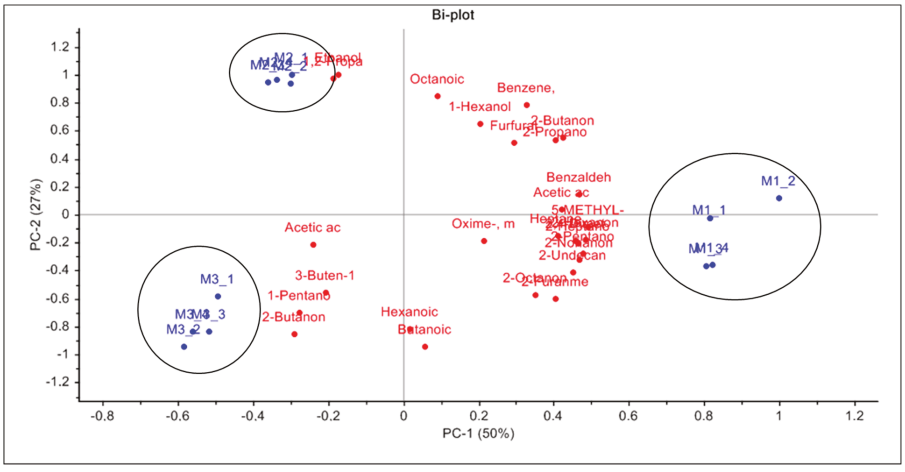

Figure 1. Principal Component Analysis (PCA) biplot of the 3 different commercial samples of mascarpone (M1, M2, and M3). For each sample, the mean (n = 4) is represented by the sample name. Score plot was given by the Volatile Organic Compound (VOC) content for each sample and loading plot of the single volatile organic compounds. The codes correspond to the samples indicated in Table 1.

In the figure, it is possible to observe the samples (scores) and variables (loadings) plots related to the first two principal components, which (cumulated) explain the 77% of the total variance (PC1, 50.0%; PC2, 27.0%) associated with the data set. A clear separation of mascarpone cheese M1 from the other samples is observable along the PC1. PC2 explains the parting between M2 and M3 mascarpone samples. The replicates belong to the same commercial mascarpone batch that is well-clustered together, while is possible to highlight a clear separation of the three samples on the biplot. Observing the loadings (i.e., the involvement of the single volatiles), it is possible to have an idea of the different influence of the diverse volatiles in justifying variance observed trends.

Table 1. List of commercial 'Mascarpone' samples analyzed in the present study. All samples (M1–M12) were investigated by PTR-ToF-MS analysis. Underlined samples (M1–M3) were evaluated also by HS-SPME GC-MS.

Sample	Claimed Characteristics	Manufacturer
M1	Mascarpone	A
M2	Mascarpone	B
M3	Mascarpone	C
M4	Mascarpone	B
M5	Mascarpone	C
M6	Mascarpone	C
M7	Mascarpone	M
M8	Mascarpone	M
M9	Mascarpone without lactose	M
M10	Mascarpone without lactose	M
M11	Mascarpone without lactose	C
M12	Mascarpone without lactose	C

2.2. PTR-ToF-MS Results and Comparison with HS-SPME GC-MS Findings

As other Direct-Injection Mass Spectrometric (DIMS) technologies, PTR-MS finds application in many sectors, from environmental sciences to food chemistry, and from biological studies to medical applications. With this regard, we recently described a tailored system, that found application in this

study, achieved connecting PTR-ToF-MS with an automated sampler, and associated custom-made data analysis applications that improve the versatility of the analytical approach in the determination of VOCs in association with i) huge numbers of samples, ii) bioprocesses monitoring, and iii) high numbers of variables to be considered [11].

PTR-MS has been already exploited to study VOCs associated to dairy products such as mozzarella cheese [22], Grana Padano, Parmigiano Reggiano, and Grana Trentino cheeses [23], liquid whey [24], butter and butter oils (by means of quadrupole-based PTR-MS analyses, sensory analyses and classical chemical analyses) [25], milk and whey powders [26], anhydrous milk fat [26], and fermented milk-based beverages (yogurt and kefir) [27,28].

All samples included in this study have been analyzed by PTR-ToF-MS. A total of 411 mass peaks were detected and extracted. Upon comparison with the blanks, 92 peaks were kept that are significantly different between various manufacturers ($p < 0.01$ with Bonferroni correction) and tentatively identified on the basis of exact mass, isotopic ration, and literature [29]. PTR-MS allowed the detection and characterization of a larger number of VOCs/VOC fragments, which was larger than the number of volatiles identified by GC. For the PTR analysis, all vials were incubated alternatively at 40 °C or at 60 °C (data not shown) for 30 min before PTR-MS analysis. The last one was the temperature at which good results were obtained by HS-SPME GC-MS. However, with PTR, even at 40 °C, the analysis was successfully performed and results were reliable. For this reason, we report the data performed at 40 °C, a temperature closer to the real mascarpone cheese testing conditions. One-way ANOVA followed by Tukey HSD test was carried out to compare and underline significant differences among the assessed mascarpone samples. For each peak, we obtained the concentration of the corresponding VOC ion in the headspace of all explored samples. Boxplots reported in Figure 2 illustrate the observed trends for 6 ions among the tested samples, as illustrative cases. In detail, the figure proposes the behaviors corresponding to the peaks at *m/z* 73.065 (tentatively identified as 2-butanone), *m/z* 75.044 (tentatively identified as propionic acid), m/z 83.086 (tentatively identified as hexanol fragment), *m/z* 87.080 (tentatively identified as 2-pentanone/isoprenol), *m/z* 98.105, and *m/z* 101.096 (tentatively identified as 2-hexanone). The intensity corresponding to the mass peak *m/z* 73.065 reaches the highest values in the delactosed samples produced by Manufacturer C, while the standard productions belonging to the same manufacturer registered the lowest values (as all mascarpone batches of Producer M) (Figure 2a). Samples from Manufacturers A and B present intermediate intensities for this peak (Figure 2a). In accordance with these results, 2-butanone was found to be variable in different types of whey [30]. In only the M2 batch did we detected a relevant intensity for the mass peak *m/z* 75.044 (Figure 2b), tentatively identified as propionic acid, a compound that can be responsible for a dairy taste/odor with a pronounced fruity lift [31].

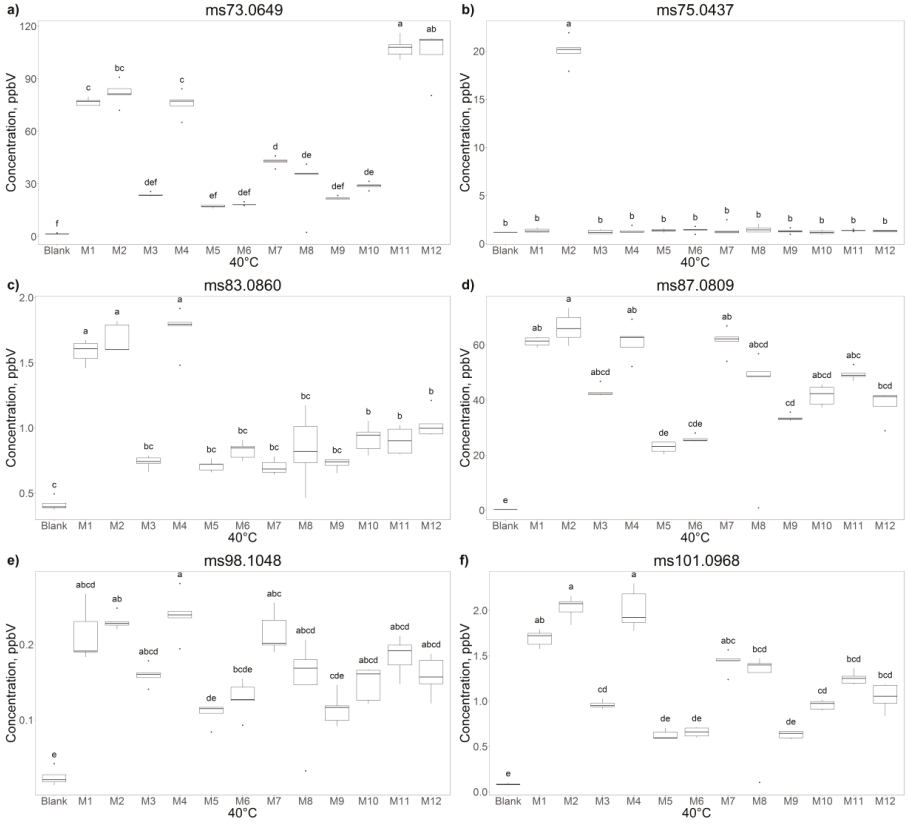

Figure 2. The boxplots indicated by letters (**a**–**f**) represent selected volatiles found in association with the different commercial mascarpone samples such as m/z 73.0649—$C_4H_9O^+$—t.i. 2-Butanone, 75.0437—$C_3H_7O_2^+$—t.i. Methyl acetate, 83.0860—$C_6H_{11}^+$—t.i. fragment of Hexanal/Hexenol, 87.0809—$C_5H_{11}O^+$—t.i. 2-Pentanone/3-Buten-1-ol, 3-methyl-, 98.1048—isotope of $C_7H_{13}^+$—t.i. Heptanal, 101.0968—$C_6H_{13}O^+$—t.i. 2-Hexanone. Different letters indicate a significant difference between different samples ($p < 0.05$, one-way ANOVA, Tukey HSD).

The mass peak *m/z* 83.086 has been found with pronounced intensities in the samples produced by the Manufacturers A and B (Figure 2c). Hexanal was included among the high-content compounds identified in samples belonging to dairy products [32] and described as having a fatty, green, grassy, powerful, penetrating characteristic fruity odor and taste [31]. A similar trend can be underlined for the intensities of mass peak *m/z* 101.096 (Figure 2f). Finally, a considerable variability can be highlighted for the intensities corresponding to the mass peaks m/z 87.080 and 98.105 (Figure 2d,e).

Other than this kind of punctual analysis, PTR analysis offers also the opportunity to depict a global analysis of molecular fingerprinting associated with the headspaces of the different samples. Considering that the present work deals with an integrated analytical approach, we propose a PTR data set selected in light of the comparison with GC data. In fact, we defined a new subset of the PTR-ToF-MS data including only the mass peaks that were found also using the HS-SPME GC-MS technique. As a result, we have a new matrix (Table 2) of twenty peaks corresponding to the masses of protonated molecular ions of compounds such as acetic acid (sour pungent, cider vinegar, slightly malty with a brown nuance; naturally occurring in various dairy products, it has a role in butter and cheese flavors), acetoin (acidic, sour, cheesy, dairy, creamy with a fruity nuance; normally occurs

in butter, milk, and cheeses), acetone (characteristic aromatic odor, pungent, somewhat sweet taste; naturally occurring in fermented dairy products), ethanol (slight, characteristic odor and a burning taste; naturally occurring in blue cheese, cheddar cheese, Swiss cheese), furfural (characteristic penetrating odor typical of cyclic aldehydes; naturally occurring in cheeses), hexanoic acid (sickening, sweaty, rancid, sour, sharp, pungent, cheesy, fatty, unpleasant odor reminiscent of copra oil; naturally occurring in cheeses, butter, milk), and octanoic acid (mildly unpleasant odor and a burning, rancid taste, also reported as having a faint, fruity-acid odor and slightly sour taste; natural component of butter fat, occurring in cheeses) [31,33].

Table 2. Volatile compounds detected by both Proton Transfer Reaction-Mass Spectrometry coupled to a Time of Flight mass analyzer (PTR-ToF-MS) and SPME/GC-MS in association with mascarpone samples.

Compound	Chemical Class	Protonated Ion	
		m/z	Sum Formula
Ethanol	Alcohols	47.049	$C_2H_7O^+$
2-Propanone	Ketones	59.049	$C_3H_7O^+$
Acetic acid	Organic acids	61.028	$C_2H_5O_2^+$
2-Butanone	Ketones	73.065	$C_4H_9O^+$
1,2-Propanediol = Propylene glycol	Alcohols	77.060	$C_3H_9O_2^+$
2-Pentanone/3-Buten-1-ol, 3-methyl-	Ketones/Alcohols	87.080	$C_5H_{11}O^+$
2-Butanone, 3-hydroxy- (B) / Butanoic acid/Acetic acid ethyl ester	Ketones/Organic acids/Esters	89.060	$C_4H_9O_2^+$
Toluene	Hydrocarbons	93.070	$C_7H_9^+$
Furfural	Furans	97.028	$C_5H_5O_2^+$
2-Hexanone	Ketones	101.096	$C_6H_{13}O^+$
Benzaldehyde	Aldehyde	107.049	$C_7H_7O^+$
5-Methyl-delta-valerolactone	Lactones	115.075	$C_6H_{11}O_2^+$
2-Heptanone	Ketones	115.112	$C_7H_{15}O^+$
Hexanoic acid	Organic acids	117.091	$C_6H_{13}O_2^+$
2,4-Dimethyl-1-heptene	Hydrocarbons	127.148	$C_9H_{19}^+$
2-Octanone	Ketones	129.127	$C_8H_{17}O^+$
1-Hexanol, 2-ethyl-	Alcohols	131.143	$C_8H_{19}O^+$
2-Nonanone	Ketones	143.143	$C_9H_{19}O^+$
Octanoic acid	Organic acids	145.122	$C_8H_{17}O_2^+$
Oxime-, methoxy-phenyl-	Oxime	152.071	$C_8H_{10}NO_2^+$
2-Undecanone	Ketones	171.174	$C_{11}H_{23}O^+$
Heptane, 2,2,4,6,6-pentamethyl	Hydrocarbons	171.211	$C_{12}H_{27}^+$

Statistical tests were performed on the new matrix in an attempt at understanding the impact of these VOCs on the characterization of the different mascarpone cheese samples. The results obtained for the twelve experimental modes were visualized by means of principal component analysis (PCA), with each point representing a distinct sample (Figure 3), maximizing explained variability in two dimensions.

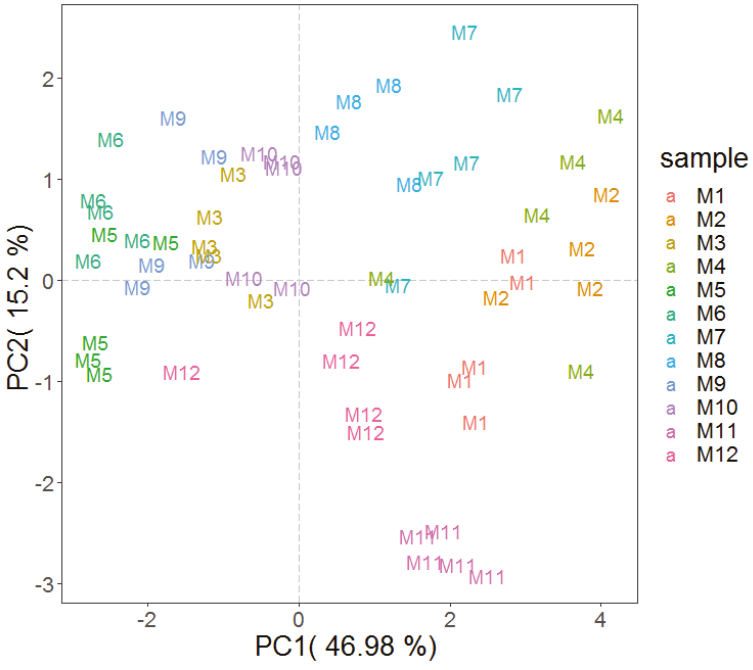

Figure 3. Analysis of mascarpone VOCs profile assessed by PTR-ToF-MS. Plot depicts the VOC profile distribution of the twelve Mascarpone over the PCA score plot defined by the first two principal components. The codes correspond to the samples indicated in Table 1.

Separation among Mascarpone samples according to the first two components accounted for about 62% of the total variance. It is possible to highlight how the replicates belonging to the same sample generally clustered together. In addition, a good separation among the different samples is also depicted. Considering all variables connoting the panel of different mascarpone cheese analyzed, it is mandatory to underline that the studied diversity in terms of different producers and classic versus delactosed was not selected in order to delve into the effect of these parameters. In fact, it was just a heterogeneous panel selected in order to provide a broad description of the overall VOCs associated with this traditional dairy production. However, it is possible to foresee some preliminary differences, such as clear groups among mascarpone cheese samples belonging to the same manufacturer and a general (more or less pronounced) separation between classic and delactosed samples within the same producer (Figure 3). These pieces of evidence suggest the need for further studies with tailored sampling in order to test the potential of a PTR-based approach as a discriminatory tool to monitor these variables. Considering the sensory changes among mascarpone cheese samples, our study confirmed the presence of a diversification comparing different batches and different producers already described in terms of spreadability [34]. In fact, Cattaneo et al. [34], studying eighteen batches from six different manufacturers, noticed differences in four viscometric parameters they selected to assess changes of rheological aptitude of mascarpone cheeses. This sensory variability calls attention to the need for versatile tools for the industrial quality control also in the case of mascarpone cheese, a topic of generally significant interest in the food industry [35,36].

In Table 3, it is possible to delve into the results for a more representative number of mass peaks, underlining significant differences among concentrations reported for 22 protonated ions out of the 92 selected after comparison with the blanks. From this analysis, it is possible to notice how the trends for selected mass peak intensities follow a certain producer-dependent behavior. It is also

clear how the probabilities to find selected mass peaks associated to given experimental variables considerably increase using the PTR-based technique, due to the potential of an untargeted approach. The opportunity to have a wide (untargeted analysis) and fast (rapid time of analysis without any sample preparation/extraction/destruction) view of the VOCs associated with mascarpone headspaces confirmed the aptitude of this analytical approach to allow rapid noninvasive quality control for the food industry (e.g., [37,38]), already explored in the dairy industry but on other matrices (e.g., [25,26]). An approach that can i) simplify the selection of mascarpone as an ingredient in the food industry and ii) boost the quality improvements in the production of this fresh cheese.

Table 3. Organic compounds associated to mascarpone headspace detected by PTR-ToF-MS. Black color indicates compounds identified also by SPME/GC-MS. For each compound, different letters indicate a significant difference between different samples according to ANOVA and Tukey HSD ($p < 0.05$). The codes correspond to the samples indicated in Table 1. In the parenthesis, the different producers.

