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Research Article

Enhanced Duration of Truffle Sauce Preservation due to Addition of Linoleic Acid

Bruno Tirillini,[1] Filippo Maggi,[2] Roberto Venanzoni,[3] and Paola Angelini[3]

[1]*Department of Biomolecular Sciences, University of Urbino, Pesaro-Urbino, Italy*
[2]*School of Pharmacy, University of Camerino, Camerino, Italy*
[3]*Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy*

Correspondence should be addressed to Paola Angelini; paola.angelini@unipg.it

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Food products based on truffles such as truffle sauces are commonly sterilized by autoclaving. With the aim of reducing sterilization durations and temperatures and therefore minimizing the formation of new molecules while maintaining sterile conditions, natural molecules with bacteriostatic action can be added. This work takes into account molecular variations in a truffle sauce with linoleic and stearic acids added at various temperatures and durations of sterilization. As controls, the sterilized truffle sauces, not additives, were taken. The SPME/GC has always detected changes in the composition of the headspace but more significant at longer durations. An increase in temperature leads to the transformation of an important fraction of total alcohol into total aldehydes. Bacteriological tests were performed on total aerobic bacteria and *Clostridium* spp. The total bacterial load, even if low, is instead present in the controls for all temperature/duration combinations. Linoleic acid is more effective than stearic acid and contributes with a concentration of 10% (w/w) to a decisive reduction of the total bacterial load at 10′/121°C and 3′/130°C. The nutritional value of truffle sauces can be increased by adding nutraceuticals, and amongst these, linoleic acid could be a valid candidate.

1. Introduction

Truffles (*Tuber* spp.) belong to the fruiting bodies of certain hypogeous ascomycetes, which may grow in ectomycorrhizal symbioses with specified shrub and tree species [1, 2]. In particular, some species of truffle, *Tuber magnatum* Pico (white truffle), *Tuber melanosporum* Vittad (black truffle), and *Tuber aestivum* (Wulfen) Spreng., are the most appreciated for their organoleptic properties, and their demand is increasing in the food markets of many countries, particularly in Italy, France, Spain, and other European countries [3–5].

For this reason, the volatile components of *Tuber* aroma have been extensively studied. Using headspace solid-phase microextraction (HS-SPME), combined with gas chromatography-mass spectrometry (GC/MS), it was possible to identify 36 compounds in *T. magnatum* [6, 7], 70 compounds in *T. melanosporum*, and 40 compounds in *T. aestivum* [8].

Truffles quickly lose their organoleptic characteristics. The large amount of bacteria present in the fruiting body leads to its rapid deterioration with loss of its edible properties [9–11].

Knowledge of the physicochemical and microbiological modifications during truffle storage is still limited [12, 13] despite *Tuber* volatiles being thoroughly investigated in relation to both truffle species identification [14–16] and to freshness evaluation [17].

To improve the shelf life of *Tuber* and preserve its sensory and structural characteristics, several methodologies have been applied.

One example of the methodologies used is the utilisation of 1.5 kGy irradiation, which safeguards some biochemical characteristics and causes a decrease in the truffle microbial flora [13]. However, standard protocols exist regarding the preparation of the fresh or preserved truffle market. Whilst truffles used for the production of a wide range of food products such as truffle sauces (homogenized with other

foods, i.e., porcini mushrooms) are immediately frozen or sterilized by autoclave at 120–130°C for 30 min. [18], fruiting bodies used in fresh products have a minimal processing and require lower temperatures (usually 4°C) in order to preserve quality and extend the shelf life of the fungus.

The conservation process of truffle sauces, however, leads to a variation of the organoleptic properties of the truffles. This is due to the autoclaving temperature, which is supposed to lead to the formation of reactions between the components of the truffle sauces with the production of new compounds, practically varying the chemical composition of the sauce. The complexity of the reactions of the matrix is not yet fully known, and one of the aims of this work was to identify the pattern of volatile compounds that are formed as a result of different autoclaving treatments. As a reference, the composition of the volatile compounds of an autoclaved truffle sauce at a lower temperature (110°C) and the shorter duration (3 min.) necessary to sterilize the product was taken into consideration.

The nutritional value of truffle sauces can be increased by adding nutraceuticals, and amongst these, linoleic acid (18 : 2) could be a valid candidate. The linoleic acid could maintain normal blood cholesterol concentrations [19] and protect the skin from UV-induced damage [20] or give a contribution to the maintenance or achievement of a normal body weight [21].

The second aim of the present work was to determine how the VOC pattern of a truffle sauce added with linoleic acid varies and its microbiological modifications as a result of autoclaving conditions, in order to select the most suitable solution to be applied in future studies related to its preserving ability. Reference was made to stearic acid (18:0), a C-18 acid which reacted slightly to the various experimental autoclaving tested [22].

The final aim of this work was to vary experimental autoclaving conditions so as to achieve minimum bacteria levels in truffle sauces after 12 months of storage.

2. Materials and Methods

2.1. Sample. The truffle sauce was taken directly from the company that produces it before industrial autoclaving and put into 100 g jars (standard sample). The truffle sauce contained other than *Tuber melanosporum* even *Boletus edulis* (1 : 1). 10 g of stearic acid or linoleic acid was added to the 100 g jars. 3 jars of each type were treated as shown in Table 1.

2.2. SPME-GC-MS. Volatile compounds were extracted by the solid-phase microextraction technique (SPME). 100 mg of sample was placed in a 4 mL vial tightly sealed at 50°C. A 100 μm thick, and 1 cm long divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) fiber was used for sampling. The fiber remained in the vials for 20 min. The fiber was desorbed on the gas chromatography (GC) on liners for SPME (0.75 mm i.d., Supelco) at 250°C for 3 min.

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on Agilent 6890 N coupled to a 5973 N mass spectrometer. The compounds were separated by a polar DB-WAX column (30 m, 0.25 μm i.d., 0.25 μm, J&W, Scientific, Folsom, CA, USA) with the following temperature program: 6 min at 45°C, then 6°C/min up to 140°C, and finally 25°C/min to 250°C.

The volatile organic compounds (VOCs) were identified by comparing the retention index and mass spectrum of the chromatographic peaks with those of standards analyzed under the same conditions (when available in the market). Retention indices were calculated using a mixture of normal alkane (C8-C30) (Supelco) diluted in hexane within a 4 mL vial sampled via the same CAR-DVB-PDMS fiber used for analysis of samples and using the same temperature program as mentioned above.

The peak assignments of the other volatile components were based on computer matching of the mass spectra obtained with the WILEY275, NIST 08, and ADAMS libraries, taking into account the coherence of the retention indices of the analyzed compounds with those reported by Adams and NIST 08 libraries [23, 24].

