

## Article

# Effect of New Methods for Inhibiting Malolactic Fermentation on the Analytical and Sensory Parameters of Wines

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**Abstract:** This study focuses on the impact of new methods for inhibiting malolactic fermentation in white wines on their analytical and sensory properties. Enological preparations with different mechanisms of effect were tested: fumaric acid, chitosan, Estaan (a preparation based on tannin inhibition), medium-chain fatty acids (MCFAs), sulphur dioxide and a control variant. Malolactic fermentation (MLF) was also performed. The samples underwent analysis through HPLC (high-performance liquid chromatography) to determine the concentrations of malic and lactic acid, as well as biogenic amines. GC (gas chromatography) analysis was used to monitor volatile substances, alongside sensory evaluation. This study demonstrated a significant influence of individual enological preparations on the aromatic profile of the examined wines. The SO<sub>2</sub> and MCFA variants exhibited the highest concentrations of volatile substances within the esters group, specifically isoamyl acetate, 1-hexyl acetate and phenylethyl acetate. Conversely, the fumaric acid and Estaan variants displayed the lowest concentrations of these esters. The most notable disparities were observed in acetoin concentration, with the MCFA variant exhibiting the lowest values. Additionally, the chitosan variant showed higher concentrations of putrescine and spermidine compared to the MCFA and fumaric acid variants, which presented the lowest levels.

**Keywords:** inhibition; malolactic fermentation; chitosan; fumaric acid; medium-chain fatty acids; tannins



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## 1. Introduction

Malolactic fermentation (MLF) is a biotechnological process performed by lactic acid bacteria, predominantly used in red wines and also in some white wines, to achieve more stable wines with improved sensory profiles [1].

However, MLF can lead to undesirable effects, such as excessive de-acidification, which can result in microbial spoilage [2], the formation of undesirable toxic molecules like biogenic amines or ethyl carbamate [3] and reduced colour intensity (up to 30% in some cases) and stability [4].

In recent years, the loss of freshness due to high pH values and excessive alcohol content has become a characteristic issue in warmer regions. Inhibiting MLF presents an intriguing method to preserve malic acid and retain wine freshness [5].

SO<sub>2</sub> is extensively used in enology to control MLF. However, its conversion into combined sulphites diminishes its long-term effectiveness and can cause health problems for sensitive individuals. Therefore, the industry aims to minimise its content [6].

Various techniques and strategies, including High Hydrostatic Pressure, Ultrasound, Pulsed Electric Fields, Pulsed Light, Ionising Irradiation, e-Beam Irradiation, Ozone and electrolysed water, as well as the addition of lysozyme, chitosan and dimethyl dicarbonate, have been described for use instead of large amounts of SO<sub>2</sub> in wines [7,8].

Lysozyme, at a dose of 500 mg·L<sup>-1</sup>, hydrolyses cell wall peptidoglycan, resulting in cell lysis and death. Its addition can aid in controlling spoilage by lactic acid bacteria

(LAB), reducing acetic acid production and the formation of biogenic amines. However, it is disadvantaged by its precipitation due to red wine phenols, significantly reducing its effectiveness in actual winemaking conditions [9–11].

In wine production, chitosan functions as an antioxidant, aiding in clarification, fining and the chelation of heavy metals [12,13]. It also has the ability to counteract the main spoilage yeast *B. bruxellensis* [14]. Its efficiency remains independent of the chemical features of the beverage, especially within the characteristic pH range of fermented beverages, and it is removed after treatment [14–16]. Chitosan reacts with anionic peptidoglycans in Gram-positive LAB bacteria, while in Gram-negative bacteria, the target is the anionic surface of the lipopolysaccharide [17]. The degree of acetylation of chitosan and the pH of the medium determine the charge density and, consequently, the level of antibacterial activity [18]. Low mechanical resistance, control of pore size and low solubility in neutral pH are among the chitosan-related limitations [19,20].

Fumaric acid, structurally similar to malic acid, acts as a competitive inhibitor for the active site of the malolactic enzyme, which results in the inhibition of MLF at a dose of 400 mg·L<sup>-1</sup>. Thanks to the low pH, it helps the wines to maintain a fresh aroma [21]. It exists as the conjugate acid of fumarate, primarily present in its ionised form at wine pH. Fumarate serves as a significant intermediate in Krebs and urea cycles, potentially absorbed by yeasts and transformed into L-malate. Additionally, it possesses antioxidant and anti-inflammatory properties in its ester forms [22–27].

Medium-chain fatty acids (MCFAs) (C6 to C12) are produced in small amounts by *Saccharomyces cerevisiae* during fermentation [28,29]. Decanoic and dodecanoic acids are the most common fatty acids in wine. At higher concentrations, they exert an inhibitory effect on LAB, with their toxicity increasing at low pH, indicating that the undissociated molecule is the toxic form. This form easily permeates membrane phospholipids, entering the cell through passive diffusion. Portions of these acids may be integrated into the plasma membrane, altering the composition and permeability. At concentrations of 20 mg L<sup>-1</sup> for decanoic acid and 5 mg L<sup>-1</sup> for dodecanoic acid, the ATPase activity of *O. oeni* is reduced by approximately 5% and 42%, respectively. The toxicity of MCFAs escalates in the presence of ethanol [30]. Sensory expression of saturated fatty acids shows an even lower number of points with an increasing number of hours (weeks). Over time, sludge particles, including dead yeast with MCFAs bound, are deposited on the bottom of the vessel, and, thus, the typical sensory expression of these acids fades away. This fact is very positive for winemaking practice, due to the low dosages added, which should not exceed [31,32] 10 mg·L<sup>-1</sup>. In a study by Babikova [31], the negative effect of saturated MCFAs was perceived only at a dose of 30 mg·L<sup>-1</sup> or higher.

Potential alternatives to using SO<sub>2</sub> are phenolic compounds (PCs), natural constituents of grapes and wines. PCs have been reported to inhibit the growth of LAB in a concentration-dependent manner [33–35].

The inhibitory effect of wine polyphenols on LAB has been extensively studied and confirmed for individual compounds against isolated bacteria, primarily *O. oeni* species [36–44]. Consequently, polyphenols have been suggested as a substitute for sulphites in controlling the growth and metabolism of LAB during winemaking. The disadvantage of tannins can be a bitter taste when using higher doses [45,46]. The inhibitory mechanism of polyphenols revolves around their ability to alter the structure of the cell membrane, causing the leakage of bacterial cell components, such as proteins, nucleic acids and inorganic ions [47,48].

