspace at 35°C for 30min. The SPME fiber was conditioned according to the manufacturer's recommendations (280°C for 30min in the GC injector). The volatile analytes absorbed to the SPME fiber were analyzed by GC-MS (Hewlett-Packard, 5890 series II plus, N.J., U.S.A.). The compounds were separated

by a CPWAX-52CB fused silica column ($50 \text{ m} \times 0.25 \text{ mm} \times 0.2 \mu\text{m}$, Agilent, Calif., U.S.A.).

GC-MS operating parameters

The injection port was heated at 250°C , in splitless mode, and helium flow rate was maintained at 1 mL/min. The SPME fiber was maintained in the injection port for 10min. The oven temperature program was held at 40°C for 4min, increased to 250°C at a rate of $8^{\circ}\text{C}/\text{min}$, and held for 5min. Detection was performed with the mass spectrometer operating in a scan mode (mass range from 35 to 400 m/z using a scan rate of 4.51 scans/s) and the ionization energy was set at 70 eV. The temperatures of the ion source and the quadrupole mass analyzer were held at 200 and 100°C , respectively. The eluted compounds were identified by their retention times and by comparison of their mass spectra with the Wiley Mass Spectral database (Wiley & Sons Inc., N.Y., U.S.A.). Triplicate samples were analyzed. Gas chromatography with flame ionization detector (GC-FID) (HP5890 series II plus) was used to quantify volatils. The compounds were separated by a capillary AT1000 column (Alltech, Ky., U.S.A.) ($25 \text{ m} \times 0.32 \text{ mm} \times 0.30 \mu\text{m}$). The analysis conditions were the same as for GC-MS. The eluted compounds were identified on their retention times as compared with the retention times of corresponding standards and after analyzing the same sample in GC-MS. The internal standard used was 2-ethylbutanoic acid at a concentration of 10 ppm. Duplicate samples were analyzed. The data was then analysed using ANOVA.

Results and discussion

Viability of *Lactobacillus plantarum* CWBI-B1419 during storage.

According to [14] stability of dried lactic acid bacteria upon storage was better in the 0.1-0.2 a_w zone. During this study, the water activity of *Lb. plantarum* CWBI-B1419 after freeze drying have some a_w less than 0.170 at 25°C . This result give us an indication that the lactic acid bacteria are well lyophilized. *Lb. plantarum* CWBI-B1419 was produced in bioreactor; the cells were harvested, added or not with 5% maltodextrine, 2% glycerol and freeze-dried. **Table 1**, shows viable counts during the process and survival after freeze-drying. A viable population of $11.88 \pm 0.03 \log_{10}\text{CFU/g}$ or $11.65 \pm 0.04 \log_{10}\text{CFU/g}$ *Lb. plantarum* CWBI-B1419 added or not with maltodextrine and glycerol were obtained after the freeze-drying process. The effect of the additives is not significant. Moisture content and water activity (a_w) were determined (**table 1**).

Table 1. Viability of *Lactobacillus plantarum* CWBI-B1419 before and after freeze-drying

	Dry matters (%)	Dry parameters		Population (log CFU/g)	Survival (%)
		Moisture (%)	a_w		
Before freeze-drying	93.8 ± 0.4^a	3.1 ± 0.3^a	–	9.4 ± 0.02^b	0.29 ± 0.02^b
After freeze-dying	93.7 ± 0.5^a	4.7 ± 0.7^a	0.165	11.8 ± 0.05^b	0.19 ± 0.04^b

The rate of dry matter in a lyophilized powder, which is nearly 94% and a water activity less than 0.2, would be necessary to ensure a level of storage stability at room temperature. These conditions are consistent with research undertaken by French's Patent FR No. 2829147, (Béal et al., 2001) which stipulates that in a dried product the following parameters: very low water activity ($a_w < 0.2$), rate of dry matter around 96% and a $T_g = T_s - 25^\circ\text{C} > 20^\circ\text{C}$, contribute to the stability of the product for an extended period during the storage.