2.2.1. Statistical Analyses. All the analyses were run in triplicate and averaged.

(1) Cluster Analyses. To see if there was any evidence of interesting natural groupings, we applied cluster analyses to the database. A hierarchical agglomerative clustering procedure, based on furthest neighbour, was carried out. All variables were measured on an interval scale using the Euclidean distance.

(2) One-Way Analysis of Variance. The hypothesis tested was that the set of mean variables were the same across groups. Levene's test was used for testing the homogeneity of variances. To determine which specific-group mean was different, a post hoc multiple comparison technique was carried out. We used Tukey's honestly significant difference (HSD). Values of $p < 0.05$ were considered as statistically significant. All statistical evaluations were performed by using the SPSS 16.0 software package for Windows.

(3) Principal Component Analyses. The database for the principal component analyses is the matrix experimental mode x VOC. The intent behind the method was an attempt to reduce the complexity of the data by decreasing the number of variables that need to be considered.

2.3. Microbiological Analyses

2.3.1. Counts of Total Aerobic Bacteria. Counts of total aerobic bacteria (TAB) were done in tryptone glucose yeast extract agar (TGYE, Merck, Milan, Italy) medium. Decimal dilutions of the sample were plated in triplicate and incubated at 30°C for 48 h. [25].

2.3.2. Counting of Sulphite-Reducing Clostridia and Clostridium perfringens. Selective enumeration was carried out in pour plates of tryptose sulfite cycloserine agar (TSCA, Merck, Milan, Italy) to which 25 g NaCl was added

Table 1: Autoclaving conditions of truffle sauce with or without the addition of fatty acids.

Sample	Autoclaving conditions				Component
	ID	Duration	Temperature (°C)	Pressure (atm)	
P1.C		10′	110	0.41	Standard sample
P1.1	A	10′	110	0.41	+stearic acid
P1.2		10′	110	0.41	+linoleic acid
P2.C		10′	121	1.02	Standard sample
P2.1	B	10′	121	1.02	+stearic acid
P2.2		10′	121	1.02	+linoleic acid
P3.C		3′	130	1.66	Standard sample
P3.1	C	3′	130	1.66	+stearic acid
P3.2		3′	130	1.66	+linoleic acid
P4.C		5′	130	1.66	Standard sample
P4.1	D	5′	130	1.66	+stearic acid
P4.2		5′	130	1.66	+linoleic acid

per litre. After incubation at 37°C for 18–24 h under anaerobic conditions, black colonies were counted and subsequently confirmed as *C. perfringens* according to ISO7937 [26], using fermentation of lactose (+), gelatin liquefaction (+), motility (−), and nitrate reduction (−) as criteria after anaerobic incubation for 24 h at 37°C.

3. Results and Discussion

Seventy-seven volatile components were identified in truffle sauce, corresponding to 86.75–99.89% of the total headspace compounds (VOCs) (Table 2). 2-Methyl-butanal is the most abundant compound and reaches the maximum concentration of 37.07% in P4.C. Other compounds are present in significant quantities such as 2-pentylfuran 14.92% in P2.2, benzyl alcohol 12.93% in P1.1, 2-butanol 11.03% in P1.C, 2-butanone 9.96 in P3.1, 1-octen-3-ol 9.46 in P1.C, and *n*-hexanal 9.16 in P2.2.

VOCs were rich in alcohols (17.46–39.9%), and in this fraction, 2-butanol (11.03 % in P1.C), 1-octen-3-ol (from 10% to 5.74%), and *n*-hexanol (10.18% in P1.2) formed the major contribution.

The variations of the VOCs classes for the various experimental conditions are reported in Figure 1. As can be seen, varying the autoclaving duration results in more or less marked variations in the reciprocal relationships of the classes of chemical compounds.

The class of furan derivatives is represented by 2-pentylfuran, 3-furaldehyde, 2-furanmethanol, and 2(5H)-furanone. Among these, the 2-pentylfuran shows the widest variation in concentration depending on the operating conditions. The highest concentration is with conditions P2.2 (14.92%), and higher than average concentrations are also found with conditions P4.2 (5.59%) and P1.2 (4.59%) and even slightly less with conditions P3.2 (2.83%). We can hypothesize that the addition of linoleic acid promotes the formation of this compound at all temperatures and durations experienced while remaining much lower and constant in all other conditions.

In all the temperature and duration conditions experienced, the addition of stearic acid (SA) causes an increase in the quantity of acids.

The addition of the two acids tends to decrease the alcohol content compared to the control but under conditions P4, the addition of linoleic acid (LA) causes a significant increase compared to the control.

Aldehydes increase with the addition of the two acids only under P2 conditions, under the other conditions they decrease by about the same amount. Under lowest conditions P1, the two acids act in the opposite way, and only the LA produces a significant increase compared to the control.

A drastic decrease of aromatics occurs under P2 conditions with the addition of LA and a little less accentuated with the addition of SA. In all cases, the addition of LA leads to a more or less great decrease compared to the control.

Under P2 conditions, the addition of SA or LA produces a significant increase in ketones, while the addition of both causes a sharp decrease of ketones at lower temperatures (P1).

The esters and sesquiterpenes do not seem to be affected by the addition of SA or LA.

The furan derivatives are strongly influenced by the addition of LA under all the tested conditions, but under conditions P2, they produce the maximum increase of these compounds.

At the lower temperature, the addition of acids produces significant decreases in monoterpenes.

LA always produces a lowering of the quantity of oxygenated monoterpenes. The SA in P4 produces a significant increase in oxygenated monoterpenes in contrast to the effect of linoleic acid.

The paraffins are drastically influenced by the addition of the two acids starting from the P2 conditions with increases due to the LA at least double compared to the SA.

At two lower temperatures, the addition of LA produces a net decrease of sulfur compounds while under P4 conditions, the addition of the two acids produces an increase in sulfur compounds.

Under P1 conditions, the addition of LA produces a decrease in ketones, alcohols, and aromatics and an increase in aldehydes and furan derivatives. The addition of SA decreases ketones and increases the aromatics and especially the acids.

Under the experimental conditions P2, there is a strong increase in furan derivatives and, albeit less so, in ketones,

Table 2: Identification and quantification of VOCs.