MM	TM	SF	M1 (A)	M2 (B)	M3 (C)	M4 (B)	M5 (C)	*p*-Value
41.039	41.039	$C_3H_5^+$	21.2 ± 0.9 ^{b}	27 ± 3 ^{c}	11.4 ± 0.6 ^{a}	21 ± 1 ^{b}	10 ± 1 ^{a}	1×10^{-13}
43.018	43.018	$C_2H_3O^+$	30.0 ± 0.7 ^{b}	44 ± 3 ^{c}	24 ± 2 ^{a}	33 ± 2 ^{b}	24 ± 5 ^{a}	3×10^{-9}
43.054	43.054	$C_3H_7^+$	11.5 ± 0.6 ^{c}	16 ± 1 ^{d}	3.5 ± 0.5 ^{a}	6.7 ± 0.4 ^{b}	3.1 ± 0.4 ^{a}	4×10^{-17}
45.033	45.033	$C_2H_5O^+$	113 ± 6 ^{b}	175 ± 12 ^{c}	81 ± 3 ^{a}	114 ± 10 ^{b}	88 ± 23 ^{a}	2×10^{-9}
47.049	47.049	$C_2H_7O^+$	8 ± 3 ^{a}	52 ± 39 ^{c}	10 ± 8 ^{a}	16 ± 1 ^{ab}	44 ± 5 ^{bc}	2×10^{-3}
55.054	55.054	$C_4H_7^+$	13.3 ± 0.4 ^{c}	14.9 ± 0.8 ^{d}	8.1 ± 0.3 ^{b}	15 ± 1 ^{d}	6.0 ± 0.4 ^{a}	7×10^{-16}
57.070	57.070	$C_4H_9^+$	6.0 ± 0.1 ^{c}	5.6 ± 0.6 ^{c}	3.9 ± 0.2 ^{b}	12 ± 1 ^{d}	2.7 ± 0.1 ^{a}	7×10^{-17}
59.049	59.049	$C_3H_7O^+$	1062 ± 35 ^{c}	976 ± 72 ^{c}	563 ± 29 ^{b}	1230 ± 116 ^{d}	355 ± 26 ^{a}	9×10^{-15}
61.029	61.028	$C_2H_5O_2^+$	10 ± 3 ^{a}	26 ± 6 ^{b}	18 ± 5 ^{ab}	11 ± 2 ^{a}	24 ± 10 ^{b}	4×10^{-4}
63.026	63.026	$C_2H_7S^+$	15.1 ± 0.3 ^{c}	16 ± 1 ^{c}	7.2 ± 0.4 ^{b}	20 ± 2 ^{d}	3.3 ± 0.5 ^{a}	4×10^{-16}
69.070	69.07	$C_5H_9^+$	6.4 ± 0.2 ^{a}	8.3 ± 0.6 ^{b}	6.0 ± 0.4 ^{a}	9.0 ± 0.8 ^{b}	6.2 ± 0.6 ^{a}	2×10^{-8}
71.086	71.086	$C_5H_{11}^+$	1.2 ± 0.1 ^{ab}	1.7 ± 0.8 ^{b}	0.7 ± 0.1 ^{a}	1.3 ± 0.1 ^{ab}	0.72 ± 0.05 ^{a}	1×10^{-3}
73.065	73.065	$C_4H_9O^+$	77 ± 2 ^{b}	82 ± 7 ^{b}	24 ± 1 ^{a}	76 ± 7 ^{b}	17.2 ± 0.9 ^{a}	1×10^{-16}
75.044	75.044	$C_3H_7O_2^+$	1.4 ± 0.2 ^{a}	20 ± 1 ^{b}	1.2 ± 0.2 ^{a}	1.4 ± 0.3 ^{a}	1.4 ± 0.2 ^{a}	1×10^{-21}
83.086	83.086	$C_6H_{11}^+$	1.6 ± 0.1 ^{b}	1.7 ± 0.1 ^{b}	0.7 ± 0.0 ^{a}	1.8 ± 0.2 ^{b}	0.71 ± 0.04 ^{a}	4×10^{-14}
87.044	87.044	$C_4H_7O_2^+$	3.7 ± 0.5 ^{bc}	3.8 ± 0.9 ^{bc}	3.0 ± 0.4 ^{ab}	4.3 ± 0.4 ^{c}	2.0 ± 0.3 ^{a}	2×10^{-5}
87.081	87.08	$C_5H_{11}O^+$	61 ± 2 ^{c}	66 ± 5 ^{c}	43 ± 2 ^{b}	61±6^{c}	23 ± 2 ^{a}	6×10^{-13}
89.060	89.06	$C_4H_9O_2^+$	2.2 ± 0.6 ^{a}	5.2 ± 0.6 ^{c}	2.9 ± 0.3 ^{ab}	2±1^{ab}	3.5 ± 0.3 ^{b}	2×10^{-6}
97.102	97.101	$C_7H_{13}^+$	2.3 ± 0.1 ^{c}	2.7 ± 0.2 ^{d}	1.8 ± 0.0 ^{b}	2.6±0.1^{d}	1.1 ± 0.1 ^{a}	4×10^{-14}
101.097	101.096	$C_7H_7O^+$	1.7 ± 0.1 ^{c}	2.0 ± 0.1 ^{d}	1.0 ± 0.0 ^{b}	2.0±0.2^{d}	0.6 ± 0.1 ^{a}	5×10^{-14}
115.113	115.112	$C_7H_{15}O^+$	28 ± 1 ^{c}	30 ± 2 ^{c}	20.5 ± 0.6 ^{b}	30±2^{c}	12 ± 1 ^{a}	7×10^{-14}
143.145	143.143	$C_9H_{19}O^+$	2.1 ± 0.1 ^{c}	2.5 ± 0.2 ^{d}	1.7 ± 0.1 ^{b}	2.4±0.2^{d}	1.1 ± 0.1 ^{a}	2×10^{-12}

This panel of 22 peaks includes only 9 masses detected also by the GC analysis, thus providing a broader overview of the diversity among samples associated with VOCs content. Comparing these findings with a recent PTR headspace analysis of other dairy product of industrial interest (milk powder, whey powder and anhydrous milk fat), the mass peaks 47.049, 63.026, 73.065, 87.081, 89.060, 101.097, 115.113, 143.145 seem to be peculiar of mascarpone headspace [26], indicating a potential role of the corresponding volatiles in shaping perceptions associated to Mascarpone consumption. Additionally, on the other hand, we found variable trends in mass peaks already detected in association with the headspaces of skim milk powder (43.018, 61.029, 87.044, 97.102), whole milk powder (41.039, 43.018, 45.033, 55.054, 61.029, 71.086, 75.044, 83.086, 87.044), whey powder (43.018, 59.049, 61.029, 75.044), and anhydrous milk fat (43.018, 43.054, 57.070, 69.070) [26]. This partial and specific overlapping, in terms of volatiles content, with the headspaces of other dairy ingredients/products, can be probably of help in the understanding of the unique sensory properties of mascarpone matrix.

Finally, in order to provide more complete information about the preliminary potential that arises from the PTR data in terms of separation of delactosed products, we propose two PCA representations, analyzing samples with or without lactose for the Manufacturers C and M, respectively (Figure 4).

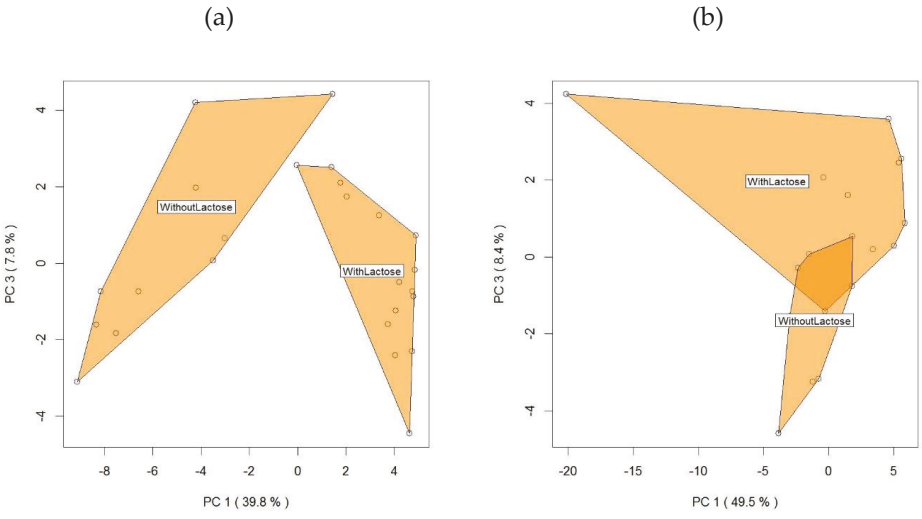

Figure 4. Analysis of mascarpone VOCs profile assessed by PTR-ToF-MS for the Manufacturers C (**a**) and M (**b**) plotted by the first and the third principal components. The labels and the selected areas indicate the separation between samples with or without lactose.

Figure 4a (Manufacturer C) and 4b (Manufacturer M) show that mascarpone samples classic and delactosed in this subset (different producers) are separated along the first and third PC (explaining 47.6% and 57.9% of the total variance, respectively). Even if preliminary, these results confirm the potential of PTR-TOF-MS analysis for the quality evaluation of lactose-free dairy products. In fact, recently, this analytical approach found application to monitor VOC variability in ultrahigh temperature lactose-free milk samples (assessing the impact of storage time and the of the use of different lactase preparations) [39]

3. Materials and Methods

3.1. Sample Selection and Preparation

A total of 12 different mascarpone batches were studied in this project that are listed in Table 1. The corresponding chemicophysical characteristics are reported in Table S1.

We obtained the samples from different local markets and stored them at 4 °C. The samples represent different manufacturers, all analyzed within the expiration date, and both plain and delactosed Mascarpone.

3.2. HS-SPME GC-MS Measurements

Aliquots of 8 mL of sample were placed in a 20 mL vials that were immediately sealed with a silicone rubber Teflon cap and crimped with aluminium seal. Then samples were heated at 60 °C and kept at the same temperature for 30 min while a polydimethylsiloxane/divinylbenzene SPME fibre (Supelco, Bellefonte, PA, USA) was exposed to the headspace over the surface of each sample in order to collect the compounds in the vapour phase. The exposure time was optimized in preliminary experimental trials. The SPME coating containing the headspace volatile compounds was inserted into the GC injection port and then thermally desorbed at 250 °C for 10 min in a 6890 GC (Agilent Technologies, Santa Clara, CA, United States). Compounds were eluted by a He gas flow of 1,4 mL/min in split mode (split 1:4) and separated using a 60 m Varian FactorFour WAXms capillary column (film thickness 0.25 mm, 0.25 mm internal diameter) (Varian, Middelburg, The Netherlands). The oven

temperature, initially set to 35 °C, was increased to 210 °C at 4 °C/min, then to 240 °C at a rate of 20 °C/min, and then this final temperature was held for 5 min. The mass spectrometer was set to electron ionization mode (MS-EI) generated at 70 eV, and mass spectra were collected in full scan mode, collecting ions from 39 to 250 *m/z*. The volatile compounds studied were identified by comparing their mass spectra and their retention times to those of reference standards analyzed at the same conditions and by comparison with spectra recorded in the Wiley 6 N mass spectral library (Wiley, Hoboken, NJ, USA) and, when needed, to literature references. Due to the lengthy HS-SPME GC/MS analysis, only four samples have been analysed by this method. For each sample, four replicates were analyzed.

3.3. PTR-ToF-MS Measurements

A commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) was used for the headspace measurements. The instrumental conditions in the drift tube were as following—drift voltage 550 V, drift temperature 110 °C, drift pressure 2.30 mbar affording an E/N value of 140 Townsend (1 Td = 10^{-17} V.cm^2). Sampling was performed with a flow rate of 40 sccm. The mass resolution (m/Δm) was at least 3800. Measurements were performed in an automated way by using a multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) as previously described [11]. The measurement order, both samples and replicates, was randomized to avoid memory effects. All vials were incubated at 40 °C for 30 min before PTR-MS analysis. Each sample was measured for 30 s, at an acquisition rate of 1 spectrum per second with an overall throughput of one sample every 5 min. The experiment was repeated at 60 °C, the temperature at which HS-SPME GC-MS provided better results. The entire experiment was repeated three times and empty vials, containing lab air, were measured together with the sample set and considered as "blanks". Data processing of PTR-ToF-MS spectra included dead time correction, external calibration and peak extraction steps performed according to a procedure described elsewhere [40]. The baseline of the mass spectra was removed after averaging the whole measurement and peak detection and peak area extraction was performed by using modified Gaussian to fit the data [41]. To determine the concentrations of volatile compounds in ppbv (part per billion by volume) the formulas described by Lindinger et al. were used by assuming a constant reaction rate coefficient (k_R=2 × 10^{-9} cm^3/s) for H_3O^+ as primary ion [42].

3.4. Statistical Analyses

Data exploration was based on Principal Component Analysis (PCA) of centered and scaled data. Analysis of variance (ANOVA) with Bonferroni correction was performed for selection of mass peaks in the sample headspace which are significantly higher than blanks. After this step, one-way ANOVA followed by Tukey's HSD ($p < 0.05$) was applied to evaluate the significant differences among mascarpone samples. All analyses were performed with core functions of R programming language (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2014) and its external packages (ChemometricsWithR, DiscriMiner, prospectr). In some cases, in order to interpret the results of the experiment, the entire dataset was divided into smaller subsets based on different criteria (e.g., producer, lactose content).

4. Conclusions

Using two complementary analytical approaches, Headspace-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME GC-MS) and Proton-Transfer Reaction-Mass Spectrometry coupled to a Time of Flight mass analyzer (PTR-ToF-MS), the present work provides a first description of Volatile Organic Compounds (VOCs). In addition, we underline the differences in VOC content susceptible to characterize the aroma of different brands and product types (classic and lactose-free). On the whole, the dominance of volatiles generally associated to floral, fruity, sweet, and nutty notes might contribute to explain the delicate sensory impression perceived by smelling this fresh dairy product. Unfortunately, the aroma profile of the present investigation cannot be discussed

in light of previous literature that is, as mentioned, very scarce. Considering the wide number of products that use mascarpone as raw material, such as the popular Tiramisù and coffee mascarpone cream, this study provides information to design future studies conceived to assess the contribution of this unripened cheese to the sensory characteristics of final products.

Supplementary Materials: The following are available online, Table S1: Monitored chemico-physical characteristics for the list of 'Mascarpone' samples analyzed in the present study.

Author Contributions: Conceptualization, F.B., and L.N.; methodology, V.L., I.K., L.C., F.B., and L.N.; software, L.C.; validation, L.C., F.B., and L.N.; formal analysis, V.L., and I.K.; investigation, V.C., V.L., and I.K.; resources, F.B. and L.N.; data curation, V.C., V.L., and I.K.; writing—original draft preparation, V.C., V.L., and I.K.; writing—review and editing, L.C., F.B., and L.N.; visualization, V.C., V.L., I.K., F.B. and L.N.; supervision, F.B., and L.N.; project administration, F.B., and L.N.; funding acquisition, F.B., and L.N. All authors have read and agreed to the published version of the manuscript.

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References

1. Carminati, D.; Perrone, A.; Neviani, E. Inhibition of *Clostridium sporogenes* growth in mascarpone cheese by co-inoculation with *Streptococcus thermophilus* under conditions of temperature abuse. *Food Microbiol.* **2001**, *18*, 571–579.
2. Franciosa, G.; Pourshaban, M.; Gianfranceschi, M.; Gattuso, A.; Fenicia, L.; Ferrini, A.M.; Mannoni, V.; De Luca, G.; Aureli, P. *Clostridium botulinum* spores and toxin in mascarpone cheese and other milk products. *J. Food Prot.* **1999**, *62*, 867–871. [PubMed]
3. Hinrichs, J. Mediterranean milk and milk products. *Eur. J. Nutr.* **2004**, *43*, i12–i17.
4. Del Prato, O.S. *Trattato di Tecnologia Casearia*; Edagricole: Bologna, Italy, 1998.
5. Donelly, C. *The Oxford Companion to Cheese*; Oxford University Press: New York, NY, USA, 2017; ISBN 978-0-19-933088-1.
6. Majchrzak, T.; Wojnowski, W.; Lubinska-Szczygeł, M.; Różańska, A.; Namieśnik, J.; Dymerski, T. PTR-MS and GC-MS as complementary techniques for analysis of volatiles: A tutorial review. *Anal. Chim. Acta* **2018**, *1035*, 1–13. [PubMed]
7. Dewulf, J.; Van Langenhove, H.; Wittmann, G. Analysis of volatile organic compounds using gas chromatography. *TrAC Trends Anal. Chem.* **2002**, *21*, 637–646.
8. Panseri, S.; Soncin, S.; Chiesa, L.M.; Biondi, P.A. A headspace solid-phase microextraction gas-chromatographic mass-spectrometric method (HS-SPME–GC/MS) to quantify hexanal in butter during storage as marker of lipid oxidation. *Food Chem.* **2011**, *127*, 886–889.
9. Biasioli, F.; Gasperi, F.; Yeretzian, C.; Märk, T.D. PTR-MS monitoring of VOCs and BVOCs in food science and technology. *TrAC Trends Anal. Chem.* **2011**, *30*, 968–977.
10. Jordan, A.; Haidacher, S.; Hanel, G.; Hartungen, E.; Märk, L.; Seehauser, H.; Schottkowsky, R.; Sulzer, P.; Märk, T.D. A high resolution and high sensitivity proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS). *Int. J. Mass Spectrom.* **2009**, *286*, 122–128.
11. Capozzi, V.; Yener, S.; Khomenko, I.; Farneti, B.; Cappellin, L.; Gasperi, F.; Scampicchio, M.; Biasioli, F. PTR-ToF-MS Coupled with an Automated Sampling System and Tailored Data Analysis for Food Studies: Bioprocess Monitoring, Screening and Nose-space Analysis. *J. Vis. Exp.* **2017**, *123*, e54075.
12. Marsili, R. Flavors and off-flavors in dairy foods. In *Encyclopedia of Dairy Sciences*, 2nd ed.; Fuquay, J.W., Ed.; Academic Press: San Diego, CA, USA, 2011; pp. 533–551. ISBN 978-0-12-374407-4.
13. Mutarutwa, D.; Navarini, L.; Lonzarich, V.; Compagnone, D.; Pittia, P. GC-MS aroma characterization of vegetable matrices: Focus on 3-alkyl-2-methoxypyrazines. *J. Mass Spectrom.* **2018**, *53*, 871–881.
14. Delgado, F.J.; González-Crespo, J.; Cava, R.; Ramírez, R. Formation of the aroma of a raw goat milk cheese during maturation analysed by SPME–GC–MS. *Food Chem.* **2011**, *129*, 1156–1163. [PubMed]