The powder produced from this lactic acid bacterium meets all these conditions and yet conservation is not optimal during storage. Apart from this, freeze-dried powders meet the above-mentioned criteria and yet the survival rate is not high during storage. According to the work of [15], a number of factors can influence the rate of survival in function of time. With regard to the other predominant factors to this loss of viability, one could cite (the water activity), probably taken into contained pure water powders, the effect of temperature and light for hermetically sealed bags (under vacuum) containing 2-3 g of powder. Loss of viability was also noted that at this level find its origin by the combined effect of temperature and oxidation of cellular constituents as unsaturated fatty acids [16]. Peroxides (R-OOH) are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation [17].

This oxidation finds its source by oxygen effect, lyophilized powders that create free radicals. These free radicals are toxic substances that lead to rupture the membrane and thus cellular cell death in the future. These primarily mitigate the adverse events and are especially responsible for significant losses during storage that biologists increasingly need antioxidant to stop or reduce the oxidation products at the level of freeze-dried powders. It is safe to say that based on our experiences and those of others; the loss of viability during storage is subject to a phenomenon of auto-oxidation, which should optimize the survival rate especially during storage at room temperature. The oxidation products are varied. Hydroperoxides from the decomposition of polyunsaturated give way to volatile secondary products that represent the same phenomenon. These compounds are also important indicators of cellular oxidation

Volatile compound analysis.

Volatile compounds from freeze-dried powders were extracted by SPME. Several types of fibers (CAR/PDMS, and CAR/PDMS/DVB) were tested under the same conditions to evaluate the more adapted one. Best results were obtained with the CAR/PDMS/DVB fiber, which is in good accordance with [18], [19]. Analysis of the volatile SPME profiles obtained for the different time points of freeze-dried cells. 109 compounds were found as volatile compounds and very low levels of these compounds as compared to volatile compounds extracted from freeze-dried *Lactobacillus plantarum* NCIMB 8826 in cereal-based substrates. Details about the peak identities and relative abundances of the volatile compounds expressed as (compound peak area/total compounds peak area) x 100 are shown in **Table 1**. Among the identified organic compound

there are acids, amides, alcohols, aldehydes, aromatic hydrocarbons, esters, furans, ketones, peroxides, and pyrans. High concentrations of ten volatile compounds were found in freeze-dried powder (**Table 2**). 1,3-butanediol, 2,3 butanediol, acetic acid, hexanal, cyclotetrasiloxane octamethyl and benzaldehyde are mainly identified. Beside these compounds, additional volatile compounds were also identified in very small amounts (**Table 2**). Hexanal is the only aldehyde formed from both the 9- and the 13-hydroperoxide of linoleic acid at least by chemical reactions. Hexenal may be the direct product of the chemical fragmentation of the 13-hydroperoxide of linoleic acid, or it may derive from 2,4 decadienal that may be a product of chemical oxidation of 9-hydroperoxy linoleic acid (HPOD) [20], [21]. Freeze-dried powder of *Lactobacillus plantarum* had known to contain mainly linoleic acid as PUFAs and the major LOX activity in tubers forms 9-derivatives, the precursors of enzymatic C-9 aldehyde formation [22]. Our study failed to detect other compound as aldehyde. However, hexenal may be the preferred product of 9-hydroperoxides from chemical reactions as well. Moreover, the SPME technique may discriminate as the fibers adsorb more preferentially the most volatile compounds. Despite this disadvantage, the technique is well-adapted to define smell perception [23]. Heptane and hexene were also identified and quantified by HPLC; benzaldehyde, pyrazine and nonadecane were identified as minor products. Except hexenal, none of aldehydes have been identified by SPME-GC-MS. The rate of hexenal has been increased during storage. Based on these results and the work carried out by [24] and [25] assuming an oxidation by enzymatic formation of hexenal in the lyophilized powder has been issued. Another group of compounds originally aroma can be generated during the treatment because of the enzymatic activity of lactic bacteria. The high concentrations of acetic acid could be explained by the aerobic metabolism of *Lb. plantarum*. [26] have showed that *L. plantarum*, during the stationary phase, under aerobic conditions and low glucose concentration, can consume the previously secreted lactic acid and an equivalent amount of acetic acid is released. Similar behaviour of *L. plantarum* in residual media was also reported by [27]. The high relative concentrations of acetic acid found in freeze-dried powder suggest that part of the lactic acid produced could have been metabolised to acetic acid. Aldehydes were found in freeze-dried *Lactobacillus* in relatively quantities (**Tables 2 & 3**). The final stages of the fermentation of bacteria, plants and yeast involve the conversion of pyruvate to acetaldehyde by the enzyme pyruvate decarboxylase (EC 4.1.1.1), followed by conversion of acetaldehyde into ethanol. The latter reaction is further catalyzed by alcohol dehydrogenase, running in the opposite direction [28]. or formed by the metabolism of lactate or by the oxidation of ethanol [29]-[31]. Branched aldehydes originate from amino acid degradation [32], i.e. 2-methyl propanal, 2-methyl butanal and 3-methyl butanal were produced from Val, Ile and Leu, respectively [33] were not detected, benzaldehyde has also been found in some trials with important concentration.