No.	Component	P1.C (%)	P1.1 (%)	P1.2 (%)	P2.C (%)	P2.1 (%)	P2.2 (%)	P3.C (%)	P3.1 (%)	P3.2 (%)	P4.C (%)	P4.1 (%)	P4.2 (%)
1	Dimethyl sulfide*	0.25^{h}	0.23^{h}	0.2^{gh}	0.22^{h}	0.19^{fg}	0.17^{f}	0.18^{fg}	0.16^{e}	0.13^{d}	0.12^{c}	0.1^{b}	0.07^{a}
2	*n*-Octane*	2.54^{g}	1.33^{cd}	1.96^{f}	0.3^{a}	1.24^{cd}	2.16^{fg}	0.76^{b}	1.6^{de}	2.61^{g}	0.89^{b}	1.34^{cd}	2.61^{g}
3	Acetone*	5.8^{c}	—	—	1^{a}	—	—	1.32^{ab}	—	—	1.47^{b}	—	—
4	2-Butanone*	1.16^{a}	0.47^{a}	1.18^{a}	1.9^{a}	8.95^{cd}	6.47^{b}	7.02^{bc}	9.96^{d}	8.1^{cd}	8.2^{cd}	6.71^{b}	6.51^{b}
5	2-Methyl-butanal*	4.43^{a}	1.44^{a}	5.71^{a}	7^{a}	23.17^{cd}	12.39^{b}	24.69^{cd}	21.21^{cd}	19.58^{c}	37.07^{e}	26.97^{d}	23.5^{chd}
6	3-Methyl-butanal*	—	—	—	2.8^{b}	—	—	—	—	—	0.39^{a}	—	—
7	Ethanol*	3.05^{c}	3.47^{cd}	1.68^{b}	0.2^{a}	4.61^{ef}	0.91^{ab}	5.09^{f}	4.02^{de}	4.25^{ef}	—	—	5.09^{f}
8	*n*-Pentanal*	0.78^{bc}	0.98^{cd}	1.12^{cd}	1.2^{d}	0.8^{bc}	0.65^{ab}	1.69^{e}	0.3^{a}	1.12^{cd}	2.39^{f}	1.3^{de}	0.99^{bbc}
9	*α*-Pinene*	0.92^{ef}	0.82^{ef}	0.48^{ab}	0.7^{de}	0.71^{de}	0.38^{a}	0.49^{bc}	0.74^{ef}	0.64^{cd}	0.95^{f}	0.67^{cd}	0.41^{a}
10	2-Butanol*	11.03^{c}	7.41^{b}	6.34^{b}	1.8^{a}	1.43^{a}	1.06^{a}	1.03^{a}	1.17^{a}	1.11^{a}	1.82^{a}	1.5^{a}	1.2^{a}
11	Camphene*	3.83^{d}	—	—	1.2^{c}	—	0.48^{ab}	—	—	0.37^{ab}	0.52^{b}	0.26^{ab}	0.55^{b}
12	*n*-Hexanal*	0.95^{ab}	—	1^{ab}	1.3^{ab}	0.59^{ab}	9.16^{c}	0.29^{a}	0.24^{a}	0.59^{ab}	0.34^{ab}	0.49^{ab}	1.01^{ab}
13	Dimethyl disulfide*	0.05^{c}	0.05^{c}	0.03^{b}	0.05^{c}	0.05^{c}	0.03^{b}	0.03^{b}	0.02^{ab}	0.01^{a}	0.03^{b}	0.03^{b}	0.01^{a}
14	*β*-Pinene*	0.23^{d}	—	—	—	0.11^{bc}	—	—	0.08^{ab}	—	0.07^{a}	0.14^{c}	—
15	Ethylbenzene*	—	—	—	1.2^{a}	—	—	—	—	—	—	—	—
16	*m*-Xylene*	—	—	—	1.8^{a}	—	—	—	—	—	—	—	—
17	*o*-Xylene*	0.21^{a}	—	—	7^{b}	—	—	—	—	—	—	—	—
18	1-Butanol*	0.32^{cd}	—	0.44^{d}	0.8^{e}	—	0.36^{cd}	0.24^{ab}	—	—	0.23^{a}	—	0.23^{a}
19	*α*-Phellandrene*	0.11^{a}	—	—	—	—	—	—	—	—	—	—	—
20	*β*-Myrcene*	0.44^{f}	0.32^{dee}	0.35^{de}	0.7^{g}	0.2^{b}	—	0.27^{bc}	—	—	0.26^{ab}	0.37^{ef}	—
21	Limonene*	0.88^{a}	0.85^{a}	1.21^{b}	1.8^{c}	0.74^{a}	0.87^{a}	0.67^{a}	0.73^{a}	0.74^{a}	0.68a	0.82^{a}	0.85^{a}
22	1,8-Cineole*	4.35^{e}	4.45^{e}	2.84^{bc}	4.7^{e}	3.72^{cd}	2.46^{ab}	3.6^{bc}	3.78^{cd}	2.93^{bc}	3.02^{cd}	4.21^{de}	2.35^{a}
23	Isopentyl alcohol*	2.38^{f}	2.36^{f}	1.28^{e}	0.5^{cd}	0.27^{bc}	0.17^{bc}	—	—	—	0.4^{bc}	0.69^{d}	0.26^{bc}
24	2-Pentyl-furan*	0.53^{a}	0.51^{a}	4.59^{bc}	0.5^{a}	0.49^{a}	14.92^{d}	0.43^{a}	0.53^{a}	2.83^{b}	0.53^{a}	0.65^{a}	5.59^{c}
25	*γ*-Terpinene*	0.68^{a}	0.83^{a}	0.59^{a}	0.7^{a}	0.6^{a}	1.68^{b}	0.73^{a}	0.82^{a}	0.72^{a}	0.64^{a}	0.73^{a}	0.67^{a}
26	(*E*)-*β*-Ocimene	1.25^{bc}	2.31^{d}	2.86^{de}	1^{bc}	0.64^{a}	2.97^{f}	0.7^{ab}	0.65^{a}	0.87^{bc}	0.56^{a}	0.71^{ab}	1.39^{c}
27	3-Octanone*	0.9^{cd}	1.08^{de}	1.57^{f}	1^{cd}	0.82^{cd}	1.37^{ef}	0.73^{ab}	0.97^{cd}	0.94^{cd}	0.63^{a}	0.91^{cd}	1.14^{de}
28	1-Pentanol*	0.54^{bc}	0.72^{d}	0.72^{d}	0.7^{cd}	0.38^{ab}	0.32^{a}	0.43^{ab}	0.43^{ab}	0.4^{ab}	0.3^{a}	0.41^{ab}	0.45^{ab}
29	*p*-Cymene*	0.16^{a}	—	0.4^{b}	0.5^{bc}	0.51^{bc}	0.55^{c}	0.43^{bc}	0.53^{bc}	0.48^{bc}	0.41^{bc}	0.46^{bc}	0.53^{bc}
30	Hexyl acetate*	—	0.08^{ab}	0.17^{c}	—	0.06^{a}	0.21^{d}	—	—	—	—	0.09^{bc}	0.1^{bc}