15. Curioni, P.M.G.; Bosset, J.O. Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* **2002**, *12*, 959–984.
16. Jung, H.J.; Ganesan, P.; Lee, S.J.; Kwak, H.S. Comparative study of flavor in cholesterol-removed Gouda cheese and Gouda cheese during ripening. *J. Dairy Sci.* **2013**, *96*, 1972–1983. [PubMed]
17. Mallia, S.; Escher, F.; Rehberger, B.; Schlichtherle-Cerny, H. Aroma-active secondary oxidation products of butter. In Proceedings of the 3rd QLIF Congress: Improving Sustainability in Organic and Low Input Food Production Systems, Stuttgart, Germany, 20–23 March 2007.
18. Peterson, D.G.; Reineccius, G.A. Characterization of the volatile compounds that constitute fresh sweet cream butter aroma. *Flavour Fragr. J.* **2003**, *18*, 215–220.
19. Wang, W.; Zhang, L.; Li, Y. Production of Volatile Compounds in Reconstituted Milk Reduced-Fat Cheese and the Physicochemical Properties as Affected by Exopolysaccharide-Producing Strain. *Molecules* **2012**, *17*, 14393–14408. [PubMed]
20. Povolo, M.; Contarini, G. Comparison of solid-phase microextraction and purge-and-trap methods for the analysis of the volatile fraction of butter. *J. Chromatogr. A* **2003**, *985*, 117–125.
21. Qian, M.; Reineccius, G. Identification of aroma compounds in Parmigiano-Reggiano cheese by gas chromatography/olfactometry. *J. Dairy Sci.* **2002**, *85*, 1362–1369.
22. Gasperi, F.; Gallerani, G.; Boschetti, A.; Biasioli, F.; Monetti, A.; Boscaini, E.; Jordan, A.; Lindinger, W.; Iannotta, S. The mozzarella cheese flavour profile: A comparison between judge panel analysis and proton transfer reaction mass spectrometry. *J. Sci. Food Agric.* **2001**, *81*, 357–363.
23. Boscaini, E.; van Ruth, S.; Biasioli, F.; Gasperi, F.; Märk, T.D. Gas Chromatography−Olfactometry (GC-O) and Proton Transfer Reaction−Mass Spectrometry (PTR-MS) Analysis of the Flavor Profile of Grana Padano, Parmigiano Reggiano, and Grana Trentino Cheeses. *J. Agric. Food Chem.* **2003**, *51*, 1782–1790.
24. Gallardo-Escamilla, F.J.; Kelly, A.L.; Delahunty, C.M. Influence of Starter Culture on Flavor and Headspace Volatile Profiles of Fermented Whey and Whey Produced from Fermented Milk. *J. Dairy Sci.* **2005**, *88*, 3745–3753.
25. van Ruth, S.M.; Koot, A.; Akkermans, W.; Araghipour, N.; Rozijn, M.; Baltussen, M.; Wisthaler, A.; Märk, T.D.; Frankhuizen, R. Butter and butter oil classification by PTR-MS. *Eur. Food Res. Technol.* **2008**, *227*, 307–317.
26. Makhoul, S.; Yener, S.; Khomenko, I.; Capozzi, V.; Cappellin, L.; Aprea, E.; Scampicchio, M.; Gasperi, F.; Biasioli, F. Rapid non-invasive quality control of semi-finished products for the food industry by direct injection mass spectrometry headspace analysis: The case of milk powder, whey powder and anhydrous milk fat. *J. Mass Spectrom.* **2016**, *51*, 782–791. [PubMed]
27. Benozzi, E.; Romano, A.; Capozzi, V.; Makhoul, S.; Cappellin, L.; Khomenko, I.; Aprea, E.; Scampicchio, M.; Spano, G.; Märk, T.D.; et al. Monitoring of lactic fermentation driven by different starter cultures via direct injection mass spectrometric analysis of flavour-related volatile compounds. *Food Res. Int.* **2015**, *76*, 682–688. [PubMed]
28. Yépez, A.; Russo, P.; Spano, G.; Khomenko, I.; Biasioli, F.; Capozzi, V.; Aznar, R. In situ riboflavin fortification of different kefir-like cereal-based beverages using selected Andean LAB strains. *Food Microbiol.* **2019**, *77*, 61–68. [PubMed]
29. Romano, A.; Capozzi, V.; Spano, G.; Biasioli, F. Proton transfer reaction-mass spectrometry: Online and rapid determination of volatile organic compounds of microbial origin. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 3787–3795.
30. Gallardo-Escamilla, F.J.; Kelly, A.L.; Delahunty, C.M. Sensory Characteristics and Related Volatile Flavor Compound Profiles of Different Types of Whey. *J. Dairy Sci.* **2005**, *88*, 2689–2699.
31. Burdock, G.A. *Fenaroli's Handbook of Flavor Ingredients*; CRC Press: Boca Raton, FL, USA, 2019; ISBN 978-1-00-069466-6.
32. Jia, W.; Wang, H.; Shi, L.; Zhang, F.; Fan, C.; Chen, X.; Chang, J.; Chu, X. High-throughput foodomics strategy for screening flavor components in dairy products using multiple mass spectrometry. *Food Chem.* **2019**, *279*, 1–11.
33. Puniya, A.K. *Fermented Milk and Dairy Products*; CRC Press: Boca Raton, FL, USA, 2015; ISBN 978-1-4665-7800-5.
34. Cattaneo, T.M.P.; Summa, C.; Bertolo, G.; Giangiacomo, R. Spreadability of Mascarpone cheese: Sensory and objective measurements. *Milchwissenschaft* **2005**, *60*, 399–402.

35. Su, W.-H.; He, H.-J.; Sun, D.-W. Non-Destructive and rapid evaluation of staple foods quality by using spectroscopic techniques: A review. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 1039–1051.
36. van den Berg, F.; Lyndgaard, C.B.; Sørensen, K.M.; Engelsen, S.B. Process Analytical Technology in the food industry. *Trends Food Sci. Technol.* **2013**, *31*, 27–35.
37. Capozzi, V.; Makhoul, S.; Aprea, E.; Romano, A.; Cappellin, L.; Sanchez Jimena, A.; Spano, G.; Gasperi, F.; Scampicchio, M.; Biasioli, F. PTR-MS Characterization of VOCs Associated with Commercial Aromatic Bakery Yeasts of Wine and Beer Origin. *Molecules* **2016**, *21*, 483. [PubMed]
38. Campbell-Sills, H.; Capozzi, V.; Romano, A.; Cappellin, L.; Spano, G.; Breniaux, M.; Lucas, P.; Biasioli, F. Advances in wine analysis by PTR-ToF-MS: Optimization of the method and discrimination of wines from different geographical origins and fermented with different malolactic starters. *Int. J. Mass Spectrom.* **2016**, *397–398*, 42–51.
39. Bottiroli, R.; Pedrotti, M.; Aprea, E.; Biasioli, F.; Fogliano, V.; Gasperi, F. Application of PTR-TOF-MS for the quality assessment of lactose-free milk: Effect of storage time and employment of different lactase preparations. *J. Mass Spectrom.* **2020**. [CrossRef]
40. Cappellin, L.; Biasioli, F.; Fabris, A.; Schuhfried, E.; Soukoulis, C.; Märk, T.D.; Gasperi, F. Improved mass accuracy in PTR-TOF-MS: Another step towards better compound identification in PTR-MS. *Int. J. Mass Spectrom.* **2010**, *290*, 60–63.
41. Cappellin, L.; Biasioli, F.; Granitto, P.M.; Schuhfried, E.; Soukoulis, C.; Costa, F.; Märk, T.D.; Gasperi, F. On data analysis in PTR-TOF-MS: From raw spectra to data mining. *Sens. Actuators B Chem.* **2011**, *155*, 183–190.
42. Lindinger, W.; Hansel, A.; Jordan, A. On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) medical applications, food control and environmental research. *Int. J. Mass Spectrom. Ion Process.* **1998**, *173*, 191–241.

Sample Availability: Samples of the compounds are not available from the authors.

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MDPI

Review

Flavor and Texture Characteristics of 'Fuji' and Related Apple (*Malus domestica* L.) Cultivars, Focusing on the Rich Watercore

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Abstract: Watercore is a so-called physiological disorder of apple (*Malus domestica* L.) that commonly occurs in several well-known cultivars. It is associated with a rapid softening of the flesh that causes a marked changed in flavor and texture. In Asia, apples with watercore are preferred and considered a delicacy because of their enhanced sweet flavor. The 'Fuji' cultivar, the first cultivar with rich watercore that is free from texture deterioration, has played a key role in the development of the market for desirable watercored apples. This review aimed to summarize and highlight recent studies related to the physiology of watercore in apples with special focus on 'Fuji' and related cultivars.

Keywords: 'Fuji'; watercore; sweetness; flavor; texture; flesh browning disorder; apple

1. Introduction

The 'Fuji' cultivar has maintained a large share of the global apple production market over the last two decades [1]. Originally, 'Fuji' was selected from cross between 'Ralls Janet' and 'Delicious' in the Tohoku region of Japan and gained popularity because it is extremely juicy and crisp with a sweet flavor similar to that of 'Delicious' [2]. 'Fuji' is also susceptible to watercore development. Watercore is a phenomenon that presents as a translucent appearance at the core and/or flesh of the fruit, and it is caused by the intercellular spaces of the affected tissue being filled with fluid. It has been reported that watercore development is related to sugar metabolism during the maturation process and fruit mineral composition. Watercored apple is prone to several physiological disorders, such as browning and breakdown during storage [3–9]. Furthermore, strains of the 'Delicious' cultivar are also highly susceptible to watercore, which is typically accompanied by changes in texture traits, such as softening and mealiness. These undesirable characteristics that commonly occur in watercored 'Delicious' strains have caused watercoring in apples to be viewed negatively.

In spite of this, watercored 'Fuji' has gradually become desirable in Japan and other Asian countries, and the palatability of 'Fuji' and watercored apples has been identified in the last decade. Today, Japanese producers and consumers generally value watercored apple owing to its excellent fruit flavor, which occurs when it fully matures on the tree. In fact, watercored apples are often advertised using phrases such as aroma-rich and pineapple-like. Furthermore, the rich watercore trait has become a breeding target with the aim of increasing the sweet flavor in apple [10]. Aprea et al. [11] proposed that apple breeding programs must take into account factors such as volatile compounds, texture

parameters, minor components, and information from sensory panels. However, perceived sweetness is difficult to be described because it is always perceived in combination with other sensory properties, which influence its evaluation. Sweetness perception is a complex and multisensory process, and only gustatory stimuli are insufficient to fully understand and predict it [12]. In this work, we focus on 'Fuji' and the related cultivars and review the mechanism of watercoring in apple palatability. We also assess various characters, such as flavor, texture, and genetic properties, employing integrated analysis of instrumental and sensory profiling.

2. Flavor Characteristics

2.1. Sensory Analysis

Although watercored apple has been extremely popular among Japanese consumers, there were little published data regarding the overall acceptance for watercored apple available. In order to characterize the unique flavor and overall acceptance, Tanaka et al. [13] conducted sensory analysis using watercored and nonwatercored 'Fuji' with 29 trained panelists. With respect to overall acceptance, watercored apples scored significantly higher than nonwatercored apples (Table 1). Overall intensities of aroma and taste and five sensory attributes were scored using a seven-point scale. Taste intensity was evaluated with nose clip. Overall aroma intensity and perception of sweet and fruity flavors were enhanced in watercored apple, whereas green and sour perception was enhanced in nonwatercored apple (Table 1). Overall taste intensity, in which the influence of aroma was eliminated by clips, was not significantly different, indicating that the contribution of aroma to the overall acceptance and characteristics of flavor was remarkably large in this case.

Table 1. Sensory evaluation for watercored and nonwatercored 'Fuji' apples.

Sample Status	Overall Acceptability	Overall Intensity		Sensory Attribute				
		Aroma	Taste	Green	Fruity	Floral	Sweet	Sour
Nonwatercored	3.0	4.0	4.1	4.3	4.0	3.1	3.9	4.0
Watercored	3.5	4.5	4.2	3.6	4.4	4.2	4.6	3.2
Significance	**	**	ns	**	*	***	**	***

Apple: Products of a commercial orchard, peeled, cored, and cut into bite-size pieces just before being served. Evaluation: a seven-point categorical scale (1–7); 29 panelists trained for quantitative destructive analysis, 10 females and 19 males. Taste was evaluated with nose clip to eliminate the influence of aroma. Significance: *, **, *** indicate significant differences at the level of $p < 0.05$, $p < 0.01$, or $p < 0.001$, respectively, using paired *t*-test; ns means not significant. Reproduced with permission from Tanaka et al. [13].

2.2. Analysis of Volatile and Water-Soluble Compounds

Volatile and water-soluble components were profiled for watercored and nonwatercored 'Fuji' and 'Koutoku', a progeny of 'Fuji' (Tables S1 and S2) [13]. In both cultivars, ethyl esters and methyl esters of fatty acids were detected in watercored fruit; their peak intensities were as large as several to several hundred times those of nonwatercored apple (Table S1). In addition, principal component analysis of the intensities of the 109 components suggested that the PC1 score was differentiated by cultivar, whereas the PC2 score was differentiated by the presence of watercore (Figure 1). The PC2 loadings suggested that ethyl esters, methyl esters, sorbitol, galactaric acid, erythronic acid, and dehydroascorbic acid were associated with watercore. Increase in sorbitol was consistent with previous reports [14–17]. This integrated profiling analysis suggested that an increase in methyl esters and ethyl esters is crucial to the attributes and desirability of watercored apple. Similar phenomena have been revealed by Dixon et al. [18] when, following a short-term exposure to hypoxic conditions, time courses of apple aroma components and odor units for 10 apple cultivars were analyzed, and their results indicated that odor unit values highly corresponded to ethyl ester levels.

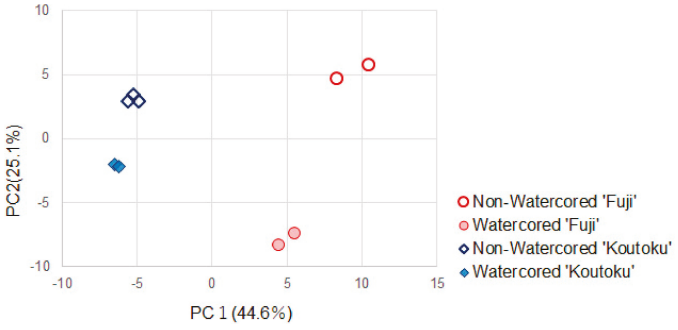

Figure 1. Principal component analysis score plots of the volatiles and solubles of fruit juice. PC1 and PC2 scores were discriminated by cultivars and watercore existence, respectively. Top 10 of PC2 loadings were (1) ethyl butanoate, (2) ethyl propanoate, (3) ethyl 2-methylbutanoate, (4) ethyl acetate, (5) ethyl hexanoate, (6) sorbitol, (7) galactaric acid, (8) methyl 2-methylbutanoate, (9) methyl acetate, and (10) ethyl tiglate. Reproduced with permission from Tanaka et al. [13].

Ethyl esters have been reported to have an apple-like, fruity, sweet aroma with an extremely low threshold value. For example, Komthong et al. [19] analyzed head-space volatiles of 'Fuji' using aroma extract dilution analysis and determined flavor dilution factor, which is the lowest dilution ratio of the volatile compounds. Then, methyl 2-methylbutanoate and ethyl 2-methylbutanoate were estimated and determined to be the most potent odorants in the volatiles based on their lowest threshold odor values. Moreover, we demonstrated that an increase in ethyl esters significantly enhanced the perception of apple-like sweetness by sensory evaluation using a series of 'Fuji' samples that had chemically modified aroma (Figure 2). Based on these data, ethyl esters appear to be potent, key flavor compounds in watercored apples.

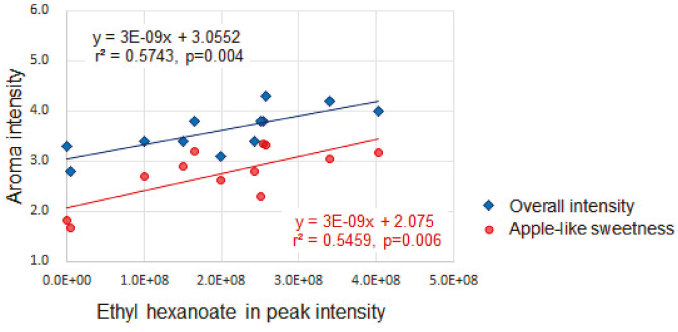

Figure 2. Ethyl hexanoate and aroma intensity in ester-enhanced 'Fuji' by incubation with ethanol mixture. Ethyl hexanoate is shown as a representative of ethyl esters because major ethyl esters of 'Fuji' correlate with one another in their peak intensities.

The difference among sugar and sorbitol contents, soluble solid contents (SSC), and gene expression related to sugars in watercored and nonwatercored tissues has been studied extensively [14,16,20,21]. Specifically, sorbitol accumulation in the watercored tissues was observed in several cases, whereas fructose, glucose, sucrose, total sugar contents, and SSC were only observed in a few cases [13,15,16,20]. Fructose, the sweetest sugar of the apple components [22,23], tended to be lower in watercored tissues. Sorbitol tends to be present at a low content and exhibits weaker perceptive sweetness compared with other sugars, even though it increases in content in watercored tissues. [23,24]. The comparison between watercored and nonwatercored tissues often found decreases in sweetness in the watercored tissues. According to Melad-Herreros [15] and Williams et al. [16], nonaffected tissues of watercored

apples, often edible parts, scored higher for fructose and sucrose than the affected tissues (Table 2), indicating that watercored apples may in fact be sweeter. Harker et al. [25] stated that two apples needed to differ in °Brix (SSC) by more than 1 before evoking a change in response to a perceived sweet taste for the median panelist. A difference of 1 °Brix corresponds to 1% difference in sucrose. Table 2 presents the estimated sweetness using two equations that defined sucrose sweetness as 1 [26,27]. Our estimation (a) took sorbitol into account based on the estimation summarized by Kitahata and Machinami [22], whereas (b), known as the total sweetness index, did not [23]. Williams et al. [17] also found that the difference between nonwatercored and nonaffected tissues of watercored apples was nearly 1. In this case, there was a perceived sweetness difference between the edible part of a watercored and that of a nonwatercored apple at a near-threshold level. These findings are in agreement with the results of sensory evaluation (Table 1) of taste intensity, which found that while watercored apples were generally evaluated a little intense, they did not differ significantly from control samples. Given these findings, the significant difference in sweetness is likely affected by components other than sugars.

Table 2. Sugar profiles and estimated perceived sweetness of various apple cultivars.

Cultivar	Watercore	Sugar Contents (g/100 g FW)				Estimated Sweetness		Ref.
		Fructose	Glucose	Sucrose	Sorbitol	(a)	(b)	
'Fuji'	absent	5.7	3.1	1.5	0.4	12.0	12.3	[14] [1]
	present (richest level)	5.5	2.2	3.4	1.2	12.7	13.2	
'Fuji'	absent	6.6	2.3	1.8	0.5	12.3	13.4	[15]
	present	5.6	1.9	1.8	1.5	11.3	11.7	
'Gloster'	absent	5.2	1.9	3.2	0.3	11.5	12.4	
	present	3.8	1.7	2.6	0.9	9.2	9.6	
'Delicious'	absent	6.4	1.7	2.2	0.2	11.9	13.1	
	present	5.1	1.5	2.0	1.0	10.2	10.7	
'Esperiega'	absent	7.2	1.51	2.8	0.8	13.7	14.7	[16]
	present (nonaffected site)	6.9	2.0	3.2	1.5	14.4	15.0	
	present (affected site)	6.3	2.2	1.5	2.9	13.0	12.6	
'Winesap'	absent	3.2	4.0	3.8	0.9	11.3	11.6	[17] [1]
	present (nonaffected site)	3.4	4.2	4.1	1.3	12.2	12.4	
	present (affected site)	3.0	3.7	3.8	1.8	11.4	11.1	

[1] Original sugar contents were converted to g/100 g FW. Estimated sweetness: (a) (1.0 [sucrose]) + (1.3 [fructose]) + (0.7 [glucose]) + (0.7 [sorbitol]) [22]; (b) = (1.0 [sucrose]) + (1.5 [fructose]) + (0.76 [glucose]) [23].

Recently, the importance of aroma components in the characteristics of flavor and preference in apple has been widely recognized. Aprea et al. [11] reported that sorbitol content correlated with perceived sweetness better than any other single sugar or total sugar content. Furthermore, their predictive model based on partial least squares regression included not only SSC but also volatile compounds and revealed that several volatiles are possibly contributing to flavor. Having a sweet taste is an important but difficult attribute to be predicted using objective measurements [25]. The contribution of sugars to the enhancement of perceived sweetness in watercored apple is likely limited, whereas the profile of aroma components varies widely and accounts for several of the unique flavor profiles. Aroma components between watercored and nonwatercored apples can be markedly different. For instance, our analysis revealed that the detected levels of most ethyl esters that created an aroma profile with characteristics similar to pineapple or ginjoshu (high-quality sake) were ten times their levels in nonwatercored apples (Table S1) [13,28–31]. Considering these profiles of flavor components and sensory attributes, the contribution of aroma components, such as ethyl esters, is crucial in producing the flavor characteristics in watercore-rich apples.