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Table 2. Relative abundances of volatile compounds detected in freeze-dried *Lactobacillus plantarum* CWBI-B1419 during storage.

Peak n°	Compounds	RT	Identification (%)				Σ trials
			Trial-1	Trial-2	Trial-3	Trial-4	
1.	Hexane	0,15	0,17	-	-	-	0,17
2.	Heptane	0,96	-	5,19	-	-	5,19
3.	Ethanol	1,11	0,79	-	-	-	0,79
4.	Acetic acid	1,60	6,89	-	13,18	-	20,07
5.	Ethanoic acid	1,65	3,42	-	6,23	-	9,65
6.	1-Butanol	1,86	0,68	-	-	-	0,68
7.	1,3-Butanediol	2,91	15,26	0,87	12,89	6,84	35,86
8.	2,3-Butanediol	2,95	6,75	6,48	19,89	-	33,12
9.	Silane triethyl(ethoxy)	2,96	-	4,31	-	-	4,31
10	Butanol trimethyl ester	2,98	-	-	-	14,67	14,67
11	1,3-Dihydroxybutane	2,99	18,54	-	-	-	18,54
12	Butane-2,3-diol	3,07	1,74	-	11,83	-	13,57
13	Ethyl 2-hydroxypropanoate	3,26	0,46	-	0,73	-	1,19
14	Cyclotrisiloxane hexamethyl	3,38	2,58	1,78	2,40	1,53	8,29
15	Pyrazine, 2,5 dimethyl	4,19	-	4,05	-	-	4,05
16	hexenal	4,79	-	-	-	18,80	18,8
17	Ethyl metoxy acetate	4,81	0,25	-	-	2,44	2,69
18	Ethanol 2- (21-methylethoxy)	4,83	-	0,21	-	-	0,21
19	1-Propene, 1-methylthio-(z)	4,84	-	-	0,42	-	0,42
20	Disiloxane ethenylpentamethyl	5,47	0,58	0,81	0,53	2,60	4,52
21	Pentamethylheptane -(2,2,4,6,6)	5,57	0,69	-	-	-	0,69
22	Cyclotrisiloxane hexamethyl	5,58	0,51	-	-	-	0,51
23	Ethanol 2,2-diethoxy	5,61	-	6,29	-	2,04	8,33
24	Cyclotetrasiloxane octamethyl	5,69	2,71	0,76	7,37	7,91	18,75
25	Trimethyl ethoxysilane	5,72	-	0,62	-	-	0,62
26	Methylanisole	5,76	-	-	-	6,11	6,11
27	Cyclotetrasiloxane	5,88	5,06	-	-	-	5,06
28	Dimethylpyrazine	5,89	-	0,91	-	-	0,91
29	Pyrazine ,2 5-dimethyl-3-(3-methylbutyl)	5,95	-	4,45	-	-	4,45
30	Benzene , 1-methoxy-4-methyl	5,96	-	-	-	3,25	3,25
31	Trimethylpyrazine	5,98	-	-	0,63	-	0,63
32	Pyrazine 3-ethyl-2,5-dimethyl	7,13	-	4,37	-	1,56	5,93
33	2-allyl-5-methylpyrazine	7,25	-	4,90	-	-	4,9
34	Tetrapyrazine	7,27	-	-	0,69	-	0,69
35	Ethoxycarbonylbenzophenone	7,60	0,23	-	-	-	0,23
36	Benzaldehyde 2,4-bis[trimethylsiloxy]	7,62	0,38	-	-	-	0,38
37	Benzaldehyde	7,65	-	-	-	1,76	1,76
38	3-Heptene	7,69	0,09	-	-	-	0,09
39	Benzeneethanol	7,70	-	-	0,96	-	0,96
40	Pyrimidine, 2-(2-nitro-3-thienyl)	7,98	-	5,55	-	-	5,55
41	Benzaldehyde 2,5-bis[(trimethylsiloxy)]	8,22	3,71	-	-	9,11	12,82
42	Benzoic acid	8,23	9,86	-	-	-	9,86
43	Benzeneacetic acid	8,25	-	-	5,09	-	5,09
44	2,3,5-Triethyl-6-ethylpyrazine	8,30	-	-	0,29	-	0,29
45	5-Methyl-1-heptene	8,69	-	-	-	2,11	2,11
46	Dodecane	8,90	0,45	0,94	-	-	1,39
47	Trisiloxane hexamethyl	9,00	-	-	0,56	-	0,56