31	Terpinolene*	—	0.25^{a}	—	—	—	—	—	—	—	—	—	—
32	*n*-Octanal*	0.45^{c}	0.55^{cd}	0.51^{cd}	0.6^{d}	0.44^{cd}	0.44^{cd}	0.44^{cd}	0.4^{cd}	0.39^{cd}	0.35^{a}	0.41^{cd}	0.45^{cd}
33	(3*Z*)-hexen-1-ol acetate	1.6^{cd}	1.81^{d}	1.15^{ab}	1.4^{cd}	1.39^{cd}	0.92^{a}	1.34^{bc}	1.44^{cd}	1.1^{ab}	1.06^{ab}	1.34^{bc}	0.91^{a}
34	2-Heptenal*	1.17^{d}	1.14^{d}	0.95^{d}	0.5^{bc}	0.5^{bc}	0.52^{bc}	0.67^{bc}	0.4^{a}	0.32^{a}	0.47^{ab}	0.69^{bc}	0.71^{c}
35	6-Methyl-5-hepten-2-one	0.07^{ba}	—	0.28^{cd}	0.4^{e}	0.16^{c}	—	—	0.17^{c}	—	0.15^{bc}	—	0.35^{e}
36	*n*-Hexanol*	3.23^{b}	3.58^{b}	10.18^{c}	1.2^{a}	0.9^{a}	0.57^{a}	1.01^{a}	0.43^{a}	0.65^{a}	0.65^{a}	1.03^{a}	0.49^{a}
37	3-Hexen-1-ol*	1.88^{g}	2.14^{g}	1.02^{de}	1.5^{f}	0.23^{a}	0.71^{b}	1.35^{ef}	1.1^{de}	0.93^{cd}	1.2^{ef}	1.33^{ef}	0.7^{bc}
38	*n*-Nonanal*	0.86^{a}	0.76^{a}	1.5^{cd}	1.8^{d}	1.3^{c}	1.4^{cd}	1.55^{cd}	1.82^{d}	1.66^{cd}	1.27^{bc}	1.4^{cd}	1.44^{cd}
39	3-Octanol*	1.15^{ab}	1.88^{c}	1.32^{b}	1.1^{ab}	1.09^{ab}	1.14^{ab}	1^{ab}	1.15^{ab}	1.03^{ab}	0.84^{a}	1^{ab}	0.99^{ab}
40	(Z)-2-Hexen-1-ol	0.5^{a}	0.71^{bc}	1.23^{e}	0.9^{cd}	0.64^{ab}	1.11^{e}	0.57^{ab}	0.64^{ab}	0.75^{bc}	0.45^{a}	0.5^{a}	1.07^{de}
41	Acetic acid*	1.77^{de}	1.97^{e}	1.34^{cd}	1.8^{de}	1.58^{de}	1.17^{bc}	1.23^{bc}	1.4^{cd}	1.2^{bc}	1.07^{ab}	1.3^{bc}	0.93^{a}
42	1-Octen-3-ol*	9.46^{de}	7.92^{cd}	5.74^{a}	10^{e}	8.45^{de}	6.22^{bc}	8.22^{de}	6.78^{bc}	6.39^{ab}	7.35^{bc}	7.98^{de}	6.24^{ab}
43	3-Furaldehyde	2.17^{bc}	2.13^{ab}	1.46^{a}	4.1^{f}	3.14^{ef}	3.59^{ef}	3.88^{ef}	3.4^{ef}	3.18^{ef}	2.54^{cd}	2.88^{de}	2.93^{de}
44	*α*-Copaene	1.05^{de}	1.23^{e}	0.9^{cd}	1.9^{g}	1.22^{de}	0.68^{a}	1.27^{ef}	0.87^{bc}	0.69^{a}	1.19^{de}	1.56^{f}	0.76^{ab}
45	Camphor*	3.14^{a}	3.17^{a}	3.04^{a}	5.9^{d}	5.28^{cd}	4.63^{cd}	5.22^{cd}	5.57^{cd}	4.74^{cd}	4.21^{bc}	4.79^{cd}	3.89^{ab}
46	1-Octanol*	1.06^{e}	—	0.94^{de}	0.8^{cd}	—	—	0.48^{b}	0.62^{bc}	0.45^{b}	0.52^{b}	—	0.64^{bc}
47	(*E*,*E*)-3,5-Octadien-2-one	—	1.39^{d}	—	0.3^{b}	0.53^{c}	0.65^{c}	0.21^{b}	0.03^{a}	0.22^{b}	—	0.69^{c}	—
48	Bornyl acetate*	—	0.48^{c}	—	—	0.29^{b}	0.54^{c}	—	0.19^{a}	0.45^{c}	—	0.32^{b}	0.53^{c}
49	2,3-Butanediol*	0.48^{e}	0.13^{a}	0.24^{cd}	0.3^{e}	0.26^{e}	0.21^{cd}	0.3^{e}	0.22^{cd}	0.16^{bc}	0.17^{bc}	0.19^{bc}	0.13^{a}
50	(*E*)-Caryophyllene*	0.76^{e}	0.94^{f}	0.5^{cd}	0.6^{de}	0.57^{cd}	0.37^{ab}	0.59^{d}	0.56^{cd}	0.41^{bc}	0.48^{cd}	0.53^{cd}	0.34^{a}
51	2-Undecanone*	—	—	—	—	—	—	—	—	—	—	—	0.15^{a}
52	Terpinen-4-ol*	0.34^{e}	0.37^{e}	—	0.3^{de}	0.25^{cd}	—	0.2^{bc}	0.19^{bc}	—	0.16^{b}	0.18^{bc}	—
53	2-Octen-1-ol*	0.42^{ef}	0.48^{f}	—	0.5^{f}	0.3^{cd}	0.34^{de}	0.26^{cd}	0.24^{cd}	0.23^{bc}	0.18^{b}	0.28^{cd}	—
54	Benzeneacetaldehyde*	—	—	—	—	—	—	—	—	—	—	—	0.24^{a}
55	2-Furanmethanol	4.51^{ab}	5.34^{bc}	3.66^{a}	7.2^{d}	5.12^{bc}	5.37^{bc}	6.43^{cd}	5.12^{bc}	5.13^{bc}	3.67^{a}	4.15^{ab}	3.88^{ab}
56	1-Nonanol*	0.53^{d}	0.72^{e}	0.47^{d}	0.3^{c}	0.08^{a}	0.2^{b}	$0.1^{b}8$	0.16^{ab}	0.13^{ab}	0.13^{ab}	0.19^{b}	0.18^{b}
57	*δ*-Terpineol*	0.13^{b}	0.28^{d}	—	—	0.17^{c}	—	—	—	—	0.04^{a}	—	—
58	*α*-Terpineol*	1.14	1.1^{de}	0.73^{bc}	1.1^{de}	0.8^{de}	0.56^{a}	0.89^{de}	0.73^{bc}	0.62^{ab}	0.72^{bc}	0.85^{cd}	0.65^{bc}
59	Borneol	0.22^{d}	0.2^{cd}	0.2^{cd}	0.2^{cd}	0.19^{cd}	0.22^{d}	—	0.16^{bc}	0.15^{bc}	—	0.12^{b}	0.17^{bc}