2.3. Mechanism of Enhanced Ethyl Ester Synthesis in Watercored Apples

Because ethyl esters are crucial in aroma and flavor profiles in apples, different analyses have already focused on the synthesis. Dixon and Hewett [18] reported that apple volatile compounds

increased in ethanol and ethyl ester concentrations after exposure to hypoxic conditions. Specifically, the synthesis of ethyl esters was high in watercore-susceptible cultivars 'Red Delicious', synonymous with 'Delicious', and 'Fuji' and low in nonsusceptible cultivars 'Golden Delicious' and 'Cox's Orange Pippin'. It has also been reported that ethyl esters from apples subjected to controlled-atmosphere (CA) storage exhibited a temporary increase in ethanol and ethyl ester concentrations. Hypoxia likely activates anaerobic glycolysis and ethanol synthesis, causing an increase in ethyl ester production [32,33]. One study found that a decrease in respiration and an increase in ethanol and acetaldehyde concentrations in watercored tissues of 'Richard Delicious', a sport of 'Delicious', shared similarity with apples that were exposed to hypoxic conditions or CA-stored [3]. Furthermore, Tanaka et al. [13] analyzed oxygen distribution within a fruit and demonstrated low-oxygen status at the watercored position (Figure 3), whereas nonwatercored fruits were flat. These phenomena support the concept that ethyl ester synthesis is enhanced under hypoxic conditions within watercored tissues, resulting in distinctive, fermented flavor.

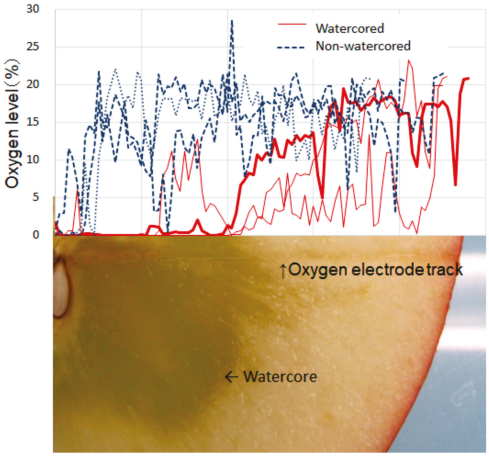

Figure 3. Oxygen distribution related to watercore of 'Fuji' apple. Three fruits were measured for each class. Thick red line corresponds to the photograph. Reproduced with permission from Tanaka et al. [13].

Questions also arise regarding discrimination of volatiles and related gene expression in a fruit associated with oxygen levels. This highly variable, cultivar-dependent response of apple cultivars to hypoxic conditions may also be associated with the physiological processes involved in the development of watercore, which has not been fully elucidated to date. To better understand the metabolism of the characteristic aroma profiles, a fusion analysis of molecular biology and metabolomics will be required.

3. Texture Characteristics

3.1. Apple Cultivars and Texture Measurement

Texture is a key factor that affects consumer preference of apple [34–36]. Texture comprises crispness, mealiness, juiciness, firmness, and other traits and has been reported to influence perceived sweetness [25,37]. Among the texture traits, crispness and juiciness are favorable for apple, whereas softness or mealiness are avoided [35]. Traditional watercore-susceptible cultivars were often accompanied by mealiness and rapid softening [5]. However, the occurrence of watercore and softening is under separate regulations, and cultivars have been developed with one but not the other [10,38]. Here, we review the studies on apple texture as it is related to watercore susceptibility.

Crispness is a sensory and integrated attribute defined as the amount and pitch of sound generated when the fruit is first bitten using the incisor [37,39,40], and it is often estimated from firmness because the two traits are highly and positively correlated [35]. Softening is usually caused by a reduction in firmness, which is typically measured using a penetrometer or sensory analysis. Cell shape, cell size, cell packing, and overall fruit anatomy as well as chemistry of the cell wall and membrane and the role of cell turgor affect firmness [41]. Among them, macromolecular network structures of cell walls, mainly comprising pectin, hemicellulose and cellulose, confer flesh cell rigidity, however, the structures are gradually lost by the cell-wall-modifying enzymes such as β-galactosidase, α-L-arabinofuranosidase, polygalacturonase, pectin methylesterase, and others. Ethylene reportedly stimulated these enzymes, subsequently causing flesh softening. Turgor reduction was also associated with firmness reduction [41,42]. Although cell membranes of apple are not typically associated with cell wall swelling and juiciness [41], so far as watercore is concerned, it may play roles in apple juiciness to some extent, as described below (Section 3.2).

Mealiness is defined as the amount of small, lumpy particles that become apparent during chewing in sensory analysis [37,39,43]. It is due to the loss of cell–cell adhesion or cell separation [37]. Iwanami et al. [38] investigated 23 cultivars and a breeding line under a time-course experiment to evaluate firmness and mealiness and divided them into four groups based on their results after 40 days of storage at 20 °C. The watercore-susceptible cultivars 'Starking Delicious' and 'Red Gold' were placed in the most rapid mealiness developing group, whereas 'Fuji' was the firmest and most nonmealy cultivar. This was consistent with other previous studies [44–46]. Iwanami et al. [47] also found that the softening performance of an apple cultivar during storage was highly dependent on the degree of mealiness and turgor reduction rate. The softening rates of all mealy cultivars were high; moreover, the softening rates of nonmealy cultivars were significantly correlated with the turgor reduction rates. In other words, nonmealy cultivars with slow turgor reduction can be expected to exhibit high storage performance. 'Fuji' had the lowest turgor reduction rate, which most likely contributed to its firmness and crispness. In addition, 'Starking Delicious', another sport of 'Delicious', surprisingly exhibited the slowest turgor reduction rate among the tested cultivars contrary to its trait of rapid softening. 'Fuji' seems to inherit the excellent trait of slow turgor reduction from the softening cultivar 'Delicious' and not from the slow softening 'Ralls Janet'. Differences in storage performance between 'Fuji' and the other 'Delicious' strains may mainly be due to differences in mealiness or nonmealiness.

A genetic contribution to watercore and mealiness in the 'Fuji'-related apples was demonstrated by Kunihisa et al. [10], who examined genomic dissection of 'Fuji' using 115 accessions of its descendants and parents. In that study, one quantitative trait loci (QTL) was detected for the following traits: degree of watercore and mealiness, acidity, and harvest day. The QTL for a high degree of watercore was detected in the middle of chromosome (chr)14, whereas the one for mealiness was detected at the middle of chr1. 'Fuji' has inherited haplotypes from both 'Delicious' and 'Ralls Janet'. The haplotype of 'Fuji' derived from 'Delicious' in the chr14 region dominantly causes watercore, whereas one in the chr1 region causes mealiness. For mealiness, another QTL associated with *MdPG1* was detected from different F_1 population [40]. So far, as 'Fuji' descendant, however, 90% of selected cultivars or superior breeding lines have inherited the haplotype of 'Fuji'-derived 'Ralls Janet' at the region of chr1 [10,48].

Sadamori, a leader of the 'Fuji' breeding team, recounted that most of the seedlings of 'Ralls Janet' and 'Delicious' generated sweet but mealy fruit in his memoir [49]. Among them (592 fruits), they found only two crisp and nonmealy fruits. One of them, which exhibited excellent flavor, was what eventually became 'Fuji' [49]. There was only a 0.3% frequency of nonmealy flesh from that cross; however, nonmealy phenotypes are more common in 'Fuji'-related accessions. Therefore, newly developed watercore-susceptible lines derived from 'Fuji' have an improved chance of possessing both excellent flavor and texture. Additional genetic information on the turgor reduction after harvest and its physiological understandings will help further improve and maintain the crispness of apples.

3.2. Watercore and Texture

Juiciness positively contributes to perceived freshness and is dependent on water content [50]. The water content of watercored apples is higher than that of nonwatercored apples, which is caused by the fluid within intercellular spaces or apoplast that causes watercore. Iwanami et al. [50] reported that both the water content of the whole fruit and apoplast tissues positively correlated with juiciness, affirming that watercored apples exhibit greater juiciness than nonwatercored apples. Although the report did not refer to watercore, the juiciest apple, 'Oyume', in their data is a cultivar that generally develops rich watercore. Maintaining the perception of freshness in apples, which are commonly stored for relatively long periods of time, is crucial for continued consumer appeal and requires appropriate storage conditions.

In order to establish a storage technique for high-quality watercored 'Fuji', Onodera et al. (2010) [51] investigated storability of apples that exhibited >30% watercoring. Time-course measurements of firmness and watercore degree were taken during 3 months of storage and 14 days of shelf time under regular atmosphere (RA). Both watercore degree and firmness decreased with time, and these exhibited significant positive correlation to each other (Figure 4). These results were in agreement with those of a previous report of Bowen and Watkins [14], which stated that flesh firmness at harvest initially tended to decrease with watercore scores and then significantly increased as watercore enhanced. These data suggest that highly watercored apples may maintain a firmer texture than lesser watercored apples for a few months after harvest. Further case examples are required.

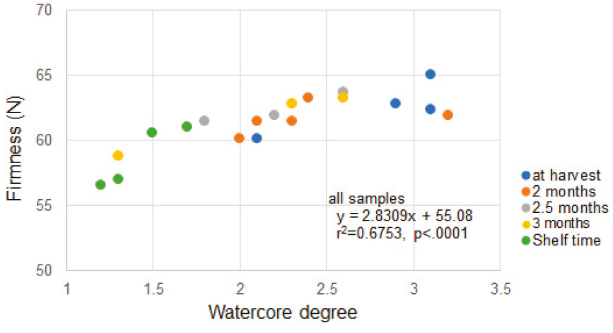

Figure 4. Watercore degree (0–4) and flesh firmness of 'Fuji' apple. Reproduced with permission from Onodera [51].

Being a major plant growth regulator and ripening hormone, ethylene is considered to be involved in the softening of apples [41,52]. Internal ethylene concentration (IEC) was measured in relation to watercore degree. Bowen and Watkins [13] reported that IEC increased with the watercore degree, whereas Argenta et al. [53] reported that IEC was higher in fruit with a low watercore score, and it decreased in fruits with a high watercore score compared with watercore-free. There have been few studies regarding the firmness of watercored apples under storage and its regulation, limiting what is currently known. As watercored apples gain popularity, further studies will likely greatly elucidate the relationship between firmness and watercoring.

4. Watercore During Storage

Watercored apples are likely to develop physiological disorders in the flesh, including watercore breakdown, internal browning, and various other disorders and, in some cases, worsen the degree of existing disorders [3–5,53–56]. These disorders often hinder the storability of apples and their use as a long-shelf life commodity. Watercore development is accompanied by photosynthetic carbohydrate accumulation in the fruit; consequently, as harvest is delayed, the degree of watercore

increases [14,57,58]. Therefore, watercore-susceptible cultivars are often harvested long before maturity at the expense of sweet flavor.

Onodera et al. [51] investigated the storability of highly watercored 'Fuji' with or without 1-methylcyclopropene (1-MCP) treatment for 3 months. Watercore breakdown did not occur until 3 months after harvest, irrespective of 1-MCP treatment and temperature settings. Another experiment in Figure 5 presents a time course of watercore degree and incidence of watercore breakdown during shelf life. The storage conditions were set at −1 °C or 2 °C for an initial 2 months and at 5 °C for 9 days followed by 20 °C for 14 days. Watercore degrees gradually decreased in all treatments during the experiment, and the incidence of watercore breakdown was detected at 14 days after storage at 20 °C. These results indicate that watercored 'Fuji' can be stored for up to 3 months under RA with refrigeration.

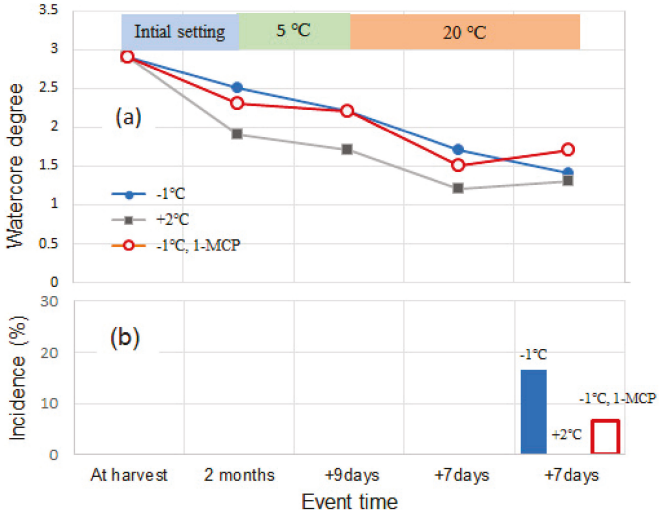

Figure 5. Time course of watercore degree (0–4) and watercore breakdown incidence (area %) in 'Fuji' apple. $n = 30$. (**a**) Watercore degree, (**b**) watercore breakdown incidence. Watercore breakdown was not observed in the apples that were initially stored at 2 °C. Reproduced with permission from Onodera [51].

Storage performance over a much longer duration than that reported by Onodera et al. [51] was reported by Kasai et al. [59] to determine which cultivars were resistant to physiological disorders and deterioration of flavor and texture. Apples of 30 cultivars were harvested at their commercial harvest time in the fall and stored under RA, CA, and 1-MCP treatment until mid-June of the next year at 0 °C followed by under RA at 20 °C for 5 days. The watercore degree and flesh browning disorder, which is regarded as a serious problem in watercored apples, were analyzed, and flesh browning disorder was found to occur in most cultivars irrespective of the presence or absence of watercore at harvest, except for 'Shuyo' and 'Ambitious'. Figure 6a presents the relationship between watercore scores at harvest and flesh browning disorder incidence after storage. Seven cultivars scored >2 in watercore, and most of them had high incidence of physiological disorders. However, the incidence in 'Fuji' was low for the score of watercore, which may have been due to the disappearance of watercore. The remaining watercore after storage and the incidence of flesh browning disorders are presented in Figure 6b. Flesh browning occurred irrespective of the remaining watercore score and storage condition; however, highly watercored remaining fruits exhibited severe flesh browning without exception. Watercore is not the only cause of the flesh browning disorder; however, prolonged, severe watercore greatly enhances the severe incidence in flesh.

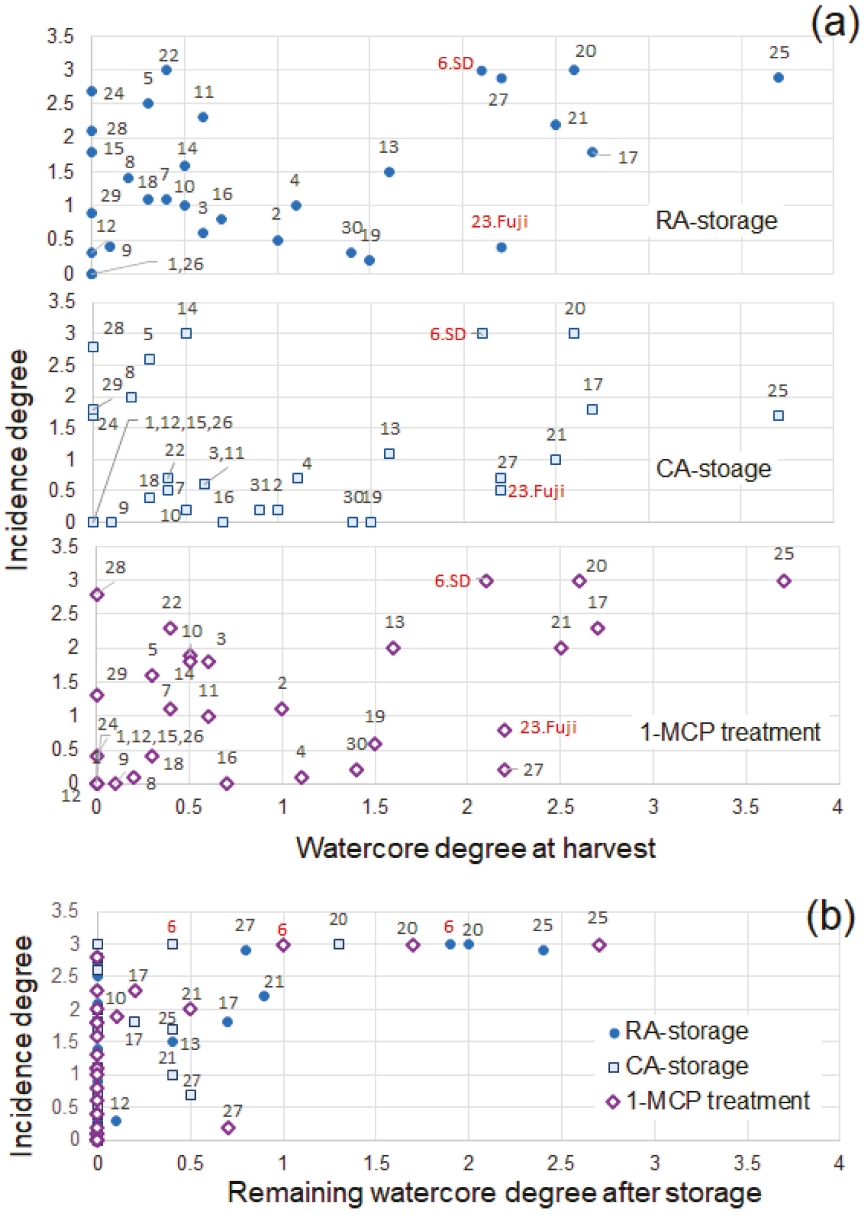

Figure 6. Watercore degree (0–4) and incidence of flesh browning disorders (0–3) in various apple cultivars. (**a**) Incidence degree to watercore degree at harvest; (**b**) Incidence degree to watercore degree after storage. $n = 10$. 1: 'Shuyo'; 2: 'Seimei'; 3: 'Shinano Sweet'; 4: 'Sekaiichi'; 5: 'Morinokagayaki'; 6: '**Starking Delicious' (SD)**; 7: 'Kitarou'; 8: 'Jona Gold'; 9: 'Koutaro'; 10: 'Yoko'; 11: 'Megumi'; 12: 'Aori 27'; 13: 'Aikanokaori'; 14: 'Mutsu'; 15: 'Shinano Gold'; 16: 'Mahe 7'; 17: 'Hokuto'; 18: 'Orin'; 19: 'Aori 15'; 20: 'Gunma Meigetsu'; 21: 'Koukou'; 22: 'Slim Red'; **23: 'Fuji'**; 24: 'Mellow'; 25: 'Koutoku'; 26: 'Ambitious'; 27: 'Romu 50'; 28: 'Granny Smith'; 29: 'Cripps Pink'; 30 'Aori 21'; 31: 'Fuji' (bagged). Reproduced with permission from Kasai et al. [59].

Storage longer than 4–5 months usually utilizes several treatments to suppress respiration and ethylene function, which results in an inhibition of aroma synthesis. This inhibits the generation of distinct, sweet aroma, which is the advantage of fresh watercore-rich apples and which cannot be produced after CA storage. In other words, watercored apples should be eaten within a few months of harvest or earlier, especially highly watercore-rich fruits.

Based on work from several previous studies, watercore development can be enhanced or inhibited using cultural techniques on watercore-susceptible cultivars. Watercore is promoted by low or high air temperatures during the preharvest period, large fruit, poor calcium concentration, high nitrogen and boron nutrition, a high leaf-to-fruit ratio, excessive fruit thinning, high or low light exposure, growth in volcanic ash soil, ethrel (ethephon) and gibberellin treatment, and girdling of the trunk and limbs [9]. Therefore, to develop rich watercore for a premium product, fruits are allowed to increase photosynthate accumulation by means of increasing the light received by the leaves and fruits and harvesting at full maturity. To maintain a long shelf life without the watercore physiological disorder, photosynthate accumulation in fruits is limited by earlier harvesting and fruit bagging. Apple producers choose one of these cultivation methods according to demand and their business policies.