Inhibiting MLF and associated undesirable LAB also contributes to reducing the concentration of biogenic amines and carbamate in wines. Even when the formation of biogenic amines is controlled by using selected bacteria strains with low histidine decarboxylase activity [49], most of these toxic compounds are produced during MLF or by uncontrolled bacterial spoilage [50].

This study's novelty lies in comparing the effect of new preparations to inhibit MLF, based on different inhibition mechanisms, fumaric acid, chitosan, Estaan (an oenological preparation based on polyphenols), and MCFA, compared to a control experiment using

SO<sub>2</sub>. The effect on volatile wine compounds was determined using GC, and the impact on sensory properties was assessed via quantitative descriptive analysis (QDA) and sensory analysis. Additionally, concentrations of biogenic amines, malic and lactic acids were determined by HPLC to evaluate the effect on MLF inhibition.

## 2. Materials and Methods

### 2.1. Design of Experiment

The experiment involved Hibernial variety grapes harvested manually on 25 October 2022. After destemming and crushing, the grapes underwent pneumatic pressing. The resulting must received a treatment of 35 mg·L<sup>-1</sup> SO<sub>2</sub> and settled statically for 24 h. From this settling, 400 L of must was obtained and fermented using active dry yeasts (a neutral strain of *Saccharomyces cerevisiae*; Oenoferm® F3, Erbslöh, Geisenheim, Germany). Initial juice parameters were as follows: sugar content—195 g·L<sup>-1</sup>; pH—3.29; TA—8.22 g·L<sup>-1</sup>; YAN—119 mg·L<sup>-1</sup>. Alcoholic fermentation occurred at a temperature range of 16–18 °C for 12 days. Subsequently, the wine was divided into smaller glass vessels (2 × 30 L) and subjected to the respective treatments. Two replicates (2 × 30 L) were prepared for each experimental variant. Experimental variants were as follows: (1) control—without undergoing treatment and MLF; (2) Estaan 4 mL·L<sup>-1</sup>; (3) fumaric acid (Protect F) 0.4 g·L<sup>-1</sup>; (4) MCFA 10 mg·L<sup>-1</sup> + SO<sub>2</sub> 40 mg·L<sup>-1</sup>; (5) control—SO<sub>2</sub> 50 mg·L<sup>-1</sup>; (6) chitosan 150 mg·L<sup>-1</sup>. After four months (8th of March), samples were taken for the needs of chemical and sensory analysis. Some of the wines were also bottled without finalisation, and on 18th of September, one more control chemical analysis was performed.

### 2.2. Determination of Basic Analytical Parameters in Wines

The basic parameters of the resulting wine (alcohol, pH, residual sugar, titratable acidity, malic acid, lactic acid, tartaric acid, acetic acid and glycerol) were determined using an Alpha FTIR analyser (Bruker, Bremen, Germany), employing the attenuated total reflection sampling technique. Prior to the initial measurement, the spectrometer underwent thorough rinsing with deionised water, and the background was established using a blank sample (deionised water). For the analyses, 1 mL samples were extracted with a syringe; 0.5 mL was allocated for system rinsing, while the remaining 0.5 mL was analysed three times. The obtained values were automatically assessed using the OpusWine 2.0. software (Bruker, Bremen, Germany) [51].

### 2.3. Determination of Individual Volatile Compounds by GC

The concentration of individual volatile compounds in the wine was determined via the extraction method using methyl *tert*-butyl ether (MTBE): 20 mL of wine was pipetted into a 25 mL volumetric flask, along with 50 µL of 2-nonanol solution in ethano serving as an internal standard (at a concentration of 400 mg·L<sup>-1</sup>), and 5 mL of a saturated ammonium sulphate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) solution. The contents of the flask were thoroughly mixed; subsequently, 0.75 mL of the extraction solvent (MTBE with an addition of 1% cyclohexane) was added. After further stirring and phase separation, the upper organic layer, along with the resulting emulsion, was transferred to a micro-test tube and centrifuged. The clear organic phase was then dried over anhydrous magnesium sulphate before GCMS analysis. The extraction and subsequent GC analysis were conducted three times. The average values and standard deviations were computed using Excel (2401) and Statistica 10. The determination took place on a Shimadzu gas chromatograph (GC-17A, Kyoto, Japan) equipped with an autosampler (AOC-5000, Shimadzu, Kyoto, Japan) and connected to a QP detector (QP-5050A, Shimadzu, Kyoto, Japan).

Identification was performed using GCsolution software (LabSolutions, version 1.20). The analysis was conducted under the following separation conditions: column: DB-WAX 30 m × 0.25 mm; 0.25 µm stationary-phase polyethylene glycol. The detector voltage was set at 1.5 kV. The sample injection volume was 1 µL, with a split ratio of 1:5. The carrier gas (helium) flow was 1 mL·min<sup>-1</sup> (linear gas velocity 36 cm·s<sup>-1</sup>), and the injection port

temperature was set at 180 °C. The initial column temperature was maintained at 45 °C for 3.5 min, followed by temperature gradients: 75 °C gradient at 6 °C·min<sup>-1</sup>, 126 °C gradient at 3 °C·min<sup>-1</sup>, 190 °C gradient at 4 °C·min<sup>-1</sup> and 250 °C gradient at 5 °C·min<sup>-1</sup>. The final temperature was sustained for 6.5 min. The analysis duration was 60 min. The detector operated in SCAN mode with 0.25 s intervals within a range of 14–264. The identification of individual compounds involved comparing the MS spectrum and retention time with the NIST 107 library [52].

#### 2.4. Determination of Individual Biogenic Amines by HPLC

Biogenic amine analyses were conducted using an ExionLC high-pressure binary gradient HPLC system coupled to a 3200QTrap LC-MSMS/MS detector (AB Sciex, Concord, Vaughan, ON, Canada). The ion source employed was a Turbo V interface equipped with an electrospray ionisation probe. Data processing was carried out using Analyst 1.5.1 software (AB Sciex, Canada). The mobile phase consisted of solvent A (3 mM HCOONH<sub>4</sub> in water) and solvent B (3 mM HCOONH<sub>4</sub> in 95% can). The pH of both mobile phases was adjusted to 2.7 with HCOOH. The flow rate of the mobile phase was 0.5 mL min<sup>-1</sup>. The gradient elution programme was as follows: 0.00 min 100% B; 3.00 min 70% B; 7.00 min 0% B; and 7.10 min 100% B. The sample injection volume was 5 µL, and the separation temperature was set at 40 °C [53]. Wine samples were centrifuged and treated as follows before analysis: 20 µL of 0.2 mM 1,6-diaminohexane solution (internal standard) and 930 µL of dilution buffer (3 mM HCOONH<sub>4</sub> in 80% acetonitrile, pH adjusted to 3.0 with HCOOH) were added to 20 µL of the sample. The treated sample was again centrifuged (3000× g; 6 min) and directly used for LC-MS analysis.