Table 2: Continued.

No.	Component	P1.C (%)	P1.1 (%)	P1.2 (%)	P2.C (%)	P2.1 (%)	P2.2 (%)	P3.C (%)	P3.1 (%)	P3.2 (%)	P4.C (%)	P4.1 (%)	P4.2 (%)
60	Verbenone	0.1^{cd}	0.13^{de}	0.21^{f}	0.1^{cd}	0.08^{bc}	0.15^{e}	0.08^{bc}	0.08^{bc}	0.11^{cd}	0.06^{a}	0.07^{ab}	0.1^{cd}
61	α-Farnesene	—	0.13^{a}	0.17^{b}	—	—	0.19^{b}	—	0.13^{a}	0.12^{a}	—	0.1^{a}	—
62	2(5H)-Furanone*	—	0.22^{c}	—	—	0.11^{ab}	0.07^{a}	0.12^{ab}	0.17^{bc}	0.9	0.06^{a}	0.11^{ab}	—
63	δ-Cadinene	—	—	0.13^{b}	—	—	0.13^{b}	—	—	—	—	—	0.08^{a}
64	1-(2-Butoxyethoxy)-ethanol	—	0.29^{c}	—	—	0.19^{b}	—	—	0.13^{a}	—	—	0.17^{ab}	—
65	2,4-Decadienal	—	0.63^{c}	0.65^{c}	—	0.21^{a}	0.47^{b}	0.26^{a}	0.26^{a}	0.46^{b}	0.16^{a}	0.21^{a}	0.48^{b}
66	Hexanoic acid*	0.12^{bc}	0.13^{cbc}	0.09	0.1^{bc}	0.12^{bc}	0.36^{d}	0.08^{ab}	0.08^{ab}	0.07^{a}	0.06^{a}	0.08^{ab}	0.06^{a}
67	Geranyl acetone	0.37^{bc}	0.44^{c}	1.34^{f}	0.2^{ab}	0.18^{a}	1^{e}	0.11^{a}	0.13^{a}	0.38^{c}	0.08^{a}	0.09^{a}	0.64^{d}
68	Benzyl alcohol*	10.16^{de}	12.93^{e}	7.73^{cd}	9^{cd}	7.73^{cd}	3.83^{a}	8.41^{cd}	9.17^{cd}	7.9^{cd}	6.85^{bc}	7.5^{cd}	5.93^{ab}
69	Dimethyl sulfone*	0.6^{e}	0.76^{f}	0.36^{cd}	0.4^{d}	0.25^{bc}	0.19	—	0.3^{cd}	0.23^{b}	—	0.27^{bc}	0.21^{b}
70	Phenylethyl alcohol*	0.17^{c}	0.19^{c}	0.14^{bc}	0.1^{ab}	0.1^{ab}	0.37^{d}	0.08^{a}	0.07^{a}	0.09^{ab}	0.06^{a}	0.1^{ab}	0.08^{a}
71	Heptanoic acid*	—	—	1.2^{c}	—	—	0.82^{b}	—	—	0.47^{a}	—	—	0.79^{b}
72	*p*-Creosol	0.62^{d}	0.7^{e}	—	0.7^{e}	0.39^{bc}	—	0.3^{b}	0.3^{b}	0.25^{a}	0.26^{a}	0.34^{bc}	—
73	Octanoic acid*	—	—	0.11^{a}	—	—	0.15^{b}	—	—	—	—	—	0.11^{a}
74	Nonanoic acid*	0.74^{bc}	0.52^{ab}	—	1^{d}	0.66^{bc}	—	0.71^{bc}	0.55^{ab}	—	0.8^{cd}	0.45^{a}	—
75	Thymol*	0.26^{d}	—	0.16^{c}	—	—	0.33^{e}	0.19^{c}	0.11^{ab}	0.11^{ab}	0.15^{bc}	—	0.1^{a}
76	Dodecanoic acid*	—	3.04^{c}	—	—	1.46^{a}	—	—	2.1^{b}	—	—	2.27^{b}	—
77	Coumarin*	0.25^{a}	0.5^{d}	0.17^{a}	0.3^{bc}	—	0.25^{a}	0.25^{a}	0.36^{c}	0.2^{a}	0.18^{a}	—	0.34^{bc}

*Compounds identified by standards and MS spectra. Compounds without the asterisk were identified by means of mass spectra and retention indices. ¯Values not detected. Values are means of three determinations. Values within a row for each compound having different letters are significantly different from each other using Tukey's and LSD test ($p > 0.05$).