48	Ethanone, 1-(3,5-dimethyl-2-pyrazinyl)	9,27	-	-	-	0,65	0,65
49	Cyclotetrasiloxane octamethyl	9,51	0,51	0,89	-	-	1,4
50	Propenylpyrazine	9,70	-	-	-	0,76	0,76
51	1,4-Di-tert-butylbenzene	9,73	-	-	0,12	-	0,12
52	1-Tetradecene	9,75	0,38	-	-	-	0,38
53	Phenethyl acetate	9,77	-	-	-	0,11	0,11
54	Cyclotetrasiloxane hexamethyl	9,82	0,38	0,64	-	-	1,02
55	2-Undecanone	10,24	-	1,21	0,32	-	1,53
56	4-Ethyl-4-methyl-1-hexene	10,42	0,32	0,87	-	-	1,19
57	Methylnonadecane	10,44	-	-	-	0,38	0,38
58	Cyclotetrasiloxane octamethyl	10,47	-	-	0,35	-	0,35
59	Pyrazine 2,5-dimethyl-3-(methylbutyl)	10,56	-	2,48	-	-	2,48
60	16-oxosalutaridine	10,66	9,90	9,46	4,61	9,35	33,32
61	Tetradecane 2-methyl	10,95	-	0,32	-	-	0,32
62	Tritriacontane	10,96	0,18	-	-	-	0,18
63	Hydroxycyclohexane	10,99	-	0,21	-	-	0,21
64	1-benzothiopyran-4-one 1-oxide	11,07	0,16	-	0,14	0,84	1,14
65	4H-1-benzothiopyran-4-one	11,08	-	0,83	-	-	0,83
66	Cyclohexane	11,11	0,34	0,50	-	-	0,84
67	Tridecane 2-methyl	11,26	0,83	1,27	0,34	1,22	3,66
68	5-Ethyl-1-nonene	11,46	0,35	0,66	-	-	1,01
69	1-Hexene	11,47	-	-	1,18	-	1,18
70	Propyl -trimethylpyrazine	11,62	-	-	0,73	-	0,73
71	Tetradecane	11,65	-	1,52	-	-	1,52
72	Silane trimethyl	11,66	-	-	-	0,42	0,42
73	Benzenepropanoic acid	11,68	-	-	0,15	-	0,15
74	Cyclohexane diethyl	11,72	-	1,13	-	-	1,13
75	Nonadecane	11,75	0,49	-	-	-	0,49
76	Trisiloxane octamethyl	11,80	-	0,39	-	-	0,39
77	Cyclohexane 1-ethyl-2, 4-dimethyl	11,92	0,16	0,63	-	-	0,79
78	Methyl decane	12,06	0,16	-	-	-	0,16
79	Naphthoic acid	12,07	-	0,40	-	-	0,4
80	Eicosane	12,09	-	-	0,43	-	0,43
81	1-Tetradecene	12,31	-	-	-	0,38	0,38
82	Cyclotetradecane	12,32	0,28	0,58	-	-	0,86
83	Undecadien-2-one	12,36	-	-	0,23	-	0,23
84	Cyclotetrasiloxane octamethyl	12,37	-	0,73	-	2,96	3,69
85	(Z)-5-Dodecenyl acetate	12,59	-	-	0,21	-	0,21
86	Spiro[4.5]decane	12,60	-	1,22	0,56	-	1,78
87	1-Decene,4-methyl	12,61	0,59	-	-	-	0,59
88	Butylated hydroxyanisole	12,62	-	1,27	-	-	1,27
89	Cyclohexadien-1-one	12,63	-	-	-	0,64	0,64
90	Z5-Decenylaceytate	12,69	-	-	0,45	-	0,45
91	Cyclopentane	12,70	-	-	-	0,6	0,6
92	2-Trideconone	12,89	-	7,81	2,45	-	10,26
93	Phosphoric acid	12,90	1,48	-	-	-	1,48
94	Cyclotetrasiloxane octamethyl	12,91	-	1,26	-	-	1,26
95	2-Tridecanol	12,98	-	-	-	0,82	0,82
96	Phenol, 2,4-bis(1,1-dimethylethyl)	13,11	0,37	0,68	2,45	-	3,50
97	2H-1-Benzothiopyran	13,13	-	-	-	1,67	1,67
98	Butylated hydroxytoluene	13,15	-	-	0,93	-	0,93
99	Phenol, 2,6-bis(1,1-dimethylethyl)	13,16	-	0,39	-	-	0,39
10	Butyl hydroxy toluene	13,20	-	-	-	0,65	0,65
10	Pentadecane, 3-methyl	13,76	0,16	0,45	-	-	0,61
10	Furan, 2-butyltetrahydro	14,15	-	0,92	-	-	0,92
10	Trisiloxane octamethyl	14,89	0,10	-	-	-	0,10
10	Mercaptoacetic acid, bis(trimethylsilyl)	14,92	-	0,35	-	-	0,35