5. Conclusions

Watercore in apple had been avoided for years due to the mealy texture and brown flesh incidence associated with it. Currently, however, watercore-rich apples are gaining popularity, mainly in Asian countries. 'Fuji', the first rich-watercored cultivar that is free from texture deterioration, greatly contributed to the paradigm shift. 'Fuji' resulted from a cross made in 1939, and though many decades have passed, the potential of 'Fuji' as a high-quality apple is still being shown by integration of diverse analytical methods, such as instrumental analysis and sensory, chemical, physiological, and genetic aspects. Still, there are many unresolved issues related to apple quality. Expanding the understanding of the nature and physiology of apple will continue to lead to improvements in apple quality by utilizing various concepts, approaches, and techniques.

Supplementary Materials: The following are available online. Table S1: Intensity of volatiles in watercored and nonwatercored 'Fuji' and 'Koutoku' apples, Table S2: Intensity of water-soluble compounds in watercored and nonwatercored 'Fuji' and 'Koutoku' apples.

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References

1. O'Rourke, D. World production, trade, consumption and economic outlook for apples. In *Apples: Botany, Production, and Uses*; Ferree, D.C., Warrington, I.J., Eds.; CABI: Oxon, Wallingford, UK, 2003; pp. 15–29.
2. Sadamori, S.; Murakami, H.; Suzuki, H.; Isizuka, S. New breeding hybrids of apple. *Bull. Tohoku Natl. Agric. Exp. Stn.* **1955**, *4*, 125–128. (In Japanese)
3. Smagula, J.M.; Bramlage, W.; Southwick, R.; Marsh, H. Effects of watercore on respiration and mitochondrial activity in Richared Delicious apples. In *Proceedings of the American Society for Horticultural Science*; Amer Soc Horticultural Science: Geneva, NY, USA, 1968; pp. 753–761.
4. Faust, M.; Shear, C.; Williams, M.W. Disorders of carbohydrate metabolism of apples (watercore, internal breakdown, low temperature and carbon dioxide injuries). *Bot. Rev.* **1969**, *35*, 169–194. [CrossRef]
5. Sharples, R. A note on the occurrence of watercore breakdown in apples during 1966. *Plant Pathol.* **1967**, *16*, 119–120. [CrossRef]
6. Marlow, G.C.; Loescher, W.H. Watercore. *Hortic. Rev.* **1984**, *6*, 189–251.

7. Kasai, S.; Arakawa, O. Antioxidant levels in watercore tissue in 'Fuji'apples during storage. *Postharvest Biol. Technol.* **2010**, *55*, 103–107. [CrossRef]
8. Tanaka, F.; Tatsuki, M.; Matsubara, K.; Okazaki, K.; Yoshimura, M.; Kasai, S. Methyl ester generation associated with flesh browning in 'Fuji'apples after long storage under repressed ethylene function. *Postharvest Biol. Technol.* **2018**, *145*, 53–60. [CrossRef]
9. Itai, A. Watercore in fruits. In *Abiotic Stress Biology in Horticultural Plants*; Springer: Tokyo, Japan, 2015; pp. 127–145.
10. Kunihisa, M.; Moriya, S.; Abe, K.; Okada, K.; Haji, T.; Hayashi, T.; Kawahara, Y.; Itoh, R.; Itoh, T.; Katayose, Y.; et al. Genomic dissection of a 'Fuji' apple cultivar: Re-sequencing, SNP marker development, definition of haplotypes, and QTL detection. *Breed. Sci.* **2016**, *66*, 499–515. [CrossRef]
11. Aprea, E.; Charles, M.; Endrizzi, I.; Laura Corollaro, M.; Betta, E.; Biasioli, F.; Gasperi, F. Sweet taste in apple: The role of sorbitol, individual sugars, organic acids and volatile compounds. *Sci. Rep.* **2017**, *7*, 44950. [CrossRef]
12. Charles, M.; Aprea, E.; Gasperi, F. Factors influencing sweet taste in apple. In *Bioactive Molecules in Food*; Mérillon, J.-M., Ramawat, K.G., Eds.; Springer International Publishing: Cham, Switzerland, 2018; pp. 1–22.
13. Tanaka, F.; Okazaki, K.; Kashimura, T.; Ohwaki, Y.; Tatsuki, M.; Sawada, A.; Ito, T.; Miyazawa, T. Profiles and physiological mechanisms of sensory attributes and favor components in watercored apple. *J. Jpn. Soc. Food Sci. Technol.* **2016**, *63*, 101–116. (In Japanese) [CrossRef]
14. Bowen, J.H.; Watkins, C.B. Fruit maturity, carbohydrate and mineral content relationships with watercore in 'Fuji'apples. *Postharvest Biol. Technol.* **1997**, *11*, 31–38. [CrossRef]
15. Zupan, A.; Mikulic-Petkovsek, M.; Stampar, F.; Veberic, R. Sugar and phenol content in apple with or without watercore. *J. Sci. Food Agric.* **2016**, *96*, 2845–2850. [CrossRef] [PubMed]
16. Melado-Herreros, A.; Munoz-García, M.-A.; Blanco, A.; Val, J.; Fernández-Valle, M.E.; Barreiro, P. Assessment of watercore development in apples with MRI: Effect of fruit location in the canopy. *Postharvest Biol. Technol.* **2013**, *86*, 125–133. [CrossRef]
17. Williams, M.W. Relationship of sugars and sorbitol to watercore in apples. *Proc. Am. Soc. Hortic. Sci.* **1966**, *88*, 67–75.
18. Dixon, J.; Hewett, E.W. Exposure to hypoxia conditions alters volatile concentrations of apple cultivars. *J. Sci. Food Agric.* **2000**, *81*, 22–29. [CrossRef]
19. Komthong, P.; Hayakawa, S.; Katoh, T.; Igura, N.; Shimoda, M. Determination of potent odorants in apple by headspace gas dilution analysis. *Lwt-Food Sci. Technol.* **2006**, *39*, 472–478. [CrossRef]
20. Gao, Z.; Jayanty, S.; Beaudry, R.; Loescher, W. Sorbitol transporter expression in apple sink tissues: Implications for fruit sugar accumulation and watercore development. *J. Am. Soc. Hortic. Sci.* **2005**, *130*, 261–268. [CrossRef]
21. Marlow, G.C.; Loescher, W.H. Sorbitol metabolism, the climacteric, and watercore in apples. *J. Am. Soc. Hortic. Sci.* **1985**, *110*, 676–680.
22. Kitahata, S.; Machinami, T. Sugar. In *Molecular Recognition of Taste and Aroma*; The Chemical Society of Japan, Ed.; Gakkai Shuppann Center: Tokyo, Japan, 1999; Volume 40, pp. 50–60. (In Japanese)
23. Magwaza, L.S.; Opara, U.L. Analytical methods for determination of sugars and sweetness of horticultural products—A review. *Sci. Hortic.* **2015**, *184*, 179–192. [CrossRef]
24. Ma, B.; Chen, J.; Zheng, H.; Fang, T.; Ogutu, C.; Li, S.; Han, Y.; Wu, B. Comparative assessment of sugar and malic acid composition in cultivated and wild apples. *Food Chem.* **2015**, *172*, 86–91. [CrossRef]
25. Harker, F.R.; Marsh, K.B.; Young, H.; Murray, S.H.; Gunson, F.A.; Walker, S.B. Sensory interpretation of instrumental measurements 2: Sweet and acid taste of apple fruit. *Postharvest Biol. Technol.* **2002**, *24*, 241–250. [CrossRef]
26. Baldwin, E.A.; Scott, J.W.; Einstein, M.A.; Malundo, T.M.M.; Carr, B.T.; Shewfelt, R.L.; Tandon, K.S. Relationship between sensory and Instrumental analysis for tomato flavor. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 906. [CrossRef]
27. Beckles, D.M. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biol. Technol.* **2012**, *63*, 129–140. [CrossRef]
28. Tokitomo, Y.; Steinhaus, M.; Buttner, A.; Schieberle, P. Odor-active constituents in fresh pineapple (*Ananas comosus* [L.] Merr.) by quantitative and sensory evaluation. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 1323–1330. [CrossRef] [PubMed]

29. Zheng, L.Y.; Sun, G.M.; Liu, Y.G.; Lv, L.L.; Yang, W.X.; Zhao, W.F.; Wei, C.B. Aroma volatile compounds from two fresh pineapple varieties in China. *Int. J. Mol. Sci.* **2012**, *13*, 7383–7392. [CrossRef] [PubMed]
30. Ichikawa, E.; Hosokawa, N.; Hata, Y.; Abe, Y.; Suginami, K.; Imayasu, S. Breeding of a sake yeast with improved ethyl caproate productivity. *Agric. Biol. Chem.* **1991**, *55*, 2153–2154.
31. Isogai, A.; Utsunomiya, H.; Kanda, R.; Iwata, H. Changes in the aroma compounds of sake during aging. *J. Agric. Food Chem.* **2005**, *53*, 4118–4123. [CrossRef] [PubMed]
32. Lee, J.; Mattheis, J.P.; Rudell, D.R. Antioxidant treatment alters metabolism associated with internal browning in 'Braeburn' apples during controlled atmosphere storage. *Postharvest Biol. Technol.* **2012**, *68*, 32–42. [CrossRef]
33. Lumpkin, C.; Fellman, J.K.; Rudell, D.R.; Mattheis, J.P. 'Fuji' apple (*Malus domestica* Borkh.) volatile production during high pCO_2 controlled atmosphere storage. *Postharvest Biol. Technol.* **2015**, *100*, 234–243. [CrossRef]
34. Hampson, C.; Quamme, H.; Hall, J.; MacDonald, R.; King, M.; Cliff, M. Sensory evaluation as a selection tool in apple breeding. *Euphytica* **2000**, *111*, 79–90. [CrossRef]
35. Iwanami, H. Breeding for fruit quality in apple. *Breed. Fruit Qual.* **2011**, 173–200.
36. Bowen, A.J.; Blake, A.; Tureček, J.; Amyotte, B. External preference mapping: A guide for a consumer-driven approach to apple breeding. *J. Sens. Stud.* **2018**, *34*, e12472. [CrossRef]
37. Harker, F.R.; Redgwell, R.J.; Hallett, I.C.; Murray, S.H.; Carter, G. Texture of fresh fruit. *Hortic. Rev.* **1997**, *20*, 121–224.
38. Iwanami, H.; Moriya, S.; Kotoda, N.; Takahashi, S.; Abe, K. Influence of mealiness on the firmness of apples after harvest. *HortScience* **2005**, *40*, 2091. [CrossRef]
39. Jowitt, R. The terminology of food texture. *J. Texture Stud.* **1974**, *5*, 351–358. [CrossRef]
40. Harker, F.R.; Johnston, J.W. Importance of texture in fruit and its interaction with flavour. In *Fruit and Vegetable Flavour*; Woodhead Publishing Limited: Cambridge, UK, 2008; pp. 132–149.
41. Johnston, J.W.; Hewett, E.W.; Hertog, M.L.A.T.M. Postharvest softening of apple (*Malus domestica*) fruit: A review. *New Zealand J. Crop Hortic. Sci.* **2002**, *30*, 145–160. [CrossRef]
42. Wei, J.; Ma, F.; Shi, S.; Qi, X.; Zhu, X.; Yuan, J. Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit. *Postharvest Biol. Technol.* **2010**, *56*, 147–154. [CrossRef]
43. Meilgaard, M.; Civille, G.; Carr, B. *Sensory Evaluation Techniques*, 2nd ed.; CRC: Boca Raton, FL, USA, 1991.
44. Nara, K.; Kato, Y.; Motomura, Y. Involvement of terminal-arabinose and-galactose pectic compounds in mealiness of apple fruit during storage. *Postharvest Biol. Technol.* **2001**, *22*, 141–150. [CrossRef]
45. Iwanami, H.; Ishiguro, M.; Kotoda, N.; Takahashi, S.; Soejima, J. Optimal sampling strategies for evaluating fruit softening after harvest in apple breeding. *Euphytica* **2005**, *144*, 169–175. [CrossRef]
46. Wakasa, Y.; Kudo, H.; Ishikawa, R.; Akada, S.; Senda, M.; Niizeki, M.; Harada, T. Low expression of an endopolygalacturonase gene in apple fruit with long-term storage potential. *Postharvest Biol. Technol.* **2006**, *39*, 193–198. [CrossRef]
47. Iwanami, H.; Moriya, S.; Kotoda, N.; Abe, K. Turgor closely relates to postharvest fruit softening and can be a useful index to select a parent for producing cultivars with good storage potential in apple. *HortScience* **2008**, *43*, 1377. [CrossRef]
48. Moriya, S.; Kunihisa, M.; Okada, K.; Iwanami, H.; Iwata, H.; Minamikawa, M.; Katayose, Y.; Matsumoto, T.; Mori, S.; Sasaki, H.; et al. Identification of QTLs for flesh mealiness in apple (*Malus* × *domestica* Borkh.). *Hortic. J.* **2017**, *86*, 159–170. [CrossRef]
49. Sadamori, S. Memories on breeding of apple 'Fuji'. *J. Agric. Sci.* **1972**, *27*, 419–421. (In Japanese)
50. Iwanami, H.; Moriya, S.; Okada, K.; Abe, K.; Kawamorita, M.; Sasaki, M.; Moriya-Tanaka, Y.; Honda, C.; Hanada, T.; Wada, M. Instrumental measurements of juiciness and freshness to sell apples with a premium value. *Sci. Hortic.* **2017**, *214*, 66–75. [CrossRef]
51. Onodera, R.; Kudo, M.; Takahashi, K.; Nakamura, Y.; Hayama, H. Long term storage of water core apple 'Fuji'. *Tohoku Agric. Res.* **2010**, *63*, 111–112. (In Japanese)
52. Tatsuki, M. Ethylene biosynthesis and perception in fruit. *J. Jpn. Soc. Hortic. Sci.* **2010**, *79*, 315–326. [CrossRef]
53. Argenta, L.; Fan, X.; Mattheis, J. Impact of watercore on gas permeance and incidence of internal disorders in 'Fuji'apples. *Postharvest Biol. Technol.* **2002**, *24*, 113–122. [CrossRef]
54. O'Loughlin, J. Storage behaviour of 'Richared' and 'Starkrimson' delicious apples. *Sci. Hortic.* **1978**, *9*, 41–46.

55. Fukuda, H. Relationship of watercore and calcium to the incidence of internal storage disorders of 'Fuji' apple fruit. *J. Jpn. Soc. Hortic. Sci.* **1984**, *53*, 298–302. [CrossRef]
56. Argenta, L.C.; Fan, X.; Mattheis, J.P. Responses of 'Fuji'apples to short and long duration exposure to elevated CO_2 concentration. *Postharvest Biol. Technol.* **2002**, *24*, 13–24. [CrossRef]
57. Kato, K.; Sato, R. The ripening of apple fruits. II. Interrelations of respiration rate, C_2H_4 evolution rate and internal gas concentrations, and their relations to specific gravity or watercore during maturation and ripening. *J. Jpn. Soc. Hortic. Sci.* **1978**, *46*, 530–540. (In Japanese) [CrossRef]
58. Harker, F.R.; Watkins, C.B.; Brookfield, P.L.; Miller, M.J.; Reid, S.; Jackson, P.J.; Bieleski, R.L.; Bartley, T. Maturity and regional influences on watercore development and its postharvest disappearance in 'Fuji' apples. *J. Am. Soc. Hortic. Sci.* **1999**, *124*, 166. [CrossRef]
59. Kasai, S.; Kobayashi, T.; Kudo, T.; Goto, S. Appropriate combinations of apple cultivars and storage technologies for long-term storage. *Hortic. Res. (Jpn.)* **2019**, *18*, 173–184. (In Japanese) [CrossRef]

Sample Availability: Samples of the compounds are not available from the authors.

molecules

MDPI

Review

Volatile Flavor Compounds in Cheese as Affected by Ruminant Diet

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Abstract: Extensive research has been conducted concerning the determination and characterization of volatile compounds contributing to aroma and flavor in cheese. Considerable knowledge has been accumulated on the understanding of the mechanisms through which these compounds are formed during ripening, as well as on the optimization of the methodological approaches which lead to their detection. More recently, particular attention has been given to the aromatic properties of milk and cheeses obtained from lactating dairy ruminants fed experimental diets, characterized, for instance, by the addition of trace elements, natural supplements, or agricultural by-products rich in bioactive compounds. The purpose of this review is to summarize the major families of volatile compounds most commonly found in these types of dairy products at various ripening stages, describing in greater detail the role of animal diet in influencing the synthesis mechanisms most commonly responsible for cheese flavor determination. A large number of volatile compounds, including carboxylic acids, lactones, ketones, alcohols, and aldehydes, can be detected in cheese. The relative percentage of each compound depends on the biochemical processes that occur during ripening, and these are mainly mediated by endogenous enzymes and factors of bacterial origin whose function can be strongly influenced by the bioactive compounds taken by animals with the diet and released in milk through the mammary gland. Further evaluations on the interactions between volatile compounds and cheese matrix would be necessary in order to improve the knowledge on the synthesis mechanisms of such compounds; in addition to this, more should be done with respect to the determination of synergistic effects of flavor compounds, correlating such compounds to the aroma of dairy products.

Keywords: lactating ruminants; milk; cheese; volatile compound; lipolysis; proteolysis

1. Introduction

Chemical stability represents the fundamental characteristic of numerous processed foods. However, in the case of cheese, reference is made to a highly dynamic product from the biochemical point of view, especially in those cheeses subjected to ripening. During this period hundreds of volatile compounds (VOCs) can be produced, thus giving rise to flavors and odors that are characteristic of each cheese variety [1].

The main biochemical pathways that occur during the cheese ripening are represented by the metabolism of residual lactose, lactate, and citrate, lipolysis which is associated to the release of free fatty acids (FFAs), and proteolysis that is responsible for casein degradation to peptides with different molecular weights and free amino acids (FAAs). In addition, all the catabolic reactions against FFAs and peptides that give rise to a wide range of VOCs should be included [2].

In the last decades several studies have been conducted with the aim of investigating the specific mechanisms responsible for the production of sapid compounds in cheese during ripening. This approach was driven by the intention to obtain information on the flavor chemistry of many cheese varieties. An aspect to which less attention has been given regards the influence of the feeding strategies administered to ruminants on the volatile profile found in ripened dairy products. It is well known that by modifying animal diet, variations in the chemical-nutritional composition can be induced in milk. Consequently, the characteristics found in milk can be transferred in cheese, making available different substrates for the metabolic functions of starter or non-starter bacteria and for the activities of lipolytic or proteolytic enzymes of endogenous origin [3]. This means that volatile and sensory characteristics of ripened cheeses are largely defined by the technological approach and the initial chemical composition of the raw material [4]. The basic dietary factors that should be considered in ruminants for their effect on milk composition are represented by the fiber content, the ratio between forage and a concentrate (generally consisting of cereal and legume flours in addition to mineral and vitamin supplements), the carbohydrate composition of the concentrate, and the lipid amount, meal frequency, and intake [5]. Over time these aspects have been extensively characterized, especially with a view to obtaining a milk with a greater predisposition to be used for the production of manufactured products [6].

Numerous studies have focused their attention on the correlation between certain variations in the chemical composition of milk and the presence in the ruminant diet of specific classes of bioactive compounds, for instance polyphenols and terpenes, which can be mostly found in plants [7,8]. In this regard a mention should be made to the work of Walker et al. [9], who discussed the most relevant aspects able to induce effects on fatty acid composition of dairy cows' milk. High intake of starch is associated with increased de novo synthesis of fat in the mammary gland, with a consequent increase in the milk of saturated fatty acids (SFAs). In contrast, dietary intake of higher concentrations of polyunsaturated fatty acids (PUFAs) was demonstrated to be effective in inducing higher concentrations of unsaturated fatty acids (UFAs), including conjugated linoleic acid (CLA). An increased intake of starch-based concentrates is instead responsible for the reduction in milk fat concentration, a phenomenon which can be attributed to variations in the balance between lipogenic and glucogenic volatile FAs of ruminal origin. However, reduced fat levels in milk are presumably dependent also on the increased production in the rumen of long-chain FAs containing a *trans*-10 double bond, specifically C18:1 *trans*-10 and C18:2 *trans*-10 *cis*-12, in response to feeding strategies characterized by increased concentrations of PUFAs and/or starch.