#### 2.5. Sensory Analysis

The sensory evaluation was conducted by a group of seven professional tasters in the sensory analysis laboratory of Mendel University in Brno. The panel of tasters, selected based on interest and availability, comprised seven enology and food science teachers and students, including five males and two females aged 25–40 years. The tasting room was specifically designed to facilitate sensory analyses under controlled conditions, following the guidelines outlined in ISO 8589 standards [54]. The wines were blind tasted in transparent glasses by seven qualified assessors from the Institut national de l'origine et de la qualité (INAO), in accordance with ISO 8586 standards [55]. A quantitative descriptive analysis was conducted to assess parameters, such as intensity of flavour, taste, body and complexity, each rated on a scale of 1–10. Additionally, the wines were evaluated using the International Union of Oenologists (UIOE) 100-point scale system. The resulting scores for each wine were averaged, and graphs were generated to present the findings.

#### 2.6. Statistical Analysis

The data underwent statistical evaluation using Statistica 12 software. ANOVA analysis was applied followed by Fisher's LSD test. Graphs were generated using this software.

### 3. Results and Discussion

#### 3.1. Determination of Basic Analytical Parameters in Wines

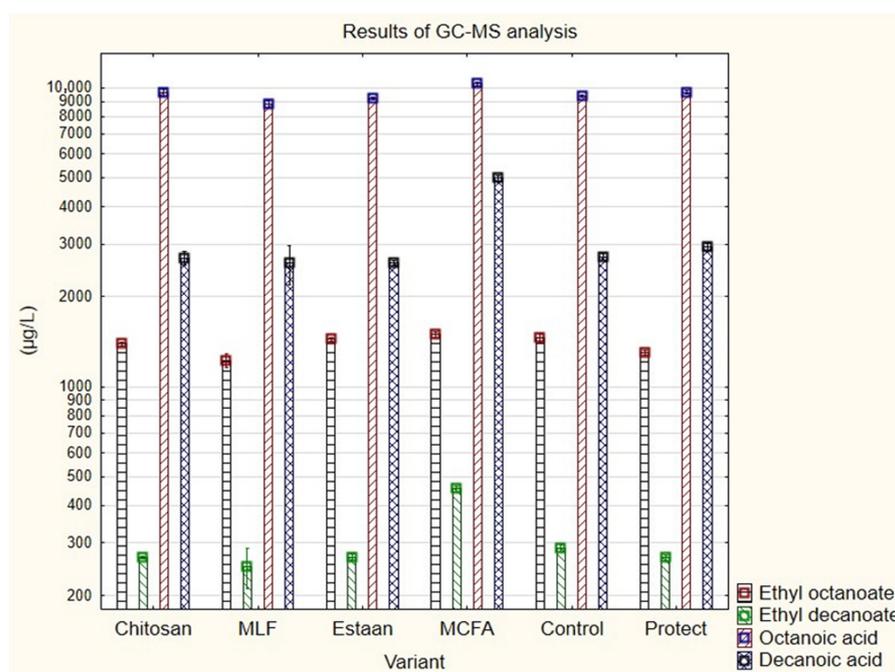
Table 1 displays the results of basic analytical parameters at two time intervals—post-malolactic fermentation (4 months after application) and after wine maturation (10 months after application). The control variant underwent malolactic fermentation. In most preparations, malolactic fermentation inhibition was achieved. The Estaan variant exhibited partial malic acid degradation post-maturation, while the chitosan variant showcased complete degradation, indicating the absence of inhibitory effects.

**Table 1.** Results of basic analytical parameters (mean ± SE). The average values (*n* = 6) are supplemented by the the contribution into homogeneous groups (a, b, c, d, e, f) according to Fisher’s LSD (least significant difference) test ( $\alpha$  = 0.05).

Variant	Term (Months)	Alcohol (% vol.)	Tit. Acids (g·L <sup>-1</sup> )	Res. Sugar (g·L <sup>-1</sup> )	pH	Malic Acid (g·L <sup>-1</sup> )	Lactic Acid (g·L <sup>-1</sup> )	Acetic Acid (g·L <sup>-1</sup> )
MLF	4	11.76 ± 0.28 a	6.13 ± 0.17 b	0.26 ± 0.19 a	3.34 ± 0.02 e	0.35 ± 0.10 a,b	1.18 ± 0.11 e	0.31 ± 0.02 b,c
	10	12.16 ± 0.02 b	6.18 ± 0.03 b	0.32 ± 0.32 a	2.98 ± 0.02 a	0.79 ± 0.18 b	1.32 ± 0.16 e,f	0.25 ± 0.02 a,b
Control	4	12.01 ± 0.06 a,b	6.89 ± 0.04 d,e	0.00 ± 0.00 a	3.26 ± 0.02 d	1.97 ± 0.14 c	0.43 ± 0.12 c	0.25 ± 0.02 a
	10	12.12 ± 0.02 b	6.61 ± 0.1 c,d	1.29 ± 0.47 b	3.14 ± 0.01 b	2.16 ± 0.23 c	0.09 ± 0.06 a,b	0.30 ± 0.02 a,b,c
Estaan	4	12.20 ± 0.04 b	7.18 ± 0.11 f	0.00 ± 0.00 a	3.24 ± 0.01 d	2.18 ± 0.12 c	0.26 ± 0.09 a,b,c	0.26 ± 0.02 a,b
	10	12.19 ± 0.04 b	5.76 ± 0.04 a	1.69 ± 0.60 b,c	3.12 ± 0.07 b	0.61 ± 0.21 a,b	0.90 ± 0.10 d	0.34 ± 0.02 c
MCFA	4	12.08 ± 0.03 b	7.10 ± 0.08 e,f	0.00 ± 0.00 a	3.24 ± 0.02 d	2.23 ± 0.08 c	0.19 ± 0.07 a,b,c	0.29 ± 0.02 a,b,c
	10	12.15 ± 0.04 b	6.60 ± 0.12 c	1.66 ± 0.67 b,c	3.16 ± 0.02 b,c	1.93 ± 0.17 c	0.06 ± 0.06 a	0.30 ± 0.02 a,b,c
Fumaric	4	12.10 ± 0.04 b	7.22 ± 0.12 f	0.12 ± 0.11 a	3.22 ± 0.02 c,d	2.27 ± 0.22 c	0.09 ± 0.05 a,b	0.29 ± 0.03 a,b,c
	10	12.15 ± 0.02 b	6.72 ± 0.11 c,d	2.45 ± 0.39 c	3.12 ± 0.02 b	2.21 ± 0.22 c	0.03 ± 0.03 a	0.32 ± 0.02 c
Chitosan	4	12.08 ± 0.02 b	7.12 ± 0.13 e,f	0.12 ± 0.12 a	3.23 ± 0.02 d	2.01 ± 0.21 c	0.33 ± 0.11 b,c	0.31 ± 0.03 b,c
	10	12.13 ± 0.04 b	6.21 ± 0.06 b	0.00 ± 0.00 a	3.00 ± 0.03 a	0.17 ± 0.07 a	1.53 ± 0.07 f	0.31 ± 0.02 b,c