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10 Dimethyl silane	14,95	-	-	1,10	-	1,10
10 Cyclohexane (3,3 dimethylpentyl)	15,09	-	1,24	-	-	1,24
10 2-Pentadecanone	15,26	-	4,55	-	-	4,55
10 Benzendicarboxylic acid	17,11	0,23	3,08	0,44	-	3,75
10 1, 13-Tetradecadiene	17,21	0,80	-	-	-	0,8

Alcohols were amongst the main volatile compounds in powder and the alcohols present at the greatest concentrations were 1,3-butanediol, 2,3-butanediol. Secondary alcohols are formed

by enzymatic reduction of methyl ketones, which are produced from fatty acids [34].

Table 3. The main volatiles compounds found in the dynamic headspace/GC analysis of freeze-dried *Lb. plantarum* CWBI-B1419 during storage.

Peak n°	Compounds names	RT	Significant effect of time	Changes during storage	Log ₁₀ of peak area		
					Storage time (days)		
					30	60	90
1.	Heptane	0.96	+	-		nd	
2.	Acetic acid ¹	1.60	++	↓	0.70	0.68	0.58
3.	Ethanoic acid	1.65	+	-		nd	
4.	Pyrazine, 2,5 dimethyl ²	2.91	+++	↑	0.95	0.95	0.98
5.	2,3-Butanediol ³	2.95	+++	↓	0.91	0.90	0.87
6.	Silane triethyl(ethoxy)	2.96	+	-			
7.	Butanol trimethyl ester ⁴	2.98	++	↑	0.56	0.60	0.66
8.	1,3-Dihydroxybutane ⁵	2.99	++	↑	0.66	0.70	0.75
9.	Butane-2,3-diol ⁶	3.07	++	↑	0.53	0.63	0.70
10.	Cyclotrisiloxane hexamethyl	3.38	+	-		nd	
11.	Hexenal ⁷	4.79	++	↑	0.67	0.73	0.79
12.	Disiloxane ethenylpentamethyl	5.47	+	-			
13.	Ethanol 2,2-diethoxy ⁸	5.61	++	↓	0.67	0.59	0.48
14.	Cyclotetrasiloxane octamethyl	5.69	+	-			
15.	Methylanisole	5.76	+	-			
16.	Cyclotetrasiloxane	5.88	+	-		nd	
17.	Pyrazine 3-ethyl-2,5-dimethyl	7.13	+	-			
18.	2-allyl-5-methylpyrazine	7.25	+	-			
19.	Pyrimidine, 2-(2-nitro-3-thienyl) ⁹	7.98	++	↑	0.50	0.65	0.70
20.	Benzaldehyde 2,5-bis[(trimethylsil)oxy]	8.22	+	-		nd	
21.	Benzeneacetic acid	8.25	+	-			
22.	16-oxosalutaridine ¹⁰	10.66	+++	↑	0.92	0.96	0.99
23.	2-Trideconone	12.89	+	-		nd	
24.	2-Pentadecanone	15.26	+	-		nd	

The results of multiple data analysis are indicated by: the significance levels $+ \leq 3$, $++ \leq 6$ and ≥ 6 . The arrows correspond with increase (↑) or decrease (↓) in-group with time. The table shows the mean values of the log-transformed peak areas for all samples after 30, 60 and 90 days of storage.