In this context, we should include all studies in which ruminant diets have been integrated with agro-industrial by-products and the effects on the chemical-nutritional composition of milk and cheeses have been evaluated. For instance, the supplementation of dairy ewes' diet with an olive crude phenolic concentrate obtained from olive oil wastewater was demonstrated to be effective in inducing in milk an increase in concentration of polyunsaturated fatty acids [10]. A similar behavior was also observed by administering dairy cows with a diet enriched with dried grape pomace, the main by-product of the wine industry; in this study the authors also evidenced an improvement of the oxidative stability of ripened cheese [11]. In addition to this, grape pomace supplementation was also demonstrated to induce in cow's milk a significant increase in concentration of lactose and β-lactoglobulin, although no effects were found for α-lactalbumin, albumin, and caseins [12]. Although the consideration may be speculative, it is conceivable that such a finding may derive from the ability of bioactive compounds of dietary origin to influence bovine gene expression. Indeed, a recent study has shown that 75 days of dietary supplementation with dried grape pomace were effective in inducing variations in the whole-transcriptome of Friesian calves. In that case the authors specifically focused their attention on the pathway of cholesterol biosynthesis, and correlated the observed molecular variations with the reduction in both serum cholesterol and lipid oxidation in carcasses [13].

More recently, a fair number of papers have been published concerning the influence of the feeding strategy on the volatile profile of ripened dairy products obtained from lactating ruminants. The objective of this review is therefore to reorganize, as much as possible, the findings in this research area, in order to obtain a clearer view on the possible correspondences between the type of administered

diet and variations in concentration of specific VOCs found in dairy products during ripening. The discussion will be performed on the individual classes of compounds (carboxylic acids, aldehydes, lactones, ketones, alcohols, esters, and phenolic compounds), also giving a nod to the relevance of specific VOCs in flavor perception and summarizing, if appropriate, the principal biochemical pathways by which flavor compounds are produced and that could be influenced by the presence of specific bioactive compounds of dietary origin.

2. Biochemical Mechanisms Responsible for the Production of Volatile Flavor Compounds in Dairy Products

The biochemical mechanisms that characterize cheese ripening can be grouped into primary and secondary events. Primary events are represented by the metabolism of residual lactose, lactate, and citrate, as well as lipolysis and proteolysis. These events are then followed by secondary biochemical mechanisms involved in the metabolism of fatty acids and amino acids, which directly contribute to the synthesis of many VOCs, credited as having a high capacity to influence the cheese flavor [1,2,14].

2.1. Metabolism of Residual Lactose, Lactate, and Citrate

Lactose is the most represented carbohydrate in milk and is converted to lactate during the cheesemaking by the lactic acid bacteria (LAB), inducing a decrease in pH. In turn, lactate can be further processed by LAB in order to release formate, acetaldehyde, ethanol, and acetate [1,2]. With regard to citrate, the residue remaining in the curd can be converted by citrate-positive LAB into acetate and lactate after cheesemaking. This event is also responsible for the production of additional volatile compounds such as acetoin, 2,3-butanediol, diacetyl, and 2-butanone [15].

2.2. Metabolism of Free Amino Acids (FAA)

The catabolism of FAAs represents the biochemical pathway mainly involved in the production of aldehydes, alcohols, carboxylic acids, amines, and sulfur compounds (Figure 1) [16,17].

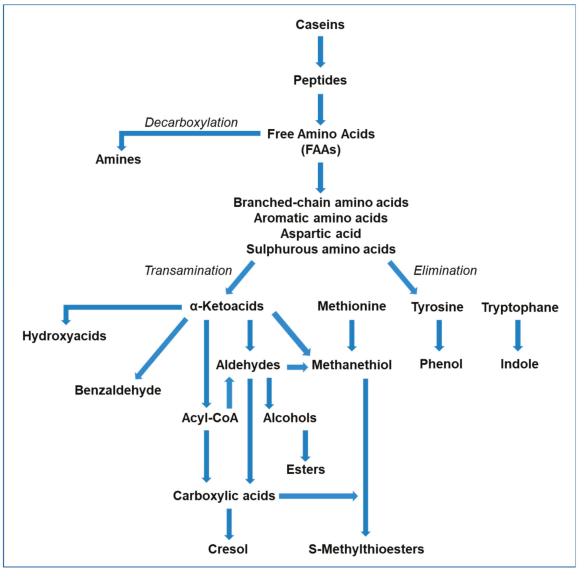

Figure 1. Schematic representation of the free amino acid (FAA) catabolism in cheese, modified from Bertuzzi et al. (2018).

The amino acid aminotransferase catalyzes a transamination reaction which leads to the conversion of aromatic amino acids, branched-chain amino acids, methionine, and aspartic acid into α-ketoacids. These compounds are then further metabolized to branched-chain and aromatic aldehydes, acyl-CoA, hydroxy acids, and methanethiol [16,18]. The production of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal is respectively due to the transamination of valine, isoleucine, and leucine, while the transamination reaction in which the substrate is represented by aspartic acid, is responsible for the release of oxaloacetate, which is in turn further converted into acetoin, diacetyl, or 2,3-butanediol [15,19]. Recently, the pivotal role of the aspartic acid transamination was demonstrated in the production of diacetyl in *Lactobacillus paracasei* [20]. Previously, in *Lactococcus lactis* var. *maltigenes* the existence of specific enzymatic pathways responsible for the production of phenylacetaldehyde and methional was observed, as a result of phenylalanine and methionine reduction, respectively [21].

With regard to aromatic aldehydes, these compounds are mainly formed starting from α-keto acids deriving from the benzaldehyde released by the spontaneous oxidation of tryptophan and phenylalanine. In this case it is therefore necessary to establish a condition causing a predisposition to a redox reaction, which is reported to be strongly influenced by the temperature, since an increase of this parameter involves catabolism acceleration [22]. Aldehydes represent the substrate of several dehydrogenases, which are able to convert such compounds to alcohols or to oxidize them into the corresponding carboxylic acids [16]. The metabolism of molds and yeast has been reported to be mainly involved in the biosynthesis of primary and aromatic alcohols, with the consequent release of corresponding carboxylic acids. In this regard, a study conducted by Yvon and Rijnen on *Geotrichum candidum* and yeasts isolated from Camembert allowed for the characterization of the mechanisms leading to the production of alcohols and carboxylic acids through FAA metabolism [23].

The excessive proteolysis in cheeses subjected to an uncontrolled ripening in terms of environmental conditions and duration leads to the formation of high concentrations of FAAs that can be decarboxylated, mainly by non-starter LAB, with the consequent release of biogenic amines, which are associated with poor flavor and potentially negative effects on consumer health. The most relevant biogenic amines are represented by histamine, tyramine, cadaverine, and putrescine, which are respectively synthesized starting from histidine, tyrosine, lysine, and ornithine [24].

In the context of FAA catabolism, a noteworthy aspect is also represented by the elimination reactions, which cleave the side chain of amino acids through a reaction catalyzed by a lyase. Over time substantial evidence has been collected about the fact that these reaction are associated to potential negative effects on flavor, as a consequence of the release of compounds such as p-cresol, phenethanol, and indole. This pathway also leads to the synthesis of methanethiol from methionine, which can be metabolized through a variety of different pathways. The major biosynthetic pathway in several strains is that of cystathionine, which involves the intervention of a cystathionine lyase. The further catabolism of methanethiol occurs through oxidative mechanisms performed by numerous LAB species, which are responsible for the production of dimethyldisulfide and dimethyltrisulfide. These compounds are reported to be characterized by low odor perception, thus markedly influencing the cheese flavor [25,26].

2.3. Metabolism of Free Fatty Acids (FFAs)

Lipolysis in dairy products is supported by the activity of lipases, microbial enzymes, enzymes of endogenous origin, and enzymes deriving from the added rennet pastes, which catalyze the triglyceride hydrolysis, with the consequent production of medium-chain (chain lengths up to 10 carbon atoms) and long-chain (chain lengths over than 10 carbon atoms) FFAs, di- and mono-glycerides, and glycerol [27].

The flavor properties of cheese are directly influenced by FFAs abundance and pH, and these parameters tend to influence each other. In presence of high pH values in cheese, the FFAs are reported to be less prone to the release of compounds capable of significantly influencing the flavor. Specifically, in this condition the FFAs are converted in non-volatile salts which induce the onset of unpleasant

"soapy" aromatic notes. When pH is low, the FFAs are present in volatile form in the dairy matrix, and their excessive increase in concentration is generally effective in inducing a rancid taste [14].

As schematized in Figure 2, methyl ketones, secondary alcohols, straight-chain aldehydes, lactones, esters, and S-thioesters represent classes of VOCs partially deriving from the catabolism of FFAs, which therefore can contribute to the formation of cheese also indirectly as precursors of aromatic compounds [18,28]. FFAs can undergo oxidation, giving origin to β-ketoacids, which are converted to the corresponding methyl ketones through decarboxylation [17,27]. The biosynthetic pathway of methyl ketones is mainly associated to biochemical mechanisms performed by molds; however, hypotheses with regard to synthesis mechanisms induced by heating milk, or, alternatively, derived from a direct esterification of β-ketoacids [28] have been proposed. With regard to ketones, their possible overestimation in the volatile profile of dairy samples can occur as a consequence of the direct conversion of the β-ketoacids in the gas chromatograph inlet [29].

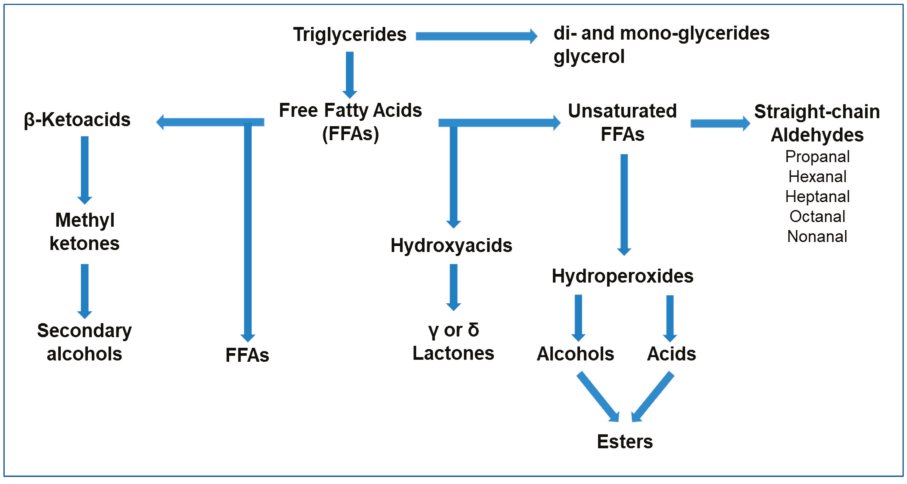

Figure 2. Schematic representation of free fatty acid (FFA) catabolism in cheese, modified from Bertuzzi et al. (2018).

The enzymatic reduction of ketones leads to the production of secondary alcohols, a mechanism mainly attributed to molds (such as *Penicillium* spp.) which are specifically responsible for the production of 2-pentanol, 2-heptanol, and 2-nonanol in blue veined cheeses [27]. Such compounds are reported to minimally contribute to the cheese flavor, although the 2-heptanol was identified as a strong odorant in Gorgonzola and Grana Padano cheese [26].

Unsaturated free fatty acids and esterified fatty acids can undergo an auto-oxidation process through non-enzymatic mechanisms, releasing straight-chain aldehydes, mainly propanal, hexanal, heptanal, octanal, and nonanal, that are responsible for the so defined "green grass-like" aroma [27].

The synthesis of esters can occur by esterification, mediated by esterases which use alcohols and carboxylic acids as substrates, or through alcoholysis, which involves the activity of acyltransferases and leads to the synthesis of esters from alcohols, acylglycerols, or acyl-CoA mainly derived from the metabolism of FAAs and FFAs. The transfer to alcohols of fatty acyl groups from acylglycerols or acyl-CoA derivatives represents the main biosynthetic mechanism adopted by LAB to obtain esters. These compounds are associated to pleasant fruity notes able to reduce the sharpness and bitterness that occur in dairy products in which an increase in concentration of FFAs and amines is observed [26]. During esterification or alcoholysis the production of S-methyl-thioesters may occur, a phenomenon mainly correlated to the presence of methanethiol, therefore strictly dependent on the metabolism of specific bacterial species such as *Micrococcaceae Brevibacterium linens*, and *Geotrichum candidum*.

S-methyl-thioesters can be alternatively released by the reaction between FFAs and methanethiol, and are commonly found on the surface of mold-ripened cheese and in blue veined cheeses, conferring strong odors with low threshold perception [30].

Lactones are produced by hydroxylated FFAs, which are integrated in milk triglycerides and released by reactions catalyzed by specific enzymes or induced by heating processes. In addition, hydroxylated FFAs can alternatively be produced by the catabolism of unsaturated fatty acids mediated by lipoxygenases and hydratases of microbial origin [28].

Unlike what has been reported for the other classes of compounds, phenols and terpenes can be identified in several varieties of dairy products, as a direct consequence of their presence in milk before the cheese-making. Phenolic compounds are mostly found in appreciable concentrations in goat and ewe milk, and while they are generally associated with pleasant aromatic notes, they tend to negatively affect the cheese flavor if present in excessive concentrations.

3. Major Volatile Flavor Compounds Found in Ripened Cheese and Influenced by Ruminant Diet

The lipolysis and the catabolism of fatty acids represent the most common biochemical mechanisms in cheese during ripening [27]. Therefore, the most represented family of VOCs in cheeses is usually that of carboxylic acids, generally composed of acids from C2 (acetic) to C10 or C12 (decanoic or dodecanoic) [31], followed by other classes of compounds such as aldehydes, lactones, ketones, alcohols, esters, and phenolic compounds. As summarized in Table 1, all these classes of compounds may undergo variations in quantity and composition, as a consequence of variations in the diet administered to ruminants.

Table 1. Summary of the most relevant variations found in different dairy products obtained from ruminants fed experimental diets.

VOC Family	Dietary Supplement (Ruminant)	Type of Dairy Product	Effects	Ref.
Carboxylic acids	Dried grape pomace (Friesian cows)	Fresh and 28-day ripened Caciotta cheese	↓ Acetic acid	[32]
	Nutrient-rich pasture (Simmental cows)	12-month ripened Montasio cheese	↓ Butanoic acid ↓ Hexanoic acid	[33]
	Dried grape pomace (Friesian cows)	28-day ripened Caciotta cheese		[34]
	Dried licorice root (Saanen goats)	Fresh and 30-day ripened Caciotta cheese	↓ Hexanoic acid	[35]
	Organic zinc (Friesian cows)	30-day ripened Caciotta cheese	↑ Butanoic acid ↑ Hexanoic acid	[36]
		5-day stored Giuncata cheese		[37]
	Extruded linseed (Saanen goats)	60-day ripened Caciotta cheese	↓ Dodecanoic acid	[38]
Aldehydes	Organic zinc (Friesian cows)	120-day ripened Caciocavallo cheese	↑ Nonanal	[39]
	Organic zinc (half-breed ewes)	90-day ripened Pecorino cheese	↑ Hexanal	[40]
	Organic selenium (Friesian cows)	30-day ripened Caciotta cheese	↓ Hexanal ↓ Heptanal	[41]
Lactones	Organic zinc (Friesian cows)	120-day ripened Caciocavallo cheese	↑ γ-nonalactone ↑ γ-dodecalactone ↑ δ-nonalactone ↑ δ-decalactone ↑ δ-dodecalactone ↑ δ-tetralactone	[39]
		30-day ripened Caciotta cheese	↑ δ-octalactone ↑ δ-decalactone	[36]

Table 1. *Cont.*

VOC Family	Dietary Supplement (Ruminant)	Type of Dairy Product	Effects	Ref.
Lactones	Organic selenium (Friesian cows)	30-day ripened Caciotta cheese		[41]
	Dried olive pomace (Friesian cows)	30-day ripened Caciotta cheese	↑ γ-dodecalactone ↑ δ-octalactone	[42]
Ketones and Alcohols	Silages (Simmental cows)	68-day, 200-day and 360-day ripened Montasio cheese	↑ acetone ↑ 2-3-butanedione ↑ 2-butanone ↑ 2-hexanone ↑ 2-heptanone ↑ 2-methyl-1-butanol	[43]
	Nutrient-rich vs nutrient-poor pasture (Simmental cows)	60-day ripened Montasio cheese	↑ 2-Propanone [1] ↑ 2-Hepta-none [1] ↑ 2-Undecanone [1] ↑ 3-Methyl-1-butanol [2]	[44]
	Organic selenium (Friesian cows)	120-day ripened Caciocavallo cheese	↑ 2-pentanone ↑ 2-nonan-2-one ↓ Hexanol	[45]
Esters	TMR + native pasture (dairy cows)	Ragusano Cheese	Geranyl acetate [3] [E]-Methyl-jasmonate[3]	[46]
	Organic zinc (Friesian cows)	120-day ripened Caciocavallo cheese	↑ Ethyl butanoate ↑ Ethyl hexanoate ↑ Ethyl octanoate ↑ Ethyl nonanoate ↑ Ethyl decanoate ↑ Ethyl dodecanoate ↑ Ethyl tetradecanoate ↑ Ethyl hexadecanoate	[39]
		30-day ripened Caciotta cheese	↑ Ethyl hexanoate ↑ Ethyl hexadecanoate	[36]
Phenolic compounds	Pasture (dairy cows)	Raw milk	↑ Toluene	[47]
	Crops (dairy cows)		↑ Ptaquiloside ↑ Genistein ↑ Daidzein	[48]

VOC = volatile compound; TMR = total mixed ration; ↑ = Increase in concentration; ↓ = Decrease in concentration. [1] Data referred to cheese obtained from cows fed the nutrient-poor pasture. [2] Data referred to cheese obtained from cows fed the nutrient-rich pasture. [3] Compounds only found in cheeses obtained from cows fed the experimental feeding strategy.

3.1. Acids

Acetic acid can be synthesized by the catabolism of lactose, citrate, and FAAs, can alternately be derived from propionic fermentation, and is associated to pungent, vinegary, and acidic notes [1,32]. Ianni et al. [34] showed a significant decrease in concentration of this compound in fresh and 28-day ripened cheeses obtained from lactating cows fed for 60 days with 5% dietary supplementation of grape pomace, the major by-product of the oenological industry. A plausible explanation for this finding probably lies in the fact that grape pomace induced in milk an increase in concentration of long-chain fatty acids, making less likely the release of short-chain free fatty acids. In this regard, the study of Harper et al. [49] is of note, in which milk fat was substituted with various vegetable lipids in Romano and Cheddar cheeses. During the ripening process of cheese slurries, low molecular weight free fatty acids were formed, although the vegetable fats did not contain these compounds. As also discussed by Urbach [29], this interesting behavior was not fully characterized by authors from a microbiological and biochemical point of view, and it is therefore possible that the behavior observed may in part have been determined by exogenous factors.