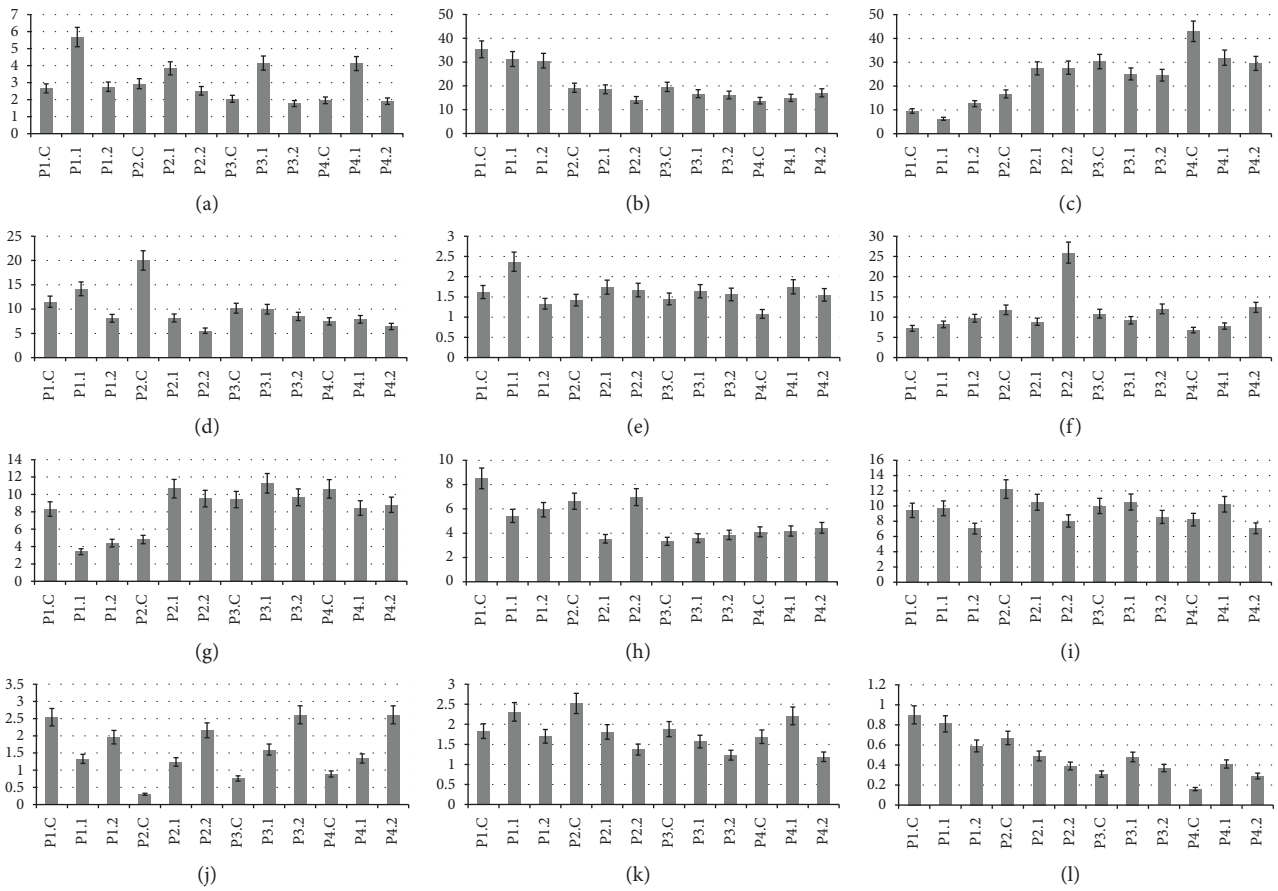

Figure 1: Classes of compounds according to Table 2. (a) Acids. (b) Alcohols. (c) Aldehydes. (d) Aromatics. (e) Esters. (f) Furan derivatives. (g) Ketones. (h) Monoterpenes. (i) Oxygenated monoterpenes. (j) Paraffins. (k) Sesquiterpenes. (l) Sulfur compounds.

paraffins, and aldehydes induced by LA. Both acids cause a drastic reduction in the aromatics and, although less so, also in oxygenated monoterpenes and alcohols.

At higher temperatures P3 and P4, there is a drastic reduction of aldehydes following the addition of LA. SA has a similar but slightly less-accentuated action. Furano derivatives instead increase on the addition of LA and decrease on the addition of SA.

From the alimentary point of view, research in databases carried out on the molecules found has shown that none of these has toxic effects at least up to 10 mg/kg of body weight [27].

3.1. Cluster Analyses. The cluster analyses of the VOC composition of the sauces without additions showed the formation of 4 distinct groups (P1.C-P2.C-P3.C-P4.C) (Figure 2) corresponding to the four experimental conditions A, B, C, and D (Table 1). The P1.C and P2.C groups, which are formed at a distance cluster of 40 from the other groups, are distinct from the P3.C and P4.C groups which are a little more homogeneous among them forming a cluster distance of 20. The compositions of the VOCs within each group are very homogeneous, with an average cluster distance of 3 (Figure 2). The addition of stearic acid to the sauce has created the formation of new groups compared to the already formed groups P1.C-P2.C-P3.C-P4.C (Figure 3(a)). The effect of temperature and duration caused the VOC composition to vary in some cases. The groups P1.1-P1.C and P2.C separate from each other at a cluster distance 42. An increase in temperature (scheme B) led to the partial fusion of groups P2.1 with P3.C and P2.C with P3.1 (Figure 3(a)). The addition of linoleic acid to the sauce has created the formation of 8 homogeneous groups distinctly from each other (Figure 3(b)). The effect of the autoclaving temperature and duration always produced a variation of the VOCs compared to the controls. The groups begin to separate at a cluster distance of 40, and the homogeneity between the components is quite high (cluster distance between more homogeneous groups (P1.2-P1.C-P2.C): 4; more heterogeneous groups (P4.C): 8) (Figure 3(b)).

According to the cluster analysis, two main group were formed, i.e., (P1.C-P1.1-P1.2-P2.C) and (P2.1-P2.2-P3.C-P3.1-P3.2-P4.C-P4.1-P4.2) at a distance cluster (ds) = 42 (Figure 4).

We may suppose that starting from 121°C for 15 min, substantial modifications occur in the chemical composition of the matrix. At lower ds (about 3), P1.C and P2.C separate from each other indicating that even under the milder duration/temperature autoclaving conditions, there was some modification in the chemical compositions.

3.2. Principal Component Analyses. Here, we opt to analyze the correlation matrix since the variances of rates for different types of VOC differ considerably. Working with the correlation matrix amounts to using the VOC rates after standardizing each to have unit standard deviation. This seems sensible since without standardization, the derived components are likely to be dominated by single variables with large variances. We choose the number of principal components according to the number of eigenvalues above one which, in the case of the correlation matrix, amounts to identifying components with more variance than the average.

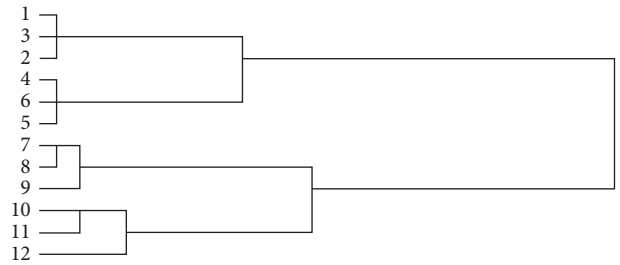

Figure 2: Cluster analysis (complete linkage clustering). Data from truffle sauces (control)*. *Ordinal numbers referred to data from experiments as indicated in Table 1: 1–3 to P1.C/4–6 to P2.C/7–9 to P3.C/10–12 to P4.C.

3.2.1. PCA for the Experimental P1.C-P2.C-P3.C-P4.C. The first principal component has a variance of 35.88; this amounts to 54.36% of the total variance. The second principal component has a variance of 22.70 and accounts for a 34.40% of the variance. The first two (three) principal components account for 88.76% (100%) of the total variance. The screen plot (not reported) shows a marked decrease in downward slope after the second or third principal component, implying that we can summarize our VOC variables by the first two principal components. The first principal component has a high positive correlation with dimethyl sulfide/dimethyl disulfide/(E)-β-ocimene/acetic acid/α-terpineol/borneol/hexanoic acid/dimethyl sulfone. The second principal is much positively correlated with n-nonanal/α-copaene/camphor. The principal component plot is given in Figure 5(a).