### 3.2. Determination of Individual Volatile Compounds by GC-MS

Figure 1 shows the concentrations of fatty acid esters and fatty acids (Figure 1). The most statistically significant differences were observed in the concentrations of ethyl decanoate and decanoic acid, which peaked in the MCFA and control variant, while the control variant exhibited the lowest values. Lipases, released from lactic acid bacteria, metabolise lipids, thereby elevating the concentration of volatile fatty acids in wine [56]. Some studies have reported an increase in octanoic acid after malolactic fermentation [57–59]. However, our study did not confirm this, as the control variant undergoing malolactic fermentation displayed the lowest concentration of these fatty acids. Other studies also support this, indicating that malolactic fermentation might reduce octanoic acid in wine [59–61].



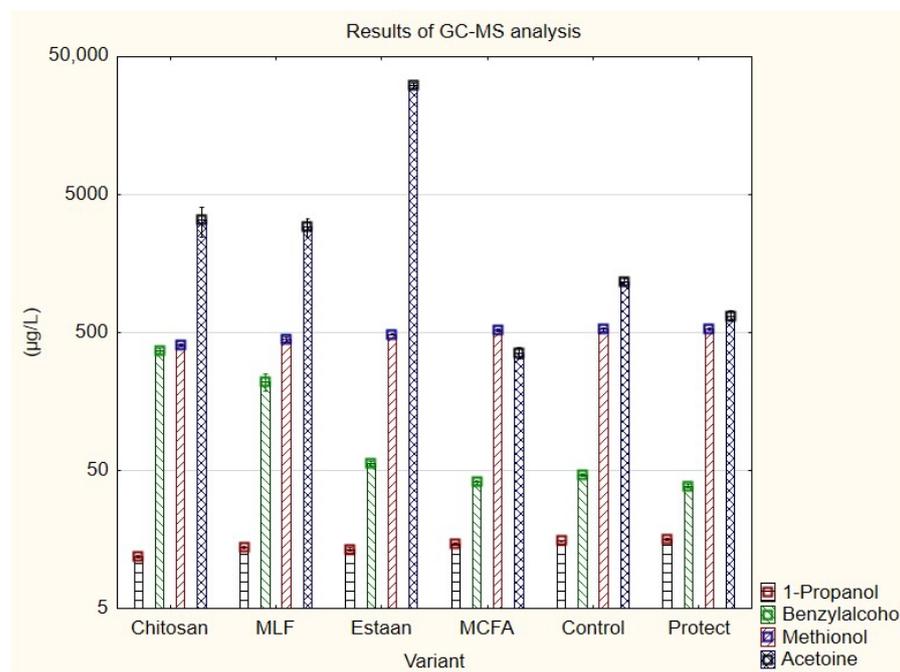
**Figure 1.** Results of GC-MS analysis (ethyl decanoate; ethyl octanoate; octanoic acid; decanoic acid). Detailed results are available in Appendix A (Table A1).

The MCFA preparation comprises octanoic, decanoic and dodecanoic acids in varying proportions. The results from a study [62] reveal that the application of the investigated

mixture of MCFA at doses of 10 and 20 mg·L<sup>-1</sup> did not lead to an increase in the content of individual fatty acids compared to the control variant. The MCFA mixture was applied at doses of 0, 10, 20 and 60 mg·L<sup>-1</sup>. GC-MS determined the content of individual fatty acids. The results showed that the use of the investigated mixture of fatty acids at doses of 10 and 20 mg·L<sup>-1</sup> did not cause an increase in the content of individual fatty acids residues. The octanoic acid content after application of the 20 mg·L<sup>-1</sup> MCFA mixture was 8.24 mg·L<sup>-1</sup> after 744 h, while the untreated control variant showed a value of 7.71 mg·L<sup>-1</sup>. The performed sensory analysis also did not show a negative effect of MCFA application on the sensory properties of wine.

Alcoholic fermentation in wine production plays a crucial role in generating higher alcohols [63]. These alcohols contribute desirable complexity when their concentration is below 300 mg·L<sup>-1</sup>, but if exceeding 400 mg·L<sup>-1</sup>, they can detrimentally affect the wine's aromatic quality, depending on the representation of individual higher alcohols [64].

Figure 2 displays the concentrations of 1-propanol, benzyl alcohol, methionol and acetoin. The variant using fumaric acid (15.9 µg·L<sup>-1</sup>) contained the highest concentration of 1-propanol, while the chitosan variant contained the least (11.8 µg·L<sup>-1</sup>). Significant differences were noted in the concentration of benzyl alcohol, with the highest concentration in the chitosan variant (363 µg·L<sup>-1</sup>) and the control (249 µg·L<sup>-1</sup>), and the lowest concentrations in the Estaan, MCFA, control and fumaric acid variants (38.5–56 µg·L<sup>-1</sup>). Differences in the concentration of volatile substances can be caused by the different activity of b-glucosidases that were released from yeast or lactic acid bacteria. An increased content of benzyl alcohol was noted in samples that undergo MLF, which can increase the content of this compound [65].



**Figure 2.** Results of GC-MS analysis (1-propanol; benzylalcohol; methionol; acetoin). Detailed results are available in Appendix A (Table A1).

Methionol, known to have a negative impact on wine, ranged from 404 to 528 µg·L<sup>-1</sup>. The lowest concentrations were in the chitosan and control variants (404 µg·L<sup>-1</sup> and 434 µg·L<sup>-1</sup>), while the highest were in the MCFA, control and fumaric acid (521–528 µg·L<sup>-1</sup>).