The characteristic aroma of freeze-dried powder can be attributed to the secondary alcohols, in particular, 2-heptanol and 2-nonanol [34], [35]. Among the 24 compounds identified as having the highest rates, ten were followed for their high levels after analysis (Table 3). During storage, the results of analysis of 30, 60 and 90 days we noticed, seven compounds with high concentrations those are: hexanal, 16-oxosalutaridine, pyrimidine, 2-(2-nitro-3-thienyl), butane-2,3-diol, butanol trimethyl ester, 1,3-dihydroxybutane and pyrazine, 2,5 dimethyl while acetic acid, 2,3- butanediol and 2,2-ethanol diethoxy experiencing decline during the same storage. This difference could be due to the fact that the mortality increased in the cells and the rate of peroxide with increasing rates in acetate and alcohol decreasing [36], [37].

(Barron et al., 2005; McSweeney et al., 2000).

This assertion is consistent with the work carried out by [38] and [39]. These alcohols are used as precursor for the formation of ethyl esters [40]. There is a link between cell death and the oxidation phenomena observed during storage and production of certain volatile compounds. Among the most important in *Lactobacillus plantarum* CBI-B1419, hexenal is a compound key product of the secondary decomposition related to membrane oxidation. Our work is consistent with those made by [34]. Products are essentially volatile compound and hexenal has the highest concentration. Methyl ketones are products of microbial β -oxidation of saturated fatty acids followed by β -keto acid decarboxylation [41].

The Results section should include the rationale or design, optimization, validation of the experiments as well as the results of the experiments.

Conclusions

The alcohols, alkanes, esters, methyl-branched and phenylalanine derivatives, ketones, and aldehydes were the most abundant groups of volatiles in freeze-dried *L. plantarum* CWBI-B1419. The amino acid catabolism and ester synthesis seemed to be more prominent than the lipid oxidation and glycolysis during 90 days storage with no access to air. Many common secondary lipid oxidation products remained stable or decreased during storage. Some of the different additives affected only the esters and pyrazines, not the lipid oxidation compounds. Even with a high content of long chained PUFA in freeze-dried cell product, the occurrence of lipid oxidation compounds of these vacuum-packed products was lower than expected. In general, the volatile production depends more on the substrate than on the microorganism. The results obtained add to the knowledge of the volatile composition of cereals and cereal-based products, and provide useful information for further development of suitable dairy probiotic used for drinks and others products.

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