3.2.2. PCA for the Experimental P1.C-P1.1-P2.C-P2.1-P3.C-P3.1-P4.C-P4.1. The first principal component has a variance of 28.85; this amounts to 40.08% of the total variance. The second principal component has a variance of 17.50 and accounts for a 24.31% of the variance. The first two (three) principal components account for 64.39% (80.41%) of the total variance. The screen plot (not reported) shows a marked decrease in downward slope after the second or third principal component, implying that we can summarize our VOC variables by the first two principal components. The first principal component has a high positive correlation with (E)-β-ocimene/n-hexanol/acetic acid/(E)-caryophyllene/terpinen-4-ol/1-nonanol/verbenone/geranyl acetone/dimethyl sulfone/phenylethyl alcohol. The second principal component is more positively correlated with n-hexanal/1-butanol. The principal component plot is given in Figure 5(b).

3.2.3. PCA for the Experimental P1.C-P1.2-P2.C-P2.2-P3.C-P3.2-P4.C-P4.2. The first principal component has a variance of 27.52; this amounts to 37.20% of the total variance. The second principal component has a variance of 16.09 and

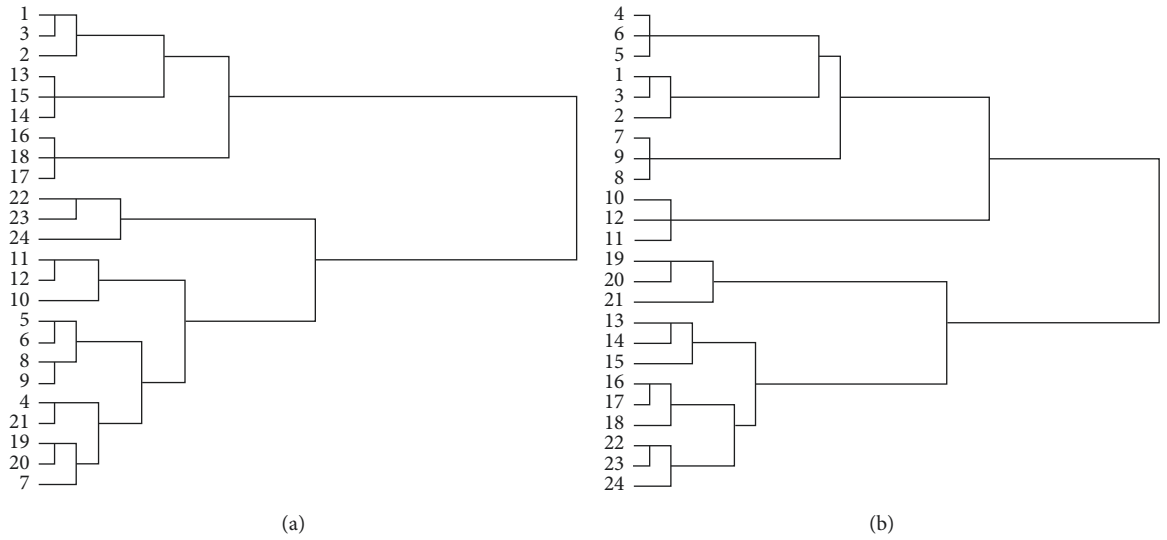

Figure 3: Cluster analysis (complete linkage clustering). Data from truffle sauces added with (a) stearic acid* and (b) linoleic acid**. *Ordinal numbers referred to data from experiments as indicated in Table 1: 1–3 to P1.1/4–6 to P2.1/7–9 to P3.1/10–12 to P4.1/13–15 to P1.C/16–18 to P2.C/19–21 to P3.C/22–24 to P4.C. **Ordinal numbers referred to data from experiments as indicated in Table 1: 1–3 to P1.C/4–6 to P1.2/7–9 to P2.C/10–12 to P2.2/13–15 to P3.C/16–18 to P3.2/19–21 to P4.C/22–24 to P4.2.

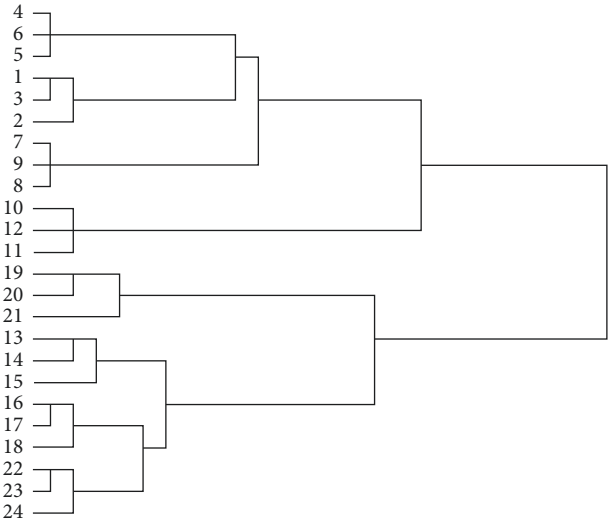

Figure 4: Cluster analysis (complete linkage clustering). Data from truffle sauces added with stearic and linoleic acids*. *Ordinal numbers referred to data from experiments as indicated in Table 1: 1–3 to P1.C/4–6 to P1.2/7–9 to P2.C/10–12 to P2.2/13–15 to P3.C/16–18 to P3.2/19–21 to P4.C/22–24 to P4.2.

accounts for a 21.75% of the variance. The first two (three) principal components account for 58.95% (76.03%) of the total variance. The screen plot (not reported) shows a marked decrease in downward slope after the second or third principal component, implying that we can summarize our VOC variables by the first two principal components. The first principal component has a high positive correlation with 3-hexen-1-ol/terpinen-4-ol/α-terpineol/*p*-creosol. The second principal component is much positively correlated with 3-octanol. The principal component plot is given in Figure 5(c).

The SPME CG/MS results from the combination of the VOC derived from the mushrooms, truffle, degradation of linoleic acid, and other compounds to form new compounds. According to [28], the monoterpenoids and sesquiterpenoids fractions were apported to the mushrooms together to *n*-hexanal, *n*-hexanol, 1-octen-3-ol, benzaldehyde, and benzyl alcohol. The solforate compounds derived from the truffle together with 2-methyl-butanal, 3-methyl-butanal, hexanal, 1-octen-3-ol, and benzaldehyde [8]. Other compounds derived from the secondary thermal degradation of linoleic acid. It is known that the lipid autoxidation initially produces peroxides or hydroperoxides [29]. The peroxide values were smaller for the free fatty acids forms of linoleic acid at all durations. Generally, the hydroperoxide content decreased during heating in a secondary oxidation status of linoleic acid, and among the numerous categories of volatiles, aldehydes are particularly represented. The saturated aldehydes were generally the mainly represented ones; moreover, their percent content was always higher. Saturated aldehydes having chain length from C5 to C7 were detected for linoleic acid. According to other authors [29], generally hexanal was the main volatile oxidation product, which is derived from the 13-hydroperoxide degradation of linoleic acid [28]. Among monounsaturated aldehydes, *trans*-2-heptenal was the most represented one for linoleic acid model systems. It has been reported that trans-2-octenal would come from the unexpected 11-hydroperoxy-9,12-octadecadienoate [30].