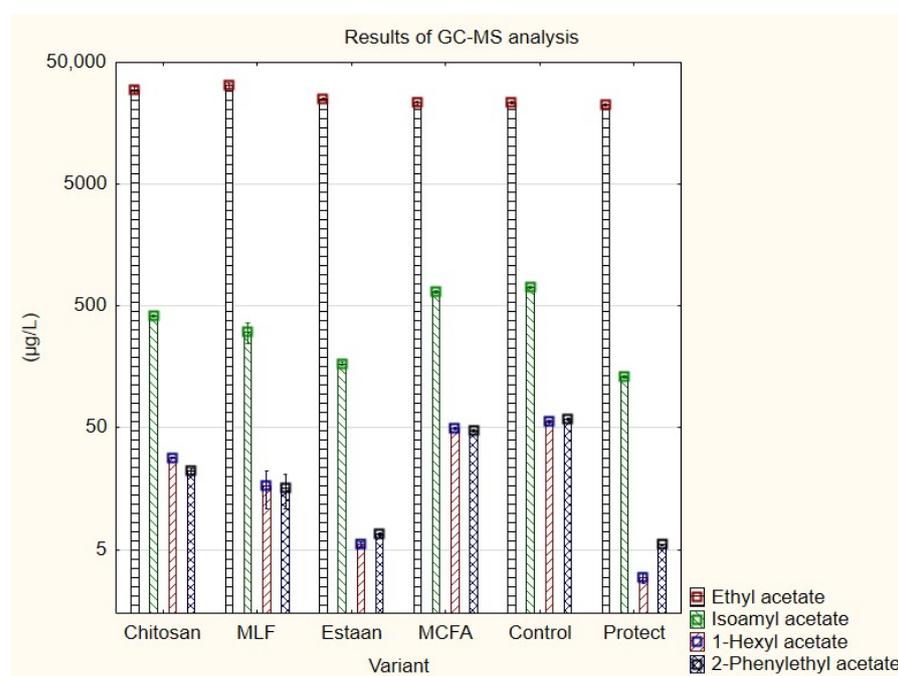
Acetoin, at higher concentrations, negatively affects wine. The variant Estaan contained the highest concentration (30,735 µg·L<sup>-1</sup>), followed by chitosan (3680 µg·L<sup>-1</sup>) and MLF (3315 µg·L<sup>-1</sup>). Lower values were found in the control (1169 µg·L<sup>-1</sup>) and fumaric acid (659 µg·L<sup>-1</sup>) variants, while the variant MCFA had the very lowest concentration at

only  $366 \mu\text{g}\cdot\text{L}^{-1}$ . The low acetoin concentration in the variant MCFA aligns with the results of the study by Licek et al. (2020) [66], indicating that the addition of MCFA results in a lower production of carbonyl compounds such as acetaldehyde or acetoin [66].

MCFA positively affects the reduction in acetoin content, responsible for buttery tones in wine aroma. This compound is formed during alcoholic fermentation [67], but higher concentrations are produced by lactic acid bacteria during malolactic fermentation [68].

According to a study by Licek [66], acetoin levels ( $3.14\text{--}14.25 \text{ mg}\cdot\text{L}^{-1}$ ) for all variants were well below the reported odour threshold of  $150 \text{ mg}\cdot\text{L}^{-1}$ . The perception threshold of acetoin is strongly influenced by the wine style [69]. Nevertheless, reducing acetoin could be another positive effect of the addition of MCFA.

Figure 3 presents the concentrations of ethyl acetate, isoamyl acetate, 1-hexyl acetate and phenylethyl acetate. The MLF ( $33,896 \mu\text{g}\cdot\text{L}^{-1}$ ) and chitosan ( $28,974 \mu\text{g}\cdot\text{L}^{-1}$ ) variants contained the highest concentration of ethyl acetate, while the fumaric acid ( $22,506 \mu\text{g}\cdot\text{L}^{-1}$ ) and MCFA ( $23,012 \mu\text{g}\cdot\text{L}^{-1}$ ) variants showed the lowest concentrations, all exceeding the odour threshold [59] ( $12,300 \mu\text{g}\cdot\text{L}^{-1}$ ). Studies have reported that ethyl acetate exhibits pineapple, fruity, solvent and balsamic scents [70]. Changes in ethyl acetate content can be caused by MLF, depending on the species or strain of lactic bacteria [65].



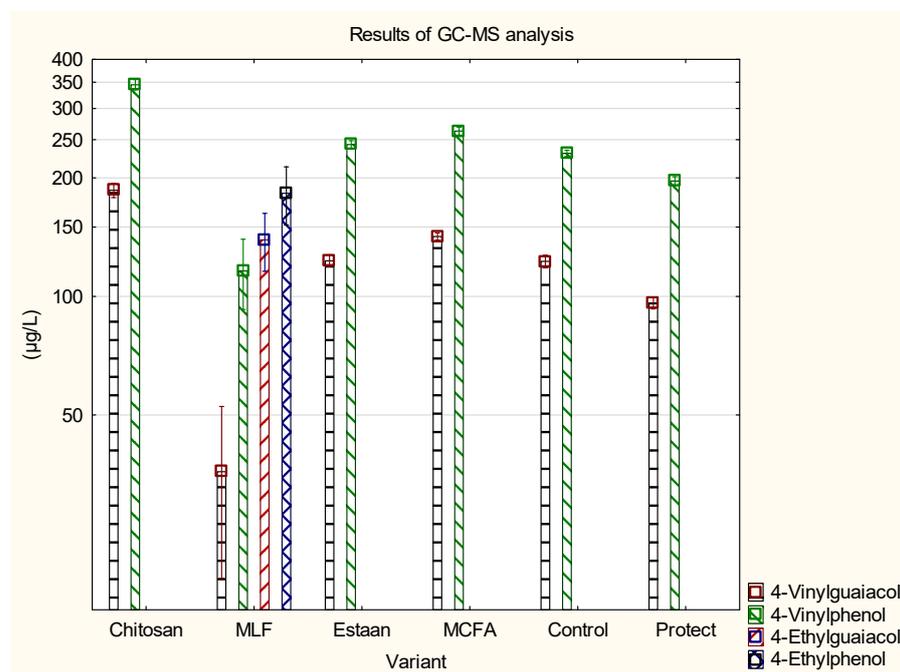
**Figure 3.** Results of GC-MS analysis (ethyl-acetate; isoamyl acetate; 1-hexyl acetate; 2-phenylethylacetate). Detailed results are available in Appendix A (Table A1).