Butanoic and hexanoic acids are considered to be the primary cause of strong and, in some cases, unpleasant odors defined as cheesy, rancid, and sweaty, and their tendency to increase in concentration during ripening in hard cheeses has been widely observed and characterized [50,51]. In a recent study conducted by Aprea et al. [33] a significantly lower concentration of both compounds was observed in ripened Montasio cheese obtained from Italian Simmental cows grazing in a pasture characterized by a nutrient-rich vegetation type. A similar behavior was also observed in other studies in which the ruminant diet was supplemented with plant matrices, which is particularly interesting from the biological point of view, due to the high content of bioactive compounds. Specifically, the reduction of butanoic and hexanoic acids in ripened cheeses was obtained by enriching the diet of Saanen goats with 1% of dried licorice root for 60 days [35], and by administering dietary grape pomace supplementation in lactating Friesian cows [34]. In contrary to the above reported, an increase in butanoic and hexanoic acid was evidenced in dairy products obtained from Friesian cows given dietary zinc supplementation. The particularity in this case lies in the fact that this finding was observed both in a 30-day ripened Caciotta cheese and in a fresh Italian dairy product, the Giuncata cheese, which was analyzed after 5 days of storage at 4 °C [36,37]. The general increase in concentration of FFAs, such as butanoic and hexanoic acids, is commonly explained by the extent of starter cell autolysis during cheese ripening, with the consequent release of enzymes, especially lipases, that promote lipolysis by cleaving the ester linkage between the fatty acid and the glycerol of the triacylglycerol [27]. The breaking of the bacterial envelope and the release of enzymatic factors into the extracellular environment is mediated by peptidoglycan hydrolases, commonly named autolysins, which are characterized by an N-terminal domain, a central catalytic domain, and a C-terminal domain containing a binding motif for zinc, which therefore represent a valuable cofactor [52]. The increase of FFAs in the presence of zinc may therefore depend by the ability of the trace element to favor bacterial autolysis in cheese. In the case of licorice root and grape pomace the opposite phenomenon was instead observed, presumably due to the ability of bioactive compounds deriving from these matrices to slow down the lipolytic action. In this regard it could be taken into account that lipase activity in cheese is strongly influenced by the concentration and type of fatty acids present in the reaction environment [3]. Indeed, the dietary intake of both licorice and grape pomace induced in milk significant variations in the fatty acid profile, with a presumable effect especially on lipases of endogenous origin. This interpretation could also be applied to the just-mentioned studies based on the use of zinc with respect to the reduced production of short-chain FFAs in dairy products, and an increase in concentration of long-chain fatty acids in milk and specifically vaccenic (C18:1 *trans*-11), oleic (C18:1 *cis*-9), linoleic (C18:2 *cis*-9, *cis*-12), and rumenic (C18:2 *cis*-9, *trans*-11) acids.

With regard to the longer chain carboxylic acids, the picture seems to appear more complex. In a study conducted on ripened goat cheese, evidence was found of a tendency for octanoic, decanoic and dodecanoic acids to increase in concentration after short aging periods (about 12 weeks), reaching concentrations well above the threshold values of aroma perception [53]. Octanoic acid is considered to be the main "goaty" compound in dairy products, and is reported to exhibit a waxy aroma that strongly contributes to the flavor of hard goat cheeses. Also, decanoic and dodecanoic acids undoubtedly influence the overall flavor of hard cheeses, and their increase is generally associated to soapy flavor [54]. With regard to the effect of the ruminant diet on the concentration of these compounds in dairy products during ripening, the study conducted by Bennato et al. [38] is noteworthy, as a reduction of dodecanoic acid was observed in a 60-day ripened cheese obtained from goats given dietary supplementation with extruded linseed. This plant matrix did not induce changes in the chemical composition of milk; the only variation was represented by the increase in concentration of linolenic acid (C18:3 *cis*9, *cis*12, *cis*15), which is known to be particularly represented in linseed. As previously reported, an effect of different acidic compositions of milk in influencing the activity of endogenous lipases during cheese ripening [3] could be hypothesized.

3.2. Aldehydes

Aldehydes are strongly flavored compounds and are commonly associated in foods to aroma defects referred to as oxidative rancidity [1,27]. These compounds are mainly released by the catabolism of FAAs and, in turn, represent the substrate for specific dehydrogenases responsible for the production of alcohols and carboxylic acids [17]. Aldehydes can also derive from non-enzymatic auto-oxidation reactions which lead to the degradation of unsaturated fatty acids, both free and esterified. These reactions do not occur with high frequency, since the cheese is characterized by a reducing environment. However, this event is responsible for the release of straight-chain aldehydes, which are reported to be associated with pleasant flavor notes [28]. The dairy matrices particularly rich in polyunsaturated fatty acids are therefore more prone to encountering oxidative phenomena able to produce aldehydes.

Recently, the enrichment of the ruminant diet with trace elements, such as zinc and selenium, resulted effective in inducing in milk, and consequently in cheese, an increase in concentration of PUFAs [39–41]. With regard to zinc, the authors specifically observed an increase in desaturation of stearic acid, and this finding was at least in part attributed to the role of zinc as a cofactor for a protease involved in the expression of stearoyl coenzyme A desaturase (SCD) in the mammary gland. SCD is reported to be an endoplasmic reticulum-bound enzyme responsible for the Δ^9-desaturation of saturated fatty acyl-CoAs. The gene expression of this enzyme is mediated by the sterol response element binding proteins (SREBPs) which are activated by a metalloprotease (Site-2 protease) that needs zinc to perform its catalytic function [55,56]. In these studies, the analysis of volatile profile in dairy products did not evidence significant variations in the amount of total aldehydes. Authors discussed this finding by advancing the hypothesis of a role of zinc and selenium in curbing the oxidative damage, a conclusion also supported by the reduction of lipid oxidation evaluated by measuring in cheese the thiobarbituric acid reactive substances (TBARS). In this regard, zinc has been reported to inhibit lipid peroxidation in biological systems by competing with prooxidant metals (i.e., Cu and Fe) for binding sites, thus decreasing their ability to transfer electrons in a particular environment [57]. In the case of selenium, its antioxidant property lies in the ability to act as a scavenger of reactive oxygen-based radicals, with a direct effect in opposing the lipid oxidation in biological systems [58]. Although no differences were found in the total aldehyde content, interesting differences were observed at the level of individual compounds. The dietary zinc supplementation induced a significant increase in concentration of nonanal and hexanal in 120-day ripened Caciocavallo cheese and in 90-day ripened Pecorino cheese, respectively [39,40], whereas the selenium supplementation administered to Friesian cows was effective in inducing a decrease in hexanal and heptanal in samples of 30-day ripened Caciotta cheese [41]. Therefore, in light of what has been reported, dietary zinc enrichment seems to induce a better effect on the aromatic properties of ripened dairy products, since nonanal and hexanal, unlike other aldehydes, are commonly associated with pleasant herbal and slightly fruity notes [28].

3.3. Lactones

The main precursors of lactones are represented by hydroxylated FFAs which are incorporated in milk fat triglycerides and released as a result of enzymatic lipolytic mechanisms or the heating process. Hydroxylated FFAs can also be produced by the activities of lipoxygenases or hydratases of microbial origin, within the catabolism of unsaturated fatty acids. A reaction of one-step transesterification is effective in synthesizing lactones from hydroxylated FFAs [28]. These mechanisms heavily and quite positively affect the cheese flavor, since lactones are associated with very pronounced fruity notes, although they have been found to also contribute in cheese to the buttery character [59]. The synthesis of lactones leads to the release of α- and β-lactones that are reported to be highly reactive and unstable, while γ- and δ-lactones are stable and have been identified in several dairy products. In Cheddar cheese, the concentration of lactones rapidly increased in the early stages of the ripening, reaching levels well above their thresholds of flavor perception. δ-Octalactone was reported to be the most represented lactone in Parmigiano Reggiano cheese, while γ-decalactone, δ-decalactone, γ-dodecalactone, and δ-dodecalactone have been found in several French blue cheeses [60].

Also in this case, the studies previously cited have highlighted an active role of the ruminant diet in inducing a change in the relative concentration of this class of volatile compounds. In Caciocavallo cheese obtained from Friesian cows given zinc supplementation, there was evidence of different lactones: γ-nonalactone, γ-dodecalactone, δ-nonalactone, δ-decalactone, δ-dodecalactone, and δ-tetralactone. All these compounds went through a significant increase in concentration at the end of the 120 days of ripening [39]. No specific studies have been conducted on the effect of zinc in the biosynthetic pathway of lactones; however, as reported in the previous paragraphs, a role of the trace element in promoting the starter cells autolysis with consequent release of lipases in the dairy environment could be hypothesized [52]. This event has been reported to be responsible for the increase in concentration of FFAs, from which hydroxyacids, precursors of γ- and δ-lactones [28], are derived. The dietary zinc supplementation was also reported to induce an increase in concentration of lactones in samples of 30-day ripened Caciotta cheese, in which the compounds involved were however limited to δ-octalactone and δ-decalactone [36]. This phenomenon, involving δ-octalactone and δ-decalactone, has also been observed in samples of 30-day ripened Caciotta cheese obtained from lactating Friesian cows given dietary selenium supplementation [41]. This finding therefore suggests a common role of trace elements in favoring the biochemical mechanisms, especially of an enzymatic type, responsible for the synthesis of this class of VOCs.

Interestingly, lactones did not seem to undergo noteworthy variations in experimentations in which the diet of dairy goats and cows was supplemented with plant matrices such as linseed, licorice root, and grape by-products, rich in compounds credited of considerable interest from a biological point of view because of their well characterized anti-inflammatory and antioxidant properties [34,35,38]. An exception to this consideration is found in the study conducted by Castellani et al. [42], who administered to dairy cows a dietary supplementation of olive pomace, a by-product of the olive oil production, rich in fiber and unsaturated fatty acids. In samples of 30-day ripened Caciotta cheese, authors observed an increase in concentration of γ-dodecalactone and δ-octalactone, together with compounds belonging to other chemical classes such as 2-octenal and 1-hexanol.

3.4. Ketones and Alcohols

Ketones and alcohols mainly derive from biochemical mechanisms involving the lysis of triglycerides and the oxidation of saturated FFAs, with the consequent production of ketoacids that are decarboxylated to ketones which, in turn, can be reduced to obtain alcohols [61]. These compounds are mainly released by molds such as *Penicillium roqueforti* and *Penicillium camemberti*, which are responsible for typical odors that characterize the aroma of ripened blue veined cheeses [27]. In order to appreciate the potential contribution of these compounds to the cheese aroma, it could be useful to consider that in water, methyl ketones are reported to be characterized by perception thresholds that are quite low, ranging from 0.09 mg·100 g^{-1} for 2-heptanone and 4.09 to 50.0 mg·100 g^{-1} for 2-propanone [28].

In many studies on the volatile profile of cheeses, these classes of compounds are present in limited concentrations, precisely due to the fact that in the manufacturing of many dairy products molds are unwanted and strongly countered [29]. In addition to this, it should be mentioned that the concentration of ketones and alcohols does not seem to be particularly related to the degree of maturation of the cheeses, with a heterogeneous condition of complicated interpretation.

Stefanon and Procida [43] conducted a study aiming to evaluate the effects of including silage in dairy cow diet on the volatile profile of Montasio cheeses. During cheese ripening, significant variations were evidenced for ketones and mostly for the amount of total alcohols, with specific changes in concentration of ethanol, isobutanol, 1-penten-3-ol, and 2-methyl-1-butanol. Authors discussed these findings by assuming a direct effect of diet composition in affecting microbial and chemical fermentations in cheese during ripening rather than a transfer of selected compounds from milk. The Montasio cheese was also the subject of the research conducted by Bovolenta et al. [44], who performed evaluations on the volatile profile of cheese obtained by using raw milk coming from Italian Simmental

cows grazing on two alpine pastures different for botanical composition. The "nutrient-poor pasture" resulted effective in inducing molding of the volatile profile of 60-day ripened cheese; in particular an overall increase in concentration of ketones, phenolic compounds, and terpenes was observed, with consequent slight effects noticed by panelists in the sensory analyses. With specific regard to terpenes, during cheese ripening differences were observed that the authors justified by taking into account the study of Belviso et al. [62] who demonstrated the ability of lactic acid bacteria isolated from cheese to influence the terpenoid biosynthesis.

An interesting behavior was recently observed in the volatile profile of Caciotta cheese obtained by enriching dairy cows diet with olive pomace. The dietary supplementation was effective in inducing a significant increase in FFAs, ester, and ketones in raw milk; however, following pasteurization and cheese-making, these differences disappeared both in the fresh and 30-day ripened dairy product [63]. Authors did not specifically investigate this phenomenon but hypothesized that the observed variations could at least in part derive from a change in the microbial population in pasteurized milk cheese, thus passing from a prevalence of lactic acid bacteria in raw milk to a greater concentration of the microbial genera used for pasteurized cheese manufacturing (*Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Propionibacterium*).

Ianni et al. [45] compared the aromatic compounds of Caciocavallo cheeses obtained from Friesian cows fed a standard diet and a diet supplemented with selenium. Although the trace element did not induce differences in the chemical composition of milk and cheese, interesting variations were identified in the volatile profile of 120-day ripened cheeses, in which an increase in concentration of two methyl ketones (2-pentanone and 2-nonan-2-one) and a decrease of an alcohol (1-hexanol) were found. In this study changes in the family of ethyl esters were also highlighted, but no evaluations were executed on the hypothetical consumer acceptability of the experimental dairy product, since no sensory analyses were performed.

3.5. Esters

Esters represent a class of VOCs indirectly involved in the metabolism of FFAs [27]. Many of these compounds are reported to have low perception thresholds and are widely associated to a pleasant aroma characterized by sweet, fruity, and floral notes; furthermore, esters are appreciated for their role in stemming the bitterness and sharpness of cheeses, very often due to the high content of amines and FFAs [64].

Carpino et al. [46] analyzed the aroma-active compounds of Ragusano cheese obtained from dairy cows fed a total mixed ration (TMR) supplemented with native Sicilian pastures, in comparison with the same cheese obtained from cows fed only TMR. In samples of Ragusano cheese derived from native pasture 8 unique volatile flavor compounds were identified, among which 2 were esters, specifically geranyl acetate and [E]-methyl jasmonate. The latter compound represents a mediator of the physiological defense mechanisms adopted by plants subjected to stress induced by herbivorous insects. Specifically, when damage occurs plants produce VOCs reported to have detrimental effects on insect physiology [65]. The physical damage of the plant tissue entails the activation of the octadecanoid-lipoxygenase (LOX) pathway, responsible for the release of a wide range of lipid-derived VOCs. Therefore, it is conceivable that a small part of these compounds can be identified in raw milk and consequently in the cheese of ruminants fed with fresh pasture. The finding concerning the identification of unique odor-active esters was also found by analyzing the volatile profile of 60-day ripened goats' milk cheese obtained from animals fed a dietary supplementation of extruded linseed, a well characterized plant matrix rich in linolenic acid (C18:3 *cis*-9, *trans*-12, *trans*-15). These esters, only detected in the "experimental" ripened cheese, were specifically the butanoic acid pentyl ester, the butyric acid 2-ethylhexyl ester, and the isopentyl hexanoate [54].

The previously mentioned addition of zinc to the diet of lactating dairy cows resulted effective in inducing noteworthy variations in volatile esters in derived dairy products. In 120-day ripened Caciocavallo cheese a marked increase in concentration of all the detected ethyl esters was shown,

specifically ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, and ethyl hexadecanoate. Interestingly, the last two compounds resulted only present in ripened cheese samples obtained from zinc feeding [39]. As previously reported, these data allow the discussion of a role of zinc in inducing an increase in lipolytic activity on the triglycerides present in the dairy matrix [52], with a consequent increase in concentration in the reaction environment of FFAs, contributing to the determination of the volatile profile not only directly, but also giving rise to other families of compounds, including esters [28]. In a 30-day ripened Caciotta cheese, the dietary zinc supplementation induced an increase in concentration of only two compounds, ethyl hexanoate and ethyl hexadecanoate, whereas no variations in this class of compounds were evidenced in Giuncata cheese, a fresh dairy product, that was analyzed after 5 days of storage at 4 °C [36,37]. In light of what has been just reported, it seems plausible that the observed increase in concentration of volatile ethyl esters is related to the length of the maturing period, although this consideration should be properly verified. As a partial support to the discussion, in one study lactating ewes were administered a zinc-enriched diet. In addition, samples of Pecorino cheese matured for 90 days showed an increase in concentration of two ethyl esters, specifically ethyl butanoate and ethyl hexanoate. In this case a slight but still significant reduction in concentration of ethyl octanoate was also reported [40].

3.6. Phenolic Compounds

Phenolic compounds are secondary metabolites of plants to which interesting properties are attributed from the biological point of view [66]. For that reason, over time great interest has been given to the development of functional dairy products containing specific phenolic compounds, such as catechin, tannic acid, hesperetin, and flavones, or natural crude compounds, for instance grape extract, green tea extract, and dehydrated cranberry powder [67].

Their presence in animal products can also derive from the direct transfer of these compounds from green herbage, or the synthesis by rumen bacteria which are reported to be mainly responsible for the lignin breakdown into monomeric phenols, through a mechanism characterized by decomposition of benzyl ether bonds of lignin polymers under anaerobic conditions [68]. Previous studies focusing on the evaluation of meat quality evidenced the presence of specific phenolic compounds in ruminant fat as a consequence of the ingestion of higher percentages in green herbage than in grain-based diets; specifically, the identification of 4-methylphenol in ruminant fat was reported to be positively affected by grazing [69,70].

As reported by O'Connell and Fox [71], the majority of phenolic volatile compounds identified in milk and dairy products are strictly related to the diet administered to ruminants, although a proportion may represent the product of FFA catabolism, preferably exploiting tyrosine as a precursor. In another study, in which lactating Friesian cows received a diet enriched with olive pomace, in pasteurized milk cheeses an increase in phenolic compounds was observed, specifically phenylacetaldehyde and 2-phenylethyl alcohol, both derived from the catabolism of phenylalanine. Authors discussed the finding by assuming a non-enzymatic Strecker degradation of phenylalanine or by enzymatic transamination of phenylalanine as an imide that is subsequently degraded to give phenylacetaldehyde, that, in turn, undergoes reduction to produce 2-phenylethyl alcohol [72].

With specific regard to cow milk, a study conducted by Villeneuve et al. [47] showed higher concentrations of toluene in samples obtained from cows on pasture, in comparison with milk samples collected from animals fed hay and silage. Authors discussed this finding by advancing the hypothesis of a greater degradation of β-carotene in forages such as silage or hay subjected to wilting and sun curing following harvesting [73]. Therefore, authors concluded that cows on pasture presumably consumed more β-carotene, explaining the significant increase in milk of toluene concentration. To better understand this finding, the study conducted by Contarini et al. [74] should be taken into account, where the effect of different heat treatments on the volatile profile of milk by applying a dynamic headspace capillary gas chromatography coupled with multivariate statistical approach was studied. Also in this work, it was assumed that the identification of toluene in raw milk was

the consequence of β-carotene degradation. Furthermore, it was evidenced that the identification of toluene in milk, together with 2-pentanone, 2-heptanone, pentanal, and 3-methylbutanal, was effective in discriminating in-bottle sterilized milk (in which these compounds are more greatly represented) from pasteurized samples.

Other studies confirmed that by feeding cattle with high levels of particular crops, other phenolic compounds may also be detected in ruminant milks, such as ptaquiloside, a norsesquiterpene from bracken (*Pteridium aquilinum*), or genistein and daidzein (derived from clover) [48].

4. Conclusions

In this review, the main biochemical mechanisms characterizing dairy products during ripening have been recalled, and the influence of different feeding strategies on the production and relative concentrations of various VOCs in fresh and ripened cheeses has been discussed.