The dimethyl sulfone is not contained in the components of truffle-like sauce but is formed following the thermal treatment [31].

Hexanal could be derived from mushroom, truffle, and linoleic acid thermal degradation, while hexanol, 1-octen-3-ol, and benzaldehyde derived from mushroom and truffle. 2-pentyl-furan and 2,4-decadienal isomers could be either

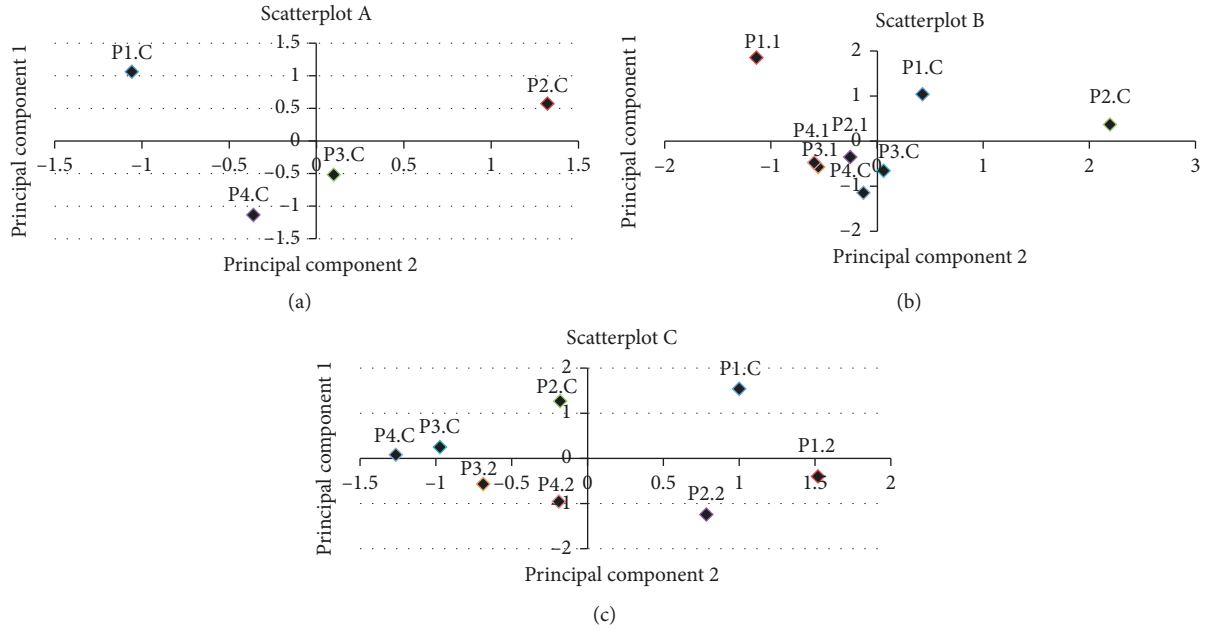

Figure 5: Scatterplot of the first two principal components: (a) P1.C/1-P2.C/1-P3.C/1-P4.C/1, (b) P1.C/1-P2.C/1-P3.C/1-P4.C/1, (c) P1.C/2-P2.C/2-P3.C/2-P4.C/2.

Table 3: Effect of various experimental autoclaving conditions and different fatty acids (10% w/w) on control of bacteria in truffle sauce.

Sample ID	Autoclaving conditions ID	Bacteriological analyses	Stearic acid	Linoleic acid	Control
P1.C		TAB (CFU/g)	260 ± 1.3	160 ± 0.8	270 ± 1.3
P1.1	A	*Clostridium* spp. (CFU/g)	<1	<1	<1
P1.2		*C. perfringens* (CFU/g)	<1	<1	<1
P2.C		TAB (CFU/g)	120 ± 1.0	<10 ± 0.2	180 ± 1.1
P2.1	B	*Clostridium* spp. (CFU/g)	<1	<1	<1
P2.2		*C. perfringens* (CFU/g)	<1	<1	<1
P3.C		TAB (CFU/g)	250 ± 0.9	150 ± 0.6	320 ± 1.4
P3.1	C	*Clostridium* spp. (CFU/g)	<1	<1	<1
P3.2		*C. perfringens* (CFU/g)	<1	<1	<1
P4.C		TAB (CFU/g)	200 ± 0.8	<10 ± 0.1	280 ± 1.4
P4.1	D	*Clostridium* spp. (CFU/g)	<1	<1	<1
P4.2		*C. perfringens* (CFU/g)	<1	<1	<1

VOCs from truffle or derived from a terminal decomposition of linoleic acid.

The low amounts of heptanal could be the chemical marker of small amounts of conjugated linoleic acids present in both truffle and mushrooms according to [30].

The fats in *Tuber melanosporum* content were about 11.30 g/100 g on dry weight, and the linoleic acid and stearic acid content was about 50% and 11% of the lipidic fraction, respectively [18]. The fats in *Boletus edulis* content was about 2.2 g/100 g on dry weight, and about 57% was the linoleic acid content, while the stearic acid content was about 2% [32].

3.3. Microbiological Analyses. The microbiological quality of samples produced with different experimental autoclaving conditions and different fatty acids is shown in Table 3. Total aerobic bacteria ranged from a mean of 10 to 320 CFU/g. It is present in all samples prepared for the different experimental conditions (A–D), although to varying degrees. *Clostridium* spp. and *C. perfringens* were not detected in any of the produce sauces tested. Linoleic acid is more effective than stearic acid, and in tests B and C, it contributes to a strong reduction of the total bacterial load (Tables 1 and 3).

All the autoclaving temperature/duration combinations to which the samples and controls were subjected proved to be effective bacteriostatic against sulphite-reducing clostridia and *Clostridium perfringens*, and this regardless of the addition made to the normal formulation of the product. The total bacterial load, even if low, is instead present in the controls for all temperature/duration combinations. Linoleic acid is more effective than stearic acid and contributes with a concentration of 10% (w/w) to a decisive reduction of the total bacterial load in tests B and D (Tables 1 and 3).

4. Conclusion

The temperature, duration, and pressure of autoclave operation for truffle sauce sterilization induce variations in the

chemical composition of the sauce as shown by headspace examination.

Compared to hot sterilized sauces, also the addition of linoleic acid has made significant variations in the composition of the headspace. The addition of stearic acid has an action much less than that of linoleic acid.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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