More significant differences were observed for other aromatic substances. Isoamyl acetate, known for its positive sensory effect on wine, was most abundant in the control ( $710 \mu\text{g}\cdot\text{L}^{-1}$ ) and MCFA ( $646 \mu\text{g}\cdot\text{L}^{-1}$ ) variants. In contrast, the MLF ( $249 \mu\text{g}\cdot\text{L}^{-1}$ ) and fumaric acid ( $130 \mu\text{g}\cdot\text{L}^{-1}$ ) variants contained the least, with concentrations below the odour threshold [60] ( $160 \mu\text{g}\cdot\text{L}^{-1}$ ). Similarly, the concentrations of 1-hexyl acetate and phenylethyl acetate were highest in the control and MCFA variants ( $56.1 \mu\text{g}\cdot\text{L}^{-1}$  and  $49.4 \mu\text{g}\cdot\text{L}^{-1}$  for 1-hexylacetate, and  $59.3 \mu\text{g}\cdot\text{L}^{-1}$  and  $47.2 \mu\text{g}\cdot\text{L}^{-1}$  for phenylethyl acetate) and lowest in the fumaric acid variant (only  $2.9 \mu\text{g}\cdot\text{L}^{-1}$  for 1-hexylacetate and  $5.5 \mu\text{g}\cdot\text{L}^{-1}$  for phenylethyl acetate). The measured values suggest that enological preparations like MCFA and control positively affect the content of significant esters, while the fumaric acid and Estaan variants contain the lowest amounts.

In a study by [71], monitoring the effect of different strains of lactic bacteria on aromatic substances, a decrease in most aromatic substances was observed in variants undergoing

malolactic fermentation, indicating that inhibiting malolactic fermentation preserves the content of aromatic substances. This was also confirmed in our study, depending on the inhibitor preparation used. For example, the fumaric acid variant contained lower amounts of isoamyl acetate, 1-hexyl acetate and phenylethyl acetate than the MLF variant, where malolactic fermentation was performed.

Significant differences among variants were also noted in the concentration of volatile phenols (Figure 4). The chitosan variant contained the highest concentration of vinylphenols ( $183 \mu\text{g}\cdot\text{L}^{-1}$  for vinylguaiacol and  $340 \mu\text{g}\cdot\text{L}^{-1}$  for vinylphenol), while the MLF variant exhibited the lowest ( $19.2 \mu\text{g}\cdot\text{L}^{-1}$  for vinylguaiacol and  $93.7 \mu\text{g}\cdot\text{L}^{-1}$  for vinylphenol), and fumaric acid showed  $96.3 \mu\text{g}\cdot\text{L}^{-1}$  for vinylguaiacol and  $196 \mu\text{g}\cdot\text{L}^{-1}$  for vinylphenol. Conversely, regarding ethylphenols, the MLF variant contained the highest concentration ( $162 \mu\text{g}\cdot\text{L}^{-1}$  for ethylguaiacol and  $213 \mu\text{g}\cdot\text{L}^{-1}$  for ethylphenol), while no ethylphenols were found in the other variants.



**Figure 4.** Results of GC-MS analysis (4-vinylguaiacol; 4-vinylphenol; 4-ethylquaiacol; 4-ethylphenol). Detailed results are available in Appendix A (Table A1).

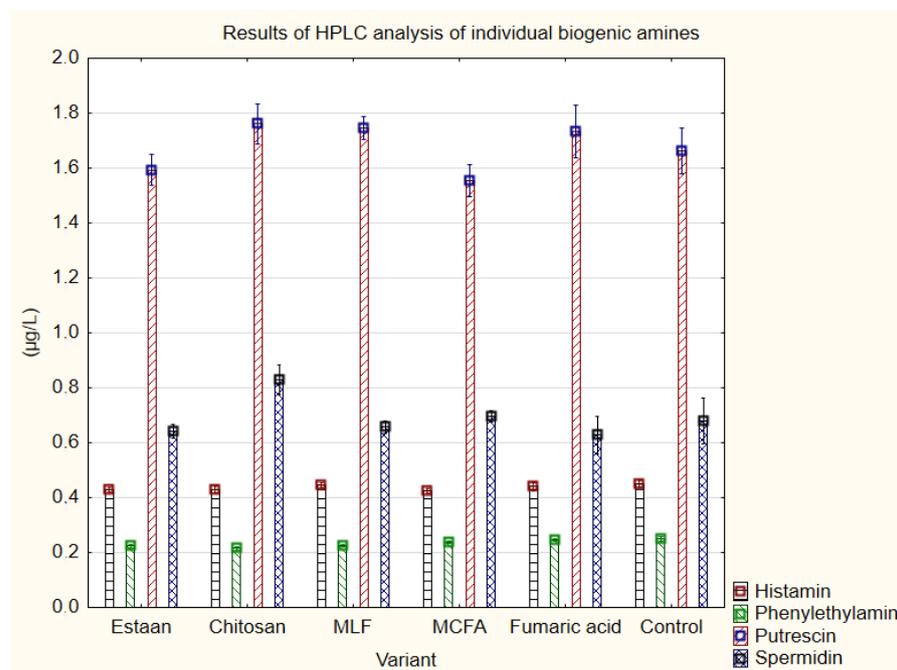
While the formation of vinylphenols might be associated with the activity of *Saccharomyces* yeasts, the creation of ethylphenols is solely attributed to the apiculate yeast *Brettanomyces*. This study's findings suggest that all the preparations employed effectively inhibited these apiculate yeasts, as ethylphenols were solely detected in the control variant [72,73].

The obtained results showcase a significant influence of individual preparations on the aromatic profile of the wine. The control and MCFA variants contained the highest concentrations of volatile substances from the ester group—isoamyl acetate, 1-hexyl acetate and phenylethyl acetate. Conversely, the fumaric acid and Estaan variants displayed the lowest concentrations of these esters. Concerning negative aromatic substances like methionol and acetoin, higher concentrations of methionol were measured in the MCFA, control and fumaric acid variants, while the lowest were found in the control and chitosan variants. The most significant differences were observed in the concentration of acetoin. The Estaan variant exhibited the highest concentration, several times exceeding the acetoin concentration of the other variants, while the MCFA variant showed the lowest values, consistent with previous studies, demonstrating that the addition of MCFA generally reduces the number of carbonyl compounds [62].

### 3.3. Determination of Individual Biogenic Amines by HPLC

Biogenic amines negatively impact wine quality and could potentially pose safety concerns for consumers [74]. Amino acids in wine act as major precursors of biogenic amines. In the presence of lactic acid bacteria, these amino acids can be metabolised to produce biogenic amines [75]. Different lactic acid bacteria species possess varying abilities to metabolise amino acids for biogenic amine production. *O. oeni*, the most commonly used lactic acid bacteria, also exhibits a high capability of producing biogenic amines [76,77].