Despite the large amount of research activity, to date the influence of certain dietary strategies on the quality of dairy products has not been well characterized, and there is a lack of findings useful to establish VOCs directly transferred from feeds to animal products that could be used for authenticity studies in order to discriminate milk samples or fresh and ripened dairy products. Furthermore, it should be also kept in mind that there is considerable variability induced by the cheese manufacturing process (heating, starter cultures type, ripening conditions), which could eliminate some of the VOCs present in milk. This remains an interesting challenge for researchers in the field of animal production.

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References

1. McSweeney, P.L.; Sousa, M.J. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Le Lait* **2000**, *80*, 293–324. [CrossRef]
2. McSweeney, P.L. Biochemistry of cheese ripening. *Int. J. Dairy Technol.* **2004**, *57*, 127–144. [CrossRef]
3. Fox, P.F.; Guinee, T.P.; Cogan, T.M.; McSweeney, P.L. Microbiology of cheese ripening. In *Fundamentals of Cheese Science*; Springer: Boston, MA, USA, 2017; pp. 333–390.
4. Buchin, S.; Delague, V.; Duboz, G.; Berdague, J.L.; Beuvier, E.; Pochet, S.; Grappin, R. Influence of pasteurization and fat composition of milk on the volatile compounds and flavor characteristics of a semi-hard cheese. *J. Dairy Sci.* **1998**, *81*, 3097–3108. [CrossRef]
5. Sutton, J.D. Altering milk composition by feeding. *J. Dairy Sci.* **1989**, *72*, 2801–2814. [CrossRef]
6. DePeters, E.J.; Cant, J.P. Nutritional factors influencing the nitrogen composition of bovine milk: A review. *J. Dairy Sci.* **1992**, *75*, 2043–2070. [CrossRef]
7. White, S.L.; Bertrand, J.A.; Wade, M.R.; Washburn, S.P.; Green, J.T., Jr.; Jenkins, T.C. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci.* **2001**, *84*, 2295–2301. [CrossRef]
8. De Noni, I.; Battelli, G. Terpenes and fatty acid profiles of milk fat and "Bitto" cheese as affected by transhumance of cows on different mountain pastures. *Food Chem.* **2008**, *109*, 299–309. [CrossRef]
9. Walker, G.P.; Dunshea, F.R.; Doyle, P.T. Effects of nutrition and management on the production and composition of milk fat and protein: A review. *Aust. J. Agric. Res.* **2004**, *55*, 1009–1028. [CrossRef]
10. Cappucci, A.; Alves, S.P.; Bessa, R.J.; Buccioni, A.; Mannelli, F.; Pauselli, M.; Viti, C.; Pastorelli, R.; Roscini, V.; Serra, A.; et al. Effect of increasing amounts of olive crude phenolic concentrate in the diet of dairy ewes on rumen liquor and milk fatty acid composition. *J. Dairy Sci.* **2018**, *101*, 4992–5005. [CrossRef]

11. Ianni, A.; Di Maio, G.; Pittia, P.; Grotta, L.; Perpetuini, G.; Tofalo, R.; Cichelli, A.; Martino, G. Chemical–nutritional quality and oxidative stability of milk and dairy products obtained from Friesian cows fed with a dietary supplementation of dried grape pomace. *J. Sci. Food Agric.* **2019**, *99*, 3635–3643. [CrossRef]
12. Chedea, V.S.; Pelmus, R.S.; Lazar, C.; Pistol, G.C.; Calin, L.G.; Toma, S.M.; Dragomir, C.; Taranu, I. Effects of a diet containing dried grape pomace on blood metabolites and milk composition of dairy cows. *J. Sci. Food Agric.* **2017**, *97*, 2516–2523. [CrossRef] [PubMed]
13. Iannaccone, M.; Elgendy, R.; Giantin, M.; Martino, C.; Giansante, D.; Ianni, A.; Dacasto, M.; Martino, G. RNA sequencing-based whole-transcriptome analysis of friesian cattle fed with grape pomace-supplemented diet. *Animals* **2018**, *8*, 188. [CrossRef] [PubMed]
14. Ardö, Y.; McSweeney, P.L.; Magboul, A.A.; Upadhyay, V.K.; Fox, P.F. Biochemistry of cheese ripening: Proteolysis. In *Cheese*; Academic Press: Burlington, MA, USA, 2017; pp. 445–482.
15. Singh, T.K.; Drake, M.A.; Cadwallader, K.R. Flavor of Cheddar cheese: A chemical and sensory perspective. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 166–189. [CrossRef]
16. Ganesan, B.; Weimer, B.C. Amino acid catabolism and its relationship to cheese flavor outcomes. 2017. Biochemistry of cheese ripening: Proteolysis. In *Cheese: Chemistry, Physics, and Microbiology*, 4th ed.; McSweeney, P.L.H., Fox, P.F., Cotter, P.D., Everett, D.W., Eds.; Academic Press: San Diego, CA, USA, 2017; pp. 483–516.
17. Kilcawley, K.N. Cheese flavour. In *Fundamentals of Cheese Science*; Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H., Eds.; Springer: Boston, MA, USA, 2017; pp. 443–474.
18. Smit, G.; Smit, B.A.; Engels, W.J. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiol. Rev.* **2005**, *29*, 591–610. [CrossRef] [PubMed]
19. Ardö, Y. Flavour formation by amino acid catabolism. *Biotechnol. Adv.* **2006**, *24*, 238–242. [CrossRef] [PubMed]
20. Peralta, G.H.; Wolf, I.V.; Bergamini, C.V.; Perotti, M.C.; Hynes, E.R. Evaluation of volatile compounds produced by *Lactobacillus paracasei* I90 in a hard-cooked cheese model using solid-phase microextraction. *Dairy Sci. Technol.* **2014**, *94*, 73–81. [CrossRef]
21. Morgan, M. The chemistry of some microbially induced flavor defects in milk and dairy foods. *Biotechnol. Bioeng.* **1976**, *18*, 953–965. [CrossRef]
22. Klačanová, K.; Fodran, P.; Rosenberg, M. The possible production of natural flavours by amino acid degradation. *Chem. Mon.* **2010**, *141*, 823–828. [CrossRef]
23. Yvon, M.; Rijnen, L. Cheese flavour formation by amino acid catabolism. *Int. Dairy J.* **2001**, *11*, 185–201. [CrossRef]
24. Schirone, M.; Tofalo, R.; Perpetuini, G.; Manetta, A.; Di Gianvito, P.; Tittarelli, F.; Battistelli, N.; Corsetti, A.; Suzzi, G.; Martino, G. Influence of Iodine Feeding on Microbiological and Physico-Chemical Characteristics and Biogenic Amines Content in a Raw Ewes' Milk Cheese. *Foods* **2018**, *7*, 108. [CrossRef]
25. Forde, A.; Fitzgerald, G.F. Biotechnological approaches to the understanding and improvement of mature cheese flavour. *Curr. Opin. Biotechnol.* **2000**, *11*, 484–489. [CrossRef]
26. Curioni, P.M.G.; Bosset, J.O. Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* **2002**, *12*, 959–984. [CrossRef]
27. Collins, Y.F.; McSweeney, P.L.H.; Wilkinson, M.G. Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. *Int. Dairy J.* **2003**, *13*, 841–866. [CrossRef]
28. Bertuzzi, A.S.; McSweeney, P.L.; Rea, M.C.; Kilcawley, K.N. Detection of volatile compounds of cheese and their contribution to the flavor profile of surface-ripened cheese. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 371–390. [CrossRef]
29. Urbach, G. The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. *Int. J. Dairy Technol.* **1997**, *50*, 79–89. [CrossRef]
30. Helinck, S.; Spinnler, H.E.; Parayre, S.; Dame-Cahagne, M.; Bonnarme, P. Enzymatic versus spontaneous S-methyl thioester synthesis in *Geotrichum candidum*. *FEMS Microbiol. Lett.* **2000**, *193*, 237–241. [CrossRef] [PubMed]
31. Faccia, M.; Trani, A.; Natrella, G.; Gambacorta, G. Chemical-sensory and volatile compound characterization of ricotta forte, a traditional fermented whey cheese. *J. Dairy Sci.* **2018**, *101*, 5751–5757. [CrossRef]
32. Beuvier, E.; Buchin, S. Raw milk cheese. In *Cheese: Chemistry, Physics and Microbiology*, 3rd ed.; Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P., Eds.; Elsevier Academic Press: London, UK, 2004; Volume 1, pp. 319–345.

33. Aprea, E.; Romanzin, A.; Corazzin, M.; Favotto, S.; Betta, E.; Gasperi, F.; Bovolenta, S. Effects of grazing cow diet on volatile compounds as well as physicochemical and sensory characteristics of 12-month-ripened Montasio cheese. *J. Dairy Sci.* **2016**, *99*, 6180–6190. [CrossRef]
34. Ianni, A.; Innosa, D.; Martino, C.; Bennato, F.; Martino, G. Compositional characteristics and aromatic profile of caciotta cheese obtained from Friesian cows fed with a dietary supplementation of dried grape pomace. *J. Dairy Sci.* **2019**, *102*, 1025–1032. [CrossRef]
35. Bennato, F.; Ianni, A.; Martino, C.; Di Luca, A.; Innosa, D.; Fusco, A.M.; Pomilio, F.; Martino, G. Dietary supplementation of Saanen goats with dried licorice root modifies chemical and textural properties of dairy products. *J. Dairy Sci.* **2020**, *103*, 52–62. [CrossRef]
36. Ianni, A.; Innosa, D.; Martino, C.; Grotta, L.; Bennato, F.; Martino, G. Zinc supplementation of Friesian cows: Effect on chemical-nutritional composition and aromatic profile of dairy products. *J. Dairy Sci.* **2019**, *102*, 2918–2927. [CrossRef] [PubMed]
37. Ianni, A.; Iannaccone, M.; Martino, C.; Innosa, D.; Grotta, L.; Bennato, F.; Martino, G. Zinc supplementation of dairy cows: Effects on chemical composition, nutritional quality and volatile profile of Giuncata cheese. *Int. Dairy J.* **2019**, *94*, 65–71. [CrossRef]
38. Bennato, F.; Ianni, A.; Innosa, D.; Grotta, L.; D'Onofrio, A.; Martino, G. Chemical-nutritional characteristics and aromatic profile of milk and related dairy products obtained from goats fed with extruded linseed. *Asian-Austral J. Anim. Sci.* **2019**, *33*, 148–156. [CrossRef] [PubMed]
39. Ianni, A.; Martino, C.; Innosa, D.; Bennato, F.; Grotta, L.; Martino, G. Zinc supplementation of lactating dairy cows: Effects on chemical-nutritional quality and volatile profile of Caciocavallo cheese. *Asian-Austral J. Anim. Sci.* **2019**, in press. [CrossRef] [PubMed]
40. Martino, C.; Ianni, A.; Grotta, L.; Pomilio, F.; Martino, G. Influence of zinc feeding on nutritional quality, oxidative stability and volatile profile of fresh and ripened ewes' milk cheese. *Foods* **2019**, *8*, 656. [CrossRef] [PubMed]
41. Ianni, A.; Martino, C.; Pomilio, F.; Di Luca, A.; Martino, G. Dietary selenium intake in lactating dairy cows modifies fatty acid composition and volatile profile of milk and 30-day-ripened caciotta cheese. *Eur. Food Res. Technol.* **2019**, *245*, 2113–2121. [CrossRef]
42. Castellani, F.; Vitali, A.; Bernardi, N.; Marone, E.; Grotta, L.; Martino, G. Lipolytic volatile compounds in dairy products derived from cows fed with dried olive pomace. *Eur. Food Res. Technol.* **2019**, *245*, 159–166. [CrossRef]
43. Stefanon, B.; Procida, G. Effects of including silage in the diet on volatile compound profiles in Montasio cheese and their modification during ripening. *J. Dairy Res.* **2004**, *71*, 58–65. [CrossRef]
44. Bovolenta, S.; Romanzin, A.; Corazzin, M.; Spanghero, M.; Aprea, E.; Gasperi, F.; Piasentier, E. Volatile compounds and sensory properties of Montasio cheese made from the milk of Simmental cows grazing on alpine pastures. *J. Dairy Sci.* **2014**, *97*, 7373–7385. [CrossRef]
45. Ianni, A.; Bennato, F.; Martino, C.; Innosa, D.; Grotta, L.; Martino, G. Effects of selenium supplementation on chemical composition and aromatic profiles of cow milk and its derived cheese. *J. Dairy Sci.* **2019**, *102*, 6853–6862. [CrossRef]
46. Carpino, S.; Mallia, S.; La Terra, S.; Melilli, C.; Licitra, G.; Acree, T.E.; Barbano, D.M.; Van Soest, P.J. Composition and aroma compounds of Ragusano cheese: Native pasture and total mixed rations. *J. Dairy Sci.* **2004**, *87*, 816–830. [CrossRef]
47. Villeneuve, M.P.; Lebeuf, Y.; Gervais, R.; Tremblay, G.F.; Vuillemard, J.C.; Fortin, J.; Chouinard, P.Y. Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. *J. Dairy Sci.* **2013**, *96*, 7181–7194. [CrossRef]
48. King, R.A.; Mano, M.M.; Head, R.J. Assessment of isoflavonoid concentrations in Australian bovine milk samples. *J. Dairy Res.* **1998**, *65*, 479–489. [CrossRef] [PubMed]
49. Harper, W.J.; Kristoffersen, T.; Wang, J.Y. Formation of free fatty acids during the ripening of fat modified cheese slurries. *Milchwissenschaft* **1978**, *33*, 604–608.
50. Zabaleta, L.; Gourrat, K.; Barron, L.J.R.; Albisu, M.; Guichard, E. Identification of odour-active compounds in ewes' raw milk commercial cheeses with sensory defects. *Int. Dairy J.* **2016**, *58*, 23–30. [CrossRef]
51. Fox, P.F.; McSweeney, P.L.; Cogan, T.M.; Guinee, T.P. *Cheese: Chemistry, Physics and Microbiology, General Aspects*; Elsevier: Oxford, UK, 2004; Volume 1.
52. Huard, C.; Miranda, G.; Wessner, F.; Bolotin, A.; Hansen, J.; Foster, S.J.; Chapot-Chartier, M.P. Characterization of AcmB, an N-acetylglucosaminidase autolysin from *Lactococcus lactis*. *Microbiology* **2003**, *149*, 695–705. [CrossRef] [PubMed]

53. Attaie, R.; Richter, R.L. Formation of volatile free fatty acids during ripening of Cheddar-like hard goat cheese. *J. Dairy Sci.* **1996**, *79*, 717–724. [CrossRef]
54. Woo, A.H.; Kollodge, S.; Lindsay, R.C. Quantification of major free fatty acids in several cheese varieties. *J. Dairy Sci.* **1984**, *67*, 874–878. [CrossRef]
55. Miyazaki, M.; Ntambi, J.M. Role of stearoyl-coenzyme A desaturase in lipid metabolism. *Prostaglandins Leukot. Essent. Fat. Acids* **2003**, *68*, 113–121. [CrossRef]
56. Smith, S.B.; Lunt, D.K.; Chung, K.Y.; Choi, C.B.; Tume, R.K.; Zembayashi, M. Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. *Anim. Sci. J.* **2006**, *77*, 478–486. [CrossRef]
57. Bray, T.M.; Bettger, W.J. The physiological role of zinc as an antioxidant. *Free Radic. Biol. Med.* **1990**, *8*, 281–291. [CrossRef]
58. Zimmerman, M.T.; Bayse, C.A.; Ramoutar, R.R.; Brumaghim, J.L. Sulphur and selenium antioxidants: Challenging radical scavenging mechanisms and developing structure-activity relationships based on metal binding. *J. Inorg. Biochem.* **2015**, *145*, 30–40. [CrossRef] [PubMed]
59. Castada, H.Z.; Hanas, K.; Barringer, S.A. Swiss Cheese flavor variability based on correlations of volatile flavor compounds, descriptive sensory attributes, and consumer preference. *Foods* **2019**, *8*, 78. [CrossRef] [PubMed]
60. Thierry, A.; Collins, Y.F.; Mukdsi, M.A.; McSweeney, P.L.; Wilkinson, M.G.; Spinnler, H.E. Lipolysis and metabolism of fatty acids in cheese. In *Cheese*; Academic Press: Burlington, MA, USA, 2017; pp. 423–444.
61. Urbach, G. The chemical and biochemical basis of cheese and milk aroma. In *Microbiology and Biochemistry of Cheese and Fermented Milk*; Springer: Boston, MA, USA, 1997; pp. 253–298.
62. Belviso, S.; Giordano, M.; Dolci, P.; Zeppa, G. Degradation and biosynthesis of terpenoids by lactic acid bacteria isolated from cheese: First evidence. *Dairy Sci. Technol.* **2011**, *91*, 227. [CrossRef]
63. Castellani, F.; Vitali, A.; Bernardi, N.; Marone, E.; Palazzo, F.; Grotta, L.; Martino, G. Dietary supplementation with dried olive pomace in dairy cows modifies the composition of fatty acids and the aromatic profile in milk and related cheese. *J. Dairy Sci.* **2017**, *100*, 8658–8669. [CrossRef] [PubMed]
64. Liu, S.Q.; Holland, R.; Crow, V.L. Esters and their biosynthesis in fermented dairy products: A review. *Int. Dairy J.* **2004**, *14*, 923–945. [CrossRef]
65. Dicke, M.; Gols, R.; Ludeking, D.; Posthumus, M.A. Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *J. Chem. Ecol.* **1999**, *25*, 1907–1922. [CrossRef]
66. Pereira, D.; Valentão, P.; Pereira, J.; Andrade, P. Phenolics: From chemistry to biology. *Molecules* **2009**, *14*, 2202–2211. [CrossRef]
67. Han, J.; Britten, M.; St-Gelais, D.; Champagne, C.P.; Fustier, P.; Salmieri, S.; Lacroix, M. Polyphenolic compounds as functional ingredients in cheese. *Food Chem.* **2011**, *124*, 1589–1594. [CrossRef]
68. Kajikawa, H.; Kudo, H.; Kondo, T.; Jodai, K.; Honda, Y.; Kuwahara, M.; Watanabe, T. Degradation of benzyl ether bonds of lignin by ruminal microbes. *FEMS Microbiol. Lett.* **2000**, *187*, 15–20. [CrossRef]
69. Knudsen, K.E.B. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim. Feed Sci. Technol.* **1997**, *67*, 319–338. [CrossRef]
70. Raes, K.; Balcaen, A.; Dirinck, P.; De Winne, A.; Claeys, E.; Demeyer, D.; De Smet, S. Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Sci.* **2003**, *65*, 1237–1246. [CrossRef]
71. O'Connell, J.E.; Fox, P.F. Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *Int. Dairy J.* **2001**, *11*, 103–120. [CrossRef]
72. Castellani, F.; Bernardi, N.; Vitali, A.; Marone, E.; Grotta, L.; Martino, G. Proteolytic volatile compounds in milk and cheese of cows fed dried olive pomace supplementation. *J. Anim. Feed Sci.* **2018**, *27*, 361–365. [CrossRef]
73. Nozière, P.; Graulet, B.; Lucas, A.; Martin, B.; Grolier, P.; Doreau, M. Carotenoids for ruminants: From forages to dairy products. *Anim. Feed Sci. Technol.* **2006**, *131*, 418–450. [CrossRef]
74. Contarini, G.; Povolo, M.; Leardi, R.; Toppino, P.M. Influence of heat treatment on the volatile compounds of milk. *J. Agric. Food Chem.* **1997**, *45*, 3171–3177. [CrossRef]

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