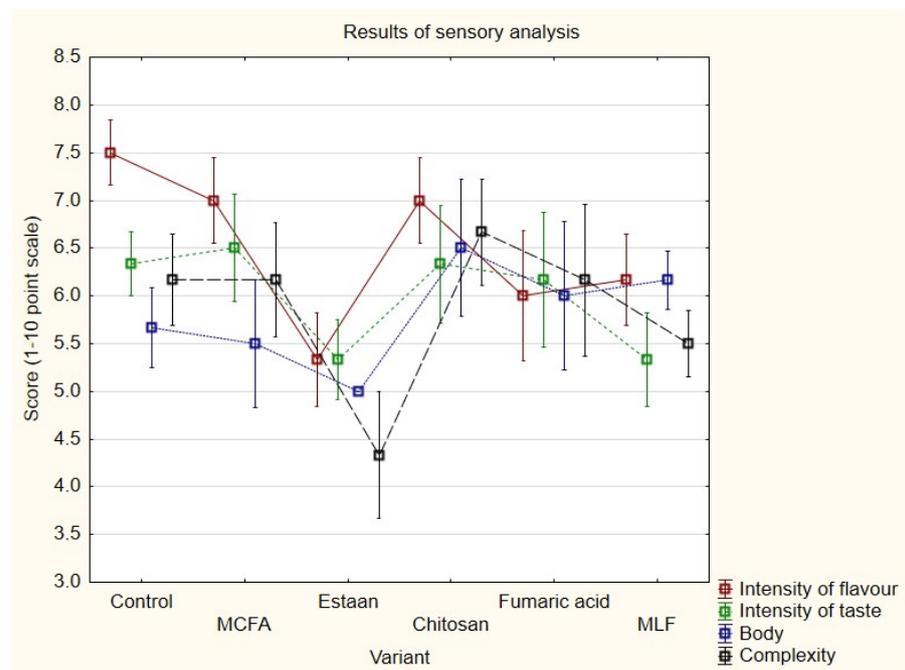
Regarding the effect on the content of biogenic amines, only a very slight, statistically insignificant impact of individual preparations was observed (Figure 5). The preparations had no discernible effect on the histamine content, as the concentration in all samples ranged from 0.43 to 0.45  $\mu\text{g}\cdot\text{L}^{-1}$ . The only slight difference was noted in the concentration of putrescine and spermidine, with their highest concentration measured in the chitosan variant (1.76  $\mu\text{g}\cdot\text{L}^{-1}$  for putrescine and 0.83  $\mu\text{g}\cdot\text{L}^{-1}$  for spermidine). This concentration was higher than that in the MLF variant, where malolactic fermentation took place, and the lowest in MCFA (1.56  $\mu\text{g}\cdot\text{L}^{-1}$  for putrescine) and fumaric acid (0.63  $\mu\text{g}\cdot\text{L}^{-1}$  for spermidine). Since the formation of biogenic amines is mainly caused by lactic bacteria, these differences may result from different mechanisms of action of individual oenological preparations on the microflora of the investigated wines [75].



**Figure 5.** Results of HPLC analysis of individual biogenic amines. Detailed results are available in Appendix A (Table A2).

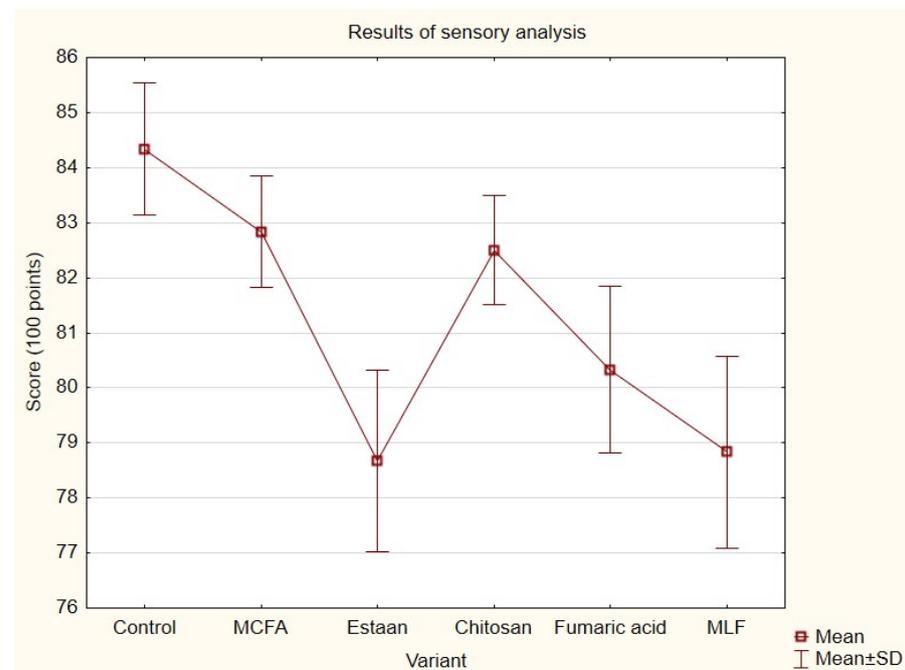
### 3.4. Sensory Analysis

Figure 6 displays the results of individual sensory characteristics, such as flavour intensity, taste, body and complexity. The Estaan variant exhibited the lowest score in all sensory characteristics. Flavour intensity was higher in the control, MCFA and chitosan variants, while it was lowest in Estaan, fumaric acid and the MLF variant. Taste intensity showed no statistically significant differences, except for the Estaan and control variants. The results for body and complexity did not show statistically significant differences between individual variants.



**Figure 6.** Results of sensory analysis (profile of wine structure). Detailed results are available in Appendix A (Table A3).

Figure 7 illustrates the results on a 100-point scale of sensory analysis. The best results were obtained in the control, MCFA and chitosan variants. These results corresponded to the results of the aromatic profile measured by gas chromatography, where the control and MCFA variants exhibited the highest concentrations of positive volatile compounds such as esters.



**Figure 7.** Results of sensory analysis (100-point evaluation). Detailed results are available in Appendix A (Table A3).

#### 4. Conclusions

This study compared the influence of individual preparations recently used to inhibit malolactic fermentation to reduce the use of SO<sub>2</sub>. All oenological preparations used to inhibit malolactic fermentation demonstrated an inhibitory effect against lactic bacteria, as measured by FTIR analysis. However, after six months of wine aging, it was observed that the Estaan and chitosan variants underwent partial to complete malic acid degradation, indicating that their inhibitory effect was not permanent.

A significant effect on the content of aromatic substances in the final wine was also demonstrated. The highest ester content was determined in the control and MCFA variants, while the highest 1-propanol content was found in the fumaric acid variant, and the highest benzyl alcohol content was noted in the chitosan variant. The most significant differences were observed in the concentration of acetoin, which negatively affects wine at higher concentrations. The concentration was lowest in the MCFA variant, while in the other variants, the concentration was several-times higher but still below the sensory detection threshold.

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#### Appendix A

**Table A1.** Detailed results of volatile compound content measured by GC-MS.

Compound (µg·L <sup>-1</sup> )	Chitosan (Mean ± SE)	MLF (Mean ± SE)	Estaan (Mean ± SE)	MCFA (Mean ± SE)	Control (Mean ± SE)	Fumaric a. (Mean ± SE)	p-Value
1-Propanol	11.8 ± 0.2 a	13.7 ± 0.4 b	13.4 ± 0.3 b	14.7 ± 0.2 c	15.5 ± 0.1 c	15.9 ± 0.3 d	p = 0.0000
Benzylalcohol	363 ± 15 c	249 ± 11 b	56 ± 2 a	41.5 ± 1.1 a	46.6 ± 1.1 a	38.5 ± 1.2 a	p = 0.0000
Methionol	404 ± 11 a	434 ± 10 a	486 ± 8 b	521 ± 11 c	528 ± 16 c	528 ± 12 c	p = 0.0000
Ethyl acetate	28,974 ± 2273 b	33,896 ± 671 c	24,921 ± 521 a	23,012 ± 581 a	23 298 ± 439 a	22,506 ± 441 a	p = 0.0000
Isoamyl acetate	415 ± 15 c	249 ± 28 b	168 ± 7 a	646 ± 12 d	710 ± 13 e	130 ± 2 a	p = 0.0000
1-Hexyl acetate	28.2 ± 0.4 c	11.3 ± 2.2 b	5.6 ± 0.2 a	49.4 ± 0.7 d	56.1 ± 0.8 e	2.9 ± 0.3 a	p = 0.0000
2-Phenylethyl acetate	21.5 ± 1.2 c	10.9 ± 1.6 b	6.7 ± 0.2 a	47.2 ± 0.8 d	59.3 ± 1.3 e	5.5 ± 0.1 a	p = 0.0000
Ethyl octanoate	1390 ± 19 c	1168 ± 32 a	1441 ± 21 c,d	1505 ± 27 d	1458 ± 45 c,d	1300 ± 22 b	p = 0.0000
Ethyl decanoate	268 ± 3 b	212 ± 3 a	269 ± 5 b	459 ± 9 d	288 ± 8 c	268 ± 6 b	p = 0.0000
Octanoic acid	9522 ± 298 b,c	8579 ± 110 a	9215 ± 98 b	10,277 ± 116 d	9374 ± 101 b,c	9659 ± 130 c	p = 0.0000
Decanoic acid	2637 ± 151 b	2197 ± 38 a	2588 ± 42 b	4965 ± 97 d	2716 ± 61 b,c	2924 ± 88 c	p = 0.0000
4-Vinylguaiaicol	183 ± 9 e	19.2 ± 0.9 a	123 ± 4 c	141 ± 3 d	123 ± 5 c	96.3 ± 3.4 b	p = 0.0000
4-Vinylphenol	340 ± 11 e	93.7 ± 8.3 a	243 ± 6 c,d	261 ± 5 d	231 ± 4 c	196 ± 5 b	p = 0.0000
4-Ethylguaiaicol	0 ± 0 a	162 ± 3 b	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	p = 0.0000
4-Ethylphenol	0 ± 0 a	213 ± 5 b	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	p = 0.0000
Acetoin	3680 ± 842 c	3315 ± 211 b,c	30,735 ± 1699 d	366 ± 29 a	1169 ± 74 a,b	659 ± 57 a	p = 0.0000

The average values (n = 6) are supplemented by the ANOVA results (p-value) and the contribution into homogeneous groups (a, b, c, d, e) according to Fisher’s LSD (least significant difference) test (α = 0.05).

**Table A2.** Detailed results of individual biogenic amines content measured by HPLC.

Compound (µg·L <sup>-1</sup> )	Estaan (Mean ± SE)	Chitosan (Mean ± SE)	MLF (Mean ± SE)	MCFA (Mean ± SE)	Control (Mean ± SE)	Fumaric a. (Mean ± SE)	p-Value
Histamin	0.43 ± 0.01 a	0.43 ± 0.01 a	0.44 ± 0.01 a	0.42 ± 0.01 a	0.45 ± 0.01 a	0.44 ± 0.01 a	p = 0.5263
Phenylethylamin	0.22 ± 0.01 a,b	0.22 ± 0.01 a	0.23 ± 0.00 a,b	0.24 ± 0.01 b,c	0.25 ± 0.01 c	0.25 ± 0.00 c	p = 0.0002
Putrescin	1.59 ± 0.06 a,b	1.76 ± 0.07 b	1.75 ± 0.04 a,b	1.56 ± 0.06 a	1.66 ± 0.08 a,b	1.73 ± 0.10 a,b	p = 0.2200
Spermidin	0.64 ± 0.03 a	0.83 ± 0.05 b	0.66 ± 0.02 a	0.70 ± 0.02 a,b	0.68 ± 0.08 a	0.63 ± 0.07 a	p = 0.1061

The average values (n = 6) are supplemented by the ANOVA results (p-value) and the contribution into homogeneous groups (a, b, c) according to Fisher’s LSD (least significant difference) test (α = 0.05).

**Table A3.** Detailed results of sensory analysis results.

Category	Control (Mean ± SE)	MCFA (Mean ± SE)	Estaan (Mean ± SE)	Chitosan (Mean ± SE)	Fumaric a. (Mean ± SE)	MLF (Mean ± SE)	p-Value
Score (100 points)	84.3 ± 1.2 c	82.8 ± 1.0 b,c	78.7 ± 1.6 a	82.5 ± 1.0 a,b,c	80.3 ± 1.5 a,b	78.8 ± 1.7 a	0.034
Intensity of flavour	7.5 ± 0.3 c	7.0 ± 0.4 b,c	5.3 ± 0.5 a	7.0 ± 0.4 b,c	6.0 ± 0.7 a,c	6.2 ± 0.5 a,b,c	0.042
Intensity of taste	6.3 ± 0.3 a	6.5 ± 0.6 a	5.3 ± 0.4 a	6.3 ± 0.6 a	6.2 ± 0.7 a	5.3 ± 0.5 a	0.453
Body	5.7 ± 0.4 a	5.5 ± 0.7 a	5.0 ± 0.0 a	6.5 ± 0.7 a	6.0 ± 0.8 a	6.2 ± 0.3 a	0.487
Complexity	6.2 ± 0.5 b	6.2 ± 0.6 b	4.3 ± 0.7 a	6.7 ± 0.6 b	6.2 ± 0.8 b	5.5 ± 0.3 a,b	0.116

The average values (n = 7) are supplemented by the ANOVA results (p-value) and the contribution into homogeneous groups (a, b, c) according to Fisher’s LSD (least significant difference) test (α = 0.05).

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